

University of Technology Sydney

**The spread of the native macroalga *Caulerpa filiformis***

**By**

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the degree of Doctor of Philosophy

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**CERTIFICATE OF ORIGINAL AUTHORSHIP**

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## **Preface**

This thesis consists of six chapters. Chapters 2 to 5 have been written as separate articles that have either been published, are in review or are in preparation for submission to peer reviewed scientific journals. These papers are included as or close to their published or submitted form, and as a result, some repetition occurs. To prevent unnecessary duplication, with the exception of Chapter 2, a single reference list has been provided at the end of this thesis.

Chapter 2 contributed to an article that has been published in *Marine and Freshwater Research* 66(11) 1018-1026 (2015). This article is inserted in the thesis in the same format as how it has been published. The rest of this work is in submission to *Marine and Environmental Research*.

The second study of this chapter, “Habitat associations of a range expanding native alga across its geographic range”, has been submitted to *Marine Environmental Research*. Authors of this manuscript are Sofie Voerman, Tim Glasby, William Gladstone and Paul Gribben. S. Voerman conducted all the experimental work and analysis and wrote the manuscript, T. Glasby, W. Gladstone and P. Gribben provided conceptual advice and guidance. Chapters 3-5 are in preparation for submission to scientific journals. Author contributions are as described above. Chapter 2 received contribution from Sam Burrell, who helped with the laboratory work.



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## Abstract

Marine coastal systems are threatened by a wide range of anthropogenic pressures. Of particular concern are increased sediment and nutrient runoff. Moreover, hotspots of anthropogenic pressures are also where invasive species tend to be. The spread of invaders can directly translate to a loss of biodiversity and associated ecosystem services. There is a growing awareness that the spread of native species can have similar large impacts on biodiversity and ecosystem function to their famous non-native counterpart. Yet the how, when and why a native species transitions to a ‘native-invader’ (*sensu* Simberloff and Rejmánek 2010) are poorly known. This is especially true in the marine environment. In New South Wales (NSW), eastern Australia, a native green macroalga from the notorious *Caulerpa* genus, *C. filiformis*, has spread both inside and outside its native range.

This study investigated the mechanisms that may have promoted the success of *C. filiformis*. The main aims were to increase our understanding on why this species has become so abundant, the habitat associations of the alga across large and local spatial scales, and the potential impacts on the seaweed community. In Chapter 2, large scale surveys showed that adult population abundance was related to sedimentation at multiple spatial scales, such as Reef Beach Index (RBI), reef width and the presence of a sediment veneer on top of the rocky reef (r+s habitat). In contrast, a low association with turf habitat was found, which is opposite to the observations for several other

*Caulerpa* invaders. A model was proposed where the adult association with r+s represents an end stage of succession after outcompeting turfing alga. Moreover, it was shown that the seaweed community showed reduced morpho-functional richness in presence of *C. filiformis*. To test the proposed model, habitat association of the recruitment stage was investigated in Chapter 3. Surveys at several locations throughout the alga's distribution showed that *C. filiformis*' recruit abundance was found to be high on turfing algae habitat and not on r+s habitat. This positive association of the recruitment stage was observed for coralline forms of turfing alga, but not for filamentous forms. Those results support the model hypothesized. Additionally, the mechanisms behind this positive association were further explored in this chapter. A laboratory experiment revealed that the high association of *C. filiformis*' recruits with coralline alga was explained by the increased attachment performance of *C. filiformis* fragments on this substrate. Namely, the increased structural complexity of coralline alga aided in rapid and strong attachment of fragments.

Finally, mechanisms that may benefit post-recruitment processes of *C. filiformis*, and in particular the role of turf habitat, were investigated. With the use of long term field observations in Chapter 5, it was shown that established *Caulerpa* appears a poor competitor, and possibly requires further disturbance to spread. For example, a laboratory experiment showed that *C. filiformis* is highly tolerant to large sedimentation rates, aided by rapid morphological plasticity (Chapter 4). This may benefit the alga indirectly if sediment more negatively affects competitors. Moreover, increased

sediment-nutrient availability was shown to promote growth rates, benefitting the alga's competitive strength (Chapter 5).

Overall, my thesis indicates that disturbance to native macrophytes indirectly promotes recruitment success by promoting turf habitat and that its continued spread is reliant on further disturbance to native communities – a model that has been demonstrated to invasive marine algae but not previously for a rapidly spreading native alga.





# **Chapter 1**

## **General introduction**



## 1.1 Research scope

Marine coastal environments are diverse and complex ecosystems involving a range of abiotic and biotic interactions. They provide many valuable ecosystem services for humans such as coastal protection and food resources (Barbier et al., 2011). Ecosystems are threatened worldwide by a variety of stressors such as climate change, habitat destruction, pollution, unsustainable fisheries and invasive species (Millennium Ecosystem Assessment, 2005). A large share of the world's population lives in coastal regions, often centred around estuaries, with population densities reaching 3 times more than the global average (Small and Nicholls, 2003, Bongaarts, 2007, McGranahan et al., 2007). High population density and associated coastal development and agriculture come with added large environmental pressures on those shallow coastal systems (e.g. sediment and nutrient runoff) (Halpern et al., 2007, Halpern et al., 2008, Worm and Lenihan, 2013). These hotspots of environmental pressures are also at greater risk from invasive species (e.g. Bulleri et al., 2011, Tamburello et al., 2014, Occhipinti-Ambrogi and Savini, 2003).

The spread of invaders can directly translate in a loss of biodiversity and associated ecosystem services (Bax et al., 2003). However, there is a growing awareness that the spread of native species can have similar impacts on biodiversity and ecosystem function as their more well-known non-native counterpart. Yet how and when a native species transitions to a 'native-invader' (*sensu* Simberloff and Rejmánek 2010) are poorly known, although their spread is often associated with altered disturbance regimes

(Simberloff et al., 2012). In this thesis, I explore the pre- and post-settlement processes that may influence the apparent rapid spread of the native alga *Caulerpa filiformis* and the potential consequences of its spread on the coastline of New South Wales (NSW), Australia.

For more than 40 years coastal ecosystems have been a focus for studies testing the effects of disturbance (natural and anthropogenic) on biotic interactions (e.g. predation, competition, facilitation) and succession of communities (e.g. Levin and Paine, 1974, McGuinness and McGuinness, 1987, Toohey et al., 2007, Dayton et al., 1984, Sousa, 1979, Connell, 1978). This is due, in part, to these ecosystems being amenable to experimental work. The great deal of ecological knowledge provides for an ideal system in which to research the mechanisms behind the success of introduced invasive species and their associated impacts (e.g. Arenas et al., 2006, Bando, 2006, Britton-Simmons, 2006, Carlton, 1996, Dunstan and Johnson, 2004, Nyberg and Wallentinus, 2005, Occhipinti-Ambrogi and Savini, 2003, Stachowicz and Byrnes, 2006, Stachowicz et al., 1999). The vast knowledge on how species dominance may change spatially and temporally with altered disturbance regimes, and what we have learned subsequently about mechanisms and traits benefitting non-natives, may also be applied to native species that show invasive characteristics.

The impacts of altered disturbance regimes are not always easy to predict and often species shifts are only noticed after they have started to occur (Hughes et al., 2013). Ecosystem shifts can be sudden and dramatic and linked to an equally sudden and dramatic disturbance such as a heatwave (Smale and Wernberg, 2013, Voerman et al.,

2013). However, in many other cases change may be slow and easy to miss, i.e. they suffer from a shifting baseline syndrome (Hughes et al., 2013, Dayton et al., 1998). This may especially be true for the often largely invisible marine environment (Pauly, 1995), and we must be careful to not assume the current state of a species' distribution and abundance is the norm (Pauly, 1995, Connell et al., 2008).

In formulating hypotheses about the mechanisms promoting the spread of native species, it is informative to first examine existing models of how novel or previously subdominant species spread and become dominant; most commonly studied in this context are non-native invasive species. The following sections first explore the scientific interest in invasive species and the concept of native invaders is presented, then the major knowledge gaps are identified. The next sections outline important processes driving non-native invasive species' distribution, abundance and impacts, and draw parallels to what may be expected for rapidly spreading native species. In particular, the roles of environment, species interactions, disturbance and invader traits in invasion success are reviewed. Finally, I introduce the spreading native macroalga *Caulerpa filiformis* and outline hypotheses regarding this species' spread.

## **1.2 Native species as invaders**

### **1.2.1. Invaders impacts.**

The spread of invasive plants or algae is recognized as a leading threat to freshwater, marine, and terrestrial systems (Pimentel et al., 2001, Tegner et al., 1996, Bax et al., 2003, Thomsen et al., 2016). Globally, the impacts and control of invasive species cost trillions of dollars annually (Olson, 2006, Lovell et al., 2006). Thus understanding the spread of invaders and the associated impacts has become a major focus of current ecology (Pimentel et al., 2001, Richardson, 2011), with a strong rise in the research output over the past 50 years (Richardson, 2011, Richardson and Ricciardi, 2013). Marine systems are one of the most heavily invaded systems on Earth (Richardson, 2011, Carlton, 1996), and invasive macroalgae have been well studied in this regard (e.g. Williams and Smith, 2007, Boudouresque and Verlaque, 2002, Nyberg and Wallentinus, 2005, Thomsen et al., 2016, Schaffelke and Hewitt, 2007, Schaffelke et al., 2006, Maggi et al., 2015). Invasive marine macroalgae can negatively impact native macroalgal communities by e.g. competition and habitat alteration (e.g. Tamburello et al., 2015, Bulleri et al., 2010), which in turn can have cascading effects on the rest of the community (e.g. Levin et al., 2002, Janiak and Whitlatch, 2012, Vázquez-Luis et al., 2008).

### **1.2.2 Native invaders, their impacts and research gaps**

Similar to non-native invaders, native species are increasing in local abundance and expanding their geographic or habitat range dramatically with impacts rivalling those of invasive species (Simberloff and Rejmánek, 2011, Carey et al., 2012, Valery et al., 2009). Such species have been referred to as native invaders (Simberloff and Rejmánek, 2011). Although not a new phenomenon (see Simberloff and Rejmánek, 2011 and references therein), native invasion is expected to increase with increasing anthropogenic pressures on our ecosystems (Davis et al., 2011). Hence it is increasingly important to improve understanding of the mechanisms underlying a native species increasing dominance and the associated impacts (Carey et al., 2012). Despite extensive anthropogenic disturbances to marine environments and the well-known impacts of invasive macroalgae, we often lack a good understanding of why, when, where, and how a native species transitions from being previously subdominant to dominant (van Kleunen et al., 2011, Carey et al., 2012), although the answers to these questions may well be similar to those found for non-native invasive species.

### **1.2.3 Invasive species definition and the inclusion of native-invaders**

The increasingly complicated web of invasion nomenclature is widely acknowledged (Lockwood et al., 2013). Numerous attempts have been made to clarify the invasion terminology and define “invasive species”, which are summarized by Valery et al. (2008) (see also Lockwood et al., 2013 for an overview). Valery et al (2008) grouped those definitions according to an invaders’ impact or its origin, but found problems with

each of those classifications (Valéry et al., 2013, Valéry et al., 2008). In essence, the authors argued, a biological invasion can be observed as a state of dominance of a single species (the invader) after its rapid expansion (Valéry et al., 2013). In the search for a definition of biological invasions that did not exclude species on their origin or impact, Valéry and colleagues (2008) formulated the following definition that is also followed in this thesis:

*“A biological invasion consists of a species’ acquiring a competitive advantage following the disappearance of natural obstacles to its proliferation, which allows it to spread rapidly and to conquer novel areas within recipient ecosystems in which it becomes a dominant population”*

Here, a novel ecosystem can also refer to a change in the historical settings of an ecosystem (e.g. an alteration of disturbance regime) that may allow for species to spread within their native ranges (i.e. native-invaders).

The observation of species dominance is equal for both native and non-native invaders, although they differ in their distribution origin (i.e. foreign or native) in relation to the invaded range (Simberloff and Vitule, 2014). Certain limiting factors that apply to non-native species, such as transport of propagules and successful settlement within a new area, do therefore not apply to native invaders (Colautti and MacIsaac, 2004). However, once non-native species have established in a new area (i.e. formed a viable, self-sustaining population), processes that determine a species’ distribution and abundance



are conceptually similar for both native and nonindigenous species (Colautti and MacIsaac, 2004).

### **1.3 Mechanisms underpinning the spread of invasive species**

Commonly recognized is the role of different invasion stages in explaining an invader's success, with each stage being equally important (e.g. Blackburn et al., 2011, Richardson et al., 2000, Colautti and MacIsaac, 2004). For example, species' abundance at a site depends on the successful establishment of an invader, which depends on successful recruitment and post-recruitment spread. Both are determined by a range of different abiotic and biotic mechanisms (Colautti and MacIsaac, 2004).

#### **1.3.1 Recruitment processes**

Once established at a site, the recruitment success of invasive species is often linked to propagule supply. For example, Wright and Davis (2006) demonstrated a higher biomass of the invasive *C. taxifolia* six months after fragment supply was experimentally enhanced. Successful recruitment of a species may also depend on the traits of its propagules, such as their size or morphology. This has been well established in the terrestrial literature for both invasive and non-invasive species (e.g. Kimmerer, 1991, Weis, 1982, Stanton, 1984, Pyšek and Richardson, 2008), and similar

observations have been made for seaweed propagules (e.g. Khou et al., 2007, Watanabe et al., 2009, Vermeij et al., 2009).

In addition, abiotic habitat features may alter, positively or negatively, recruitment success. For example, the attachment strength of propagules may differ greatly between soft or hard substrata (Lüning, 1990, Thomsen et al., 2004). Biotic interactions often also play an important role in recruitment success. For example, intact native canopies can restrict the recruitment of invasive algae (Davies et al., 2010, Arenas et al., 2006, Ceccherelli et al., 2002, Britton-Simmons, 2006, Bulleri et al., 2016b, Vermeij et al., 2009). While disturbance (i.e. removal) of native macroalgal canopies by biotic processes (e.g. grazing, disease, predation or competition) or abiotic factors (e.g. storms, desiccation, pollution) may open up space allowing for invader recruitment (Thompson and Schiel, 2012b, Valentine and Johnson, 2003, Vadas et al., 1992).

In other cases, biotic assemblages may facilitate invasion. For example, algal turfing species can increase recruitment success of invasive macrophytes as has been demonstrated for *Undaria pinatifida*, several *Caulerpa* species and *Codium fragile* spp. *Fragile*. It is believed that their complex three-dimensional structure benefits propagule entrapment and attachment of recruits (Thompson and Schiel, 2012b, Watanabe et al., 2009, Bulleri and Benedetti-Cecchi, 2008, Ceccherelli et al., 2002), although studies to confirm this theory are still missing.

### 1.3.2 Post-recruitment processes

Post recruitment spread is similar to recruitment success a function of biotic and abiotic processes. Distributions of native or invasive seaweeds are strongly determined by abiotic conditions acting at multiple spatial scales. At large spatial scales (i.e. kilometres) variation in sea surface temperature can be important, whilst at a local scale (hundreds of metres) suitable substrate (e.g. rock vs soft sediment) is important for shaping boundaries in macroalgal distributions (Lüning, 1990, Infantes et al., 2011). At a smaller scales (m), abundance may be determined by e.g. depth, changing surface slope or rugosity (Toohey, 2007, Bischof et al., 1998, Somsueb et al., 2001, Fowler-Walker and Connell, 2007, Toohey et al., 2007, Harlin and Lindbergh, Korpinen et al., 2007).

Invasive macroalgae often occur over broad latitudinal ranges that cover large environmental changes. Their survival across those wide ranges appears linked to their high tolerance to environmental variation occurring at multiple spatial scales (Nyberg and Wallentinus, 2005). For many marine invaders, the ability to tolerate a broad spectrum of environmental conditions is often linked to high morphological plasticity (Smith, 2009, Stewart, 2008). Although examples of morphological plasticity of macroalga in their non-native distributions are not well studied (Peteiro and Freire, 2014, van Kleunen et al., 2011b), similar observations have been made for several other non-invasive species (e.g. Arenas et al., 2002, Monro et al., 2007, Norton et al., 1981,

Charrier et al., Yñiguez et al., 2010, Kaandorp and Kübler, 2001). Thus we may also expect rapidly spreading native species to exhibit similar high morphological plasticity.

Spreading invaders may encounter several native competitors that may vary in the strength of their interaction. Those biotic interactions with native assemblages may be especially strong for vegetatively expanding invaders, in which horizontal spread directly depends on competition with neighbours (Minchinton and Bertness, 2003, Amsberry et al., 2000). Biotic resistance to invasion has largely focussed on the role of high biodiversity in preventing the recruitment of invasive plants (e.g. Rejmánek, 1996, Stachowicz et al., 2002, Stachowicz et al., 1999, Kennedy et al., 2002). However, increasingly recognized is the role of individual species or functional groups in controlling the spread of invasive species (Davies et al., 2010, Arenas et al., 2006, Ceccherelli et al., 2002, Britton-Simmons, 2006, Bulleri et al., 2016b). Additionally, in certain cases, the loss of native macroalgal canopies may promote alternative native habitats that may promote invader post-recruitment spread. For example, the loss of otherwise resisting canopies and subsequent dominance of turfing alga promoted the horizontal expansion of *Caulerpa cylindracea* (Gennaro and Piazzini, 2014). And similar observations of turfing alga promoting horizontal spread of invasive *Caulerpa* spp. have been made by other authors (Bulleri and Benedetti-Cecchi, 2008, Bulleri et al., 2009). Whether a species may expand into neighbouring habitat will depend on the competitive dominance of the invader (Glasby, 2013), which may change as a function of neighbouring assemblage.

Those post-recruitment native/invasive algal interactions are also strongly mediated by disturbance regimes (e.g. Valentine and Johnson, 2003, Bertocci et al., 2015, Vaz-Pinto et al., 2013, Tamburello et al., 2015). Disturbance may affect the invader directly by altering its competitive strength. For example, Lapointe and Bedford (2011) demonstrated that the abundance of the non-native *Acanthophora spicifera* was enhanced with proximity to a stormwater outfall due to increased competitive advantage of the invader over native species, mediated by the increased nutrient availability. Alternatively, disturbance may affect the invader indirectly by removing competitors completely or by altering the outcome of competitive interactions (e.g. Gennaro and Piazzì, 2014).

Disturbance regimes can affect biotic relationships across multiple spatial scales, and thus may influence patterns of invader abundance over similar scales. For example, in the Mediterranean large scale distribution of the invasive *Caulerpa cylindracea* is positively associated with urbanization caused by reduced resistance of the degraded seaweed assemblages close to urban areas. And at a local scale the species was more abundant on degraded habitat (seagrass dead matte) (Bulleri et al., 2011). Other examples of disturbance regimes that may vary across larger spatial scales are sources of sediment and nutrient runoff associated with agriculture and urban development (Gorman et al., 2009). At a local (within site) scale different rates of sedimentation associated to altered hydrodynamics may alter the invaders success for example (Díaz-Tapia et al., 2013).

Finally, the spread of the invader may in turn affect the abundance and composition of recipient communities. Salvaterra et al. (2013) demonstrated that invasion by the non-native *Sargassum muticum* caused drastic changes in the native seaweed assemblage. In contrast, the invasion of *Undaria pinatifida* was shown to have little effect on sub-canopy algae, with its presence following disturbance to native canopies rather than replacing them actively (South and Thomsen, 2016).

A recent focus in ecology is whether invasive species, including marine macrophytes, are passengers or drivers of ecological change (South and Thomsen, 2016, Bulleri et al., 2010, Ceccherelli et al., 2014, Didham et al., 2005, Tamburello et al., 2015). And specifically whether observed invaders impacts may be compounded by the effects of disturbance on the native assemblage (MacDougall and Turkington, 2005, Didham et al., 2005). Whilst disturbance is a key process promoting the early stages of an invader's spread, suggesting the passenger model, post-recruitment processes may cause change to assemblages without the requirement of further disturbance. This suggests that invasive macrophytes may transition from passengers to drivers of change without the requirement of further disturbance to promote their spread. For example, Bulleri et al. (2010) showed that although *C. cylindracea* benefitted from disturbance to otherwise resistant macroalgal canopies (i.e. a passenger of change), once established, its presence further drove species interactions (i.e. a driver of change). The question of whether native spreading algae can become drivers of change once established has received little attention.

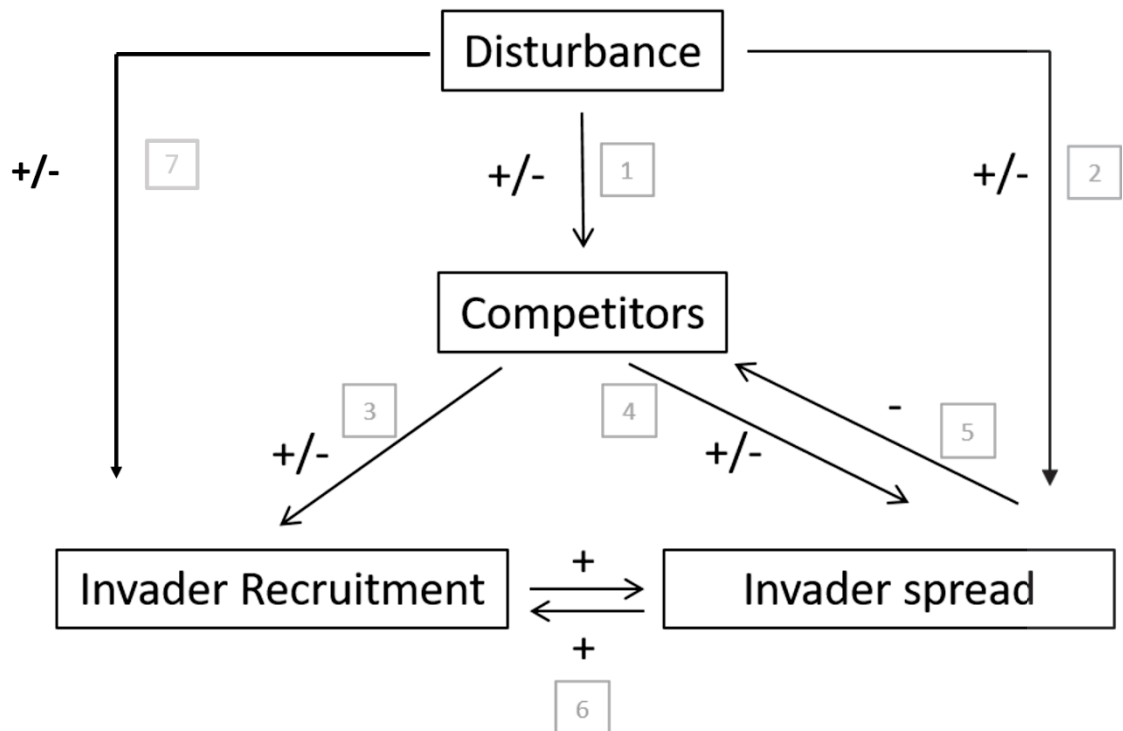


Fig. 1. 1. Schematic overview of processes affecting an invader's abundance. Positive interactions are indicated with +, negative interactions with -. Different numbers indicate the following processes: Disturbance may (1) affect competitors or (2 & 7) affect the invader directly. In turn different competing species can inhibit or enhance invader recruitment success (3) or invader horizontal spread (post-recruitment spread) (4). Invader spread may in turn affect competitors (driver model) (5). Additionally, invader recruitment success may affect invader abundance by horizontal expansion. And, vice versa, invader spread abundance may affect recruitment abundance via increased propagule supply (6).

Although the literature on non-native invaders is large, mechanisms affecting the pathways of native-invaders' recruitment and subsequent horizontal spread remain largely untested. This thesis is set up in a similar structure to test the mechanisms affecting multiple stages of a native species' spread. The factors effecting non-native invaders success as described above (summarized in Fig. 1.1) form an equally adequate model to test factors affecting the spread of a native invader.

## **1.4 *Caulerpa filiformis* as a native invader**

### **1.4.1 Spread of the alga**

The family Caulerpaceae contains some of the most invasive macroalgal species worldwide (Williams and Smith, 2007) and mechanisms affecting their establishment and potential impacts are well documented (Glasby, 2013, Piazzini et al., 2016, Williams and Smith, 2007, Gribben et al., 2013, Wright and Davis, 2006, Wright and Gribben, 2008). Similarly, a native alga from this genus, *Caulerpa filiformis*, has drastically spread within its native range along the coast of NSW, Australia (Glasby et al., 2015) (Fig. 2). *C. filiformis* was first recorded in Botany Bay by Lucas in 1927 (Cummings and Williamson, 2008, Davis et al., 2005, Lucas, 1927), and its historical distribution is considered to be Port Stephens to Wollongong, a distance of 250 km (Glasby et al., 2015). *C. filiformis* was noted as rapidly spreading and becoming more abundant during the 1970s (May, 1976), so much so that it has become one of the dominant algal species in many locations (Schaffelke and Hewitt, 2007; Voerman, unpublished data, Glasby et al., 2015), thereby seemingly replacing important habitat forming macroalgae.





Fig. 1. 2. A) Detail of *C. filiformis* patch at Kingsley Beach (32°47'17.29"S; 152° 6'15.41"E) and B) Example of *C. filiformis* patch indicated by the white dotted contour at Towradgi, NSW, Australia (34°23'8.51"S; 150°55'4.14"E) © GoogleEarth.

#### 1.4.2 Current state of knowledge of *C. filiformis*' impacts and biology.

Other species from this genus are among some of the most invasive species in the world (Cummings and Williamson, 2008), which may be a good predictor for the successful invasive behaviour of *C. filiformis* (Kolar and Lodge, 2001). Prominent examples include the tropical green alga *Caulerpa taxifolia* which is spreading in the Mediterranean and eastern Australia and has severe impacts on native biota (McKinnon et al., 2009, Gribben et al., 2009a), and *Caulerpa cylindracea* (ex. *racemosa*) which may negatively affect seagrass habitats in the Mediterranean (Pacciardi et al., 2011, Ceccherelli and Campo, 2002). *C. filiformis* co-occurs with important habitat formers of the Great Southern Reef, such as the kelp *Ecklonia radiata* and *Sargassum* spp. (Bennett et al., 2016, Coleman and Wernberg, in press). *C. filiformis* is structurally very different from co-occurring species, and has the potential to greatly alter biodiversity. Intertidal patches of *C. filiformis* harbour a very different macrofauna

community compared to two main other habitat forming seaweeds, *Sargassum* spp. and *Hormosira banksii* (Lloyd et al., in prep.), and are shown to have spill-over effects to fauna in neighbouring habitats (Lanham et al., 2015). In the subtidal, *C. filiformis* appears to have large negative effects on sea-urchin abundance and behaviour (Bradley, personal communication) and possibly also affects fish communities (Cummings and Williamson, 2008; Bradley, personal communication). Moreover, Zhang et al. (2014) showed that epibiotic diversity in cleared patches inside *C. filiformis* patches was reduced compared to those inside *Sargassum* spp. habitat. The same authors also showed the potential negative effect *C. filiformis* may have on its competitors (*Sargassum* spp.) by reducing their photosynthetic abilities, potentially resulting from the alga's ability to trap sediment.

*C. filiformis* spreads vegetatively by horizontal expansion of its stolon network and by vegetative propagules, and can be highly successful in doing so (Khou et al., 2007, Zhang et al., 2014). Khou et al. (2007) demonstrated that recruitment success of *C. filiformis* may vary with propagule size, but did not investigate how this may interact with suitability of substrate. Also, Zhang et al. (2014) demonstrated that horizontal spread of the alga is highly efficient in occupying newly created space in an intertidal study.

### 1.4.3 Challenges

Although there is evidence *C. filiformis* increased in abundance both within and outside its historical distribution (see Glasby et al., 2015), we do not know how the current distribution of the alga varies across large or local (within site) scales and its habitat associations. Nor do we know the reasons behind the current success of *C. filiformis*. A change in nutrient regimes has been suggested as a potential reason for its observed proliferation (May, 1976), but the potential benefit of increased nutrient availability has not been tested. Other disturbances such as SST change or coastal development commonly observed to alter algal abundances may also be responsible for the species' current success. Additionally, we do not know how its distribution is shaped by recruitment and post recruitment dynamics or their interactions, and the mechanisms that may affect their respective success. For example, it is not known whether *C. filiformis* is similarly capable of encroaching into intact neighbouring assemblages like it has been shown to do into cleared space (Zhang et al., 2014), or how this may vary among different neighbouring assemblages. Finally, although the alga may drastically affect associated flora and fauna in comparison to other macroalga, we do not know how *C. filiformis* affects the abundance of competing algae and if *C. filiformis* is capable of actively outcompeting those species (i.e. becoming a driver of spread *sensu* MacDougall and Turkington, 2005).

## **1.5 General aims**

The over-arching objectives of this thesis are to determine (i) the habitat associations of *C. filiformis* within and across sites, (ii) the mechanisms behind the observed distribution patterns, and (iii) the effects of its spread on other alga species and how this may vary among sites across its range. This information will increase our understanding of the alga's current success in NSW, Australia. The separate studies within this thesis examine a range of scientific questions concerning various components of species invasion. Chapter 2 and 3 explore distribution patterns across both large and local spatial scales, while chapters 3-5 investigate processes affecting the success of different life stages.

The specific aims are:

### **Chapter 2) Habitat associations of *C. filiformis* across multiple spatial scales**

The aims of this chapter were to describe the large- and small-scale habitat associations of the alga, and to determine the biotic and abiotic factors that explain the distribution and abundance of *C. filiformis* throughout its range. To do this local scale survey sampling (i.e. transects) was used at several locations.

### **Chapter 3) Mechanisms behind the recruitment success of *C. filiformis***

This chapter aimed to investigate mechanisms underpinning the recruitment success of *C. filiformis*. Large scale surveys (100s of kms) were used to investigate abundance and distribution of recruits. Experimental work was used to test the effect of different recipient habitats in recruitment success.

### **Chapter 4) Morphological variation, tolerance and plasticity to sediment disturbance**

The aim of this chapter was to investigate how *C. filiformis*' morphology varies across its distribution in relation to both large and local scale environmental variation, and to test its tolerance to environmental change. Large scale surveys were done across the species entire (~800 km) distribution in NSW. Experimental work tested the alga's tolerance to sediment disturbance and the role of morphological plasticity in this.

### **Chapter 5) The roles of nutrient disturbance and neighbouring habitat in *C. filiformis* post-recruitment spread**

This chapter investigated the direct effect of nutrient disturbance on the horizontal expansion rates of *C. filiformis* among different neighbouring habitats. An experimental study was used to test for the effects of nutrient disturbance and the effects of turf habitat in promoting *C. filiformis* growth. Expansion rates of *C. filiformis* beds were

surveyed for one year at three different sites across its distribution. Although this chapter investigated the role of different neighbouring habitats in the spread of *C. filiformis*, it simultaneously provided information on the ability of the alga to alter neighbouring assemblages as an established population (i.e. be a driver of change).

## **Chapter 2**

### **Habitat associations of *C. filiformis* across multiple spatial scales**





**Part I) Range and habitat associations of the native macroalga**

***Caulerpa filiformis* in New South Wales, Australia**

This study has been published in *Marine and Freshwater Research* 66(11), 1018-1026 (2015), and is included in its published format.



## Range and habitat associations of the native macroalga *Caulerpa filiformis* in New South Wales, Australia

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**Abstract.** *Caulerpa filiformis* is a green seaweed found in New South Wales (NSW, Australia), South Africa, Mozambique and Peru. It has been suggested that the abundance of the species has increased in NSW over recent decades. Extensive aerial and diver surveys identified a 500-km northerly extension to the range of *C. filiformis* in NSW (to 28°21'S) compared with previous records. The alga has a disjunct distribution with small isolated populations around rocky headlands in far northern NSW, but then no apparent populations for 350 km southwards. The far northern populations could be the result of recent human-mediated transport (a species introduction), or were simply not detected previously. The increased distribution around the previous northerly limit is likely a natural range expansion. The distribution of *C. filiformis* in NSW and globally seems confined to a temperature range of ~16–23°C. We found no relationship between abundance of *C. filiformis* and human population or oceanic chlorophyll-*a* (a surrogate for nutrient availability). We demonstrate that *C. filiformis* is predominately subtidal, being found along sections of coastline where there is a mixture of rocky reefs and beaches. It is argued that sand movement may have facilitated increases in abundance of *C. filiformis*.

**Additional keywords:** biogeography, Chlorophyta, distribution, seaweed, sedimentation, temperature.

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### Introduction

The distributional range of a species may be limited by a variety of factors including climate, dispersal capability, habitat availability, competition with other species, predation and disease (Gaston 2009). Changes in distribution of a species due to the aforementioned factors are termed range shifts (which could be either expansions or contractions), whereas those mediated by humans are generally termed introductions or invasions (Sorte *et al.* 2010). Anthropogenic influences on the distributions of habitat-forming species such as macroalgae are receiving renewed attention of late, particularly in relation to effects of climate change and invasive species (e.g. Lima *et al.* 2007; Sorte *et al.* 2010; Harley *et al.* 2012; Smale and Wernberg 2013). To understand current distributional patterns and help interpret tests for anthropogenic impacts on these, it is critical to have a good understanding of historical species distributions (Bolton *et al.* 2004; Connell *et al.* 2008; Mead *et al.* 2013).

Opportunistic species can respond positively to physical disturbances that create space and there are many examples of range expansions of native species being correlated with high

levels of physical disturbance (Sorte *et al.* 2010; Carey *et al.* 2012). For example, the incremental range expansion of the brown macroalga *Fucus serratus* in northern Spain appears to be related to the species' ability to rapidly colonise cleared patches (Arrontes 2002). Similarly, physical disturbance is potentially facilitating the spread of the native green macroalga *Caulerpa filiformis* on intertidal rocky shores in south-eastern Australia (Zhang *et al.* 2014). Like other members of the Caulerpaceae, *C. filiformis* grows quickly and reproduces asexually from fragments, traits that have contributed to this family being overly represented in lists of invasive marine algae (Williams and Smith 2007).

*Caulerpa filiformis* (Suhr) Hering has a restricted global distribution, being found in South Africa and southern Mozambique (Coppejans *et al.* 2005), northern Peru (Ramirez and Santelices 1991) and New South Wales (NSW) in south-eastern Australia (Millar and Kraft 1994). Belleza and Liao (2007) suggested that *C. filiformis* is found off Chile and Peru, but they cited no references and there appears to be no evidence for the species occurring in Chile (S. Navarette and E. Macaya,

pers. comm.). *C. filiformis* has, however, been discovered recently in central Peru (P. Gil, pers. comm.). In all locations, *C. filiformis* is associated with intertidal or shallow subtidal rocky shores and can also occur in sandy sediments (Coppejans *et al.* 2005), possibly growing to a depth of ~25 m in some locations (Ramírez and Santelices 1991).

The first published record of *C. filiformis* in NSW described the alga (referred to as *C. ligulata*) as being 'abundant' on mud-covered rocks at Sandringham in Botany Bay and drift fragments were found at Balmoral in Port Jackson, Sydney (Lucas 1927). Lucas (1927) also referred to older herbarium specimens of *C. filiformis* from an unknown location and various earlier records are available in the National Herbarium of New South Wales (A. Millar, pers. comm.). The south-eastern Australian populations of *C. filiformis* are genetically distinct from those in South Africa and are thus considered native (Pillmann *et al.* 1997), whereas the affiliations of Peruvian *C. filiformis* are unknown. May (1976) suggested that *C. filiformis* had increased in abundance in NSW since Lucas (1927), purportedly due to sewage pollution, but provided no empirical data to support either claim. Rather, she described comments from uncited herbarium collections that recorded the abundance of *C. filiformis* as 'rare' or 'occasional' at some locations around Sydney in the 1920s, 1930s and 1960s, and 'dominant' or 'very prevalent' at these or other locations in 1946, 1969 and 1974 (May 1976). It is difficult to get a clear picture about changes in the abundance of a species from such subjective and irregular estimates, especially for a genus that can change in abundance over short periods (e.g. seasonally) (Sánchez-Moyano *et al.* 2007; Glasby 2013). Nevertheless, observations of increased abundances of *C. filiformis* in NSW have subsequently been made by others (Davis *et al.* 2005; Cummings and Williamson 2008; Zhang *et al.* 2014).

Here we describe the present distribution of *C. filiformis* in NSW based on state-wide surveys over 8 years, and provide quantitative estimates of its abundance at a variety of sites. We investigate this local distribution in the context of the global distribution of the species and test hypotheses about associations between *C. filiformis* and a variety of environmental and anthropogenic factors.

## Materials and methods

### *Distribution of C. filiformis*

The range of *C. filiformis* in NSW has most recently been described as Port Stephens (32°45'S, 152°12'E) to Jervis Bay (35°00'S, 102°50'E) (Davis *et al.* 2005). Our surveys extended well beyond this documented range. Specifically, aerial surveys from a helicopter covered the entire NSW coast and were done at altitudes of between 40 and 250 m on sunny days when there was low swell (<1.5 m) and wind speed of <30 km h<sup>-1</sup>. Flights were made on multiple days in Spring (September–November) each year from 2009 to 2013. Being bright green, *C. filiformis* was obvious from the air and field surveys (intertidal and subtidal) at multiple sites demonstrated that the species was rarely mistaken for another species, and never incorrectly identified when in patches larger than ~4 m<sup>2</sup>. Aerial surveys were augmented with ground searches of rocky shores and observations made while diving at a variety of sites along the open coast and in estuaries

(data from estuaries not presented here). *C. filiformis* specimens collected from outside of the species' previously documented range were registered with the National Herbarium of New South Wales. Coordinates of sites where *C. filiformis* was present were recorded spatially in an ESRI geodatabase such that its distribution could be represented as isolated points, or, more commonly, segments of coastline.

### *Patterns of distribution v. environmental and anthropogenic factors*

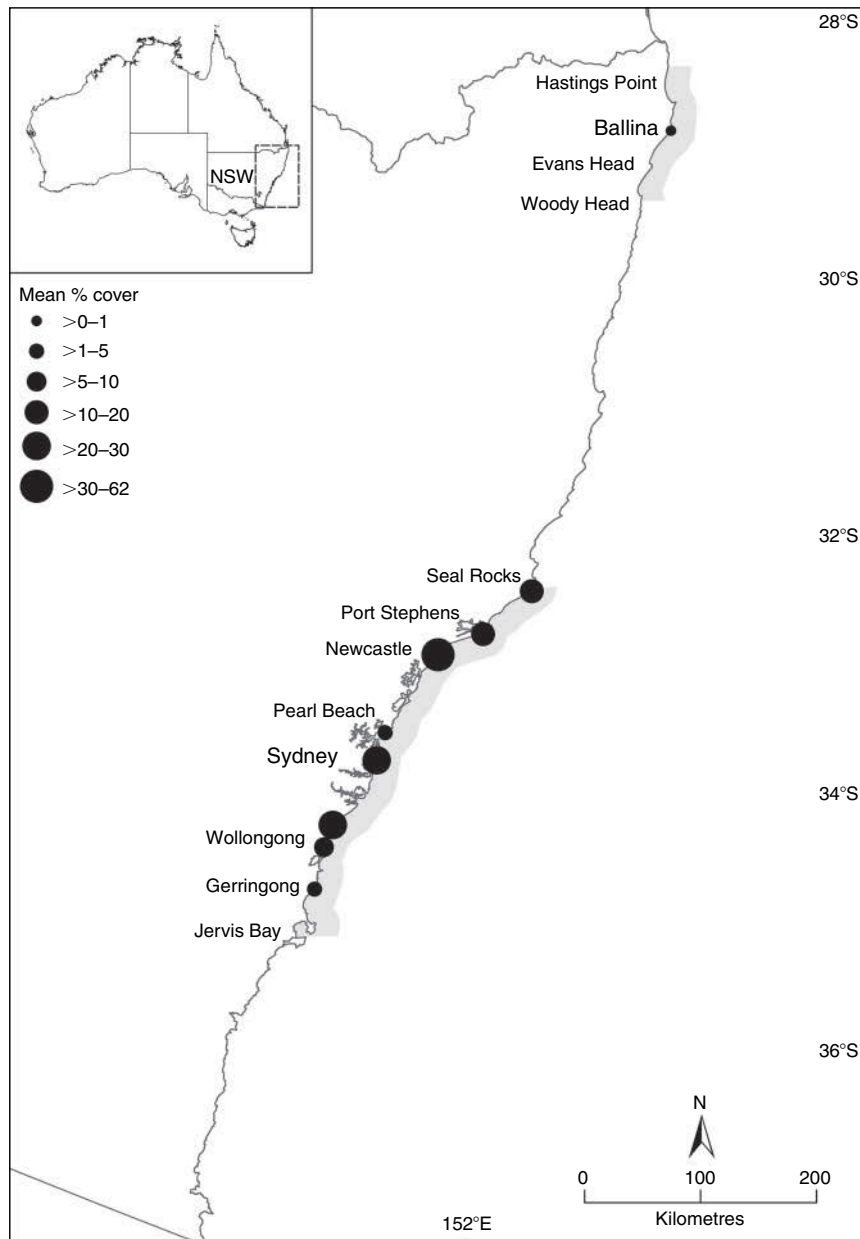
To investigate the hypothesis that the presence of *C. filiformis* is related to temperature, the global distribution of *C. filiformis* was compared with mean sea surface temperatures (SST) derived from satellite imagery (NOAA National Oceanographic Data Center, World Ocean Atlas), averaged over the years 2001, 2005, 2009 at a resolution of 550 × 550 km.

Within NSW, we examined the occurrence of *C. filiformis* along the open coast in relation to beaches and rocky reefs. We mapped the observed distribution of *C. filiformis* (in both intertidal and shallow subtidal habitats) along the open coast of NSW and then, for each 5-km section of coastline, calculated a reef: beach index (RBI) for sections of coast with or without *C. filiformis*. The RBI was calculated as (reef length – beach length) ÷ (reef length + beach length), and its values ranged from –1 (100% beach) to +1 (100% reef), with 0 indicating that the length of beach and reef were the same. The minimum feature size for beaches and headlands was 25 linear metres (i.e. no beach or rocky platform smaller than this was mapped). The RBI was compared between sections of coast with *C. filiformis* present ( $n = 54$ ) v. absent ( $n = 286$ ) using a one-factor PERMANOVA (as described below). The association between *C. filiformis* and shoreline complexity was also examined by calculating shoreline sinuosity for areas with v. without *C. filiformis*. Sinuosity was calculated for each 5-km section of coastline (at approximate high tide mark) as the total shoreline length (km) ÷ 5; thus a value of 1 indicates a straight shoreline, with increasing values corresponding to increasing complexity.

We also examined the relationship between *C. filiformis*, the width of subtidal rocky reefs and distance from shore at the scale of 20-m sections of coastline. At each of six subtidal sites between Ballina and Wollongong (Fig. 1), the presence of *C. filiformis* in 50 × 50-cm quadrats (3 m apart) was recorded along five transects (5 m apart) running perpendicular to the shore. The total number of quadrats per site ranged from 25 to 65 according to the width of reef. The shallow edge of the reef was the low intertidal and the deep edge of the reef was defined as the point at which sand extended for >10 m along a transect. Sites ranged in depth from 0.5 to 6.5 m below mean low water and were sampled between August and December 2013.

### *Abundance estimates of C. filiformis*

Abundances of *C. filiformis* were estimated from aerial imagery of 10 intertidal and 17 subtidal rocky reef sites in NSW. Examples of imagery are available as Supplementary material. Sites extended south from Ballina to Gerringong (Fig. 1). Percentage cover of *C. filiformis* was estimated from aerial photographs taken from a helicopter using a Nikon D3



**Fig. 1.** Range of *C. filiformis* (thick grey line) in NSW, Australia, ascertained from aerial, land and in-water surveys during 2009–13. Relative percentage covers of *C. filiformis* (mean over 5 years) at representative subtidal sites are indicated by size of circles.

(12 megapixel) in good weather conditions (as described above). The camera was mounted vertically and operated by a passenger, enabling photographs to be taken when there were no breaking waves obscuring the rock platform. Images of intertidal sites were taken at low tide from an altitude of 40 m and had a resolution of 0.7 cm. Six replicate images ( $\sim 20 \times 30$  m), arranged haphazardly, were taken per low shore intertidal site. Images of shallow subtidal ( $\sim 1$  to 8 m deep) sites were taken during mid-high tide from a higher altitude (185 m) to maximise light penetration and hence clarity of subtidal features, resulting in images of 3-cm resolution covering  $\sim 88 \times 132$  m.

Five replicate images, arranged haphazardly, were taken per subtidal site.

All aerial images were subsampled using the software package Coral Point Count (Kohler and Gill 2006). Each intertidal image was subsampled with a single  $4 \times 5$ -m grid with 80 regularly spaced points, and positioned to avoid large crevices and rock pools ( $>1$  m<sup>2</sup>). Subtidal images were subsampled with a single  $40 \times 40$ -m grid with 80 regularly spaced points. Sizes of grids, number of sampling points and numbers of replicate images per site were determined from pilot studies (T. M. Glasby, unpubl. data). Numerous habitats were

sampled in each image, but only data on *C. filiformis* are presented here.

#### Abundance of *C. filiformis* in intertidal v. subtidal habitats

Abundances of *C. filiformis* were compared between intertidal and subtidal habitats at multiple sites in NSW. We also tested for changes in percentage cover of *C. filiformis* over time at these sites using PERMANOVA, with the factors Habitat (fixed, intertidal v. subtidal), Time (random, 4 years) and Site (random, nested within Habitat). Four years (ranging from 2009 to 2013) were analysed, but these differed slightly according to habitat and site because sampling was not possible in each site for all years. Data were analysed using 9999 permutations of Euclidean distance similarities and Type III sums of squares with fixed effects summed to zero. Non-significant interaction terms ( $P > 0.25$ ) were pooled to increase the power of other tests in the model. PERMANOVA is capable of constructing pseudo- $F$  tests for all terms, even for mixed models, by calculating a linear combination of mean squares and using fractional multipliers for individual mean squares. For example, for the above design, the denominator for the pseudo- $F$  test for Habitat is  $0.9 \times \text{Time} \times \text{Habitat} + 0.9 \times \text{Site}(\text{Habitat})$ .

#### Abundance of *C. filiformis* v. environmental and anthropogenic factors

Quantitative estimates of percentage cover of *C. filiformis* from aerial photos were used to test for associations with human population and oceanic chlorophyll-*a* (Chl-*a*) concentrations. Percentage covers of subtidal *C. filiformis* (averaged over 4 or 5 years of sampling) were correlated against the natural log of human population (Australian Bureau of Statistics 2011 Census) estimated for local government areas within ~5 km of the sampling site to test the hypothesis that abundance of *C. filiformis* is correlated with human population.

Chl-*a* is a surrogate for phytoplankton biomass (Klemas 2011), which in turn can be affected directly by the availability of nutrients, especially nitrogen (Rabalais 2002). As an indirect test of the hypothesis that abundance of *C. filiformis* is enhanced by nutrients (one-tailed test), we examined associations between *C. filiformis* cover and oceanic Chl-*a* concentration derived from MODIS Aqua satellite imagery (NASA reprocessing R2013.0 processed to Level 2 with the Garver–Seigel–Maritorena Algorithm: Maritorena *et al.* 2002). MODIS data were gridded to a rectangular projection, binned to regular 2-km pixels along the coast of NSW and then averaged over the last 12 years. Information in pixels within 2 km of the coast was too noisy to interpret (due to effects of the land), so these were excluded and only pixels 2–6 km off the coast were used. Pixel grids were therefore 2 km long  $\times$  4 km wide. Only those grid cells adjacent to subtidal sites where *C. filiformis* was sampled were used to test for relationships between *C. filiformis* abundance and Chl-*a* (i.e. 16 sites).

## Results

### Distribution of *C. filiformis*

The identified range of *C. filiformis* in NSW is shown in Fig. 1. Small populations of *C. filiformis* were discovered at Hastings Point, Ballina, Evans Head and Woody Head, the former being

500 km north of the previously documented northerly limit (i.e. Port Stephens). In all three far northern locations, *C. filiformis* was growing on shallow (<2 m) subtidal rocky reef. In addition, *C. filiformis* was found growing on subtidal (~0.5–6 m) rocky reefs at Seal Rocks, 45 km north of the previous northern limit (Fig. 1). *C. filiformis* was common on reefs from Port Stephens south to Newcastle. It was patchily distributed between Newcastle and the northern outskirts of Sydney, and found on most headlands in the greater Sydney area (Fig. 1). Some of the largest beds of *C. filiformis* (intertidal and subtidal) occurred around Port Stephens, Newcastle, Sydney and along the 25-km stretch of coastline north of Wollongong (Fig. 1). On the basis of our surveys, the southern limit of *C. filiformis* is Jervis Bay (sheltered locations inside the bay). In general, *C. filiformis* was least abundant at the margins of its distributional range (Fig. 1).

The latitudinal range of *C. filiformis* in NSW (28°21'S–35°05'S) is similar to that in southern Africa (Mozambique to South Africa, ~25°S–34°S), but very different from that in Peru (~5°S–13°S). However, the SSTs of all these regions (at the resolution of 500  $\times$  500 km) are similar. Mean SSTs for the areas of southern Africa where *C. filiformis* is found are 15.9°C in the south and 23.6°C in the north (including southern Mozambique), whereas in Peru the SST range is 19.0–20.5°C. In NSW, SST for the *C. filiformis* range is 19.5–23.0°C. Within NSW, we were also able to obtain annual SST satellite data at a resolution of 2  $\times$  2 km (MODIS data over 12 years). At this spatial scale, mean SST for the distributional range of *C. filiformis* was 16.7–23.0°C. The lower temperatures identified at this latter resolution are likely a consequence of cool water upwellings close to the coast influencing mean temperatures far more so than at the coarser resolution.

Along the NSW open coast, *C. filiformis* was associated with sections of coastline that had a mixture of rocky reefs and beaches (Fig. 2). The RBI for 5-km sections of coast with *C. filiformis* was significantly greater than for sections without *C. filiformis* (pseudo- $F_{1,338} = 8.55$ ,  $P = 0.003$ ), with the latter sections having a RBI comparable to the entire NSW coast (Fig. 2). The southern coastline of NSW (Jervis Bay to the

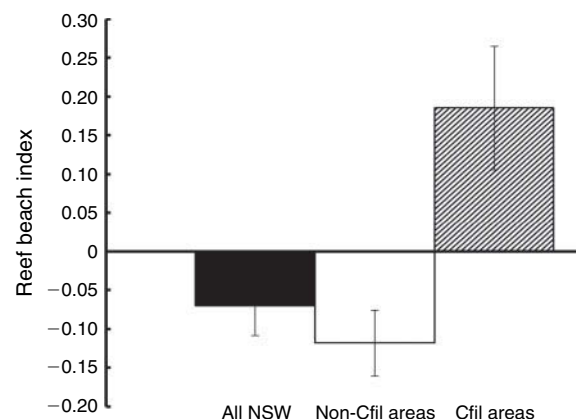
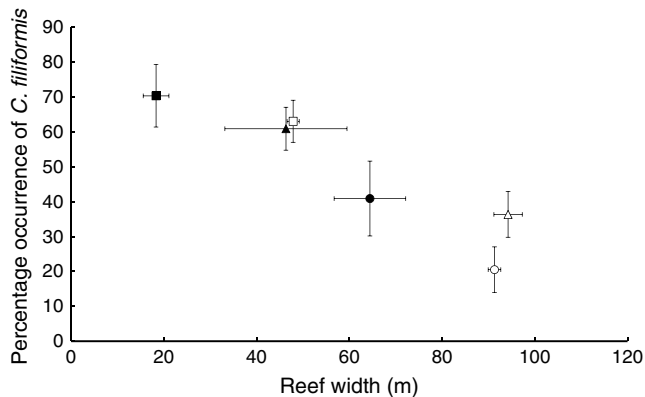


Fig. 2. Associations of *C. filiformis* with reef or beach in NSW. Reef: beach index (RBI) ranges from -1 (100% beach) to +1 (100% reef). Mean ( $\pm$ s.e.) RBI is plotted for contiguous 5-km sections along the entire coast (all NSW), sections without *C. filiformis* (non-Cfil areas) and sections with *C. filiformis* present (Cfil areas).





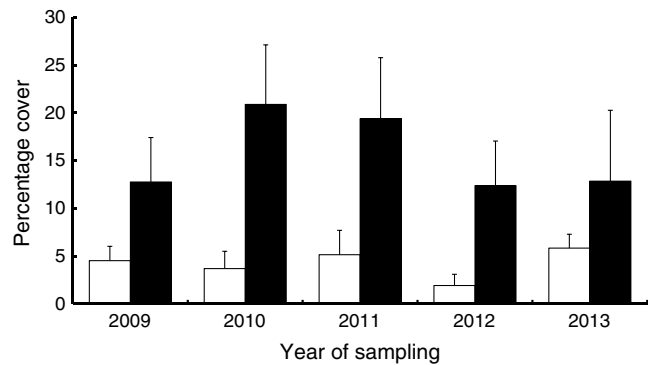
**Fig. 3.** Association between the occurrence of *C. filiformis* in  $50 \times 50$  cm quadrats and the width of subtidal rocky reef. Points are averages per site from 25 to 65 quadrats, with error bars representing the standard error among quadrats (vertical bars) or among reef width measurements from five transects (horizontal bars). Sites, from north to south, are Woody Head (filled circle), Seal Rocks (open circle), Port Stephens (filled square), Bronte (open square), Coogee (filled triangle) (latter two both in Sydney) and Wollongong (open triangle).

border) (Fig. 1), where there was no *C. filiformis*, was dominated by rocky reef, as reflected by a RBI ( $0.26 \pm 0.06$  s.e.) that was greater than for the entire coast ( $-0.07 \pm 0.04$ ) and greater than for the sections of coast that supported *C. filiformis* populations ( $0.18 \pm 0.08$ ). Conversely, the  $\sim 350$ -km section of coastline between Seal Rocks and Woody Head, where there was also no *C. filiformis* (Fig. 1), had a smaller RBI ( $-0.44 \pm 0.06$ ) than the average for the entire coast, indicating a lack of shoreline rocky reef. The occurrence of *C. filiformis* was not clearly related to the complexity of the shoreline, with mean sinuosity in areas with *C. filiformis* ( $1.58 \pm 0.06$ ) being similar to that in areas without *C. filiformis* ( $1.52 \pm 0.04$ ).

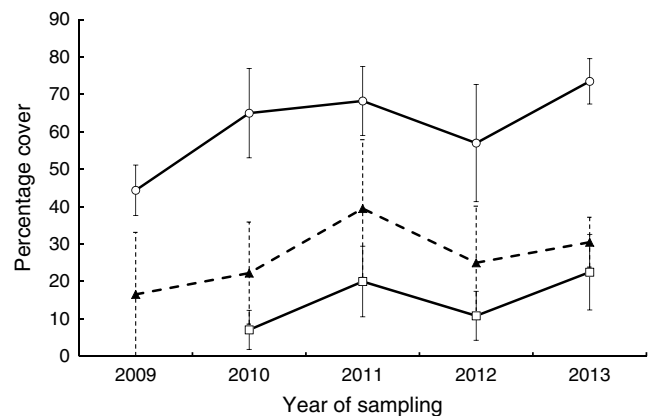
At the scale of 20-m-long subtidal reef sites, the presence of *C. filiformis* in  $50 \times 50$  cm quadrats was negatively correlated with the width of the nearshore reef ( $r = -0.927$ ,  $P < 0.01$ ) (Fig. 3). That is, *C. filiformis* was more prevalent on narrow reefs than on wider reefs. There were, however, no significant relationships between the prevalence of *C. filiformis* and depth ( $r = 0.022$ ,  $P > 0.7$ ), or distance from shore ( $r = -0.082$ ,  $P > 0.1$ ).

#### Intertidal v. subtidal habitats

In intertidal habitats, *C. filiformis* was always found low on the shore, often in rock pools or crevices, on vertical walls, or closely associated with rocks in the corners of sandy beaches. When *C. filiformis* appeared to be growing on sand in low intertidal areas, closer inspection often revealed that the alga was attached to rock buried by sand. In some cases, mats of live *C. filiformis* were found buried under 20–40 cm of sand in the corners of beaches, with no exposed fronds visible within 90 cm of the buried parts of the alga. There was typically little organic material in these sandy sediments and hence no obvious signs of anoxia. In subtidal habitats, *C. filiformis* was generally seen growing on rocky reefs or on the sand–rock interface down to a depth of  $\sim 8$  m. Similar to intertidal habitats, *C. filiformis* was sometimes found attached to subtidal rock buried by up to 25 cm



**Fig. 4.** Percentage cover ( $\pm$ s.e.) of *C. filiformis* for intertidal (white) and subtidal (black) habitats during 2009–13. Means are averages across sites: 10 intertidal sites sampled per year (except 2013, 2 sites only), 10–14 subtidal sites sampled per year.



**Fig. 5.** Subtidal sites that showed a (non-significant) trend for increasing percentage cover ( $\pm$ s.e.) of *C. filiformis* over time: open circle, Nobbys Headland at Newcastle; solid triangle, Long Reef; open square, Seal Rocks (not sampled in 2009).

of sand. The mean percentage cover of *C. filiformis* at subtidal sites over 5 years ( $16.27\% \pm 1.49$  s.e.) was significantly greater than at intertidal sites ( $4.22\% \pm 0.68$ ) ( $F_{1,26} = 3.19$ ,  $P = 0.03$ ). These differences between habitats were consistent among years (Year  $\times$  Habitat: pseudo- $F_{3,454} = 1.85$ ,  $P = 0.14$ ) (Fig. 4) and were closely related. That is, the cover of the alga in subtidal and intertidal habitats was significantly correlated ( $r = 0.63$ ,  $P < 0.001$ ) when compared across the 32 sites where *C. filiformis* was sampled in both habitats. *C. filiformis* cover in both habitats fluctuated significantly over time (pseudo- $F_{3,454} = 3.44$ ,  $P = 0.02$ ), but pairwise tests demonstrated that the pattern was stochastic, with no consistent increase (or decrease) over time when averaged over all sites (Fig. 4).

Although there were no significant patterns in coverage of *C. filiformis* over time, even when examined separately for intertidal v. subtidal habitats (Year  $\times$  Site(Habitat): pseudo- $F_{60,394} = 0.75$ ,  $P = 0.92$ ), there were trends at some sites. Specifically, at three subtidal sites (Nobbys Headland in Newcastle, Long Reef and Seal Rocks) there was a trend for increasing abundance over time (Fig. 5), but no apparent trends

for increases or decreases over time at other subtidal sites or at any intertidal site.

#### Associations between abundance of *C. filiformis* and human disturbances

There was no significant linear correlation between human population and subtidal cover of *C. filiformis* ( $r=0.14$ ,  $P>0.40$ , 27 sites), or subtidal and intertidal covers combined ( $r=0.11$ ,  $P>0.20$ , 43 sites). The intertidal and subtidal sites with the greatest percentage cover of *C. filiformis* were, however, those with the greatest human population (Newcastle, Sydney, Wollongong) (Fig. 1). Using satellite data, we also tested for a positive association between ocean nutrients (Chl-*a*) and subtidal abundance of *C. filiformis*, but this was not significant ( $r=0.35$ ,  $P>0.09$ ). This near-significant association was driven by one site (Nobbys Headland at Newcastle), which had a particularly high Chl-*a* value (presumably related to the very large Hunter River and surrounding agricultural land). When this outlier was removed from the analysis, the relationship between abundance of *C. filiformis* and Chl-*a* concentration became slightly negative and was non-significant ( $r=-0.13$ ,  $P>0.32$ ).

#### Discussion

This study has documented a 500-km northerly extension to the range of *C. filiformis* in NSW (to 28°21'S) compared with previously published records (Davis *et al.* 2005), although we acknowledge that the alga may have always been present, but not observed, in northern NSW. *C. filiformis* has a disjunct distribution with a few small populations around rocky headlands in far northern NSW (stretching over 100 km), but then no apparent populations for some 350 km southwards until Seal Rocks (Fig. 1). Our aerial surveys confirm the observation by Davis *et al.* (2005) that the current southern limit of *C. filiformis* in NSW is Jervis Bay (35°05'S). Thus the apparent geographical range of *C. filiformis* in NSW (and therefore Australia) is ~800 km from Hastings Point to Jervis Bay (Fig. 1). We demonstrated that *C. filiformis* is primarily a subtidal species that grows on rocky reefs. The species is predominately found along sections of NSW coastline where there is a mixture of rocky reefs and beaches. This association between *C. filiformis*, rocks and sand has been reported in South Africa (Leliaert *et al.* 2000), and at small (50 × 50 cm) spatial scales in NSW in intertidal habitats (Zhang *et al.* 2014).

During this 5-year study, there was significant variation in the abundance of *C. filiformis* among years in subtidal and intertidal habitats. There were trends for increases over time at some subtidal sites (in Sydney and north to Seal Rocks) (Fig. 5), whereas there were no trends for increases or decreases over 5 years at subtidal sites south of Sydney or at any intertidal sites. There is reasonable anecdotal evidence that the abundance of *C. filiformis* has increased over the last 40 years at various sites in NSW. May (1976) reported that *C. filiformis* was not present at north Narrabeen or north of Avalon, or on headlands just south of Port Jackson. Subtidal populations of *C. filiformis* now occur at these locations down to ~5 m, although it is possible that these were not seen by May who may have focussed on the low intertidal zone. Nevertheless, our anecdotal observations and

those of other ecologists suggest that the abundance of *C. filiformis* has increased markedly in subtidal areas over the last 20–30 years at many locations around Sydney (B. Curley, P. Steinberg, pers. comm.), Port Stephens (P. Scanes, pers. comm.), Bulli to Austinmer (T. Prichard, pers. comm.), but apparently not just further south around Wollongong (A. Davis, pers. comm.).

What might have caused increases in the abundance and range of *C. filiformis*? We found no evidence to support the suggestion by May (1976) that human inputs of nutrients led to more *C. filiformis*. Specifically, there was no positive association between abundance of *C. filiformis* and human population or nearshore oceanic Chl-*a* (average over the last 12 years) at spatial scales of a few kilometres. Chl-*a* was measured as a surrogate for nutrient availability and, as such, our results do not disprove the hypothesis that nutrients could enhance the growth of *C. filiformis*, particularly at more localised spatial scales. Furthermore, nearshore nutrient inputs in the Sydney region would likely have been greater 20 years ago, before the decommissioning of shoreline sewage outfalls. Past nutrient pollution from these sewage outfalls may have been responsible for the disappearance of the brown macroalga *Phyllospora comosa* from the Sydney region over the last 50 years (Coleman *et al.* 2008). This loss of *P. comosa* could have provided additional space for the co-occurring *C. filiformis* to colonise, although *P. comosa* is still abundant to the north and south of Sydney where *C. filiformis* is also abundant. Another possible explanation for changing distribution and abundance of *C. filiformis* relates to the species' tolerance of burial by sand, as documented for other species of *Caulerpa* (Williams *et al.* 1985; Glasby *et al.* 2005). Depending on how long (and how much) *C. filiformis* is buried, it is possible that sand movement could influence our perception of the abundance of the alga in areas where sand movement is great.

Sand may provide *C. filiformis* a competitive advantage over co-occurring macroalgae (such as *Sargassum* spp. and *Ecklonia radiata*). There is some evidence that *C. filiformis* is more tolerant of sand than is *Sargassum* spp. on low shore intertidal reefs in NSW (Zhang *et al.* 2014). Losses of *Sargassum* spp. on rocky reefs in Queensland, Australia, have been suggested to be related to the susceptibility of the genus to sedimentation and limited dispersal capabilities (Phillips and Blackshaw 2011). Certainly, disturbances such as sand movement and storms could generate vegetative fragments of *C. filiformis* (Khou *et al.* 2007) and create space on rocky reefs that can be colonised quickly by *C. filiformis* (Zhang *et al.* 2014). Littler *et al.* (1983) concluded that sand inundation on rocky intertidal platforms advantages opportunistic species tolerant of burial and *C. filiformis* has both traits. Unlike other macroalgae, species of *Caulerpa* can absorb sediment nutrients through rhizoids (Williams 1984; Chisholm *et al.* 1996), which might help explain the apparent dominance of *C. filiformis* in sandy habitats. Moreover, sand inundation might also have indirect positive effects on *C. filiformis* by limiting grazing (D'Antonio 1986). Despite producing potentially toxic secondary metabolites, there is evidence that *C. filiformis* is grazed by a variety of species, although perhaps less so than co-occurring macroalgae (Davis *et al.* 2005; Cummings and Williamson 2008).



Connell *et al.* (2008) discussed how the deposition or accumulation of sediment on rocky reefs can lead to losses of canopy-forming algae and increases in turfing species (often filamentous algae, but also coralline algae and ephemeral genera such as *Ulva*). *C. filiformis*, like turfing algae, may be advantaged by increased sediment deposition on rocky reefs, which can be exacerbated by land clearing, industrial discharges or perhaps increased storm activity (Airoldi 2003; Airoldi and Beck 2007). Our finding that the prevalence of *C. filiformis* is negatively associated with the width of subtidal reefs supports the notion that interactions between sand and reef are beneficial to *C. filiformis*, with such interactions likely influencing a greater proportion of a narrow than a wide reef. Although wide reefs would theoretically have more surface area available for *C. filiformis* to colonise, the alga was more prevalent on narrow reefs. Natural patterns of longshore sand movement, which are driven by wave direction and can vary over decadal or multi-decadal timescales as a consequence of ENSO (El Niño–Southern Oscillation) processes (e.g. Goodwin *et al.* 2006), may have important influences on *C. filiformis* and other rocky reef species (Engledow and Bolton 1994). If so, the abundance of *C. filiformis* could vary in (as yet) unpredictable ways over decades, and differently at different locations along the coast as a function of wave climate. Whatever the cause, a likely consequence of *C. filiformis* (and sand) spreading over rocky reefs is reduced biodiversity (Airoldi 2003; Balata *et al.* 2007; Zhang *et al.* 2014). It is, however, important to note that a positive association between *C. filiformis* and sand could be driven by *C. filiformis* accumulating sediment (Piazzi *et al.* 2007), although this is unlikely at large spatial scales (e.g. 5-km sections of shoreline), or there could be no causal relationship between the two. More experimental work is required to investigate the associations between *C. filiformis* and sand, particularly in subtidal habitats (e.g. Balata *et al.* 2007).

Sea water temperature is related to the distribution of seaweeds over scales of hundreds of kilometres (Bolton and Anderson 1990). Global comparisons of the distribution of *C. filiformis* and SST demonstrate that the current distribution of the species in NSW covers a SST range similar to that for *C. filiformis* in South Africa. Thus, if temperature is a limiting factor, it seems unlikely that the distributional range of *C. filiformis* in NSW has the scope to alter much under current conditions. Climate change could, however, affect SSTs and the frequency and intensity of storms (Dowdy *et al.* 2014), which could in turn influence the distribution of *C. filiformis* and other interacting species. On the east coast of Australia there already appears to have been a poleward shift in the distribution of the habitat-forming brown algae *Ecklonia radiata*, *Phyllospora comosa* and *Durvillaea potatorum* over recent decades (Millar 2007). Yet the pattern documented here for *C. filiformis* is in the opposite direction and apparently unrelated to temperature.

The occurrence of *C. filiformis* in far northern NSW, some 500 km north of its previous northerly limit, is intriguing. It is possible that small populations of *C. filiformis* have long been present in these far northern NSW locations, but have just not been sampled previously. Alternatively, these few northerly populations may have resulted from more recent human-mediated transport, e.g. by fragments transported on the anchor of a boat, as documented for *Caulerpa taxifolia* (West *et al.*

2007). If so, this would be classified as a native species introduction (Sorte *et al.* 2010). It seems less likely that fragments dispersed naturally from southern populations by currents given the distances involved and the low survivorship of fragments over short periods, at least in laboratory conditions (Khou *et al.* 2007). Moreover, the dominant direction of the east Australia current is southerly, although northerly transport of nearshore NSW waters over short distances is possible in some areas (Roughan *et al.* 2011). The apparent absence of *C. filiformis* between Seal Rocks and Woody Head (350 km) presumably relates to the paucity of rocky headlands, as evidenced by the particularly small RBI calculated for this region. The occurrence of *C. filiformis* at Seal Rocks, ~45 km north of the species' previous limit (Port Stephens) seems more likely to be a natural range expansion (Sorte *et al.* 2010) than a human-mediated introduction. Dispersal modelling demonstrates that northerly transport of passive particles (e.g. fragments of *C. filiformis*) in nearshore currents between Port Stephens and Seal Rocks is likely in summer and winter (Roughan *et al.* 2011).

This study has documented a considerable extension of the range of *C. filiformis* in NSW, Australia. Moreover, we concur with previous assertions that the abundance of *C. filiformis* in NSW has increased, at least over the last two decades. We did not, however, find any support for the suggestion that this increase in abundance is due to human activities such as nutrient addition, at least at the large spatial scales examined. We suggest that sand movement over rocky reefs may be facilitating the spread of *C. filiformis*. The current distribution of *C. filiformis* in NSW incorporates a temperature range similar to that occupied by *C. filiformis* in southern Africa. This study has provided the most comprehensive baseline of *C. filiformis* distribution and abundance in NSW and can be used to assess future changes that might occur in response to changing climatic conditions or specific anthropogenic disturbances.

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**Part II) Local scale habitat associations across *C. filiformis*  
geographic range**



## 2.1 Abstract

Like non-native invasive macrophytes, some native macrophytes are becoming locally dominant and are spreading rapidly outside their traditional distributions. Whilst the mechanisms underpinning the spread of native macrophytes are poorly known, we may expect them to be similar to those described for non-native invasive species. The spread of invasive macrophytes on rocky shores may be linked to alternate species such as turfing algae which may in turn promote invaders. Other environmental characteristics (e.g. reef slope and rugosity, water depth) also influence macrophyte distributions. In New South Wales, Australia, the green alga *Caulerpa filiformis* is undergoing a range expansion and has transitioned from a subdominant to a dominant alga on several rocky shores around the Sydney coastline. Here we surveyed the substrata, environmental characteristics and macrophyte communities associated with *C. filiformis* abundance at six sites covering its known distribution (approx. 700 km). We tested the following predictions: 1) *C. filiformis* cover differs among substrata and is greatest on turfing substrate; 2) *C. filiformis* cover is positively related to environmental variables linked to increased sedimentation (e.g. reduced reef size, surface slope, increased rugosity and distance from shore); 3) occurrence of *C. filiformis* is related to a change in macrophyte community structure and a reduction of macrophyte diversity and abundance. Substrate associations were highly consistent among sites, and contrary to our prediction *C. filiformis* was most commonly associated with rock or rock+sand substrate, less frequently associated with turfing algae substrate, and largely absent from sand substrate. *C. filiformis* cover was negatively correlated with reef size, which explained most of the variation observed, although distance from shore, reef slope, and water depth were also correlated to *C. filiformis* cover. Algal communities often differed in the presence of *C. filiformis* and algal morpho-functional richness correlated negatively with *C. filiformis*' cover although results varied among substrata. Especially *Sargassum* spp. were in lower abundance in the presence of *C. filiformis*. Our results suggest that processes acting at the site and quadrat scale (possibly linked to sedimentation regimes) are likely important determinants of *C. filiformis* cover. Our study may help identify

areas vulnerable to further spread (such as narrow reefs with high sediment disturbance), and the potential negative response of recipient macrophyte communities to *C. filiformis* continued spread (reduced diversity and abundance).



## 2.2 Introduction

There has been an increase in scientific focus on the understanding of altered species' abundances following environmental change such as climate change and pollution (e.g. Bruno et al., 2009, Roff et al., 2015, Vergés et al., 2014). Environmental change can transition ecosystems to alternate phase-states dominated by a single or few highly adaptive or tolerant species (Jauni et al., 2015, e.g. Davis et al., 2000, Levine and D'Antonio, 1999, Roff et al., 2015). Such native species benefit from environmental change in ways similar to those described for non-native invasive species that come to dominate communities (Valery et al., 2009), and are also referred to as native-invaders (Carey et al., 2012, Simberloff et al., 2012). Despite their predicted increasing prominence (Davis et al., 2011), how their abundance may vary locally across habitats or how associated communities may change in their presence, remain not well understood (Carey et al., 2012). This is especially true for the marine environment.

Rocky-shores have been a focal ecosystem for studying the distribution and abundance of non-native marine invaders (e.g. Occhipinti-Ambrogi and Savini, 2003, Valentine and Johnson, 2003, Bando, 2006). This knowledge may aid in the understanding of the mechanisms behind altered dominance of native spreading species. In these ecosystems, the loss of native macroalgal canopies may allow for alternative substrata that in turn promote invader' abundance (e.g. Thompson and Schiel, 2012a, Bulleri and Benedetti-Cecchi, 2008, Gennaro and Piazzzi, 2014, Vaz-Pinto et al., 2013, Mulas and Bertocci, 2016). For example, nutrient stress has caused shifts from a dominance of canopy forming algae to an impoverished state dominated by turfing algae. This secondary substrate in turn promoted the abundance of the non-native macroalga *Caulerpa cylindracea* (Gennaro and Piazzzi, 2014). Similarly, the proliferation of native macroalgae may also be linked to alternate substrata (i.e. turf).

In addition to the effects of substrate type on algal abundance, local or small-scale environmental variables are also important determinants for their establishment and spread (Toohey, 2007, Bischof et al., 1998, Somsueb et al., 2001, Fowler-Walker and Connell, 2007, Toohey et al., 2007). For example, algal density often declines with increasing water depth and diminishing light levels (Korpinen et al., 2007). Additionally, surface rugosity or surface slope can drastically affect algae' abundance (Toohey et al., 2007, Harlin and Lindbergh), e.g. by altering small-scale hydrodynamics and increasing rates of sedimentation (Toohey, 2007). Despite the obvious importance of local environmental variables in shaping algal abundance, few studies have investigated the importance of small-scale environmental characteristics on further promoting or inhibiting the spread of invasive species (but see Watanabe et al., 2009, Infantes et al., 2011).

The often-negative impacts of non-native invasive macro-algae are well-documented (e.g. Gribben et al., 2009b, Byers et al., 2010, Levin et al., 2002), and the response of native communities to these invaders can be highly variable at both local (i.e. among habitats within sites) (Ceccherelli et al., 2002, Bulleri et al., 2011) and larger spatial scales (i.e. among sites) (Bulleri et al., 2011, Gribben et al., 2015, Johnson, 2008, Tamburello et al., 2015). Whilst the effects of terrestrial native-invasive species can rival those of non-native invasive species (Carey et al., 2012), we know very little about the potential effects of spreading native macroalgae nor how they may vary across spatial scales. We may expect them to be equally negative and similarly variable across space.

The family Caulerpaceae contains some of the most invasive macroalgal species worldwide (Williams and Smith, 2007) and their establishment and impacts have been well documented (Glasby, 2013, Piazzini et al., 2016, Williams and Smith, 2007, Gribben et al., 2013). On shallow rocky shores in New South Wales (NSW), Australia, the native alga *Caulerpa filiformis* has become more locally abundant and has spread outside its known historic distribution (see Glasby et al., 2015 for an overview). *Caulerpa filiformis* is now the dominant habitat-forming species on many shallow rocky reefs

along the coast, where it can form extensive monospecific stands of >1000 m<sup>2</sup> (Glasby et al., 2015, Voerman et al. unpub. data). Although *C. filiformis* is predominantly subtidal (Glasby et al., 2015), intertidal experiments show that *C. filiformis* is able to trap high levels of sediment (Zhang et al., 2014), to which it may be highly tolerant (Glasby et al., 2015). At larger spatial scales, *C. filiformis* cover is high on small reefs that have beaches nearby (Glasby et al., 2015). These results suggest sediment may be important in determining the large-scale distribution of *C. filiformis*. However, little is known about the role of local-scale processes in determining *C. filiformis* cover within reefs. If increased sedimentation is indeed positively associated with *C. filiformis* cover, we may predict *C. filiformis* cover will increase with increasing distance from shore (i.e. closer to the reef edge/sand interface), increasing rugosity and reduced slope where less sand accumulates, and expect those local scale variables to be similarly important in accounting for variation in the species' cover as those changing over larger spatial scales (i.e. reef size).

The replacement or displacement of other native algae by *C. filiformis* may have severe implications for biodiversity of both flora as fauna (Zhang et al., 2014, Lanham et al., 2015). For example, on the rocky intertidal shores *C. filiformis* is a functionally distinct habitat for mobile epifauna with potential spill over effects to neighbouring resident alga (Lanham et al., 2015). Unexplored however is the effect of *C. filiformis* on the abundance and composition of subtidal algal assemblages. We may predict a negative relationship between *C. filiformis* cover and that of competitors, and an associated change in macroalgal community composition and diversity.

This study used large-scale biogeographic surveys on subtidal reefs throughout *C. filiformis*' entire distribution to determine whether the cover of *C. filiformis* differed among substrata, which habitat variables best explain its local distribution, and the relationships between *C. filiformis* cover and co-occurring native algae. We tested the following predictions: 1) *C. filiformis* cover is higher on turf substrate compared to other substrata available; 2) *C. filiformis* cover is positively related habitats with increased sand disturbance (reduced surface slope, increased rugosity and distance from

shore, and negatively correlated to increasing water depth and reef width); 3) presence of *C. filiformis* is related to changes in algal community composition and a reduced diversity.

## **2.3 Materials and Methods**

### **2.3.1 Study species**

*Caulerpa filiformis* is a large green alga, with leaf-like blades reaching up to 75 cm (Voerman unpublished data). *Caulerpa filiformis* spreads via asexual fragmentation (Khou et al., 2007), and once established it uses creeping stolons with root-like structures called rhizoids to anchor to the substrate. It has a disjunct distribution along ~ 700 km of the warm temperate coast of eastern Australia where it is primarily subtidal down to ~ 7 m but also occurs in low intertidal areas (Glasby et al., 2015). The alga is predominantly observed on rocky reefs, but can also occur independently on sandy substrate (Voerman, pers. observations).

### **2.3.1 Sampling**

We sampled subtidal rocky reefs at sites ( $n = 6$ ) throughout the known distribution of *C. filiformis* (Fig. 2.1). All sampling was conducted between August and December 2013. Sites were sampled in a random order to avoid potential influence of seasonal changes in the algal assemblage. Sites ranged from urban (Sydney metropolitan area) to pristine areas, however no anthropogenic influence on *C. filiformis* has been demonstrated (Glasby et al., 2015), and was not further investigated in this study. At each site, we sampled five transects each five meters apart. Transects ran perpendicular to the shoreline from the low-tide mark to  $> 6$  m distance beyond the edge of the reef (i.e. onto sand). Thus, all transects covered a similar depth range (between +0.5-6.5 m compared to low tide level) but varied in length from 30 to 60 m because of varying reef widths.

At two sites (Seal Rocks and Wollongong), transects stopped at 30 m without reaching the end of the reef, due to unfavourable weather conditions hindering the safety of the divers. For the same reason only 4 transects were wampled at Seal Rocks. The GPS location of each transect was marked with a handheld GPS (© Garmin eTrex 10) and direction of the transect was recorded *in situ* with a dive-compass. We placed quadrats (0.25 m<sup>2</sup>) at 3 m intervals along the entire length of each transect and took photographs of all quadrats for later analysis (see below) (n = 363 quadrats in total).

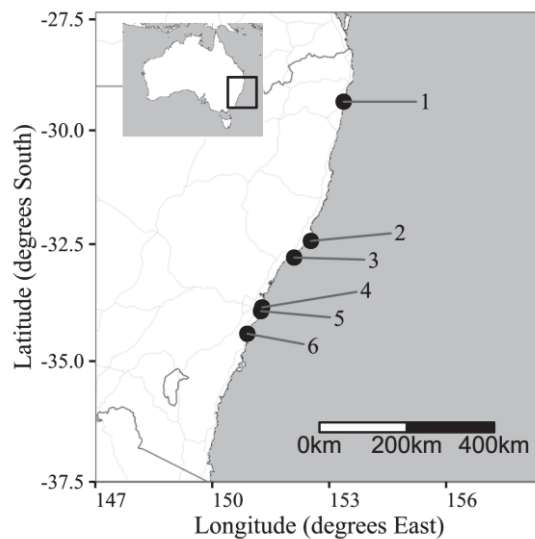


Fig. 2.1. Location of sampling sites in NSW, Australia (inset). Site numbers represent 1) Sharkies Cove; 2) Seal Rocks; 3) Kingsley Beach; 4) Bronte; 5) Coogee; 6) Wollongong. Note that Bronte and Coogee share one symbol. Those sites are 2.5 km apart. Map tiles were sourced from StamenDesign (2016).

### 2.3.2 Relationship between *Caulerpa filiformis* and the substrate occupied

For each quadrat, substrate types were identified *in situ*. Substrate types sampled were “rock” (rocky substratum without sediment present), “rock + sand” (“r+s”; rocky substratum covered with a sand layer 1-25 cm thick), “turf” (dense assemblages of mostly geniculate corallines and filamentous algae covering the rocky reef, < 5 cm in height, which could contain small amounts of sediment) and “sand” (the sandy ocean floor outside the rocky reef). There was also a fifth substrate type, “pebble” (rocks < 50

cm diameter), but this was relatively rare and removed from the analysis (< 2% of the quadrats). For each quadrat, the dominant substrate was identified in the field defined as the surface occupying > 80% of each quadrat. Quadrats without a dominant substrate type were excluded from subsequent analyses (3% of the quadrats). Photos were then used to estimate the percent cover of *C. filiformis* per quadrat using image analysis software with 100 equally distributed sampling points (ImageJ; Rasband, 2008).

A two-factor Univariate PERMANOVA (Permutational Multivariate Analyses of Variance) (PERMANOVA; Anderson, 2001) was used to determine whether the percent cover of *C. filiformis* differed among Substrata (fixed factor with 4 levels; rock + sand, rock, turf and sand) and Site (random factor, 6 levels). The analysis was based on a Euclidean distance matrix of untransformed *C. filiformis* cover data. Pairwise post-hoc tests were used to investigate differences among levels of significant factors and their interaction. P-values were calculated using 9999 permutations and type III sum-of-squares was used for this Univariate PERMANOVA test and those described below. All distance based analyses were conducted in PRIMER 6 (Primer-E Ltd, Plymouth UK) with PERMANOVA+ extension (Anderson et al., 2008, Clarke and Gorley, 2006).

### **2.3.3 Relationship between *C. filiformis* and environmental variables**

For each quadrat, the variables depth, distance from shore along the transect, slope and surface rugosity were determined *in situ*. Slope and rugosity were estimated visually. Slope ranged from 0° (horizontal) to 90° (vertical) and was estimated in 10° increments. Rugosity was classified as either: “low” (i.e. smooth or few small crevices < 2 cm deep), “intermediate” (several small to intermediate crevices up to 5 cm deep) or “high” (several crevices > 5 cm and a highly complex structure). For analysis, intermediate and high rugosity were combined to form a “high rugosity” level because of low replication within these classes. A linear regression model was used to determine if *C. filiformis* cover was associated with each of the environmental variables individually. The model

investigated the influence of the aforementioned local scale variables (depth, slope and rugosity, distance from shore) and large-scale variable reef width in predicting *C. filiformis* % cover. Reef width was calculated from the average length of the transects within each site. The edge of the reef was defined as where the reef meets the sandy ocean floor (with sandy substratum extending > 10 m from the edge of the reef). The length of the reef was calculated with Google Earth by the known GPS locations of the starting points of each transect and their directions. This method was used as some transects did not reach the end of the reef (see above) and thus length of the tape measure could not be used. For the linear regression model only quadrats on rock substrate with *C. filiformis* present were selected to exclude possible correlation between environmental variables and substrate types (n = 64). Additionally, the highest variation in levels of environmental variables was present for this substrate and thus harboured the most information. Two observations were identified as outliers by the Outlier Test ( $p < 0.05$ ) and removed from further analysis (Fox et al., 2016). Multi-collinearity of the environmental variables was examined using a Draftsman plot and Spearman correlation matrix. No variables were highly correlated (all correlation values < 0.3), thus all were included in the model. Partial-regression plots showing the relationship between one predictor variable and *C. filiformis* cover while adjusting for other predictor variables were produced in R (Fox et al., 2016). The variables best explaining the variation in *C. filiformis* cover were selected with a Bayesian Information Criterion (BIC) backward selection procedure. The relative importance of each of the predictor variables in explaining variation in *C. filiformis* cover was calculated following the LMG procedure for the model including all investigated variables and the best model selected (Lideman et al., 1980, Grömping, 2012). All linear regression model analyses and other non-distance based univariate tests in this study were conducted in R and associated packages (R Core Team, 2015, Fox and Weisberg, 2011, Wickham, 2009).

### 2.3.4 Relationships between *Caulerpa filiformis* occurrence and other macroalgae

Using each photo-replicate (see methods described above), we determined the identity and % cover of macroalgae in each quadrat. Macroalgae were identified to the lowest reliable taxonomic level or morpho-functional group (e.g. geniculate corallines) when more precise identification was not possible from the photographs (Steneck and Dethier, 1994, Balata et al., 2011). *C. filiformis* was excluded from the community data. Algal morphological groups and species identified were: Encrusting algae: crustose coralline algae (species unidentified); Geniculate (Gen.) corallines (e.g. *Corallina* spp. and *Amphiroa* spp.); Brown filamentous (fil.) turf: (e.g. *Sphacelaria* spp.); Green filamentous turf (species unidentified); Small browns (foliose & fleshy ochrophyta <15cm high, e.g. *Zonaria* spp., *Dictyota* spp., *Lobophora* sp., *Padina* spp., *Colpomenia* sp.); Small greens: (foliose & fleshy chlorophyta < 15cm high, e.g. *Ulva* spp., *Codium* spp.); *Sargassum* spp.; *Ecklonia radiata* (kelp).

A three-factor PERMANOVA was used to explore the relationships between algal community structure and the factors *C. filiformis* occurrence (fixed factor; 2-levels, present/absent), Site (random factor; 6 levels) and Substrate (fixed factor; 4 levels). Within sites, only substrata with at least 3 quadrats in which *C. filiformis* was present or absent were included in the analyses (see Fig. 2.4 for available substrata within sites). This test was based on a Bray-Curtis dissimilarity matrix based on untransformed macroalgal % cover data. Pairwise post-hoc tests were used to investigate differences among levels of significant factors. One-way SIMPER analyses were used to identify which alga contributed most to the differences in community structure for significant factor levels or their interaction.

We then used the same three-factor PERMANOVA design described above to determine the relationship between *C. filiformis* occurrence, Site and Substrate and richness of the earlier defined morphological groups. The analysis used a Euclidean



distance matrix of richness of the associated algal community in each quadrat (thus excluding *C. filiformis*).

## 2.4 Results

### 2.4.1 Relationship between *Caulerpa filiformis* and the substrate occupied

#### *Relationship between Caulerpa filiformis and the substrate occupied*

*Caulerpa filiformis* was present on all substrata investigated, and was found in 80%, 54%, 34% and 9 % of the quadrats on r+s, rock, turf and sand substrata sampled respectively (n = 46, 119, 92 and 92). When present on r+s substrate, *C. filiformis* was connected to the bedrock with its stolons and rhizoids, while buried with sand. When found growing on sand, *C. filiformis* was never present as an independent patch on this soft substratum, and always an extension of an individual connected to the nearby rocky reef (up to 0.5 m away).

The cover of *C. filiformis* reached 100% in some quadrats and was greatest on r + s substrate and least on sand (Fig. 2.2). The mean cover of *C. filiformis* varied significantly with the interaction of Site and Substrate ( $p < 0.01$ ) (Table 2.1a). This interaction was not caused by different patterns among substrata within sites, but in part by variation among sites in the magnitude of difference of *C. filiformis* cover among substrata (Fig. 2.2). The patterns among substrata in *C. filiformis* cover were highly consistent among sites (r+s > rock > turf > sand), although some substrata were not present at all sites (Fig. 2.2). Also contributing to the observed interaction was the variation in *C. filiformis* cover among sites on rock and r+s, but not on sand and turf substrate (Table 2.1a). On rock substrate *C. filiformis* cover was high at sites with narrow reefs (Site 3 = 4 = 5,  $p > 0.05$ ), and lower at some sites with wider reefs (Site 1 = 2,  $p > 0.5$ ). The one exception to this was the widest reef (Site 6), which was not different from other sites for rock substrate (Fig. 2.2) ( $p > 0.05$ ).

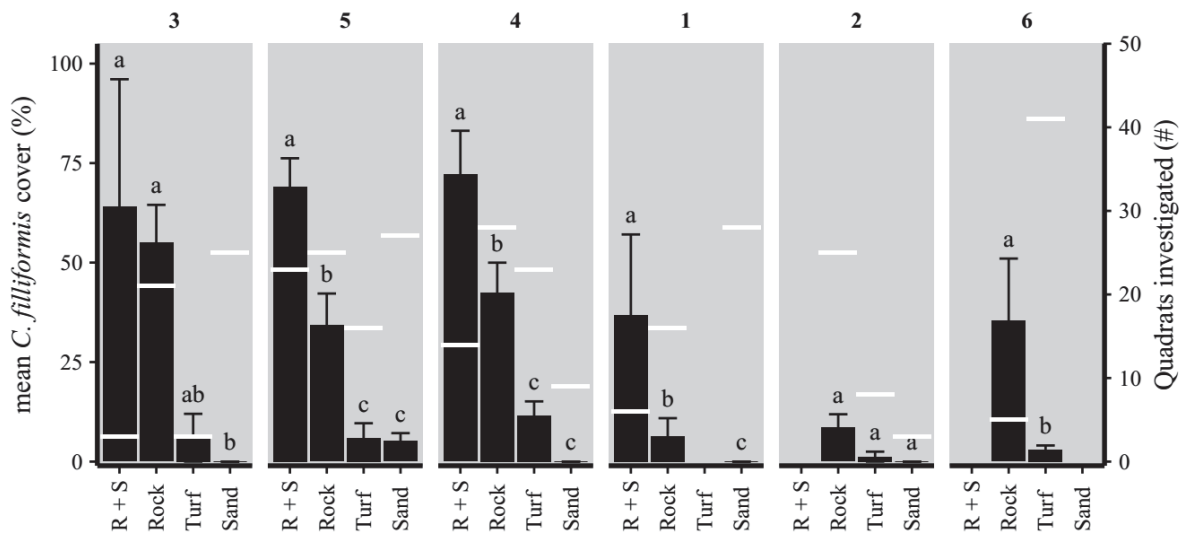


Fig. 2. 2. Mean percent ( $\pm$  SE) cover of *Caulerpa filiformis* (left y-axis) on different Substrata (below panels) (r+s, rock, turf and sand) at different Sites (above panels). Different letters indicate significant differences in *C. filiformis* cover among Substrata within Sites ( $p < 0.05$ ). White bars represent the area investigated (right y-axis). Sites are ordered by reef width, starting with the narrowest reef on the left. Note that only Substrata with  $n \geq 3$  quadrats/site have black columns with SE present.

Table 2. 1. Results of PERMANOVA tests performed to evaluate how **A)** the cover of *Caulerpa filiformis* varied with Site, Substrate and their interaction based on a Euclidean distance matrix and how **B)** community (excl. *C. filiformis*) structure and **C)** morpho-functional richness (excl. *C. filiformis*) differed among levels of *C. filiformis* presence, Site, Substrate and their interactions based on a BC and Euclidean distance matrix, respectively.

	df	Ms	Pseudo-F	<i>p</i>
<b>A) <i>C. filiformis</i> cover</b>				
Site	5	3277.7	4.9284	<b>0.001</b>
Substrate	3	26208	18.633	<b>0.001</b>
Si x Su	11	1725.3	2.5941	<b>0.003</b>
Residual	329	665.08		
<b>B) Community structure</b>				
Site	3	15356	12.517	<b>0.001</b>
Substrate	5	25844	5.3662	<b>0.001</b>
<i>C. filiformis</i> Presence	1	1822.3	0.76057	0.486
Si x Su	11	7127.9	5.8102	<b>0.001</b>
Si x Pr	5	3169.3	2.5834	<b>0.001</b>
Su x Pr	3	1806.3	0.59391	0.710
Si x Su x Pr	7	2895.5	2.3603	<b>0.001</b>
Residual	313	1241.7		
<b>C) morpho-functional richness</b>				
Site	3	15.406	18.532	<b>0.001</b>
Substrate	5	18.73	6.8129	<b>0.010</b>
<i>C. filiformis</i> Presence	1	0.81198	0.25839	0.671
Si x Su	11	3.9845	4.7930	<b>0.001</b>
Si x Pr	5	4.6709	5.6188	<b>0.001</b>
Su x Pr	3	0.96363	0.38893	0.724
Si x Su x Pr	7	2.4268	2.9193	<b>0.012</b>
Residual	313	0.83401		

Significant *p*-values are in bold.

## 2.4.2 Relationship between *C. filiformis* cover and environmental variables

*C. filiformis* was present over the entire depth range sampled (+ 0.5 - 6.5 m), over the full length of the transects (ranging a distance 0-54 m from shore), on a wide range of slopes (from horizontal to vertical structures), and on low and high rugosity surfaces.

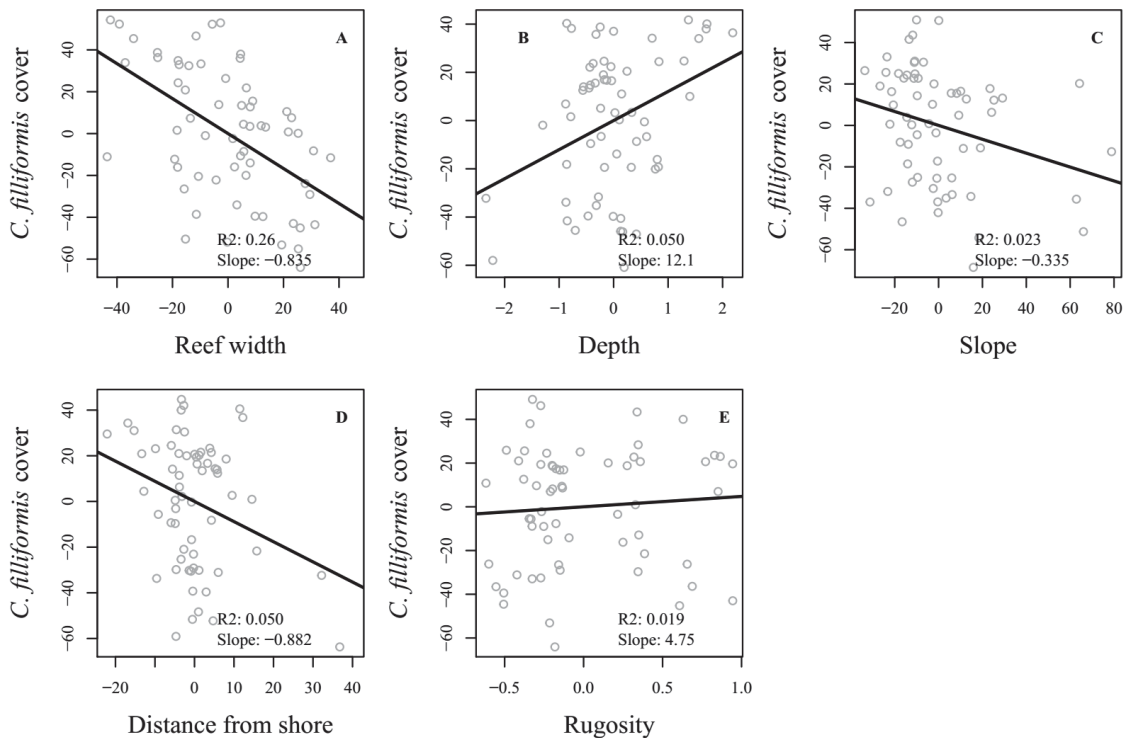


Fig. 2. 3. Partial-regression plots of relationship between **A)** Reef width, **B)** Depth, **C)** Surface slope, **D)** Distance from shore, **E)** Surface rugosity and *C. filiformis* cover. Only quadrats on rock with the alga present were selected. The line indicates the estimated relationship by the full model. Relative R<sup>2</sup> contribution based on the LMG method and estimated coefficient for each relationship are shown in the respective figures. The x- and y axis of the plots represent variation in x and y values after all other predictor variables are accounted for. See Table 2.2 for model summary.

The variable that contributed most to the variation in *C. filiformis*' cover on rock was the reef width (R<sup>2</sup> 0.26, Fig. 2.3a). *Caulerpa filiformis*' cover was negatively correlated with increasing reef width ( $p < 0.001$ ; Table 2.2; Fig. 2.3a). Cover was positively associated with depth ( $p < 0.01$ ; Table 2.2; Fig. 2.3b) and negatively associated with surface slope ( $p < 0.05$ ; Table 2; Fig. 3c) and with distance from shore ( $p < 0.05$  Table 2.2; Fig. 2.3d). No correlation was found to rugosity ( $p = 0.57$ ; Table 2.2; Fig. 2.3e). The best model selected by the BIC procedure excluded rugosity as a variable and explained 39.5% of the variation in *C. filiformis* cover, and included both large scale

(reef width) and local scale (depth, slope and distance from shore) variables. Differences in *C. filiformis* cover were for the largest part accounted for by reef width, which contributed for most (69%) of the variation explained by the model. Local scale variables accounted for 13%, 5.6% and 13% of the variation for depth, slope and distance from shore respectively.

Table 2. 2. Modelled relationships between environmental variables (reef width, depth, surface slope, distance from shore and surface rugosity) and *C. filiformis* cover. N = 62 observations. The table shows the full model estimated individual relationship between the environmental variable and each of the morphological characteristic investigated; Standard Error; t-value for individual predictors relationships and f-value for the entire model and *p*-value for each predictor variable individually and for the full model including all investigated variables and the most parsimonious model selected by the BIC procedure (which excluded variable surface rugosity).

	Slope	SE	t / f	<i>p</i>	BIC
(Intercept)	91.34	11.15	8.19	<b>&lt;0.001</b>	
Reef width	-0.84	0.18	-4.56	<b>&lt;0.001</b>	
Depth	12.05	4.31	2.80	<b>0.007</b>	
Slope	-0.34	0.16	-2.14	<b>0.037</b>	
Distance from shore	-0.88	0.38	-2.31	<b>0.025</b>	
Rugosity	4.75	8.36	0.57	0.572	
Full model		28.29	7.423	<b>&lt;0.001</b>	432.92
Best model		28.12	9.308	<b>&lt;0.001</b>	429.15

Significant *p*-values are in bold.

### 2.4.3 Relationships between *Caulerpa filiformis* occurrence and other macroalgae

Algal community relationships were dependent on an interaction among *Caulerpa* occurrence, Substrate and Site ( $p < 0.01$ , Table 2.1b). Post hoc PERMANOVA-test results revealed that on rock substrate at Site 4 ( $p < 0.05$ ,  $t = 1.69$ ), Site 5 ( $p < 0.01$ ,  $t = 2.10$ ) and Site 1 ( $p < 0.05$ ,  $t = 1.91$ ) the algal community was significantly different in the presence of *C. filiformis*. The algal community was different on the r+s substrate at

Site 4 ( $p < 0.01$ ,  $t = 2.73$ ). No differences were observed for any of the other substrata within sites ( $p > 0.1$ ).

The morpho-functional groups that contributed most to community dissimilarity in the presence of *C. filiformis* differed among substrata and sites. However, the percentage covers of most algae were smaller in the presence of *C. filiformis*, irrespective of their location (Table 2.3). The notable exception was Site 1, one of the widest reef surveyed, where *Sargassum* spp. and geniculate corallines were less abundant, but small brown algae were more abundant in quadrats amongst *C. filiformis* (Table 2.3).

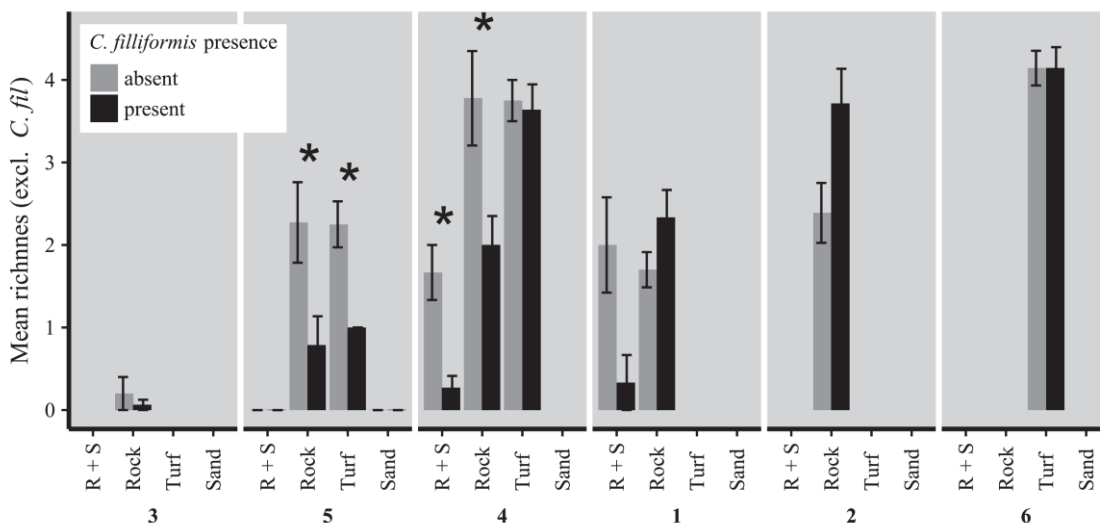


Fig. 2. 4. Mean ( $\pm$  SE) richness of the macroalgal community (excluding *C. filiformis*) per Substrate (below panel) per Site (above panel). Sites are ordered as per Fig. 2.2. Symbols indicate significant ( $p < 0.05$ ) differences between samples with *Caulerpa* absent (grey bars) and *Caulerpa* present (black bars). Note that only habitats with  $n \geq 3$  quadrats with *Caulerpa* present and absent have bars and SE present.

Richness of the associated algal community was not consistently lower in the presence of *C. filiformis* (Fig. 2.4), indicated by a significant three-way interaction between *C. filiformis* presence, Substrate and Site (Table 2.1c). Post hoc test results revealed a significant decrease in algal richness where *Caulerpa* was present on rock substrate at Site 4 and Site 5 (two narrow reef sites,  $p < 0.05$ ). Richness was also lower in the

presence of *C. filiformis* on r+s substrate at Site 4 ( $p < 0.05$ ). Turf substrate at Site 5 is marginally un-significant ( $p = 0.05$ ).

Table 2. 3. Morpho-functional group contributions to differences between samples with or without *C. filiformis* present as identified by SIMPER analyses per Substrate per Site. Columns represent mean percent cover of the main algal species in the absence of *Caulerpa filiformis*, average dissimilarity compared to cover in the presence of *C. filiformis*, dissimilarity/standard deviation and contribution to the total dissimilarity of community structure between groups of *Caulerpa* presence. Only results for Substrate x Site levels with significant *Caulerpa* presence effects on community composition as detected by the PERMANOVA post-hoc test are presented (Table 2.1B). Species are ordered by highest contribution to dissimilarity. Cut off for low contributions was 90%. Bold values indicate reduction in average percent cover in presence of *C. filiformis*. Algal morpho-functional groups identified were: Encrusting: crustose coralline algae; Gen. corallines: geniculate corallines (e.g. *Corallina* spp. and *Amphiroa* spp.); Brown fil. turf: brown filamentous turf as *Sphacelaria* sp.; Small ochrophyta (ochrophyta <15cm high, e.g. *Zonaria* spp., *Dictyota* spp., *Lobophora* sp., *Padina* spp., *Colpomenia* sp.); Small chlorophyta: (chlorophyta < 15cm high, e.g. *Ulva* spp., *Codium* spp.); *Sargassum* spp., *E. radiata*: kelp *Ecklonia radiata*.

Site (Substrate)	Species	Mean cover (%) <i>Caulerpa</i> absent	Average dissimilarity	Dissimilarity/ SD	Dissimilarity contribution (%)
1 (rock)	<i>Sargassum</i> spp.	87	<b>-21</b>	1.11	73
	Small ochrophyta	0	6	0.76	14
	Gen. corallines	3	<b>-1</b>	0.76	9
4 (rock)	Brown fil. turf	14	<b>-7</b>	0.81	32
	Encrusting algae	11	<b>-11</b>	0.72	24
	Small ochrophyta	14	<b>-13</b>	0.77	17
	<i>Sargassum</i> spp.	10	<b>-6</b>	0.55	14
	Gen. corallines	4	<b>-3</b>	0.51	9
5 (rock)	<i>Sargassum</i> spp.	37	<b>-20</b>	1.09	35
	<i>E. radiata</i>	20	<b>-7</b>	0.8	25
	Gen. corallines	17	<b>-16</b>	0.72	17
	Small ochrophyta	11	<b>-10</b>	0.77	12
	Encrusting algae	7	<b>-4</b>	0.79	8
5 (r+s)	<i>Sargassum</i> spp.	54	<b>-49</b>	3.48	90



## 2.5 Discussion

Understanding a spreading species' association to substrata and small scale environmental variables within a site, how these relationships vary across its spatial distribution and if potential impacts to associated macroalgal community varies across those different spatial scales, may aid in predicting which areas are most vulnerable to future colonisation. For *C. filiformis*, substrate associations appeared highly consistent among the different reefs investigated. For example, *C. filiformis* had higher cover on rocky substratum (both with and without a cover of sediment) at all reefs sampled. Also consistent at each site, although contrary to our predictions, *C. filiformis* had lower mean cover on turf substrate. Reef width was the most important environmental variable explaining its cover across reefs, but within reef characteristics were also important in determining its cover at the smaller (quadrat) scale. We also found that the associated macroalgal community was different in the presence of *C. filiformis*, linked to a reduced percent cover of most algal species and reduced diversity, although results varied among sites and substrata investigated.

### 2.5.1 Relationship between *Caulerpa filiformis* cover and the substrate occupied

The loss of canopy forming algae can promote the establishment of turfing algae, which in turn favours the recruitment of *Caulerpa* spp. on rocky reefs (e.g. Airoidi, 1998, Gennaro and Piazzzi, 2014, Bulleri and Benedetti-Cecchi, 2008, Piazzzi et al., 2016). The similarly low cover of *C. filiformis* on turfing algae observed across sites may have

occurred for two reasons. Firstly, turf is not a suitable substrate for the recruitment and growth of *C. filiformis* fragments, contradicting findings for other species of *Caulerpa* from other parts of this world (Bulleri and Benedetti-Cecchi, 2008). Or secondly, turf is a high-quality substrate for colonisation and growth, and *C. filiformis* quickly outcompetes turfing algae, resulting in post-recruitment populations of *C. filiformis* attached to rocky substratum in which turf is now excluded. Positive effects of turfing algae on the spread of the invasive *C. cylindracea* in the Mediterranean can result from turfing algae having high attachment quality and increased nutrient availability in the sediment trapped by the turfing algae (Piazzi et al., 2003, Bulleri and Benedetti-Cecchi, 2008). Although the colonisation of turfing algae by *Caulerpa* spp. does not always negatively affect turf cover, and even favours the abundance of algal turfs in some cases (Bulleri et al., 2010, Bulleri et al., 2016a), the cover of turfing algae was lower in the presence of *C. filiformis* at most sites investigated in this study. Experimentally manipulating *C. filiformis* fragments on different substrata will be important for determining the mechanisms underlying the patterns we observed.

Interestingly, the rock + sand substratum predominantly occurred in quadrats where *C. filiformis* was present, suggesting that *C. filiformis* is trapping sediment rather than the sediment being present prior to colonisation by *C. filiformis*. This is in line with the observations by Zhang et al. (2014) who showed that bare plots created in *C. filiformis* trap more sediment than bare plots created in nearby *Sargassum* spp.. In our study, the sediment layer in *C. filiformis* was up to 25 cm thick. Once established, trapping and tolerating high sediment loads may be a key mechanism aiding the spread of *C. filiformis* as demonstrated for other *Caulerpa* spp. (Piazzi et al., 2005, Glasby et al.,

2005, Piazzzi et al., 2007) as high sediment loads may remove competitors freeing up space for colonisation (Airoldi 1998; Zhang et al. 2014). For example, sediment disturbance may directly reduce the abundance of competing species without negatively affecting that of the invasive and sediment tolerant *C. cylindracea* (Piazzzi et al., 2005). Sedimentation may indirectly alter the outcomes of species interactions with *C. filiformis*, by reducing the competitive ability of co-occurring species. Alternatively, *Caulerpa* spp. can take up nutrients through their root like structures (Williams and Fisher, 1985), thus *C. filiformis* may benefit from nutrients available in the trapped sediment. Regardless, the ability to trap and withstand sedimentation may be a key positive feedback promoting the demography of *C. filiformis*.

### **2.5.2 Relationship between *C. filiformis* cover and environmental variables**

On rocky substratum, variation in *C. filiformis* cover was only partly accounted for by the environmental variables investigated in this study. Other processes affecting both local and large scale variation in algal abundance, such as changing hydrodynamic, grazing and competition with other sessile species, may also contribute to observed patterns in *C. filiformis* cover. In this study, the large scale variable, reef width, was by far the most important in accounting for variation of *C. filiformis* cover, with higher cover on narrower reef sites (see also Glasby et al. 2015). Narrower reefs may be more prone to sediment deposition than larger reefs, possibly due to increasing edge effects with narrow reef width (Wiens, 1985). Alternatively, larger reefs may contain larger, more stable patches of algae increasing invasion resistance (Dunstan and Johnson,

2004). Larger reefs may also contain higher biodiversity as they offer more niches (Bowden et al., 2001), which in turn can increase community stability and thereby reduce invasion success (e.g. Stachowicz et al., 1999, Levine and D'Antonio, 1999). Indeed, we observed higher taxonomic richness on wide compared to narrow reefs. Clearly, understanding how reef size influences sedimentation fluxes and interactions between *C. filiformis* and the resident algal communities will be an important avenue for future research.

Although accounting for relatively little of the variation observed, within a reef also local environmental variables were important in accounting for variation in the species' dominance, a pattern that is in agreement with several other studies of seaweed distribution (e.g. Lüning, 1990, Díaz-Tapia et al., 2013). At sites where it was present, the percentage cover of *C. filiformis* decreases with increasing distance from shore. This a pattern that was not observed by Glasby et al. (2015), although they only documented the presence of the alga across reefs. Thus, although the species may settle equally over the reef, higher cover closer to the shoreline may have resulted from increased negative effects of physical disturbance of co-occurring alga from wave action and/or suspended sediments creating space for horizontal spread by *C. filiformis*. Alternatively, and opposite to what was hypothesized, sand deposition may be higher closer to the shoreline where the reef meets the sandy beach positively affecting the horizontal spread of the alga (Santos, 1993).

Algae, including those from the *Caulerpa* genus (Marín-Guirao et al., 2015), are generally negatively affected by a reduced irradiance. However, the cover of *C. filiformis* also increased with increasing depth. Because we only surveyed to a depth of approximately 6 m, it is likely that some other process explains this pattern. For example, increasing water depth may promote sediment deposition to the benefit of *C. filiformis*. Reduced cover of *C. filiformis* with increasing slope (at the scale of within quadrats) may also have resulted from associated changes in boundary layer water flow, which may reduce sediment deposition and/or fragment contact time with the rock surface limiting their ability to successfully recruit to the rocky surface. However, hydrodynamic processes including those responsible of sediment movement and deposition are highly complex (Madsen et al., 2001), and we currently have no information on how they influence cover of *C. filiformis* at small scales and, importantly, across reefs.

### **2.5.3 Relationships between *Caulerpa filiformis* occurrence and macroalgal communities**

Relationships between algal morpho-functional richness and community structure and presence of *C. filiformis* were highly variable among sites and substrata considered. This pattern may be related to the total cover of *C. filiformis* that changed as a function of reef size and substrate type. For example, at the wider and intermediate reefs with lower percent covers of *C. filiformis* (Site 2, Seal Rocks, Site 3, Sharkies Cove and Site 6, Wollongong), richness of the remaining algal community did not decrease in the

presence of *C. filiformis*. This was in contrast to communities at narrow reef sites (Site 4 & 5) in which algal richness decreased with increasing *C. filiformis* cover at certain substrata. Algal community structure followed a similar pattern, with only significant differences with *C. filiformis* cover observed on narrow reef sites. An exception to this pattern was Site 3, a narrow reef site with high *C. filiformis* cover, where algal communities did not differ in the presence of *C. filiformis*. This may have resulted from the low cover and diversity of other algal species at this site, irrespective of the presence or absence of *C. filiformis*.

Similarly, within sites, changes in community structure and reduced morpho-functional algal richness in the presence of *C. filiformis* also only occurred on substrata on which *C. filiformis* obtained high cover (e.g. rock or r+s substrate) but not on turf or sand, where *Caulerpa* occurred in low cover. An absence of negative relationships on sand and turfing substrata may reflect their general unsuitability as substrata for algae. However, on turf substrate, high algal diversity was observed in quadrats with up to 50% cover of *C. filiformis*. Either *C. filiformis* never obtains percent covers higher than this on turf substrate, or this reflects a threshold above which *C. filiformis* quickly outcompetes turfing algae such that this substrate is not present at higher *C. filiformis* covers. Piazzì et al. (2001) showed strong effects of *C. cylindracea* on turfing algae, and suggested the advantageous conditions inside turf beds (e.g. nutrient concentrations) as a potential mechanism behind their negative relationship. Regardless, the varying biotic associations across different spatial scales highlights the importance of a multi-scale approach to understanding the potential mechanisms underlying a species distribution and effects on recipient communities.

The morpho-functional algal groups accountable for the observed reductions in richness was variable among substrata and sites, thus no single group was consistently absent from quadrats with *C. filiformis* present. Moreover, when changes in community structure were observed in the presence of *C. filiformis*, they were related to a reduction in the percent cover of all co-occurring groups with the exception of small brown algae at Site 1, which increased in the presence of *C. filiformis*. Although the mechanisms driving the negative relationships between *C. filiformis* cover and algal competitors of subtidal rocky substrata are unknown, on intertidal rocky shores, physical removal of competitors and sediment entrapment appear important for the recruitment and spread of *C. filiformis* (Zhang et al., 2014). Indeed, competition for space is one of the primary resources limiting algal distributions (Lüning, 1990), and the removal of competitors can facilitate an invaders' spread. However, the photosynthetic condition of fronds of the alga *S. linearfolium* interacting with *C. filiformis* was suppressed relative to those distant from *C. filiformis* suggesting some direct effect of *C. filiformis* on competitors (Zhang et al. 2014). Zhang et al. (2014) showed that algal species had lower recovery in cleared plots within *C. filiformis* beds versus within *Sargassum* spp. beds.

Understanding the mechanisms behind the patterns we observed will be an important avenue for future research.

Our study suggests that processes acting at multiple spatial scales may influence the establishment and spread of *C. filiformis* throughout NSW, and that the same processes affect the potential impacts on the macroalgae community. The challenge remains to experimentally determine how processes operating at the scale of reef (reef width and sedimentary processes) interact with quadrat scale processes (depth, slope, rugosity,

distance from shore and interactions with competitors) to determine the susceptibility of new areas to colonisation and the associated impacts.



## Appendix 2

### Appendix 2. Metadata of transects.

site ID	Name	Gps first transect	GPS final transect	Transect direction	# transects	Mean depth (m) (+- SE)	Max transect length (m)
1	Sharkies Cove	29°21'24.07"S, 153°21'40.41"E	29°21'23.36"S, 153°21'40.09"E	SSW	5	1.544 (0.08)	90
2	Seal Rocks	32°25'51.70"S, 152°31'31.05"E	32°25'50.99"S, 152°31'31.15"E	NNW	4	1.67 (0.11)	99
3	Kingsley Beach	32°47'18.42"S, 152° 6'11.84"E	32°47'19.05"S, 152° 6'11.61"E	SE	5	1.88 (0.06)	90
4	Bronte	33°54'18.97"S, 151°16'10.46"E	33°54'19.55"S, 151°16'11.04"E	NE	5	3.83 (0.15)	189
5	Coogee	33°55'23.00"S, 151°15'29.17"E	33°55'23.67"S, 151°15'29.36"E	NEE	5	2.05 (0.07)	234
6	Wollongong	34°25'5.68"S, 150°54'11.60"E	34°25'5.92"S, 150°54'12.35"E	NE	5	1.1 (0.1)	99



## **Chapter 3**

### **Influences of habitat and fragment characteristics on the recruitment of *C. filiformis***



### 3.1 Abstract

Habitat suitability is an important determinant in the success of invasive macroalga. Disturbance can alter resisting macroalgal canopies (e.g. kelp or *Sargassum* spp.) to alternative habitats (turf) that may in turn promote invaders' recruitment. Similarly, alternate habitat may be predicted to also facilitate the recruitment of native invading alga.

A native species of the notorious *Caulerpa* genus, *C. filiformis*, has spread within and outside its native range in New South Wales, Eastern Australia. In contrast to observations for other invasive *Caulerpa* species, adult beds were highly associated with rocky substrate with a sediment veneer, rather than turf habitat as seen for other species of the genus. A model was proposed where this observation may represent an end state of succession following the successful recruitment on this substrate.

Here, habitat associations of *C. filiformis* recruits were investigated at several sites throughout its distribution. In line with the predictions, recruit abundance on turf substrate was higher than what was expected compared to other substrate types. However, this was only true for geniculate coralline forms of turfing alga, and not filamentous forms. Moreover, no recruits were found of soft sediment.

A laboratory experiment showed that fragments attached faster and stronger on turf assemblages aided by increased structural complexity of the substrate, and not by the properties of the sediment found among turfing alga. On the other hand, attachment

strength on soft sediment was very low. Fragment morphology was also important in determining recruitment success, but there was a significant interaction with substrate investigated and time since the start of the experiment. The presence of a stolon may benefit fragments in early stages of attachment on certain substrata, but fragments consisting of only a frond were more successful after 4 days.

Thirdly, a field experiment was conducted where the recruitment success of *C. filiformis* fragments was tested in different habitats. It was predicted that large erect and canopy forming species (*Sargassum* spp. and *Ecklonia radiata*) would resist recruitment compared to bare rock, while turf would promote recruitment success. Following our predictions, turf habitat showed highest fragment retention, however, results were highly weather dependent and fragment retention was generally low under periods of high swell.

This study showed the importance of suitable habitat for recruitment in the spread of a native alga, and demonstrated the mechanisms behind the facilitative role of turf habitat in the recruitment success of *C. filiformis*.

### 3.2 Introduction

Rocky-shores have been a focal ecosystem for understanding the effects of disturbance that alters substrate availability on the success of non-native invasive marine species (e.g. Occhipinti-Ambrogi and Savini, 2003, Valentine and Johnson, 2003, Bando, 2006). In such ecosystems, the loss of native macroalgal canopies may open up space allowing for invader recruitment, while intact canopies resist invasion (Thompson and Schiel, 2012b, Valentine and Johnson, 2003, Vadas et al., 1992, Britton-Simmons and Abbott, 2008). Alternatively, the loss of native macroalgal canopies may promote alternative native habitats that in turn enhance invader recruitment. For example, turfing algae may promote recruitment of several non-native invasive macroalgae, such as the non-native *Undaria pinatifida*, several *Caulerpa* species and *Codium fragile* spp. *fragile* (Thompson and Schiel, 2012b, Gennaro and Piazzini, 2014, Watanabe et al., 2009, Bulleri and Benedetti-Cecchi, 2008). Similarly, the recruitment success of invasive native macroalgae may also be affected by inhibiting intact canopies, opening up space, or promoting alternate habitats.

Secondly, attributes of the spreading species themselves are also important in their recruitment success. Large reproductive output (i.e. propagule supply) is typically related to the recruitment success of invaders (Lockwood et al., 2005). Although similar observations have been made in marine environments, they have received much less attention (Johnston et al., 2009 and ref therein, Dayton et al., 1984). Similarly, we may predict propagule supply to be important for the distribution of recruits of spreading

native macroalgae, which may change with nearby adult abundance (if the species has limited propagule dispersal).

Based on terrestrial literature, the successful recruitment of a species may also depend on the traits of its propagules, such as their size or morphology (e.g. Kimmerer, 1991, Weis, 1982, Stanton, 1984, Pyšek and Richardson, 2008). Asexual reproduction from fragments is a common reproductive strategy among macroalgae, especially amongst some of the world's most invasive species (Williams and Smith, 2007). However, we know surprisingly little about which attributes may alter the successful recruitment of algal fragments. Fragment morphology appears important in influencing recruitment performance, with larger, more developed fragments often having enhanced recruitment success (Khou et al., 2007, Watanabe et al., 2009, Walters et al., 2002, Walters and Smith, 1994).

The family Caulerpaceae contains some of the most invasive macroalgal species worldwide (Williams and Smith, 2007), yet recruitment success can vary greatly among habitats (e.g. Bulleri and Benedetti-Cecchi, 2008, Marín-Guirao et al., 2015). On shallow rocky shores in New South Wales (NSW), Australia, the native *Caulerpa filiformis* is becoming more locally abundant and spreading outside its known historic distribution (see Glasby et al., 2015 for an overview). *C. filiformis* is now the dominant habitat-forming species on many shallow rocky reefs along the coast, where it can form extensive monospecific stands of > 1000 m<sup>2</sup> (Glasby et al., 2015, Voerman et al. unpub. data). The species spreads vegetatively by fragmentation, and can be highly successful doing so (Khou et al., 2007). Moreover, Glasby et al. (2015) also attributed the spread



400 km outside its native range to its successful vegetative reproduction. Rapid and successful establishment of propagules is important in the success of any macroalga (e.g. Wright and Davis, 2006), especially on exposed shores such as those that *C. filiformis* inhabits. However, little is known about the processes affecting the initial stage of recruitment of this species. *C. filiformis* competes for space with canopy forming kelp *Ecklonia radiata* and several *Sargassum* spp. (Voerman et al., Chapter 2). Empty space created by disturbance to those canopies is typically followed by recruitment of turfing algae (Connell, 2007, Toohey et al., 2007), and turf is a dominant habitat along *C. filiformis* its distribution (Chapter 2a). Thus, kelp, *Sargassum* spp. and turfing algae habitats are likely most important in defining *C. filiformis* recruitment success. Additionally, Khou et al. (2007) showed that a wide variety of morphologies and sizes of *C. filiformis* fragments can be found on exposed coasts in NSW, including fragments with or without stolons, and that settlement success may vary with fragment morphology. Similar to non-native invasive *Caulerpa* species, we may expect recruitment success to differ between recipient habitats.

The overall aim of this study was to determine how the recipient habitat and the morphology of *C. filiformis* fragments influence its recruitment success. First, we conducted large scale surveys at sites throughout *C. filiformis*' distribution to determine the abundance of *C. filiformis* recruits in different habitats (within sites), and relationships between number of recruits and adult density (among sites). We predicted that the number of recruits would increase with adult bed size. We hypothesised that the number of recruits would vary among habitats and in particular be greatest on turf and bare rock habitat and least amongst intact kelp and *Sargassum* spp. habitats. In

laboratory experiments, we then experimentally investigated the potential mechanisms influencing settlement success (attachment speed and strength). Here we tested the hypotheses that both the physical properties of the turfing algae, properties of the sediments contained in the turf and fragment morphology influence settlement success. Thirdly, we explored the effect of different recipient habitats on recruitment success by investigating the retention success of fragments placed in different habitats in the field. The expectation was that *C. filiformis* fragments would recruit more successfully to turf compared to bare rock, kelp and *Sargassum* habitats.

### **3.3 Materials and methods**

#### **3.3.1 Study species**

*Caulerpa filiformis* (Suhr) Hering is a large green alga, with leaf like blades up to 75 cm in length (Voerman et al. unpublished data). Established fragments consist of a dense network of creeping rhizomes with rhizoid clusters which attach the alga to the substrate (Khou et al., 2007). It has a disjunct distribution along ~ 700 km of the warm temperate coast of eastern Australia where it is primarily subtidal down to ~ 7 m but also occurs in low intertidal areas (Glasby et al., 2015). *C. filiformis* spreads via asexual fragmentation (Khou et al., 2007), with often thousands of fragments suspended in the water column at a single site (Voerman et al. unpublished data). Fragments consist of simple or branched blades, without rhizome or rhizoids present, and are predominantly 5-15 cm in length (Voerman unpubl. data). *C. filiformis* has a siphonous body structure, which allows for

rapid growth, wound healing and fast fragment propagation (Smith and Walters, 1999, Walters and Smith, 1994).

### **3.3.2 Density of *C. filiformis* recruits in different habitats**

To investigate the density and distribution of recruits across and within reefs, 6 sites throughout the distribution of *C. filiformis* were sampled during August-December 2013 (Fig. 3.1). Recruits were defined as small individual fragments (< 5 cm diameter of the individuals, with a maximum of 5 fronds). Whilst size is not a measure of recruitment, individual fragments of this size have not yet expanded horizontally, and likely represent fragments that have attached to the substrate relatively recently. Recruits were enumerated in transects (1 m wide) placed perpendicular to the shoreline from the low-tide mark to > 6 m beyond the edge of the reef (i.e. onto sand). Transects covered a similar depth range (between +0.5-6.5 m compared to low tide level) but varied in length from 30 to 60 m depending on the width of the reef. At two sites (Seal Rocks and Wollongong), transects stopped at 30 m without reaching the end of the reef due to unfavourable weather conditions hindering the safety of the divers. At all sites 5 transects were conducted, with the exception of Seal Rocks where 4 transects were investigated. For each recruit encountered we noted the substrate to which it was attached, which could be abiotic (e.g. rock) or biotic (e.g. encrusting algae) (Table 3.1). Red geniculate coralline algae and filamentous forms are often seen to be part of the same assemblage of turfing algae (Connell et al., 2014), but as the functioning of both forms may differ we investigated them separately in this study (Table 3.1).

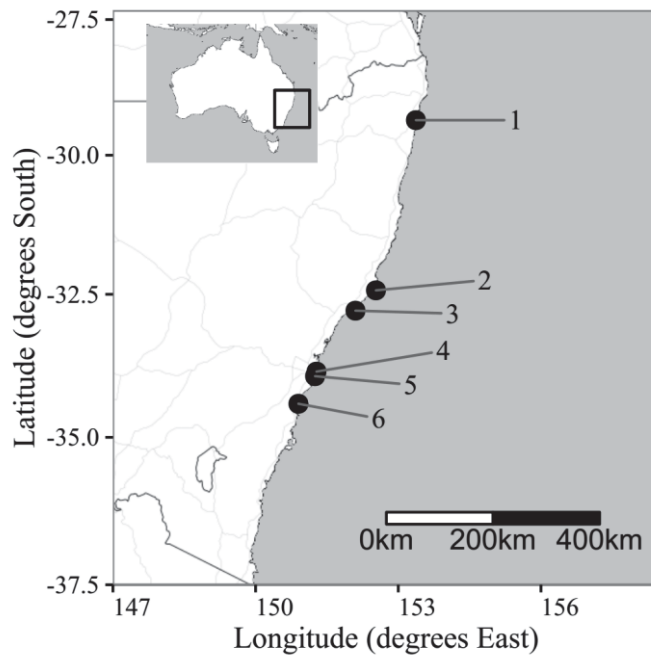


Fig. 3. 1. Map of 6 sampling sites and study site Malabar, NSW, Australia (inset). Site numbers represent 1) Sharkies Cove; 2) Seal Rocks; 3) Kingsley Beach; 4) Bronte; 5) Coogee; 6) Wollongong. Map tiles were sourced from StamenDesign (2016).

To determine recruit densities as a function of area of individual habitats sampled at a site, 0.25 m<sup>2</sup> quadrats were positioned at 3 m intervals along the transects. Each quadrat was photographed and the percent cover of the habitats described was determined. Other habitats were present (e.g. pebble and sessile fauna), but they were rare (< 2% of sampled area) and no recruits were observed on those habitats and thus they were excluded from further analyses. Additionally, no recruits were observed on the sand outside the reef and the presence of new recruits in dense adult *C. filiformis* beds could not be determined so these habitats were also not investigated further. The percent cover of the individual habitats in the quadrats were used to estimate total area of each habitat sampled by the full transects at each site. Recruit densities were calculated for each of the habitats investigated at a site (number of recruits on a certain habitat observed divided by the estimated area investigated).

To investigate differences in recruit density among sites, recruit densities were calculated for the available reef at a site. The available reef consisted of the area of reef sampled by the transects minus the extrapolated area covered by adult *C. filiformis*. Associations between recruit density and adult *C. filiformis* density per site (% of rocky reef investigated covered by adult *C. filiformis*) were examined with a linear model and tested for significance with an ANOVA test. All statistics were conducted in R (R Core Team, 2015).

To test for differences in recruit density among habitats within sites, the observed recruit densities were compared to that of their expected densities under equal association with the available habitats, and  $\chi^2$  goodness-of-fit tests were used to test for equal abundances of recruits across habitats per site ("XNomial" Package, Engels, 2015). Habitat associations were investigated at only four of the sites as no recruits were observed at Site 3, Kingsley Beach or Site 1, Sharkies Cove (Fig. 3.1 & 3.2).

Table 3. 1. Main substrata encountered when surveying for *C. filiformis* recruits.

Substrate	Description
<b>Abiotic</b>	
Bare rock (r)	Empty rocky substrate with no or little sediment present
Rock+ sediment (r+s)	Rocky substrate with a 1-25cm sediment veneer present
Sand	Sandy substrate outside the rocky reef
<b>Biotic</b>	
Red geniculate corallines (corallines)	Red geniculate coralline algae of < 5 cm height of predominantly <i>Corallina</i> spp. This habitat could have some filamentous forms present as well, but this was < 50% of its cover.
Filamentous turf	Filamentous brown and green turfing algae of < 5 cm height, species unidentified
Small foliose brown algae	Foliose brown algae <15 cm in height which included <i>Dictyota</i> spp, <i>Zonaria</i> spp. and <i>Padina</i> spp.
Encrusting algae (encrusting)	Encrusting algae forming covering the rocky substrate
Kelp habitat (kelp)	Bare rock sometimes partly covered with encrusting algae under a 100% <i>Ecklonia radiata</i> canopy
Sargassum habitat (Sargassum)	Bare rock or rock with a dense cover of turfing algae, with sometimes little sediment present, under a 100% <i>Sargassum</i> spp. canopy

### 3.3.3 Attachment performance associated with substrate types and fragment characteristics

Results suggested an important role of turf substrate in promoting fragment recruitment but the opposite for sand. Here we investigated the effects of turf and sand on the attachment performance of *C. filiformis* recruits. More specifically, for turf substrate we investigated both the role of the physical properties of the turfing algae (structure) and

the properties of the sediments inside the turf. We created a number of substrate treatments: natural turf attached to rock with sediment removed and replaced (r+t+s), natural turf on rock with the sediment removed (r+t-s), rock with turf scraped off (r-t), naturally occurring bare rock (r), artificial rock with turf, artificial rock (ceramic tile with rough surface facing up), and sand (s). R+t+s tested for the overall effect of turf sediment on attachment performance which was expected to be enhanced in comparison to r+t-s. R+t-s tested for the overall effect of turf tissue and was expected to be enhanced compared to r-t. Bare rock was a handling control for the scrapping of turf from the r-t treatment and performance was expected to be similar. Artificial turf tested for the effect of the physical structure of turf and was expected to be equal to the natural turf treatment and enhanced compared to an artificial rock treatment. The sand treatment tested for the attachment performance on soft sediment, and attachment performance was expected to be lower on sand in comparison to any of the other substrata.

Small (5-10 cm widest diameter) relatively flat rocks were used as substrata for this experiment and collected from the intertidal region at Seal Rocks (-32.430981, 152.525168) before being transported in seawater to the research facility at DPI Port Stephens. They were kept in large tanks with flow through estuarine water (~34 ppt). Tanks were aerated and kept under 12:12 h light:dark cycle at ambient temperature for 4 days until the start of the experiment. Turf consisted of an assemblage of small red, green and brown algae < 4 cm tall, but red geniculate corallines were most dominant ( $\pm$  50% cover). R+t+s received equal amounts of sediment as observed in the field, and

was removed and replaced as part of the sediment was lost during transport. In the laboratory, we made sure all sediment was removed from the rocks with turf to create the r+t-s treatment, mixed, and added in naturally occurring amounts to the turf to form the r+t+s treatment. Part of the rocks with turf were carefully cleaned with a heavy duty tile brush to form the r-t treatment. Naturally occurring bare rocks were not treated to form the rock treatment. Artificial rock consisted of a kitchen tile with the ceramic part facing upwards. This side consisted of an even surface with small scale pores and a small fish-scale structure with +/- 1mm surface relief. A ~4 mm thick heavy duty scourer, 70% nylon and 30% polyester ©MrClean, glued onto artificial rock, which was similar in size and structure to natural turf assemblages, was used to create the artificial rock + turf treatment. Sand was sourced adjacent to the turf, and a 2 cm thick layer was created to form the s treatment. Single habitat treatments covered the base of shallow plastic trays (150 x 95 x 60 mm) with openings added on the side to allow for water flow.

To test for the effect of fragment morphology on fragment performance, intact *C. filiformis* was collected from Newcastle (-32.925618, 151.793215) from large boulders around the low tide mark and transported to the laboratory in seawater. Two fragment types were made: simple or complex. Simple fragments consisted of a blade with the rhizome removed. Complex fragments consisted of a frond with 2 cm rhizome attached to it. Rhizomes were selected to have 2-4 rhizoid clusters present. Blades were 8-15 cm in length. Fragments were kept in aerated seawater with constant flow for 48 h to allow for wound healing. Wet weight of all fragments was determined prior to the start of the



experiment. Fragment weight did not differ between substrate or complexity treatments at the start of the experiment ( $F_{1,55} = 0.93, p > 0.1$  and  $F_{6,55} = 1.75, p > 0.5$  for substrate and complexity treatments respectively).

Each fragment was then placed in the centre of a tray (n = 5 replicate trays per Treatment \* Complexity combination). Trays (n = 70 in total) were divided over 10 larger tanks so that every large tank had each of the seven treatments present following a randomized block design. Tanks were kept under the same conditions as described above. Attachment success was tested every second day by carefully shaking the treatment tray and checking for movement of the fragments.

The effects of Substrate and Complexity on attachment success were tested with a biased reduction Brglmer model for binomial data ("brglm" Package, Kosmidis, 2013) and type II ANOVA test ("Car" Package, Fox et al., 2016) for each sampling day. Initial wet weight was added as a covariate to the model to account for possible effects of biomass, and tank was added as blocking factor. Significant treatment effects were further investigated with Post Hoc Lsmeans comparisons ("lsmeans" Package, Lenth, 2016). No adjustment was used as we chose to reduce the chance of type II error by the low number of replicates for binomial data used in this experiment.

Fragment performance was also investigated in terms of growth rate, growth of rhizoid clusters and attachment strength. To investigate differences in growth rates among complexity and substrate treatments, we measured total fragment wet weight at the start

and end (day 10) of the experiment. To investigate production of new rhizoid clusters, final number of rhizoid clusters attached to the substrate were counted and adjusted for initial rhizoid clusters of complex fragments. To investigate attachment strength among fragment complexities and substrata, attachment force was tested at the end of the experiment for all fragments that had attached. Force was measured by attaching a clasp with two wooden stirring sticks to the rhizome, to spread the force over the full rhizome and avoiding breaking the tissue, attached to a pull balance.

The effect of Substrate and Complexity on change in number of rhizoid clusters (end minus start number), attachment strength and biomass change (end minus start wet weight) was tested with general linear models with the same predictor variables and covariates as described above. Model assumptions were tested with the GVMMLA package (Pena and Slate, 2004), no adjustments were made as all assumptions were met. Only fragments that had attached were used to test for differences in attachment strength and number of rhizoid clusters attached to the substrate. Type II ANOVA tests were conducted and significant treatment effects were further investigated with Post Hoc Lsmeans comparisons (Lenth, 2016).

#### **3.3.4 Retention of fragments in different habitats**

Here we test whether patterns of recruit density and attachment performance differed across the habitats quantified in Table 3.1. We conducted a field experiment at Malabar in which we placed fragments in different subtidal habitats (Turf, *Sargassum*, Kelp and

Rock) (Fig. 1). Fragments were placed in 25x25 cm plots (n = 8 plots for Turf and *Sargassum* and n = 4 plots for Kelp and Rock; 5 fragments/ plot in 2015 and 7 fragments/plot in 2016).

All plots were placed in patches of habitat (1 plot/patch), and were > 1 m apart. Habitats were interspersed and > 4 m<sup>2</sup> each, except for the kelp plots which were all positioned inside the same large kelp (> 20 m<sup>2</sup>) patch. Plots were positioned on horizontal surfaces and were positioned at a similar depth (1.5-3 m below low tide level). Turf treatment consisted of a natural assemblage of a dense cover of mainly red geniculate coralline species such as *Corallina* spp. and some brown filamentous turf (species unidentified) < 5 cm tall. Kelp habitats consisted of 100% canopy cover of *Ecklonia radiata*, with plots placed underneath the canopy on bare rock with a low cover of encrusting algae.

*Sargassum* habitats consisted of a mix of dense, erect *Sargassum* spp. with a cover of 80-100%. Plots consisted of 4 stainless steel bolts on each corner of a 25 x25 cm to which plastic mesh (25 x 25 mm mesh size) (©Gardenmaster) was attached to the bolts close to the rock surface. Although the presence of mesh may have resulted in extra protection from e.g. frond sweeping or wave action, potentially confounding the results of this experiment, this was necessary for retaining the fragments in the respective habitats to allow for fragment settlement. As fragments were only attached loosely to the mesh the effects of the mesh were kept minimal.

*C. filiformis* was collected from a large patch adjacent to the experimental site and transported to the lab where we created fragments consisting of single, unbranched,

blades, each 10 cm in length. Fragments were kept in aerated ocean water with constant flow at a 12:12 h light:dark cycle at 21°C for 3 d to allow for wound healing. Fragments were then checked for full wound healing, transported back to the site where we added intact fragments underneath each mesh plot. Fragments were loosely attached to the mesh with two thin (< 0.5 mm wide) strips of duct tape. We conducted the experiment twice, once during January 2015 and again in January 2016. The 2015 experiment received 5 fragments per plot. By the low retention success of fragments, the number of fragments/ plot was increased for 2016 experiment, and plots received 7 fragments each.

For the 2015 experiment, plots were revisited every 3-6 d during the first 2 weeks, and then every 2 weeks until the experiment stopped after 8 weeks. The 2016 experiment was revisited every 2-9 d, depending on ocean conditions permitting sampling, and ran for 3 weeks. Each time the plots were revisited, the number of fragments still present were counted. Differences in fragment retention among habitats were tested with ANOVA (R Core Team, 2015) for each sampling date. Post-hoc comparisons based on least-squares means with Bonferroni adjustment were performed to investigate differences among habitats ("lsmeans" Package, Lenth, 2016). As fragment retention was very low, surface cover of *C. filiformis* did not increase in the plots and was not further investigated.

### 3.4 Results

#### 3.4.1 Distribution of recruits in the field

Recruits were found at four of the six reefs sampled and absent from Site 1, Sharkies Cove, and Site 3, Kingsley Beach. At sites where recruits were found, densities ranged from 5 (Site 5) to 13 (Site 2) individuals per 100 m<sup>2</sup> (Fig. 3.2). Opposite to what was expected, observed recruit numbers were not associated with adult cover at a reef ( $F_{1,4} = 3.23, p > 0.15$ , Fig. 3.2). However, when Sharkies Cove (Site 1) is removed from the analysis, a nearly perfect negative relationship is observed (slope =  $-2.3 \times 10^{-3}$ ,  $F_{1,3} = 4536, p < 0.001, r^2 = 99.93$ ).

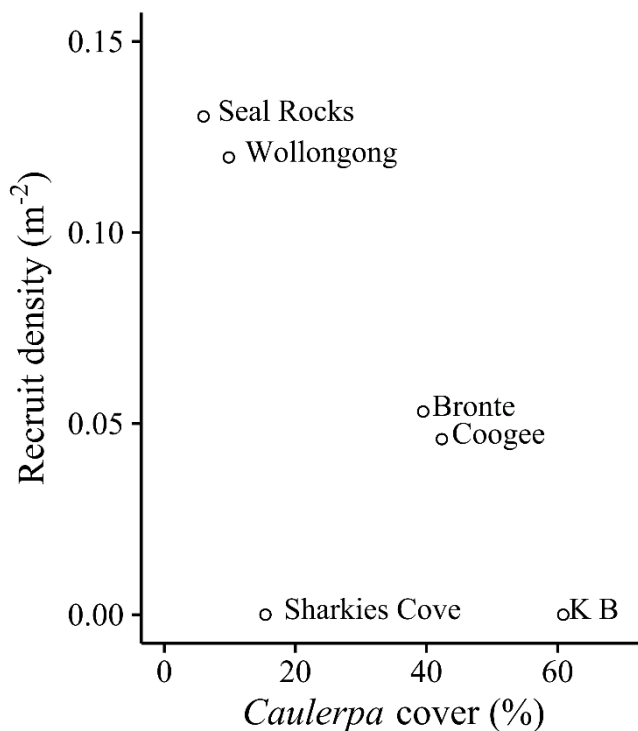


Fig. 3. 2. Associations between adult *Caulerpa* cover (%) of entire reef area investigated and recruit density (individuals/m<sup>2</sup>) on the available rocky reef investigated (that is reef area investigated minus adult *Caulerpa* cover) at a site. K B = Kingsley Beach.

Although recruits were found in a broad range of habitats, within sites, the density of recruits varied among habitats ( $p < 0.001$ ). As per our hypothesis, we observed more recruits on turf habitat than other habitats, however results depended on the guild of turf investigated. Namely, recruit density was up to 7 times higher than what was expected by chance given the proportions of each habitat per site in coralline habitat (Fig. 3.3). This result was consistent across all sites. Conversely, there was no positive association between *C. filiformis* fragments and filamentous forms of turfing algae. Also in line with our hypotheses was the observed low association with kelp habitat, with no recruits observed in this habitat. Similarly, recruit density was lower than expected in *Sargassum* habitat at nearly all sites except Site 5, Coogee (Fig. 3.3). No recruits were observed on encrusting algae or on rocky substrate when a sediment veneer was present (r+s, Fig. 3.3).

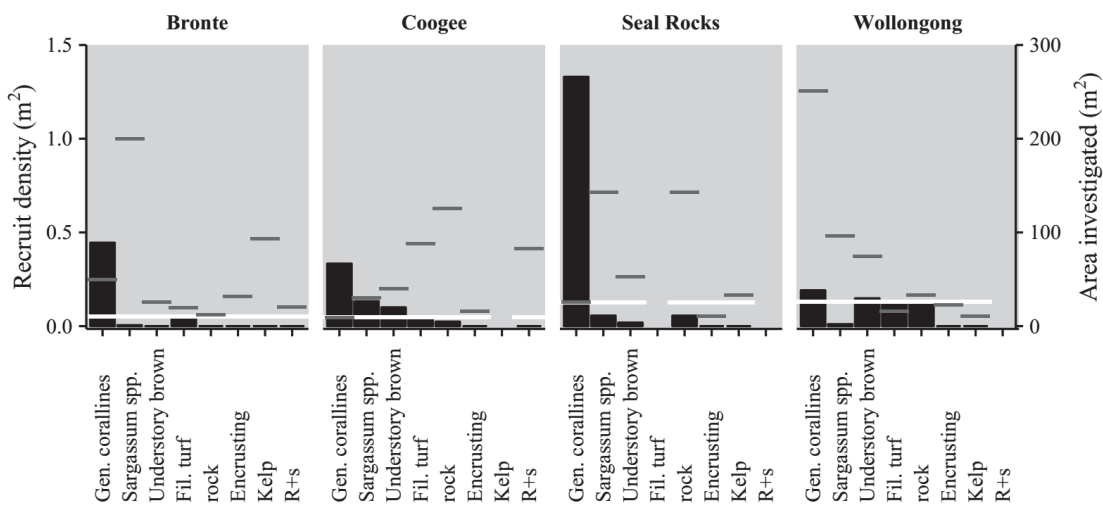


Fig. 3. 3. Observed (bars) and expected (white line) density (left y-axis) of recruits per habitat per site. No bar is present when the habitat is absent at a site. The estimated area investigated (right y-axis) for each habitat per site is indicated with grey lines. See Table 3.1 for habitat descriptions and abbreviations.

### **3.4.2 Attachment performance associated with substrate types and fragment characteristics**

Fragments in some replicates had attached after two days, and attachment was as high as 100% at the end of the experiment for some substrates (Fig. 3.4). Attachment success varied with Substrate and with Complexity, but results varied with day sampled (Table 3.2). Day 2-4 showed an interaction between Substrate and Complexity (Fig. 3.4a-b), while this was not the case at day 6-10 (Fig. 3.4c) (Table 3.2).

As predicted, attachment success was higher on natural turf treatments (with or without sediment) across most sampling times and complexities (Fig. 3.4). Fragment attachment to artificial turf equalled the two natural turf treatments, but only for the last two sampling times (Fig. 3.4c). The overall high attachment success for treatments with structure present (r+t+s, r+t-s and artificial turf) and lower attachment success on flat surfaces (artificial rock, r-t and rock) across all times suggests a key role of structure in promoting fragment attachment. Interestingly, sand showed a high attachment success, but this was only apparent at the end of the experiment, indicating a low overall attachment speed. There was very little attachment of *C. filiformis* fragments to natural rock without turf, the handling control (natural bare rock), or the artificial rock treatment (Fig. 3.4), with all showing similarly low attachment success across all days.

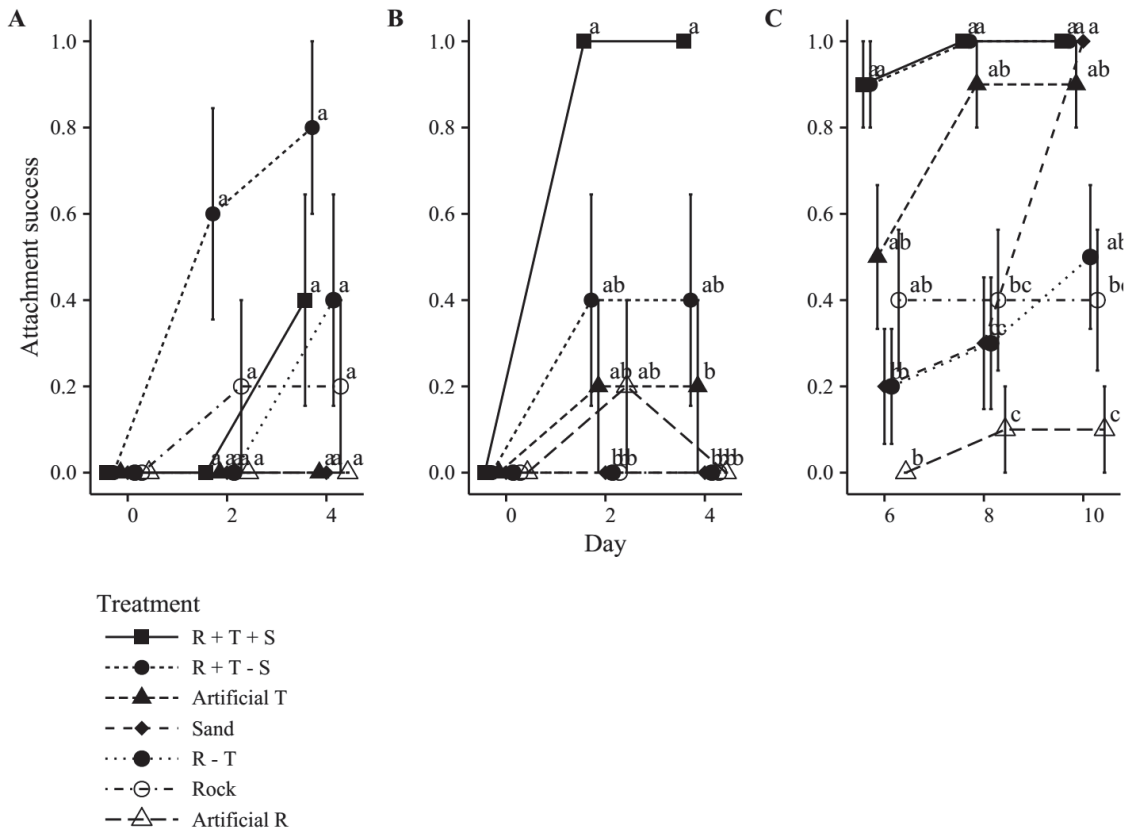


Fig. 3.4. Attachment success ( $\pm$  SE) of fragments per substrate at different days from the start of the experiment as proportion of the initial addition of fragments. Results are shown for Simple (A) and Complex (B) fragments for days 2-4 due to a significant Complexity \* Substrate interaction during this period (see Table 3.2) ( $n=5$ ). Results are averaged over the levels of Complexity (C) for days 6 – 10 because no significant interaction between Substrate and Complexity was found ( $p > 0.05$ ) during this later stage of the experiment (see Table 3.3) ( $n=10$ ). Different letters indicate differences among levels of Substrate per day ( $p < 0.05$ ). Data is spread along the x-axis ( $\pm 2$  days) for better visualization.

Following our predictions, complex fragments did show increased attachment success, however, complexity effects were only apparent at the start (day 2) on certain substrata and at the end of the experiment (day 8 and 10) (Table 3.2). At day 2, attachment success was higher for complex fragments on r+t+s substrate, but no differences between complexities were found for other substrata ( $p < 0.05$ ) (Fig. 3.4a-b). However, the opposite was true for day 8 ( $0.51 \pm 0.09$  &  $0.62 \pm 0.08$ ) and day 10 ( $0.66 \pm 0.08$  and  $0.74 \pm 0.07$ ) success for simple and complex fragments across all substrates



respectively) where simple fragments were more successful, irrespective of substrate (Table 3.2).

Complex fragments showed minimal growth of new rhizoid clusters, whereas simple fragments did show increased rhizoid production (Mean increase of 0.45 and 2.41 rhizoid clusters; SE 0.40 & 0.40 for simple and complex fragments respectively) (Table 3.3), resulting in no difference in total number of rhizoid clusters at the end of the experiment between complexities ( $F_{1,29} = 0.090, p > 0.7$ ), despite differences in initial numbers of rhizoid clusters. Similarly, there was no difference in attachment strength among complexities (Table 3.3).

Table 3. 2. ANOVA (type II) test results of main effects (factors Substrate and Complexity) on attachment success of fragments based on a Bias reduced GLM. The logarithmic model contained main effects Substrate (7 levels), Complexity (2 levels), their interaction and Container and initial biomass added as covariates, and was conducted for each sampling day during the experiment (day 2-10).

day	factor	SS	Df	<i>F</i>	<i>p</i>
2	Complexity	3.76	1.00	8.39	<b>&lt;0.01</b>
	Substrate	21.43	6.00	7.96	<b>&lt;0.001</b>
	Container	-6.45	1.00	-14.37	1.00
	Start weight	-8.88	1.00	-19.80	1.00
	Complexity * Substrate	7.86	6.00	2.92	<b>0.02</b>
	Residuals	24.24	54.00		
4	Complexity	0.14	1.00	0.27	0.60
	Substrate	29.73	6.00	9.75	<b>&lt;0.001</b>
	Container	-6.29	1.00	-12.39	1.00
	Start weight	-6.04	1.00	-11.89	1.00
	Complexity * Substrate	7.53	6.00	2.47	<b>0.03</b>
	Residuals	27.43	54.00		
6	Complexity	0.15	1.00	0.21	0.65
	Substrate	38.04	6.00	8.54	<b>0.00</b>
	Container	-0.11	1.00	-0.14	1.00
	Start weight	-5.09	1.00	-6.86	1.00
	Complexity * Substrate	2.25	6.00	0.50	0.80
	Residuals	40.09	54.00		
8	Complexity	2.73	1.00	4.93	<b>0.03</b>
	Substrate	50.23	6.00	15.12	<b>&lt;0.001</b>
	Container	0.60	1.00	1.07	0.30
	Start weight	-6.95	1.00	-12.55	1.00
	Complexity * Substrate	-1.07	6.00	-0.32	1.00
	Residuals	29.90	54.00		
10	Complexity	2.35	1.00	5.19	<b>0.03</b>
	Substrate	51.75	6.00	19.08	<b>&lt;0.001</b>
	Container	-0.11	1.00	-0.24	1.00
	Start weight	-7.47	1.00	-16.52	1.00
	Complexity * Substrate	-3.54	6.00	-1.31	1.00
	Residuals	24.41	54.00		

Significant *p* values are indicated in bold ( $p < 0.05$ ).

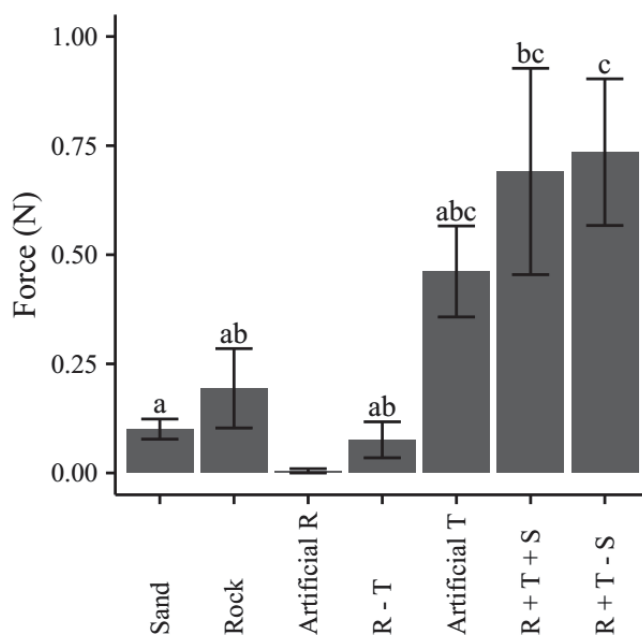


Fig. 3. 5. Attachment strength (N) ( $\pm$  SE) per Substrate pooled across Complexity. Only fragments that had attached at day 10 were selected. Fake rock was not included as it only had one attached fragment at day 10. All other treatments had  $n \geq 3$  fragments (see Fig. 3.5). Different letters indicate significant differences between levels of Substrate ( $p < 0.05$ ).

Also, no differences in the number of rhizoid clusters were observed among substrata (Table 3.3). However, attachment strength did differ among substrata (Table 3.3), indicating the importance of substrate complexity in improving attachment strength. Similar to the observed pattern for attachment success, attachment strength was higher on all substrata with structure (Fig. 3.5). However, opposite to the high final attachment success for sand, attachment strength on this substrate was low (Fig. 3.4c & Fig. 3.5).

Contrary to our predictions, we did not find an increased attachment performance (both attachment success and strength) when sediment was present in turf. Additionally, no

change in growth rates between any of the substrate treatments was observed (Table 3.3), indicating no direct benefit of turf sediment in those first days of settlement.

Table 3. 3. ANOVA (type II) results for differences in fragment characteristics among levels of Complexity (2 levels), Substrate (7 levels) and their interaction (n = 5). Initial biomass and container are added as covariates in the model. Only attached fragments were selected to test for differences of change in rhizoid clusters and force.

variable	factor	SS	Df	F	<i>p</i>
New rhizoid Clusters (#)	Complexity	45.77	1.00	13.88	<b>&lt;0.001</b>
	Substrate	32.09	6.00	1.62	0.18
	Container	15.87	1.00	4.81	<b>0.04</b>
	Start weight	0.23	1.00	0.07	0.79
	Complexity * Substrate	9.06	5.00	0.55	0.74
	Residuals	95.63	29.00		
Attachment strength (N)	Complexity	0.11	1.00	0.50	0.49
	Substrate	3.77	6.00	2.84	<b>0.03</b>
	Container	0.36	1.00	1.64	0.21
	Start weight	1.27	1.00	5.72	<b>0.02</b>
	Complexity * Substrate	0.61	5.00	0.55	0.74
	Residuals	6.43	29.00		
Growth (g)	Complexity	0.46	6.00	1.38	0.24
	Substrate	0.16	1.00	2.93	0.09
	Container	0.46	1.00	8.27	<b>0.01</b>
	Start weight	0.10	1.00	1.80	0.19
	Complexity * Substrate	0.63	6.00	1.88	0.10
	Residuals	3.03	54.00		

Significant *p* values are indicated in bold ( $p < 0.05$ ).

### 3.4.3 Fragment retention in different habitats in the field

For the 2015 experiment, fragment retention varied with Substrate at each sampling date during the first month of the experiment ( $p < 0.05$ ), however patterns among habitats

differed among times (Fig. 3.6). After 6 days, turf habitat had higher retention compared to rock substrate ( $p < 0.05$ ) as was predicted, but no differences were apparent between turf and either Kelp or *Sargassum* habitat ( $p > 0.05$ ). After 9 days, turf had a higher retention rate than both rock and kelp habitat ( $p < 0.05$ ), but no differences with *Sargassum* habitat were found ( $p > 0.1$ , Fig 6a). Additionally, *Sargassum* habitat had higher retention success compared to rock habitat that day ( $p < 0.01$ , Fig. 3.6a). At 12 days turf habitat had higher rates of fragment retention than all other habitats ( $p < 0.05$ , Fig. 3.6a). During those initial 12 days of the experiment nearly all fragments (90%) remained on turf habitat, and this was the most successful habitat for fragment retention.

Two weeks after the addition of fragments, ocean conditions changed from a period of low to moderate swell (up to 1 m) to high swell ( $> 1.5$  m) (Appendix 3.1). During this high wave period, 67% of the residing fragments across all habitats (72 at day 12) were dislodged from the mats (Fig. 3.6b). Dislodgement was especially severe on turf habitat as no fragments were retained whereas most fragments in both kelp and *Sargassum* habitat were retained, resulting in a lower fragment retention on turf compared to both *Sargassum* and kelp habitat ( $p < 0.01$ ), opposite to the previous pattern observed. The high swell period continued resulting in additional loss of fragments, and fragment retention was similarly low across all habitats at the final two sampling dates ( $p > 0.05$  and  $p > 0.3$  for day 44 and day 59 respectively). The 2016 experiment was characterized by frequent high swell during the entire study period, comparable to the second half of the 2015 experiment (Appendix 3.1). Similarly, fragment retention was low. After 2 days, 42% of all the fragments had disappeared across all habitats, and

fragment retention success did not differ among substrates ( $p > 0.05$ ) (Fig. 3.6b). After 7 days, fragment retention was greatest in *Sargassum* habitat and least on rock ( $p < 0.05$ ). The last 2 sampling dates (day 16 and day 21) had a significantly higher retention rate in *Sargassum* habitat compared to kelp and turf habitat ( $p < 0.05$ ).

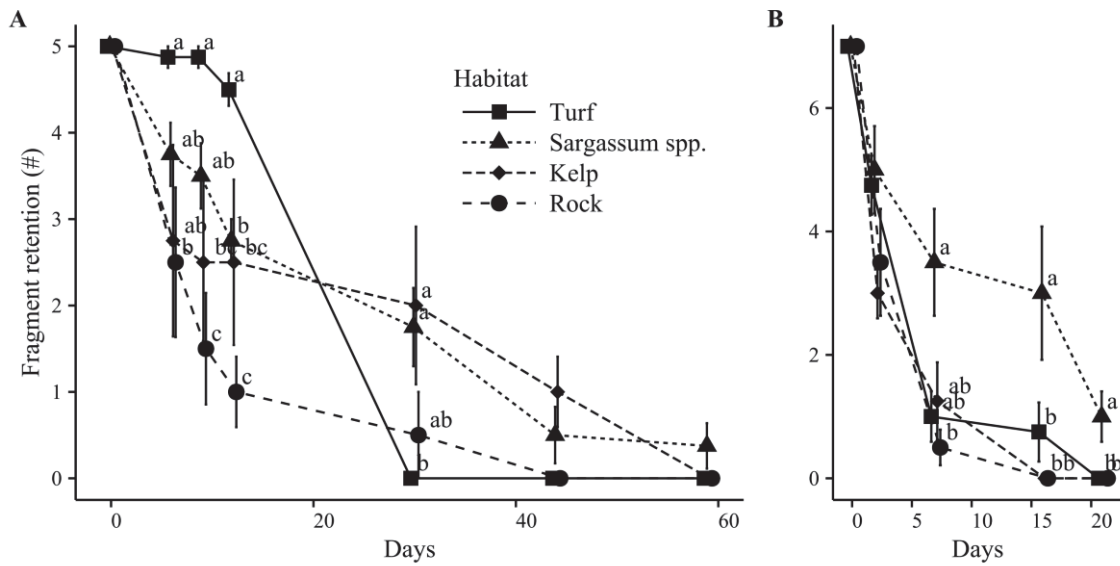


Fig. 3. 6. Fragment retention success ( $\pm$  SE) per Substrate at different days since the initial addition of **A)** 5 fragments per mat at 16/02/2015 or **B)** 7 fragments per mat at 11/01/2016 ( $n = 4$  mats for rock and kelp habitat and  $n = 8$  for turf and *Sargassum* habitat). Different letters indicate differences among substrates ( $p < 0.05$  with Bonferroni adjustment) per time period. Note the different scales of the y-axes.

### 3.5 Discussion

Disturbance can reduce a habitat ability to resist invasion. Moreover, it can enhance alternate habitats that promote the successful recruitment of invasive species. Our

results suggest that these same processes may also facilitate the spread of a native alga that is becoming more locally abundant and expanding its distribution. The recruitment success of *C. filiformis* was higher on turfing habitat – a dominant habitat of disturbed rocky shore environments (Connell et al., 2014, Toohey et al., 2007) - compared to other native habitats and that this may be related to the structural complexity of the turf.

Our prediction that recruit density was positively related to the size of resident to adult beds was not upheld. Instead, the opposite pattern was apparent. One site (Sharkies Cove) was clearly an outlier and when removed, there was a strong negative association between recruit abundance and adult bed size. This site was characterized a very small area of rocky reef, and was highly dynamic with lots of sand movement (Voerman et al. unpubl. data), which may have negatively altered recruitment success, irrespective of altered propagule pressure with adult bed size. The lack of a positive relationship may indicate that propagule supply is not limiting in recruitment success, and other processes may be behind the observed relationship. Indeed, it is not uncommon to find 100s of suspended fragments at a reef where the species is present (authors personal obs.). However, while post-recruitment processes may be most important at a site where the alga has successfully arrived, it is important to consider that the role of propagule pressure in recruitment across reefs where *C. filiformis* is still absent is likely more important (Glasby et al., 2015). The negative association observed may be caused by post-recruitment processes. Small reefs have high adult cover likely due to increased sedimentation disturbance indirectly favouring the horizontal spread of the species (Glasby et al. 2015; Chapter 5). The conditions favouring adult abundance may have

resulted in the low observed recruit abundance as they are not present in the recruitment stage. Biotic interactions affecting post settlement processes (i.e. *Caulerpa*-turf interactions) and how they may change according to environmental conditions (i.e. sediment disturbance) are an important avenue for further study.

The higher density of *C. filiformis* on turf was remarkably consistent across sites and is similar to patterns previously described for non-native invasive *Caulerpa* spp. e.g. (Bulleri and Benedetti-Cecchi, 2008, Piazzini et al., 2003). Contrary to the observed high abundance of *C. filiformis* fragments amongst coralline turf, the opposite was observed for filamentous turf. Scientists typically lump many species into the category “turf” which often includes corallines but also filamentous form (for a review see Connell et al., 2014). This is unsurprising as geniculate coralline algae and filamentous turf often form dense assemblages of a mix of species and morphological forms (Connell et al., 2014). However, here we have found that composition of the turf assemblage may matter in the ecological role of turf assemblages, and may be of increasing importance when relative cover of the different forms may change under environmental change (Short et al., 2014).

The laboratory experiments supported the field experiment with fragment performance (attachment success and attachment strength) being greatest on natural or artificial turfing (predominantly coralline) substrata. Attachment on turf was rapid, highly successful and very strong. The experiment showed that this appears to be related to the structural complexity of the turf, and there was little influence of sediment on



attachment success. This is similar to the observations of Bulleri and Benedetti-Cecchi (2008) who found no effects of sediment on recruitment success of *C. cylindracea* in the field when sediment that was normally found in turf habitat was added to barrens. Those authors suggested that trapping of algal fragments may be enhanced by the structural complexity of the turf, as also observed for other vegetatively spreading alga such as *Dictyota* spp. and *Codium* spp. (e.g. Herren et al., 2006, Watanabe et al., 2009). Our results show that turf also promotes attachment performance following entrapment, and both mechanisms have likely resulted in the high recruit abundance on coralline turf that was found in the field.

No recruits were observed on soft sediment in this study. The laboratory experiment showed that this is not caused by the inability of fragments to attach, but possibly by the slow attachment speed and the very low attachment strength. In addition, the highly mobile state of sand may also have resulted in the absence of *C. filiformis* on this substrate along the exposed shoreline of NSW (Glasby et al., 2015), but not in sheltered bays (authors' personal observations).

The presence of rhizome and rhizoid structures may aid vegetatively spreading algae in their recruitment success (Khou et al., 2007, Smith and Walters, 1999).

Counterintuitively, the presence of those structures did not always increase overall attachment success of fragments in our study. However, the laboratory experiment showed some indication of increased success of complex fragments, but only during the initial days after fragment addition and only for one substrate type (rock with turf and

sediment). The initial positive association supports the observations by Khou et al. (2007) who showed a much higher attachment success for complex fragments of *C. filiformis* during the first 48 hours of settlement. Contrary to this, our results show that simple fragments had greater attachment success after 8 days compared to fragments with stolon present. This may be because of *Caulerpa* being single celled and having a high capacity to reallocate resources (Vroom and Smith, 2001). As Khou et al. (2007) demonstrated, *C. filiformis* appears to be highly plastic and can partition energy towards rhizoid production when in contact with an adequate surface. Similarly, this study observed simple fragments with higher investment in rhizoid production compared to fragments which had rhizoid structures already in place. This selective production of rhizoids of simple fragments anywhere along its tissue may aid successful attachment once rhizoids are sufficiently developed. Despite the benefits of complex fragments, the possible advantage of more rapid attachment in the first few days of settlement may be most important in the overall recruitment successful of this species when considering the highly turbulent environment in which the species occurs.

In line with both the field observations and the laboratory results we found higher retention rates of fragments inside turf beds. However, those results were not consistent across time, as a low fragment retention success was observed during both the second half of the 2015 experiment and the 2016 experiment. Other processes have likely influenced attachment success. For example, during periods of large waves or storms there may be little fragment success because fragments are not in place long enough to attach strongly (Khou et al., 2007). Although our experiment shows that attachment

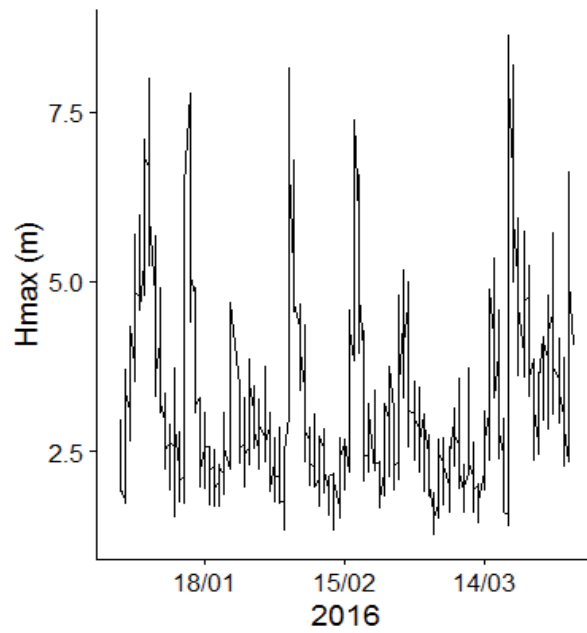
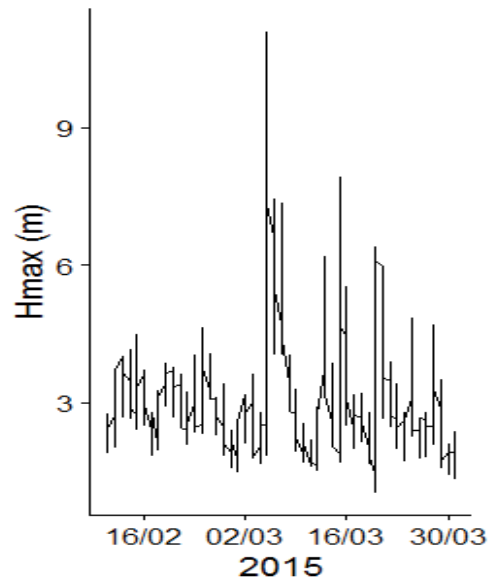
strength on turf is relatively high after only 10 days of settlement, the forces created by wave action can be excessive (Masteller et al., 2015). During those high wave periods, the habitats that may buffer near-ground wave action (i.e. kelp or *Sargassum* beds) (Komatsu and Murakami, 1994, Masteller et al., 2015) may have reduced fragment loss, although overall success in those habitats was relatively low. However, those same high wave action events may have initially created fragments (Watanabe et al., 2009). Taking into consideration the high turf association from field observations and the laboratory results, it appears that more benign conditions may determine recruitment success.

Finally, although the high association with turf habitat of *C. filiformis* recruits became apparent in this chapter, Chapter 2 showed an opposite pattern for adult beds. It was hypothesized in Chapter 2 that the high association of adults with rock and r+s, and not with turf as observed for other species of the genus, was an end stage of succession whereby turf is outcompeted by adult *C. filiformis*. Thus, results of this chapter supported this hypothesis. However, it is still unknown whether *C. filiformis* is a strong competitor that can overtake turf habitat rapidly as suggested in Chapter 2.

In conclusion, this study showed that suitable substrate together with a window of opportunity of calm weather may determine the fate of fragments. *C. filiformis* appears highly plastic in its allocation of resources, so that the possible benefits of more developed fragments are only temporary. Turf may facilitate recruitment due to its increased structural complexity aiding in more rapid, successful and stronger attachment, while canopy forming species may resist recruitment. The loss of canopy

forming algae together with increasing dominance of turfing algae worldwide may also facilitate the spread of highly adaptive native species.

### Appendix 3



Appendix 3.1, Max wave height recorded by the Office of Environment and Heritage's (OEH) Sydney offshore Waverider buoy during the fragment-retention experiments in 2015 and 2016. Wave data was collected and provided by the Manly Hydraulics Laboratory.



## **Chapter 4**

### **Morphological variation, tolerance and plasticity to sediment disturbance of *C. filiformis***





## 4.1 Abstract

A broad environmental tolerance allows many invasive species to colonise different habitats across large spatial scales and may similarly aid native species to proliferate within and outside their known home-ranges. For invasive species, a broad environmental tolerance is often aided by high morphological plasticity, however, whether rapidly spreading native species also benefit from morphological plasticity is unknown. *Caulerpa filiformis* is a native macroalga that has increased in abundance both within and outside its historic range in New South Wales, Australia. The alga's large and local distribution is associated to sediment disturbance. Sediment layers on the rocky reef potentially benefit the alga directly by promoting its growth by increased nutrient supply and the ability of the alga to take up nutrients from the sediment with its rhizomatic structures. Alternatively, this may indirectly benefit the alga if *C. filiformis* is tolerant to sediment disturbance by more negatively affecting competitors. This study first investigates *C. filiformis* morphological variation in the field. Secondly, we investigate the tolerance of the species to sedimentation and investigated the role of morphological plasticity in this, and simultaneously explored the potential benefit of sediment to the alga's growth. The specific predictions were that *C. filiformis* 1) shows high morphological variation associated to multiple environmental variables across its large (~latitude) and local scale (~depth and substrate) distribution; 2) is highly tolerant to sedimentation aided by morphological plasticity and 3) shows increased growth rates under sedimentation. The alga's morphology was highly variable in the field, with more elongated and thinner blades with increasing depth; and longer fronds when found

growing on the reef associated to a sediment veneer. Frond length decreased further south, but latitude explained the least of the variation observed compared to local scale environmental variables (depth and substrate). The alga appears highly tolerant to high levels of sedimentation, aided by increased investment in vertical growth, irrespective of the sedimentation frequency or intensity applied. No benefit of sediment presence on the alga's growth was found. Sediment may benefit the alga indirectly by more negatively affecting competitors who do not tolerate sediment disturbance. This study shows the role of rapid morphological plasticity in the high tolerance of the alga to sediment disturbance, increasing our understanding on the alga's affiliation to sediment disturbance.

## 4.2 Introduction

Invasive species are one of the largest global threats to marine coastal ecosystems (Halpern et al., 2008). Numerous studies have attempted to identify the traits that make species successful invaders, and macroalgae have been well studied in this regard (e.g. Williams and Smith, 2007, Boudouresque and Verlaque, 2002, Nyberg and Wallentinus, 2005). Invasive macroalgae can often be found over broad latitudinal ranges which encompass considerable climatic variation, and their successful spread appears linked to their high tolerance to such variation (Nyberg and Wallentinus, 2005, Stewart, 2008). For example, the distribution of the highly invasive brown alga *Undaria pinnatifida* from cold-temperate to cold-tropical environments across a large depth range (intertidal – 18 m) has been linked to its wide tolerance to variations in wave action, temperature, light, and salinity (Floc'h et al., 1991, Fletcher and Farrell, 1999, Russell et al., 2008).

Less well known are species that invade their native ranges in their native range (native-invaders *sensu* Simberloff and Rejmanek, 2010), despite their impacts rival those of their non-native counterpart (Simberloff and Rejmánek, 2010). Native species can become to dominate communities following disturbances that alter competitive outcomes (Simberloff et al., 2012). Like non-native invaders benefit from a high tolerance to environmental change in their spatial distribution, tolerance to temporally changing abiotic conditions will aid native species in their proliferation following disturbance (Simberloff et al., 2012).

A tolerance to changing abiotic conditions is often aided by high morphological plasticity (Smith, 2009). There are several examples of morphological plasticity of macroalga in their non-native distributions (but see Peteiro and Freire, 2014, Wright, 2005, Phillips and Price, 2002), and similar observations have been made for species in their native ranges (e.g. Arenas et al., 2002, Monro et al., 2007, Norton et al., 1981, Charrier et al., Yñiguez et al., 2010, Kaandorp and Kübler, 2001). For example, the red macroalga *Asparagopsis armata*, which is native to Australia and New Zealand but invasive in other countries, can tolerate shading by investment in morphological elongation and suppression of branching (Monro et al., 2007). Similarly, species from the highly invasive *Caulerpa* genus are often found to show high and rapid morphological plasticity (e.g. Svedelius, 1906, Meinesz, 1979, Peterson, 1972, Calvert, 1976, Rico and Guiry, 1996), aided by their siphonous body structure that allows for rapid energy partitioning (Walters and Smith, 1994, Vroom and Smith, 2001). For example, morphological traits of *Caulerpa prolifera* differed with water depth and associated reduced light levels: individuals had fewer fronds per stolon length and thinner, less branched and longer blades at deeper, low light conditions compared with a location of bright light and increased wave action (Collado-Vides, 2002). This is a typical growth response observed under low light conditions (Rico and Guiry, 1996), and similar patterns can be expected for other species.

Morphological variation in macrophytes may occur locally, i.e. in response to changing light levels ~ depth, but may also occur at larger spatial scales associated to changing environmental conditions such as temperature, salinity or nutrient levels (e.g. Henkel et

al., 2007, Fowler-Walker et al., 2005, Hay et al., 1983, Rice et al., 1985, Kalvas and Kautsky, 1998). For example, a strong morphological gradient in the kelp *Egregia menziesii* along 1040 km of US Pacific coast is highly correlated with temperature, wave action and nutrient concentrations. However, few studies have incorporated multiple levels of spatial variation in shaping algal morphology (but see Fowler-Walker et al., 2005, Wernberg et al., 2003), but we may expect them to be equally important.

In New South Wales (NSW), Eastern Australia, the native *Caulerpa filiformis* has spread both within and outside its known historic distribution, and is now the dominant alga at many rocky reefs along the coastline (Glasby et al., 2015). *C. filiformis* has a broad depth distribution (shallow intertidal to ~ 8 m water depth) and now occurs across 700 km of coastline, from cold tropical to temperate waters. Thus, like its congeners, it appears tolerant to a broad range environmental conditions operating at multiple spatial scales. Although we know little about how environmental conditions affect the morphology of *C. filiformis*, we predict that the alga is characterized by large morphological variation within site (i.e. across a depth gradient and between substrata types) and among sites (i.e. along a latitudinal gradient).

The spread of *C. filiformis* has been linked to sedimentation disturbance. The cover of *C. filiformis* along the NSW coast is greatest where there is a high ratio of sand to reef (Glasby et al., 2015). Similarly, *C. filiformis* appears to trap more sediment than adjacent *Sargassum* spp. on intertidal reefs (Zhang et al., 2014), whilst in the subtidal, beds of *C. filiformis* also contain high levels of sediment compared to other subtidal

algae (Voerman et al. in review). Whilst sedimentation generally has negative effects on marine macrophytes (e.g. Balata et al., 2007, Díaz-Tapia et al., 2013, Airoidi, 2003), *Caulerpa* spp. appear highly tolerant to sedimentation (Glasby et al., 2005, Piazzini et al., 2005, Piazzini et al., 2007). *C. filiformis* high tolerance to sediment may be aided by a high morphological plasticity. Despite the increasingly acknowledged role of phenotypic plasticity in affecting a species' ability to survive changing environmental conditions (Smith, 2009), little is known about how macrophytes respond to changing sedimentation levels. We predict that high sedimentation may result in an increase investment in frond production (elongation) that may allow access to light, similar to observed algal shade- response (Monro et al., 2007, Collado-Vides, 2002, Calvert, 1976, Peterson, 1972). In addition, *C. filiformis* may benefit from sedimentation as the rhizoids structure have been shown to be able utilise nutrients available in the sediment for other species of the genus (Williams and Fisher, 1985).

In this study, we used a large-scale biogeographic survey on subtidal reefs to document the range of environmental conditions over which *C. filiformis* occurs and to test whether the species' morphology varies at multiple spatial scales (among sites over a latitudinal gradient and over a depth gradient and in association with a sediment layer). We tested the following predictions: 1) *C. filiformis*' morphology changes with latitude, 2) morphology changes with depth, and more specifically individual thalli would have fewer fronds as a function of stolon length, and fronds would be longer but less branched with increasing depth, and 3) a sediment layer is associated with longer fronds and an overall increase in biomass if sedimentation provides additional resources that

are limiting. Secondly, using a laboratory experiment, we tested the effects of sedimentation in explaining the observed morphological patterns by exposing fragments of *C. filiformis* to different levels of sedimentation. Again, we predicted that increasing sedimentation would have positive effects on biomass, and that increasing sedimentation would result in increased investment in frond elongation.

## 4.3 Materials and methods

### 4.3.1 Study species

*Caulerpa filiformis* is a large green alga with a disjunct distribution along ~ 700 km of the warm temperate coast of eastern Australia where it is primarily subtidal down to ~ 8 m but also occurs in low intertidal areas (Glasby et al., 2015). It has a siphonous body structure and spreads vegetatively by horizontal expansion of a creeping rhizome. Rhizoid clusters, distributed along the rhizome, aid in attaching the alga to the substrate (Fig. 4.1). Upright fronds are characterized by a short, circular, stipe at the base, but predominantly consist of a flat blade (Stegenga et al., 1997). Fronds can vary from simple, single forms to highly complex (branched) forms (Khou et al., 2007). More complex branched forms have two major morphotypes: Y-branching, whereby the blade branches into two equally sized parts, and secondary branching, whereby like the base of the main frond a ringed stipe structure creates the base of a new blade (Fig. 4.1). Both branching types can occur at any position along another blade or along the ringed structures just described. Morphotypes can occur on separate fronds or together on the same fronds (Voerman pers. obs.).



#### 4.3.2 Morphological variation in *C. filiformis*

We determined morphological variation of *C. filiformis* within reefs at 8 sites across its distribution, covering a latitudinal range of ~ 700 km (Fig. 4.2). Sampling took place during spring and summer months August-December 2013. Sampling was not stratified by depth, sediment depth, or substrate type because these were highly variable among sites. Instead a regression approach was used to test for relationships between morphology and several physical variables. At each site, 5 quadrats (20 x 20 cm) were haphazardly placed within one large *C. filiformis* patch (> 20 m<sup>2</sup>). Quadrats were positioned at least 20 cm from the edge of the patch on horizontal surfaces without any nearby large topographic structures to avoid potential influence of edge effects or shading by other structures. Quadrats were a minimum of 1 m apart. For each quadrat, depth (in relation to low tide level) and the thickness of sand on the rock substrata were measured *in situ*. A ruler was used to classify sand conditions within each quadrat by taking the mean of 3 readings for each quadrat. Two classes of sand cover were determined: a class of absent, irregular or < 1 cm full cover of sand and a class of full cover of > 1 cm sand thickness. After taking the measurements, *C. filiformis* was removed within each quadrat by scraping it from the rock substratum and placing in a labelled plastic bag for transport to the lab in seawater. Morphological characteristics were determined (see Table 4.1 for a list of morphological variables measured). This was completed within 3 hrs of sampling.

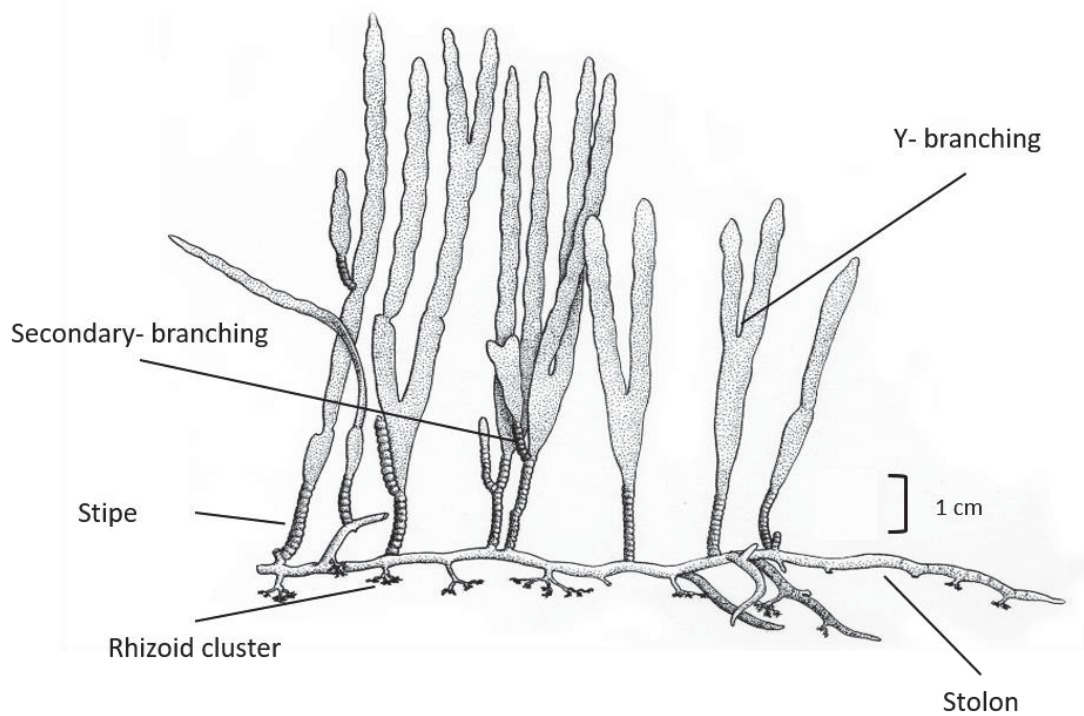


Fig. 4. 1. Schematic representation indicating the two branching morphotypes (Y-branching and Secondary branching) of *C. filiformis* fronds. Fronds consist of a small, circular stipe and long single or branched blades. Drawing adapted from Anderson et al. (2016).

For each quadrat, we determined total algal biomass after dry blotting which was converted to a  $\text{kg}\cdot\text{m}^{-2}$  measure. Fronds per quadrat were also determined and used to convert density of fronds to per  $\text{m}^2$  by the known biomass of the sample. As it was difficult to obtain complete, intact stolons by their highly dense network, we selected the 20 largest fragments sections (i.e. fractions of the dense *Caulerpa* mat with the longest stolon length intact) obtained from each quadrat and determined the density of fronds and rhizoid clusters as a function of stolon length. Morphological characteristics

of fronds were examined by photographing individual fronds and using image analysis (Rasband, 2012). For each fragment, we measured the maximum frond length (to the nearest 0.001 mm). For the longest frond per fragment, we recorded the number of y-branches and secondary-branches present and measured blade width (to the nearest 0.001 mm) (Fig. 4.1). Branching was counted by following the tip of the frond to the base and counting the number of either branching type on the way. A complexity density measure to test for reduced complexity with increasing depth was then calculated for each branching type by dividing the count data by the length of the frond. Blade width (flat side) was measured for a single point half way along the longest frond, while avoiding the position of a y-branching as blade width was usually slightly wider around this point.

For some quadrats, we could not retrieve all the algae because of a very thick sediment layer (2 samples only). Those samples were excluded from frond and rhizoid density and biomass analyses (described below). Additionally, at Seal Rocks, three quadrats were positioned on pebble substrate and were also removed from further analysis as this substrate was relatively rare.

Relationships between each of the aforementioned morphological characteristics and environmental variables (sand thickness, water depth and latitude), were analysed with linear models. Latitudinal data was obtained with Googlemaps © for each sampling site. For analysis, latitudinal data was centred around 32.55°S, the centre of the sampling sites, to give a more meaningful estimate of model intercepts for the investigated

morphological traits. Multi-collinearity of the environmental variables was examined using variance inflation factors (VIF) (Fox et al., 2016). No variables were highly correlated (all VIF < 1.3), thus all were included in the models.

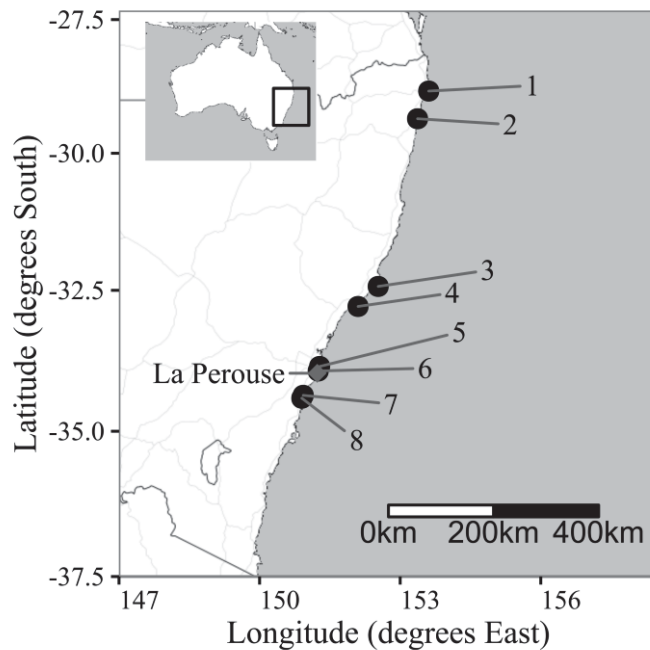


Fig. 4. 2. Location of sampling sites in NSW, Australia (inset). Black circles represent sites sampled for the morphological study. Site numbers represent 1) Ballina; 2) Sharkies Cove; 3) Seal Rocks; 4) Kingsley Beach; 5) Bronte; 6) Coogee; 7) Bellambi; 8) Wollongong. Grey diamond represents sampling site for experimental study. Map tiles were sourced from StamenDesign (2016).

Analyses were conducted on mean values/quadrat although for a few of the variables we only used a subset of the data collected above. For example, for each quadrat, average blade length, width, y- and secondary branching density were calculated from the 10 longest fronds in a quadrat to select for fully grown fronds. Additionally, for each quadrat, only fragments with stolon > 4 cm were selected (n = 231) to obtain an average

value for blade and rhizoid cluster density per sample, as smaller fragments often had either structure missing. This excluded the use of one quadrat in the analysis of frond or rhizoid density as no stolons > 4cm were present.

Model assumptions were tested with the GVMLA package (Pena and Slate, 2004), and variables were transformed where necessary. Partial-regression plots showing the relationship between one predictor variable and each morphological characteristic while adjusting for the influence of other predictor variables based on variable residuals were produced to visualize the relationships (Fox et al., 2016). The relative importance of each of the environmental variables in explaining variation of each morphological characteristic investigated was calculated following the LMG procedure (Lideman et al., 1980, Grömping, 2012). All statistical analyses were performed in R with associated packages (R Core Team, 2015, Fox et al., 2016).

Table 4. 1. Summary description of morphological characteristics and abbreviations analysed for field and experimental study.

Characteristic	Description
<b>Field data</b>	
Fronde length (cm)	From where the frond meets the stolon to a blade tip furthest away from the frond base.
Blade width (mm)	From around half way the frond length, and where no y-branching occurred
Secondary-branching density (2nd-branching D) ( $\text{cm}^{-1}$ )	Density of secondary-branches on the frond
Y-branching density (y-branching D) ( $\text{cm}^{-1}$ )	Density of y-branches on the frond
Fronde density (D) ( $\text{cm}^{-1}$ )	Density of fronds along the stolon
Rhizoid density (D) ( $\text{cm}^{-1}$ )	Density of rhizoid clusters along the stolon
Surface density (D) (fronds $\text{m}^{-2}$ )	Converted from number of fronds per 20x20 cm quadrat
Biomass ( $\text{kg m}^{-2}$ )	Converted from wet weight per 20x20 cm quadrat
<b>Experimental data</b>	
Survival (%)	Percentage of fragments (n=5) alive at end of experiment
Growth (g)	Change in wet weight between start and end of experiment
Final dry weight (g)	Dry weight at end of experiment
Delta y-branching (count)	Difference in total y-branches of the two initial fronds between start and end of experiment
Delta 2nd-branching (count)	Difference in total 2nd-branches of the two initial fronds between start and end of experiment
Fronde density	As above
Rhizoid density	As above
Stolon length (cm)	Length of the stolon at end of experiment

### 4.3.3 Effects of sedimentation on fragment morphology

We conducted a laboratory experiment to determine the effects of sedimentation (fixed factor; 5 levels) on *C. filiformis* survival and morphology. *C. filiformis* fragments (~ 20 L) were collected from a single subtidal patch at La Perouse at ~ 0.5 m below low tide level (Fig. 4.2) and transported to the laboratory in seawater. Sediment (~ 40 L) was sourced adjacent to the *C. filiformis* patch and consisted primarily of coarse sand that was typical of the sand *C. filiformis* interacts with (Glasby et al., 2015). In the laboratory, we selected fragments with 2 fronds with only one branching, a stolon length of 4-6 cm with 2-4 rhizoid clusters present. Fronds were then pruned to 10 cm length each. Newly created fragments were kept afloat in aerated seawater at 21°C under a light:dark cycle of 12:12 h to allow for wounds to heal. After 48 h, each fragment's blotted dry weight was determined (to the nearest 0.001g) and photographed for later morphological analysis. Fragments were then selected at random and attached to the back side of ceramic tiles (n = 1 fragments/tile; n = 100 tiles in total) using 2 loose fitting elastic bands. Five tiles were then placed in each of 20 large tanks (90 L) with 50 cm seawater depth. Tanks were aerated and filtered seawater was replaced twice weekly over the entire duration of the experiment. Tanks were kept under a 12:12h light:dark cycle. Fragments were allowed to settle onto the tiles for 7 d, after which the sedimentation treatments began (n= 4 tanks/ treatment).

Sediment treatments consisted of 1) a no sediment control; 2) 0.5 cm applied weekly; 3) 1 cm applied weekly; 4) 2 cm applied weekly to test for differences among

sedimentation intensities and 5) 1 cm twice weekly to test for the effect of frequency of sediment applied. Sediment was applied by carefully sprinkling sediment equally over the water surface to establish an equal sediment cover on the bottom. Sediment applied was not further touched thus it accumulated at different rates per treatment.

Sedimentation treatments lasted for 4 weeks, after which the fragments were collected. For each tank, we measured fragment survival (% survival of 5 initial fragments). Dead fragments were excluded from further analysis. Each fragment's blotted dry weight was measured and growth rate per fragment was calculated (final – initial blotted dry weight). We then photographed the fragments again and determined changes in the number of y- and secondary branches of both initial fronds combined and final stolon length of each fragment. Again, frond and rhizoid density along the stolon were calculated. Additionally, frond length and y- and secondary branching density (/length of frond) were calculated for the longest frond per fragment, as per methods described above. Finally, fragments were dried at 40 °C for 62 h and dry weight was measured. For all variables, a tank average was calculated.

One-way ANOVA tests were used to determine the effects of sedimentation on each morphological variable separately. Test assumptions were investigated by methods described above, and morphological characteristics were transformed where necessary. Significant treatment effects were further investigated with Post Hoc LSmeans comparisons with Bonferroni adjustment (Lenth, 2016).



## 4.4 Results

### 4.4.1 Morphological variation of *C. filiformis*

The morphology of *C. filiformis* varied widely from highly branched, thick and short to highly elongated, narrow forms up to 73 cm in length (Table 4.2, Fig. 4.3). All environmental variables explained variation in *C. filiformis* morphology, although the relationships were dependent on the morphological characteristic investigated (Table 4.2, Fig 4.3). Overall, local scale variables that changed within the reef (depth and sand presence) contributed most to the explained variation of morphological characteristics, while latitude explained less when significant environmental relationships with morphological traits were found (e.g. frond length, blade width and y-branching density) (Table 4.2).

Fronde length increased with water depth, but the opposite pattern was found for frond width and y- branching density (Table 4.2, Fig. 4.3b; e). In line with our predictions, there was a trend for reduced density of blades per stolon, and a reduced overall density with increasing depth, however both relationships were non-significant ( $p = 0.056$  and  $0.085$  respectively, Table 4.2, Fig. 4.3 n; t). Similarly, a sand layer was associated with increased frond length and reduced y- branching density, although no association with any of the other variables was observed. At a broader scale, both frond length and blade width decreased further south (Table 4.2, Fig. 4.3c). Latitude was not significantly associated with any of the other characteristics investigated (Table 4.2).

Table 4. 2. Modelled relationships between environmental variables (sand, depth and latitude) and different morphological characteristics investigated. The table shows estimated individual relationship between the environmental variable and morphological characteristic investigated; Standard Error; t-value for individual predictor relationships and f-value for the entire model, P-value and estimated R<sup>2</sup> for each predictor variable individually and the full model. R<sup>2</sup> values for individual predictor variables were calculated following the LMG method. Latitude was centred around 32.55 degrees.

	Slope/ <i>df</i>	SE	t/ <i>f</i>	<i>p</i>	R <sup>2</sup>
<b>Fronnd length</b>					
(Intercept)	12.964	4.399	2.947	<b>0.006</b>	
Sand present	16.581	4.426	3.746	<b>0.001</b>	0.26
Depth	8.659	2.828	3.062	<b>0.005</b>	0.14
Latitude	-2.916	1.115	-2.615	<b>0.014</b>	0.07
model	<i>df</i> 31	12.830	9.592	<b>&lt;0.001</b>	0.48
<b>Blade width</b>					
(Intercept)	5.967	0.234	25.447	<b>&lt;0.001</b>	
Sand present	0.035	0.236	0.149	0.882	0.00
Depth	0.064	0.151	0.427	0.672	0.05
Latitude	-0.565	0.059	-9.510	<b>&lt;0.001</b>	0.72
model	<i>df</i> 31	0.684	34.87	<b>&lt;0.001</b>	0.77
<b>Y-branching density</b>					
(Intercept)	0.108	0.010	11.216	<b>0.000</b>	
Sand present	-0.023	0.010	-2.341	<b>0.026</b>	0.14
Depth	-0.018	0.006	-2.879	<b>0.007</b>	0.17
Latitude	0.004	0.002	1.663	0.106	0.03
model	<i>df</i> 31	0.028	5.35	<b>0.004</b>	0.34
<b>2nd-branching density (sqrt)</b>					
(Intercept)	0.172	0.029	5.954	<b>&lt;0.001</b>	
Sand present	-0.034	0.029	-1.159	0.255	0.04
Depth	-0.030	0.019	-1.634	0.112	0.05
Latitude	0.014	0.007	1.914	0.065	0.07
model	<i>df</i> 31	0.084	2.113	0.119	0.17
<b>Fronnd density (sqrt)</b>					
(Intercept)	-0.298	0.076	-3.900	<b>0.001</b>	

Sand present	-0.122	0.076	-1.605	0.122	0.09
Depth	0.027	0.056	0.491	0.628	0.03
Latitude	0.012	0.023	0.536	0.597	0.02
model	<i>df</i> 23	0.181	1.271	0.308	0.14
<b>Rhizoid density</b>					
(Intercept)	1.563	0.151	10.384	<b>&lt;0.001</b>	
Sand present	-0.153	0.150	-1.019	0.319	0.03
Depth	-0.203	0.110	-1.843	0.078	0.12
Latitude	0.030	0.045	0.665	0.513	0.02
model	<i>df</i> 23	0.357	1.543	0.230	0.17
<b>Surface density</b>					
(Intercept)	8502.800	1157.600	7.345	<b>&lt;0.001</b>	
Sand present	1023.700	1281.900	0.799	0.432	0.02
Depth	-1310.100	729.900	-1.795	0.085	0.10
Latitude	189.700	300.000	0.632	0.533	0.01
model	<i>df</i> 24	3262.000	1.157	0.347	0.13
<b>Biomass</b>					
(Intercept)	7.556	2.249	3.360	<b>0.003</b>	
Sand present	3.613	2.490	1.451	0.160	0.08
Depth	1.776	1.418	1.253	0.222	0.08
Latitude	0.205	0.583	0.351	0.729	0.02
model	<i>df</i> 24	6.336	1.734	0.187	0.18

Significant *p*-values are in bold

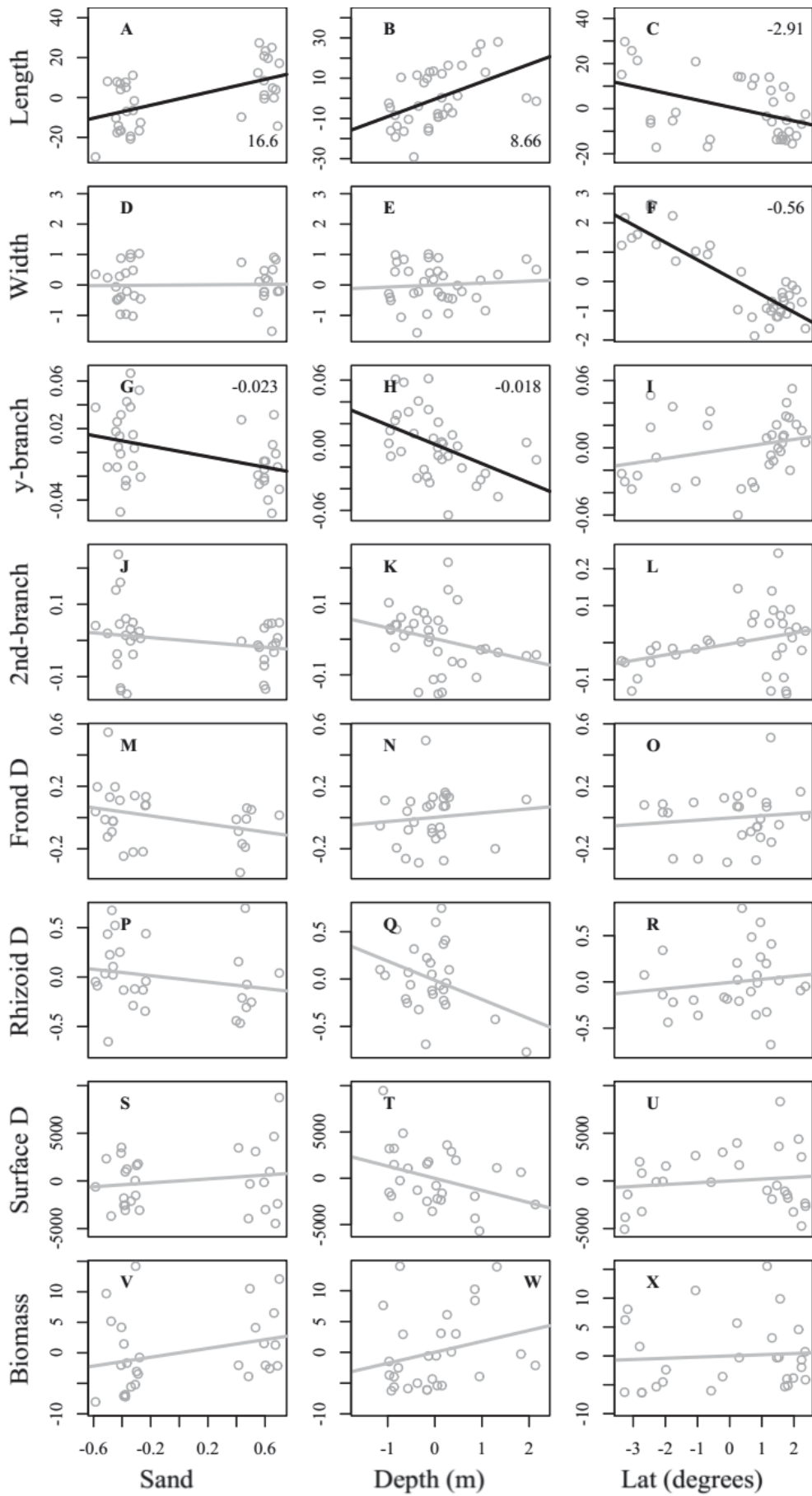


Fig. 4. 3. Added variables plots for linear models investigating the relationship between environmental variables (column 1: sand presence, column 2: depth and column 3: latitude) and each morphological characteristic (rows) investigated. Morphological characteristics investigated were frond length (cm), blade width (mm), y-branching complexity ( $\text{cm}^{-1}$ ), secondary branching complexity ( $\text{cm}^{-1}$ ), frond density ( $\text{cm}^{-1}$ ); rhizoid density ( $\text{cm}^{-1}$  stolon), surface density (fronds  $\text{m}^{-2}$ ), biomass ( $\text{kg m}^{-2}$ ). Lines represent the modelled relationship when the other two predictor variables are kept constant. Solid black lines indicate significant relationships ( $p < 0.05$ ) while grey lines are non-significant trends (see Table 4.2 for results). Inset values represent the relationship (graph slope) between the environmental variable and morphological characteristic when significant as indicated by the model (see also Table 4.2). For further description of characteristics investigated and transformations applied to comply with model assumptions a see Table 4.1.

#### 4.4.2 Effects of sedimentation on fragment morphology

*C. filiformis* was highly tolerant of sedimentation treatments, irrespective of sedimentation frequency or intensity. Overall average survival was 88% ( $\pm 3.8$ ) after 4 weeks. Changes in biomass and final dry weight were similar across all treatments (Table 4.3).

The presence of sediment affected the development of some traits compared to the no sediment control (Table 4.3; Fig. 4.3). Although surprisingly, the different sediment treatments (intensity or frequency) had little effect on most of the morphological traits measured (Table 4.3). Frond length, total secondary branching, and density of secondary branching all increased with the addition of sediment, although stolon growth was reduced with the addition of sediment. Frond length was the only variable that varied significantly among the different types of sediment addition treatments.

Specifically, the highest sedimentation treatments (with a sum of 2 cm sediment per week) showed increased blade elongation in comparison to the control, while the two lowest sedimentation rates (0.5 and 1 cm weekly) did not (Table 4.3, Fig. 4.3). There was no significant difference in frond length associated with how frequently the sediment was applied (i.e. 2 cm once per week vs 1 cm applied twice per week; Fig. 4.3).

Table 4. 3. ANOVA test results for differences in morphological characteristics between sedimentation treatments (n = 4). Transformations applied to conform with test assumptions are indicated. Mean value and standard error (SE) are indicated for ns relationships. For post-hoc test results and means per treatment for significant relationships see Fig. 4.3.

	<i>df</i>	MS	<i>F</i>	<i>p</i>	Mean (SE)
<b>Survival</b>					
Treatment	4	226.67	1.0625	0.424	88.0
Residuals	10	213.33			(3.8)
<b>Delta wet weight</b>					
treatment	4	0.040506	0.6979	0.611	0.569
Residuals	10	0.058037			(0.059)
<b>Dry weight (sqrt)</b>					
treatment	4	0.001624	0.9577	0.471	0.233
Residuals	10	0.001696			(0.011)
<b>Fronnd length (sqrt)</b>					
treatment	4	0.15991	8.5791	<b>0.003</b>	
Residuals	10	0.01864			
<b>Delta 2nd- branching</b>					
treatment	4	20.7672	11.434	<b>&lt;0.001</b>	
Residuals	10	1.8162			
<b>Delta y-branching (sqrt)</b>					
treatment	4	0.22182	1.8468	0.197	1.62
Residuals	10	0.12011			(0.23)
<b>2nd-branching density</b>					
treatment	4	0.047108	12.879	<b>&lt;0.001</b>	
Residuals	10	0.003658			
<b>y-branching density</b>					
treatment	1	0.001316	2.1781	0.164	0.0306
Residuals	13	0.000604			(0.0066)
<b>Stolon length</b>					
treatment	4	57.349	28.398	<b>&lt;0.001</b>	
Residuals	10	2.019			
<b>Blade density</b>					
treatment	4	0.01189	1.318	0.328	0.441
Residuals	10	0.009021			(0.026)

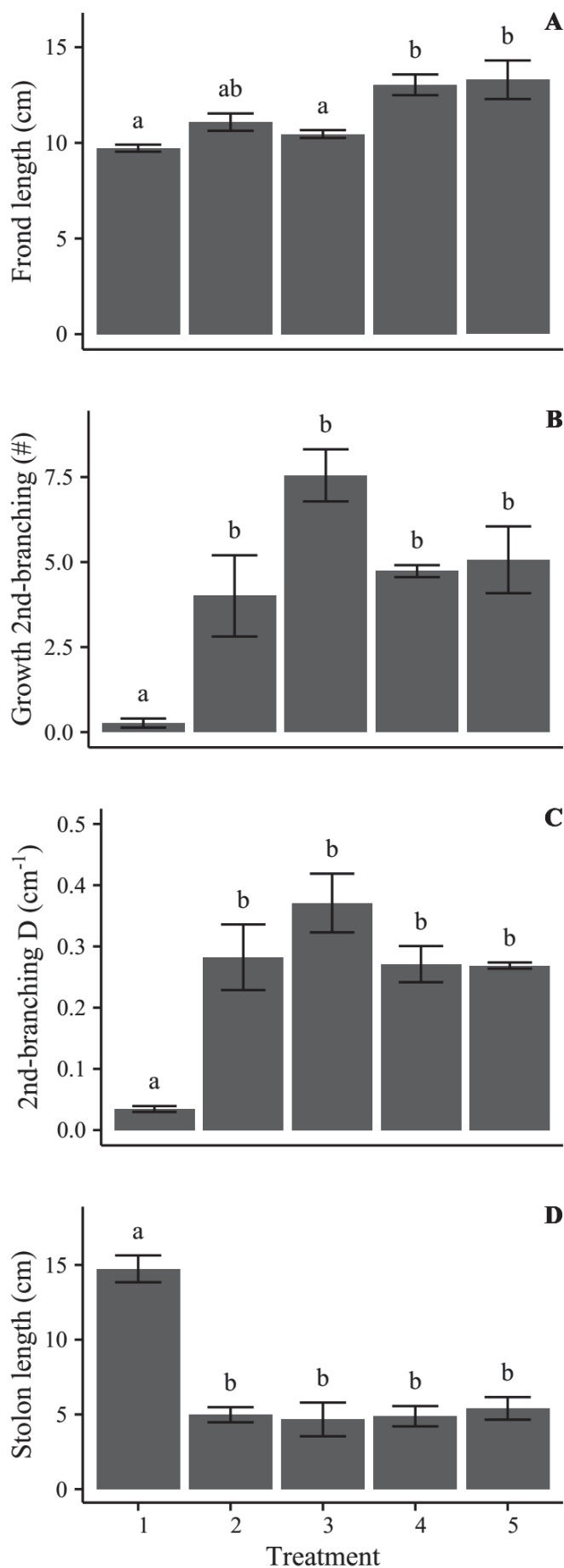


Fig. 4. 4. Mean ( $\pm$  SE) ( $n = 4$ ) **A**) frond length (cm), **B**) growth secondary branching (count), **C**) density of secondary branching (branching cm<sup>-1</sup> blade) and **D**) stolon length (cm) per sedimentation treatment. Different letters indicate differences between treatments as indicated by the post hoc test result ( $p < 0.05$  with Bonferroni adjustment) when significant treatment effects were found (Table 4.3). Treatments consisted of 1) a no sediment control; 2) 0.5 cm applied weekly; 3) 1 cm applied weekly; 4) 2 cm applied weekly and 5) 1 cm applied twice weekly.



## 4.5 Discussion

High phenotypic plasticity indicates high capacity to adjust to variable environmental conditions (Monro and Poore, 2005, Collado-Vides and Robledo, 1999, Stewart, 2008), and may be a general trait of invasive species (Davidson et al., 2011, Smith, 2009, Pyšek et al., 2009). Here we show that several morphological traits of the native alga *C. filiformis* showed high variation in relation to environmental variables occurring at local (water depth, sedimentation) and biogeographic (latitude) scales. Such morphological plasticity may in part explain the alga's increased abundance associated with sediment movement and the spread outside its historical distribution.

The spread of *C. filiformis* has been correlated to sedimentary processes acting at multiple spatial scales (Glasby et al., 2015, Chapter 2). Littler et al. (1983) described species distribution and abundance in sediment disturbed intertidal rockpools in the light of different life strategies, ranging from highly opportunistic species that quickly occupied newly created space following mortality due to burial, to long lived and highly tolerant species. For example, they found that the marine plant *Phyllospadix scouleri* dominated the lower sand-inundated pools, a species that tolerates sand by its large size and rhizomatic root system able to stabilize sediment. Similarly, *C. filiformis* appears highly tolerant to sedimentation as observed in this study, and similarly appears to persist under recurrent or continuous sediment stress for long periods of time, as patches appear highly stable through time (Glasby et al., 2015; Chapter 5).

Morphological adaptations suggested to aid a macroalga's tolerance to sedimentation include tough thalli, including those of some turfing algae such as *Corallina* spp. (Daly and Mathieson, 1977) or vegetative growth and the ability to regenerate damaged tissue from a basal thallus such as *Ulva* spp. (Daly and Mathieson, 1977, Kamermans et al., 1998, Mei and Schiel, 2007). However, here we observed that *C. filiformis*' high tolerance may result from morphological plasticity to changing sedimentary conditions. Although to our knowledge morphological plasticity in response to sedimentation in marine macroalgae has not been documented, terrestrial (e.g. Yu et al., 2004), and aquatic clonal plants (Duarte et al., 1997, Vermaat et al., 1997, Li and Xie, 2009) show increased investment in upright parts by elongation of e.g. increasing stem, internode and leaf sheet length to outgrow the sediment layer (see Yu et al., 2004 and references therein). Those patterns are similar to our field observations where we observed a similar increase in frond length but an overall reduction in complexity under sediment presence, thus prioritizing vertical vs lateral direction of growth of fronds.

In line with the field observations, our manipulative experiment demonstrated that increased frond length and decreased stolon length were a response to increased sedimentation (regardless of amount or frequency). A similar observation was made by Duarte et al. (1997), who showed small sediment layers could trigger a morphological response of highly plastic seagrass species. However, contrary to the field observations, the laboratory experiment showed increased investment in secondary branching with increased sedimentation. Duarte et al. (1997) also showed the importance of frond branching to outgrow the sediment layer of sediment tolerant seagrasses. It is not clear

why the field observations differed from the experimental observations, but perhaps our experiment did not run long enough to elicit similar responses to the patterns observed in the field sampling or levels of sedimentation used were still low in comparison to those that can be observed in the field. Alternatively, field observations were confounded by other factors not tested for in this study, such as hydrodynamics or the additional effects of sediment turbulence reducing light penetration when dissolved in the water column.

The laboratory experiment also indicated that sediment burial resulted in minimal investment in horizontal expansion (stolon growth). A full energy partitioning into upright parts may have resulted in the reduced horizontal expansion under sedimentation stress. Alternatively, the horizontal investment can be discussed in the light of searching for more suitable habitats as first described for terrestrial clonal plants (Hutchings and de Kroon, 1994), but similar observations have been made for vegetatively spreading algae (e.g. Collado-Vides, 2002). The “guerrilla” growth form of increased horizontal spread rates observed in absence of sediment may be an indication of searching for better (sandier) grounds. Daly and Mathieson (1977) classified certain algae under a “sand-loving” or psammophytic group, which implies some direct benefit from sediment presence. Although there are indications that *Caulerpa* spp. can benefit from sediment by utilizing available nutrients, in this study, field observations did not show increased biomass in association with sediment presence, nor did the experimental work show increased growth rates when sediment was present. The sediment surrounding *C. filiformis* beds consists of a very coarse, oligotrophic sand, thus with

limited potential as nutrient source. Littler et al. (1983) argued that another group of sand-associated species could be described as a stress-tolerant group, which because of their physiological, behavioural and morphological adaptations are able to survive burial where otherwise superior competitors could not. In this context, *C. filiformis* may be placed in the latter group.

The ability of *C. filiformis* to respond to and tolerate different sediment conditions may have played a crucial role in its current distribution and abundance, as many of the algae that it co-occurs with are known to be negatively affected by sedimentation. For example, performance of *Sargassum* spp. may be negatively affected by sedimentation (Zhang et al., 2014). Also, the abundance of *E. radiata* is negatively associated with sediment cover (Wernberg et al., 2005), while small algal species may be especially vulnerable to the negative effects of burial by sand (Schiel et al., 2006, Díaz-Tapia et al., 2013). Similarly, Piazzini et al. (2007) showed that the dominance of *Caulerpa cylindracea* was highly associated with sediment presence, suggested to be trapped by the invader itself, and under those altered conditions only other sediment tolerant competitors persisted in the alga's presence while most others were excluded. Tolerance to sedimentation may be increasingly important if sedimentation intensity and frequency changes with predicted future storm patterns (for a review see Connell, 2007).

The observational study also indicated that at increased water depth fronds were longer, narrower and less branched. Those observations of stimulated vertical growth direction

with increasing depth and reducing light levels have been observed for several other macroalgae (e.g. Collado-Vides, 2002, Wing et al., 2007), and have been especially well established for the *Caulerpa* genus (for a review see Rico and Guiry, 1996). Elongated fronds may optimize photosynthesis to allow for persistence in low light conditions, which quickly attenuates with depth (Iino, 2006). Additionally, self-shading by increased branching complexity at shallow environments may also benefit to protect from UV damage in very shallow or intertidal environments (Hay, 1981, Hay, 1986). Alternatively, high wave action (as found at shallower water depths) has been associated with a morphological response towards sturdier and smaller blades that reduce drag (Wing et al., 2007, Wernberg and Thomsen, 2005, Fowler-Walker et al., 2006). The individual role of changing light levels and changing wave action with depth on *C. filiformis* morphology patterns cannot be separated from this study however.

The observed decrease in frond length with increasing latitude in our study is in line with what has been observed with other algae in eastern Australia (Fowler-Walker et al., 2005, but see Mabin et al., 2013). Although significant, large scale variation was the least important in explaining *C. filiformis* morphology and overall a lot of the variation remained unexplained. Our results may be similar to those of Wernberg et al. (2003) who observed limited correlation between geographic separation and kelp morphology, suggesting that responses to environmental variables operate at different spatial scales. Morphological plasticity to environmental variables not investigated in this study, such as variable wave action at both local and large spatial scales (Kaandorp and Kübler,

2001, Wing et al., 2007, Wernberg and Thomsen, 2005), may have contributed to the high morphological variation not accounted for.

In conclusion, *C. filiformis* has a high morphological variation in association with a range of environmental variables and appears highly plastic. This morphological plasticity may explain why it appears highly tolerant to sedimentation, and its current spread along the NSW coastline. What remains is to experimentally investigate how competition between *C. filiformis* and co-occurring algae may be altered under different sedimentation regimes.

## **Chapter 5**

**The roles of nutrient disturbance and neighbouring habitat in  
*C. filiformis* post-recruitment spread**





## 5.1 Abstract

Coastal systems are under increasing anthropogenic pressure, and increased runoff of sediment and nutrients into shallow coastal systems is of particular concern. Increased nutrient concentrations may promote the expansion of some macroalgal species, including invasive species, at expense of others. This can be caused by direct benefits of increased nutrient availability that give the opportunistic species a competitive advantage, or indirectly by nutrient disturbance negatively affecting competitors. In rocky reef ecosystems, the loss of large alga may allow for alternate habitats (i.e. turf) that can promote invaders horizontal spread, potentially by the nutrient rich sediment trapped inside turf assemblages.

*Caulerpa filiformis* is a native alga that has spread within and outside its native range. This study investigated mechanisms that may contribute to its current success. It was suggested that its early proliferation followed increases in urban discharge into shallow coastal urban area around Sydney, New South Wales, Australia. This study experimentally investigated the potential benefits of sediment nutrient availability in the alga's growth rate. Simultaneously, the potential facilitative role of turf habitat was investigated, and hypotheses about the mechanism behind its potential facilitative traits (i.e. sediment nutrient availability) were tested.

Secondly, this study investigated the role of different neighbouring habitats in inhibiting or allowing *C. filiformis* horizontal expansion. The movement of *C. filiformis* patches

bordering with different neighbouring habitats for the duration of 1 year at 3 different sites was investigated. It was hypothesized that intact canopies and erect alga forms (kelp and *Sargassum* spp.) inhibit horizontal spread, while turf habitat does not. Also, the presence of a sediment veneer was expected to inhibit horizontal spread. The experimental study showed that *C. filiformis* growth rates were enhanced with increased nutrient availability in the sediment, however, very high nutrient levels reduced fragment growth and success. The sediment trapped inside the turf assemblage did not increase nutrient availability, and did not enhance *C. filiformis* growth rates. Also in the field, no support for the facilitative role of turf in horizontal expansion of the alga was found, as spread rates were highly stochastic, and no clear pattern among site, season, or bordering habitat was found. There was some support for the hypothesized inhibiting role of *Sargassum* and kelp as expansion into those habitats was only observed in combination with a retreat of those habitats. Expansion was observed onto rock with a sediment veneer present. As the extent of the sediment layer fluctuated throughout the year, *C. filiformis* horizontal expansion may have followed a sand retreat. This study showed that *C. filiformis* is likely not a dominant competitor, and may depend on disturbances such as sedimentation or eutrophication to become to dominate communities.

## 5.2 Introduction

Coastal ecosystems are under increasing anthropogenic pressure, ranging from large scale effects of global climate change such as sea surface temperature (SST) rise to localized disturbances associated with coastal development (Worm and Lenihan, 2013). Of particular concern is the increasing runoff of sediment and nutrients from terrestrial sources affecting coastal regions worldwide (Thrush et al., 2004, Bouwman et al., 2005). Nutrient disturbance may cause large shifts in macroalgal composition, benefitting highly opportunistic, fast growing species (e.g. Lapointe, 1997, McCook, 1999, Velasco et al., 2006, Borowitzka, 1972). In similar ways, nutrient disturbance has been shown to benefit invasive species. For example, Lapointe and Bedford (2011) demonstrated that the abundance of the non-native *Acanthophora spicifera* was enhanced with proximity to a stormwater outfall due to increased competitive advantage of the invader over native species in conditions of increased nutrient availability.

Alternatively, these kinds of disturbances may benefit invaders indirectly by the loss of intact canopies. For example, intact, native assemblages including erect species tend to resist horizontal spread of adjacent *Caulerpa cylindracea* (Ceccherelli et al., 2000), while on the other hand, disturbed canopies allow for the invader's horizontal spread by freeing up resources such as space and light (Bulleri et al., 2016b, Ceccherelli et al., 2014). Moreover, in certain cases disturbance may indirectly benefit invaders via the promotion of alternate habitats. For example, nutrient disturbance led to the loss of erect algae and the dominance of turfing algae which in turn promoted the spread of invasive

*Caulerpa* spp. (Bulleri and Benedetti-Cecchi, 2008, Ceccherelli et al., 2002). Thus, macroalgal interactions may vary from resisting to facilitating *Caulerpa*'s vegetative expansion, depending on the species involved. In some cases, previously subordinate native species benefit from disturbance in ways similar to those described for non-native invasive species, and come to dominate communities (Carey et al., 2012, Simberloff et al., 2012). But whether the horizontal spread of native species is altered by their different competitors, has received little attention.

Although the role of turfing algae in promoting horizontal expansion of invasive *Caulerpa* spp. has been demonstrated frequently, the underlying mechanisms have not been investigated in detail (but see Bulleri and Benedetti-Cecchi, 2008). Authors have suggested that nutrient-rich sediment trapped inside the turf may benefit the spread of invasive *Caulerpa* species (Gennaro and Piazzzi, 2014). Nutrient uptake through the rhizomatic system of species of *Caulerpa* is important for their growth (Cuhel et al., 1984, Williams and Fisher, 1985, Larned, 1998, Chisholm et al., 1996), and a positive relationship between *Caulerpa* species' abundance and sediment nutrient availability has been frequently demonstrated (e.g. Ceccherelli and Cinelli, 1997, Malta et al., 2005, Larned, 1998, Velasco et al., 2006). Turfing algae are widely known for their ability to trap sediment (for a review see Airoidi, 2003), and sediment trapped in turf on coral reefs can have high nutrient contents (Wilson et al., 2003). Thus, we may predict that turfing species promote *Caulerpa* spp. growth through the nutrients in trapped sediments, although both nutrient availability in the turf surrounding *C. filiformis* and its benefits on the alga remain to be determined.

On shallow rocky shores in New South Wales (NSW), Australia, the native alga *Caulerpa filiformis*, is becoming more locally abundant and spreading outside its known historic distribution (see Glasby et al., 2015 for an overview). *C. filiformis* is now the dominant habitat-forming species on many shallow rocky reefs along the coast, where it can form extensive monospecific stands of >1000 m<sup>2</sup> (Glasby et al., 2015, Voerman et al. unpub. data), and an increased understanding of mechanisms that may promote or impede its spread is required. The historical expansion of *C. filiformis* was thought to be caused by high levels of raw sewage being pumped into the nearshore coastal around the Sydney metropolitan region with increased nutrient levels benefitting the alga (May, 1976). Despite the observation that the current large scale distribution of the alga is not related to large scale nutrient variation (measured by chl a distribution), a relationship to more localized runoff cannot be excluded (Glasby et al., 2015). We may predict that the alga can benefit directly from increased nutrient availability.

The post-recruitment spread via vegetative growth (stolon extension) is acknowledged as an important trait in determining the success of invasive species of *Caulerpa* (Wright and Davis, 2006, Williams and Smith, 2007, Smith and Walters, 1999, Bulleri and Benedetti-Cecchi, 2008), including *C. filiformis* (Zhang et al., 2014). Biotic interactions with native assemblages may be especially strong for those vegetatively expanding invaders, where horizontal spread directly depends on competition with neighbours (Minchinton and Bertness, 2003, Amsberry et al., 2000). In similar ways to its non-native congeners, we may expect the successful horizontal spread of *C. filiformis* to be affected by its neighbouring habitats, which may vary from resistant to facilitative.

*C. filiformis* co-occurs with large erect *Sargassum* spp. and the canopy forming kelp *Ecklonia radiata* (Zhang et al., 2014; Voerman et al. in review, Lanham et al., 2015), that have been shown to drastically affect understory species (e.g. Kennelly, 1989, Toohey et al., 2007, Sanchez et al., 2005), and may also inhibit horizontal spread of the alga. Also in the systems *C. filiformis* occurs at, cleared space following the loss of *Sargassum* spp. and kelp is rapidly occupied by turfing algae (Toohey et al., 2007, Kennelly, 1987c), that similar to other *Caulerpa* spp. may promote *C. filiformis* spread. On rocky reefs, *C. filiformis* recruits are more often associated with turfing algae and fragments attach more successfully (in terms of time to attach and attachment strength) to this habitat (Chapter 2). However, the total abundance of the alga is low in association to turf habitat (Chapter 2). It was hypothesized that this discrepancy may be caused by turf being a very suitable habitat that is quickly outcompeted by *C. filiformis* after successful recruitment, in line with observations of facilitative interaction in the Mediterranean (Bulleri and Benedetti-Cecchi, 2008, Ceccherelli et al., 2002). Although sediments in turf did not promote fragment attachment, it remains to be determined whether turf nutrients can facilitate post-settlement growth of *C. filiformis*. Alternatively, the opposite may be true that turf is a very unsuitable habitat and inhibits *Caulerpa* expansion, however, those hypotheses have not been tested yet.

Additionally, current large and local scale observations demonstrated a strong association of the alga's distribution and abundance with sediment disturbance (Glasby et al., 2015; Voerman et al. in review). Sediment among *C. filiformis* stands may be a result rather than a cause of the alga's local scale distribution as the alga is thought to be

able to trap the sediment in its dense network of stolons and upright fronds (Zhang et al., 2014; Voerman et al. in review). Experimental work showed no direct benefit of sediment on the alga's growth and even reduced horizontal expansion of the alga under sediment disturbance, however, this may be a short-term stress response (Chapter 4). It has not been investigated yet if sediment presence on a reef can impede further horizontal spread but we may predict it too reduce *C. filiformis*' horizontal expansion rates.

This study aimed to increase understanding of the mechanisms that promote or continue to promote the post-recruitment spread of *C. filiformis*. First, we investigated the direct impact of nutrient disturbance in the sediment on the alga's growth by a laboratory experiment. Simultaneously the laboratory experiment was used to understand whether turf promoted or inhibited the expansion of *C. filiformis* by examining the growth of *C. filiformis* on turf. For expediency, at the same time a possible mechanism for quick growth of *C. filiformis* on turf (sediment nutrient availability) was investigated. Finally, this study aimed to investigate the role of different neighbouring habitats in facilitating or inhibiting the alga's horizontal spread under field conditions. The boundaries between established *C. filiformis* and three competing species (*Ecklonia radiata*, *Sargassum* spp. and turf) and rock with a sediment veneer (no competitors) were followed for one year. We tested the hypotheses that horizontal spread is promoted by turfing algae and inhibited by stands of *E. radiata* and *Sargassum* spp.. The hypothesis that horizontal expansion of *C. filiformis* is limited under sediment presence was also tested.

## 5.3 Materials and methods

### 5.3.1 Effects of sediments, nutrient availability and turf on algal growth

A laboratory experiment was conducted at the Port Stephens Fisheries Institute to test whether turving algae influence the growth of *C. filiformis*, and if so, whether this is due to the physical structure of the turf or the sediments in the turf (specifically elevated levels of nutrients in turf sediments). *C. filiformis* was exposed to 6 different surfaces for 6 weeks. Treatments included intact, natural turf (without sediments) (NT), artificial turf only (AT), artificial turf + turf sediment (TS), artificial turf + control sediment (biologically inert sediments) (CS), artificial turf + low nutrients sediment (+LN) and artificial turf + high nutrients sediment (+HN). These treatments allowed separation of the potential biological and structural effects of turf on fragment success whilst simultaneously testing for the effects of sediment quality on algal growth.

“Natural turf” treatment consisted of dense assemblages of algal turf (predominantly red geniculate *Corallina* spp., and filamentous forms, all <5 cm tall), attached to small rocks (approx. 10cm diameter). Turf was cleared of sediment by gently shaking the rocks while submerged in seawater. The “artificial turf” treatment was created by gluing a ~4 mm thick heavy duty scourer (70% nylon and 30% polyester ©MrClean, with a dense network of filaments) to the top of a ceramic tile (10 x 20 cm). “Turf sediment” consisted of a layer of the turf sediment removed from natural turf and added to the artificial turf to entirely cover the artificial turf similar to conditions in the field. The “sediment control” treatment consisted of Sulphuric acid washed (©VWR prolabo)



sand, again added to artificial turf. Two nutrient treatments were created by adding 19:9:12 N:P:K slow release © Osmocote coated fertilizer with 2% magnesium oxide and micro elements (Fe, Cu, B, Zn, Mn and Mo). The “high nutrient” treatment received 0.2 g Osmocote/g of dry clean sediment and the “low nutrient” treatment received 0.02 g Osmocote/g of clean sediment. Osmocote was pre-soaked in filtered seawater for 1 week to allow for Osmocote activation prior to addition to the sediment treatments (Worm et al., 2000). Again, both sediment-nutrient treatments were added to the artificial turf.

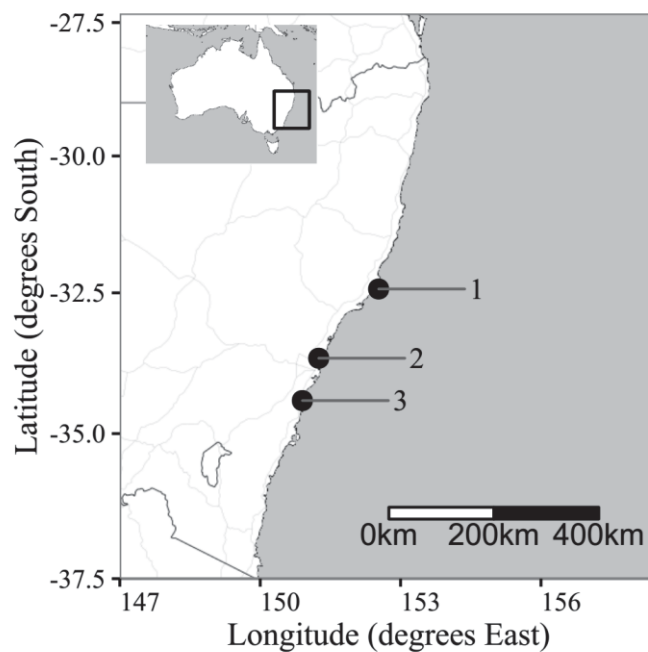


Fig. 5. 1. Location of study sites in NSW, Australia (inset). Black circles represent sites sampled for the morphological study. Site numbers represent 1) Seal Rocks; 2) Mona Vale and 3) Wollongong. Map tiles were sourced from StamenDesign (2016).

Experiments were set up in early February 2016. The natural turf and artificial turf+natural sediment treatments were established from turf with intact sediment collected from Seal Rocks (Fig. 5.1). *C. filiformis* was collected from Newcastle (-32.926° Lon; 151.793° Lat) and taken to the laboratory within 2 h and kept in aerated filtered seawater. On the day of collection, fragments of *C. filiformis* were created by cutting stolons such that each consisted of a 2 cm stolon with 2 fronds (10-15 cm high) and 2-4 rhizoid clusters. After stolons were cut, the fragments were kept floating in aerated filtered seawater for 48 h at a light:dark cycle of 12:12 h to allow for wound healing. Following this, the fragments were carefully blotted dry and the initial weight measured (to the nearest 0.001 g). A single fragment was then added to individual natural turf and artificial turf (tile) treatments without any sediment yet present. Fragments were held in place by two loose plastic bands. Replicate tanks (90 L) (n = 3 tanks/per treatment) received 3 tiles with each tile containing one fragment. Total fragment biomass did not vary among treatments at the start of the experiment ( $F_{5, 24} = 1.4, p > 0.2$ ). Fragments attached to the substratum were left for 7 days after which time the different sediment treatments were applied. Each tank with sediment treatments received 1 kg of sediment, resulting in a layer of ~0.4 cm above the tile surface, filling the layer of artificial turf similar to natural conditions observed in the field. Aquaria were aerated, and kept under a light:dark cycle of 12:12h at ambient temperature. Tanks contained oceanic water which was refreshed daily with a slow drip surface supply.

Nutrient levels in each treatment were examined to test if potential differences in growth were associated with different nutrient concentrations in the sediment, and to

investigate whether turf sediment altered nutrient availability. Sediment pore water was sampled 2 weeks after sediment was added to the aquaria. Dissolved nutrients in the pore water are bioavailable and are in equilibrium with concentrations in the sediment, thus are a good measure of altered nutrient availability with different sediment properties (Boström et al., 1988, Andrieux-Loyer et al., 2008). Sediment pore water in each aquarium (250 mL) was sampled using a 2 mm  $\varnothing$  needle attached to a 250 mL Syringe combining 9 randomly located sampling points within the artificial turf-sediment layer amongst *C. filiformis* rhizoids. Both syringe and sampling tubes were rinsed 3 times with tank water prior to sampling to avoid cross contamination. The tank water was used as pore water quantities were limited. Sampling took place 20 hours after water replacement. Samples were immediately frozen ( -80°C) and later transported to the Southern Cross EAL laboratory on ice (overnight) for sample processing. Pore-water nitrate, nitrite, ammonia (together total N) and phosphate (P) concentrations were analysed with ICP-MS or ICP-OES mass-spectrometry following APHA (2012) protocols.

To examine whether treatment results (growth rates and tissue nutrient content, see below) could be influenced by altered nutrient concentrations in the water column resulting from diffusion from the sediment treatments, or if results could be attributed to different nutrient concentrations in the sediment alone, water nutrient concentrations were also investigated. Water samples (250 mL), positioned 5 cm above the substrate, were taken in a similar manner and at the same time as the sediment pore-water collection above. Clean sediment was not investigated as it was assumed to be equal to

the artificial turf treatment. Water nutrient concentrations in the high nutrient treatment were not investigated by the following reasons: Preliminary data (3 days after sediment was added to the aquaria) analysed one randomly chosen high nutrient treatment and sediment control replicate for water and sediment pore water nutrient levels by methods described above. Preliminary data showed that nutrient concentrations in the high sediment nutrient treatment were much increased in the water column: water column nutrient levels measured were 7.25 mg/L nitrogen (N) (nitrate, nitrite and ammonia) and 2.02 mg/L P for the high nutrient treatment. While control sediment showed much lower water column values of 0.56 mg/L N and < 0.005 mg/L P. At the same time, preliminary observations showed *C. filiformis* fragments had high mortality, so we did not collect water column nutrients for this high nutrient treatment at 2 weeks as they were clearly enhanced.

To investigate differences in growth rates and tissue nutrient uptake of *C. filiformis*, change in the wet weight, final dry weight, carbon (C), nitrogen (N) and phosphate (P) tissue concentrations were analysed for data pooled within tanks. During the course of the experiment some fragments had detached from the substrate (9/90 fragments, 5 in the high nutrient treatment and 4 in the low nutrient treatment. There were never less than 2 fragments/ tank). Those were removed from further analysis. Fragments were removed from the aquaria and rinsed in clean seawater water. The thalli were blotted dry and weighed for final wet weight. Differences with initial wet weight per fragment were calculated and an average growth rate/tank was calculated. Samples were then dried at 40°C for 62 hours and sent to EAL laboratories for analysis of total dry weight,

and tissue C, N and P content. Average individual dry weight per tank was subsequently determined. Tissue P analysis followed the methods of Rayment and Lyons (2011). Total Carbon and nitrogen were analysed with an LECO TrMac CNS analyser. C:N and C:P ratios were calculated, as a measure of nutrient limitation of the alga.

One factor Analysis of Variance (ANOVA) was used to test the effect of treatment on water column and sediment pore water nutrient concentrations, tissue dry weight, and tissue characteristics among substrate treatments. Assumptions of ANOVA were investigated with the GVMMLA package (Pena and Slate, 2004), and transformations were applied where necessary to conform with the homogeneity of variance assumption. Where significant treatment effects occurred, differences were further investigated with Post Hoc LSmeans comparisons with Bonferroni adjustment (Lenth, 2016). All statistical analyses were performed in R with associated packages (R Core Team, 2015, Fox et al., 2016).

### **5.3.2 Effect of neighbouring habitats on *C. filiformis* spread**

A field study was replicated at three sites (Fig. 5.1) to investigate the influence of neighbouring habitats on the post-recruitment horizontal spread of *C. filiformis* and to test the hypotheses that horizontal spread is promoted by turfing algae and inhibited by stands of *E. radiata* and *Sargassum* spp. and sand. The boundary edges were marked between *C. filiformis* and three algal habitats (n = 5 marked boundaries/neighbour/site,

apart from at SR where  $n = 4$ ) that it commonly co-occurs with: turf (dense cover of predominantly red geniculate *Corallina* spp., < 5 cm tall and including filamentous species), *Sargassum* spp. (80-100% *Sargassum* canopy cover, > 40 cm tall, with bare rock, encrusting or some turfing algae as understory), kelp (100 % *Ecklonia radiata* canopy cover with predominantly bare rock and encrusting algae under the canopy), and 100% sand cover on rocky reef. Sand was not investigated at Seal Rocks due to the difficulty of experimental set up (drilling) on volcanic rock.

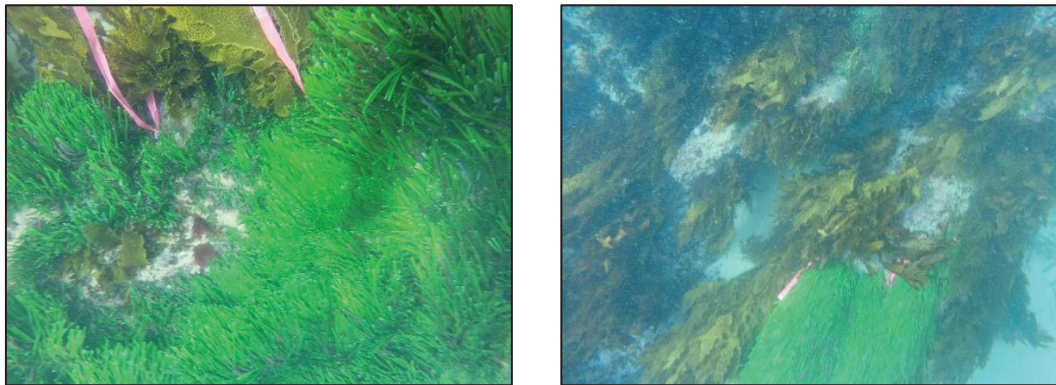


Fig. 5. 1. Examples of *C. filiformis* patch bordering with kelp habitat at  $t = 0$  at Wollongong. Bolts with flagging tape for easy identification mark a 50 cm stretch of border.

Because of the time-consuming nature of setting up boundaries (see below) all sites were established between April and July 2014. However, the full set-up of boundaries at each site only took 3-10 days. At each site boundaries were established in a random order across all neighbouring treatments. For each boundary, a 50 cm section of the border between *C. filiformis* and its neighbouring habitat was marked with 2 stainless steel bolts (8 x 80 mm, 316 marine grade stainless steel) drilled into the rock substratum beneath the algae (Fig. 5.2). The boundary position was recorded by GPS. Borders were only established where patches of *C. filiformis* and its neighbouring habitat were

both  $> 4 \text{ m}^2$  to remove the influence of early spread processes and across a similar depth range (2-4 m below low tide level). Borders were visited every 3 months after the final day of experimental set up, weather permitting. Some bolts were lost due to sediment deposition or movement of large boulders during storm events. Thus, not all replicates were located and sampled at all sampling times. Surveying was stopped at all sites in May 2015 (one year after set-up) as no replicates were found at Seal Rocks.

At each sampling time, photos were taken of the border for later analysis. Photos were later analysed for the position of the border in relation to the start of the experiment. Image processing software (ImageJ) was used to measure (in cm) the distance of the patch border perpendicular to the mid-point between the bolts. The known distance (50cm) between bolts was used as reference for length measurement. Positive values represent expansion of *C. filiformis* while negative values represent a retreat.

We used one-factor ANOVA to test for differences in the effect of neighbouring habitat on the spread of *C. filiformis* as described above for each survey. Analyses were conducted on untransformed data as model assumptions were met.

## 5.4 Results

### 5.4.1 Effects of sediments, nutrient availability and turf, on fragment growth

As expected, porewater N and P nutrient concentrations were highest in the high nutrient addition treatment, followed by the low nutrient treatment, clean sediment and turf sediment (Fig. 5.3a, b; Table 5.1). The low nutrient treatment had significantly greater N concentrations than the clean and turf sediments. A similar but non-significant trend was apparent for P (Fig 5.3b). Contrary to our prediction, N and P concentrations in turf sediment were not significantly greater than in the clean sediment (Fig. 5.3a, b; Table 5.1). Porewater in the high nutrient treatment was increased by  $30 \times 10^3$  % N and  $15 \times 10^3$  % P; and in the low nutrient treatment by 178% N and 207% P in comparison to the clean sediment treatment.



Table 5. 1. ANOVA test results for differences in *C. filiformis* growth, sediment porewater- and water-nutrient concentrations between treatments. Tissue values are compared among all treatments. N = 5, except for P content for which some samples had not enough tissue available for analysis, n = 2. Sediment values are compared between 4 treatments with sediment present (see fig. 5.3) (n = 5), water values are compared between treatments control (no sediment), turf sediment and low nutrient sediment (n = 5). Transformations applied to confirm with test assumptions are indicated. For post hoc test results for significant relationships see Fig. 5.2-5.4.

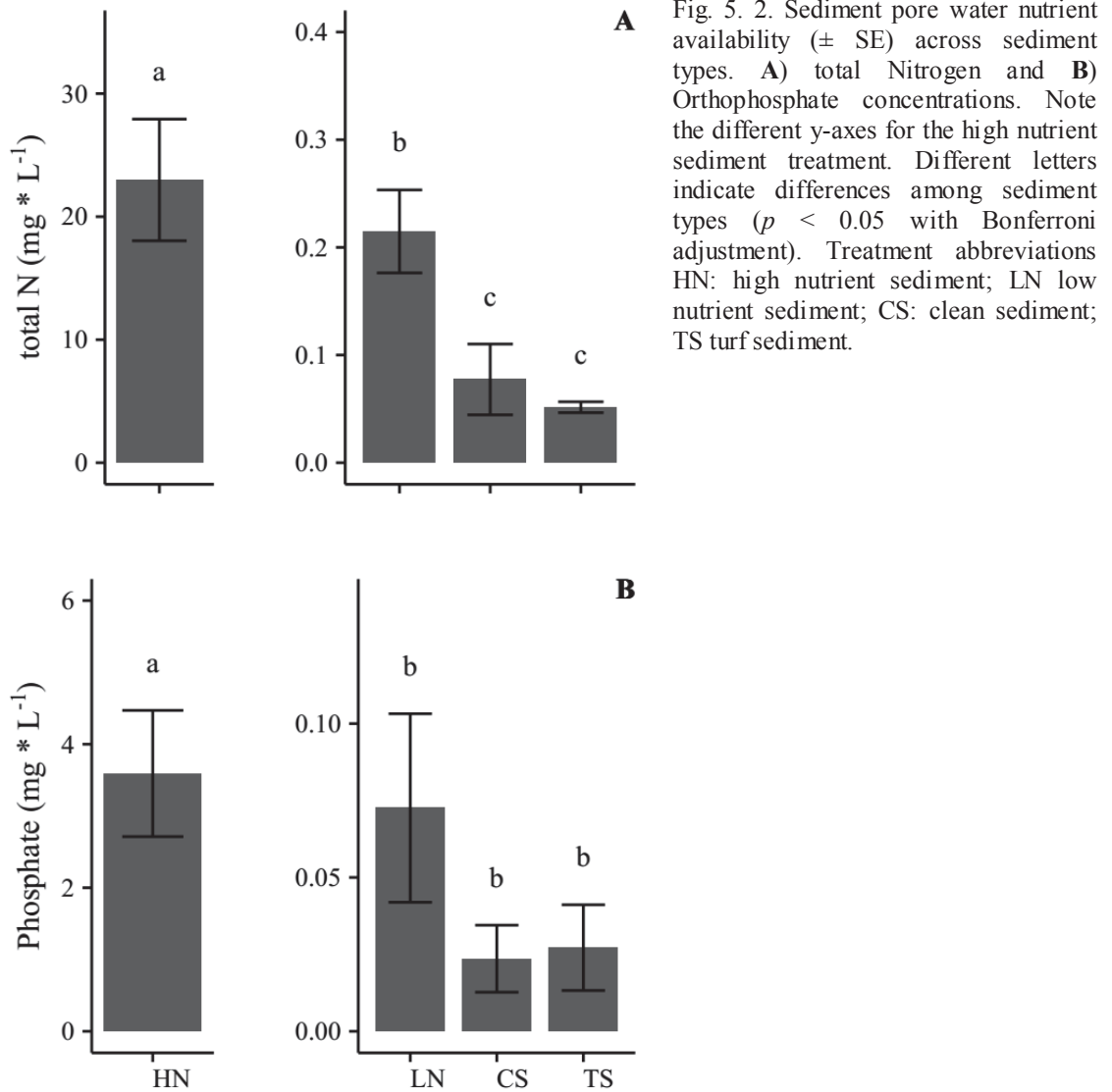
	MS	df	F	p
<b>Sediment pore water nutrients</b>				
Total N (mg/L) (log)				
treatment	119.96	3	179.22	<0.001
Residuals	3.57	16		
Orthophosphate (mg/L) (log)				
treatment	93.573	3	38.788	<0.001
Residuals	12.866	16		
<b>Water column nutrients</b>				
Total N (mg/L)				
treatment	0.0001872	2	1.0108	0.39
Residuals	0.0011112	12		
Orthophosphate (mg/L)				
treatment	1.92E-05	2	3.3488	0.070
Residuals	3.44E-05	12		
<b><i>C. filiformis</i> growth</b>				
Final dry weight (g)				
treatment	0.65169	5	4.3117	<0.01
Residuals	0.72550	24		
Delta wet weight (g)				
treatment	92.337	5	20.771	<0.001
Residuals	21.338	24		
Tissue C content (% dry weight)				
treatment	749.79	5	4.096	<0.001
Residuals	255.32	24		
Tissue N content (% dry weight)				

treatment	3.9202	5	6.3126	<b>&lt;0.001</b>
Residuals	2.9898	24		
<hr/>				
Total Phosphorus content (% dry weight) (sqrt)				
treatment	0.033656	5	17.11	<b>&lt;0.001</b>
Residuals	0.008262	21		
<hr/>				
C:N ratio				
treatment	979.15	5	15.222	<b>&lt;0.001</b>
Residuals	308.75	24		
<hr/>				
C:P ratio				
treatment	409780	5	21.025	<b>&lt;0.001</b>
Residuals	81859	21		
<hr/>				
Significant <i>p</i> -values are in bold.				

Although preliminary data showed enhanced nutrient concentrations in the water column for the high nutrient treatment, no differences were found water column concentrations of nutrients among the low nutrient, control sediment and turf sediment treatments (Table 5.1). Average concentrations of Total N were  $0.038 \pm 0.0098$  mg/L, and Total P were  $0.0074 \pm 0.0014$  P mg/L across those 3 treatments.

Final dry weight and change in wet weight of *C. filiformis* fragments followed similar patterns to sediment pore water nutrient availability (Fig. 5.4; Table 5.1). Growth rates in the laboratory experiment were generally low. *C. filiformis* grew significantly more in the low nutrient addition treatment compared to the high nutrient (which caused *C. filiformis* to shrink) (Fig. 5.4b). The low nutrient treatment growth was also elevated in comparison to the remaining treatments (control, turf, clean sediment and turf

sediment), amongst which growth rates were low and not significant different (Fig. 5.4b). Although a similar trend is observed for the final dry weight measure, this was not significant (Fig. 5.4a).



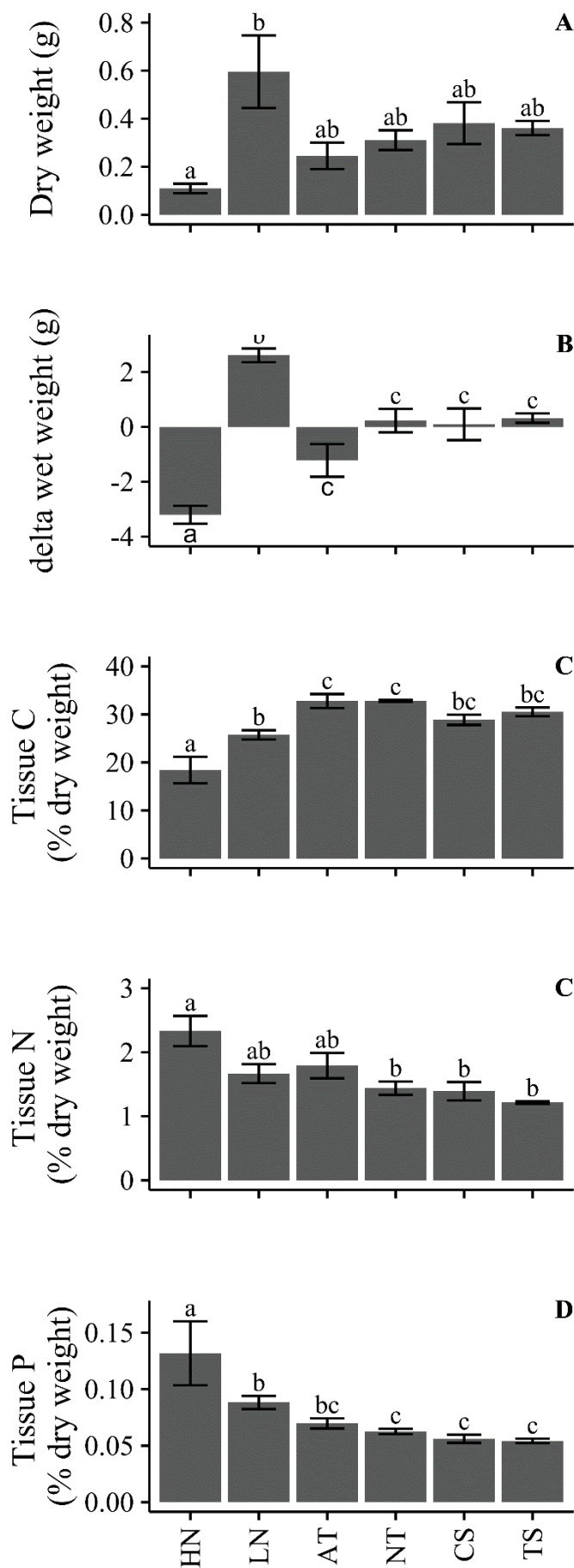


Fig. 5. 3. Growth responses ( $\pm$  SE) of *C. filiformis* to different substrata (n=5). **A**) Average final dry weight; **B**) average change in wet weight between the start and the end of the experiment (6 week period); **C**) tissue N and **D**) tissue P concentrations. Different letters indicate differences among sediment types ( $p < 0.05$  with Bonferroni adjustment). Treatment abbreviations HN: high nutrient sediment; LN low nutrient sediment; AT: artificial turf; NT: natural turf; CS: clean sediment; TS turf sediment.

*C. filiformis* was able to utilise nutrients from the sediment. *C. filiformis* tissue concentrations were typically larger in the nutrient treatments (Table 5.1, Fig. 5.4c and d). Correspondingly, relationships among treatments for C:N and C:P tissue ratios strongly followed the opposite patterns to N and P showing a strong reduction in C:N and C:P at high nutrient loads, somewhat reduced for low nutrient loads and no difference among the remaining treatments (Fig. 5.4c and d; Fig. 5.5).

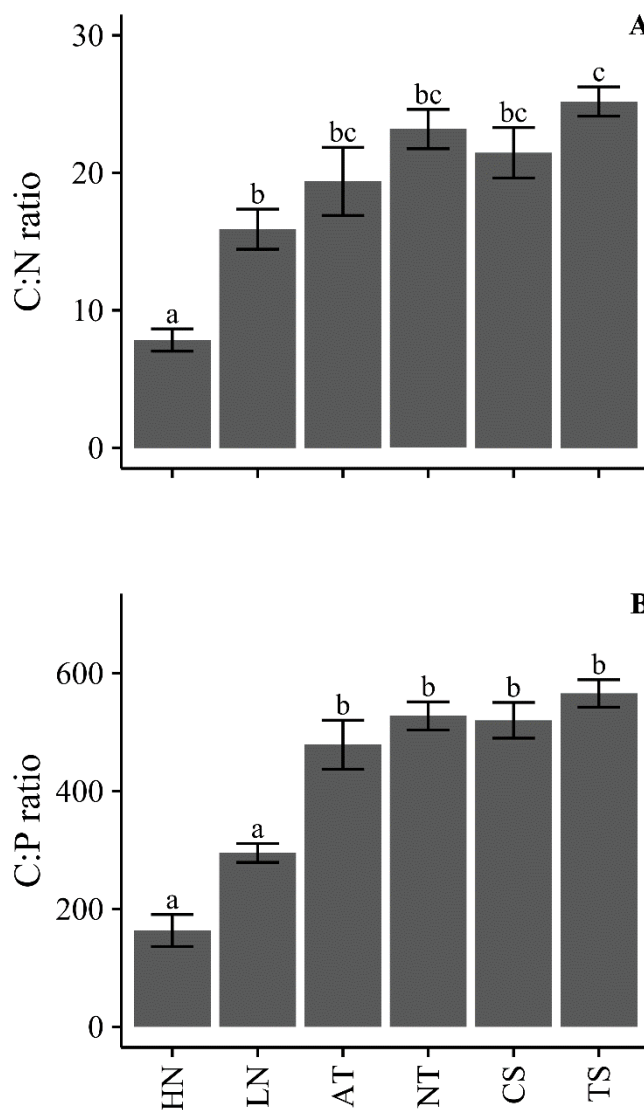


Fig. 5. 4. Tissue a) C:N and b) C:P ratio ( $\pm$  SE) per sediment treatment ( $n = 5$ ). Different letters indicate differences among sediment types ( $p < 0.05$  with Bonferroni adjustment). Treatment abbreviations HN: high nutrient sediment; LN low nutrient sediment; AT: artificial turf; NT: natural turf; CS: clean sediment; TS turf sediment.

#### 5.4.2 Effect of neighbouring habitats on *C. filiformis* expansion

The horizontal spread of *C. filiformis* was highly variable both temporally and spatially, and varied from a 1 m expansion over 3 months (*Sargassum* habitat at Seal Rocks) to a 2.8 m contraction (adjacent to kelp habitat at Seal Rocks); Fig. 5.6. In no case was a general expansion of *C. filiformis* into a habitat observed. The only significant difference in expansion rates of *C. filiformis* among treatments was at Mona Vale after 3 months, with higher expansion into turf habitat, but by the end of the 12 month study ( $F_{3,10} 5.37, p < 0.05$ ), there had been no overall change in *C. filiformis* borders ( $p > 0.05$ ) (Fig. 5.6).

When expansion into kelp or *Sargassum* habitat was observed, this was always associated with the absence of those canopies with newly created space sometimes colonized by turfing alga, although the retreat of competing algae was not quantified. However, when expansion was observed into turf habitat, turf was still present and *C. filiformis* encroached through the turf assemblage.

In the occasions that *C. filiformis* did regress from boundaries, there was no subsequent expansion of the neighbouring algae across the border, except for turfing alga. Instead, the space was often colonized by turf although this was not quantified.

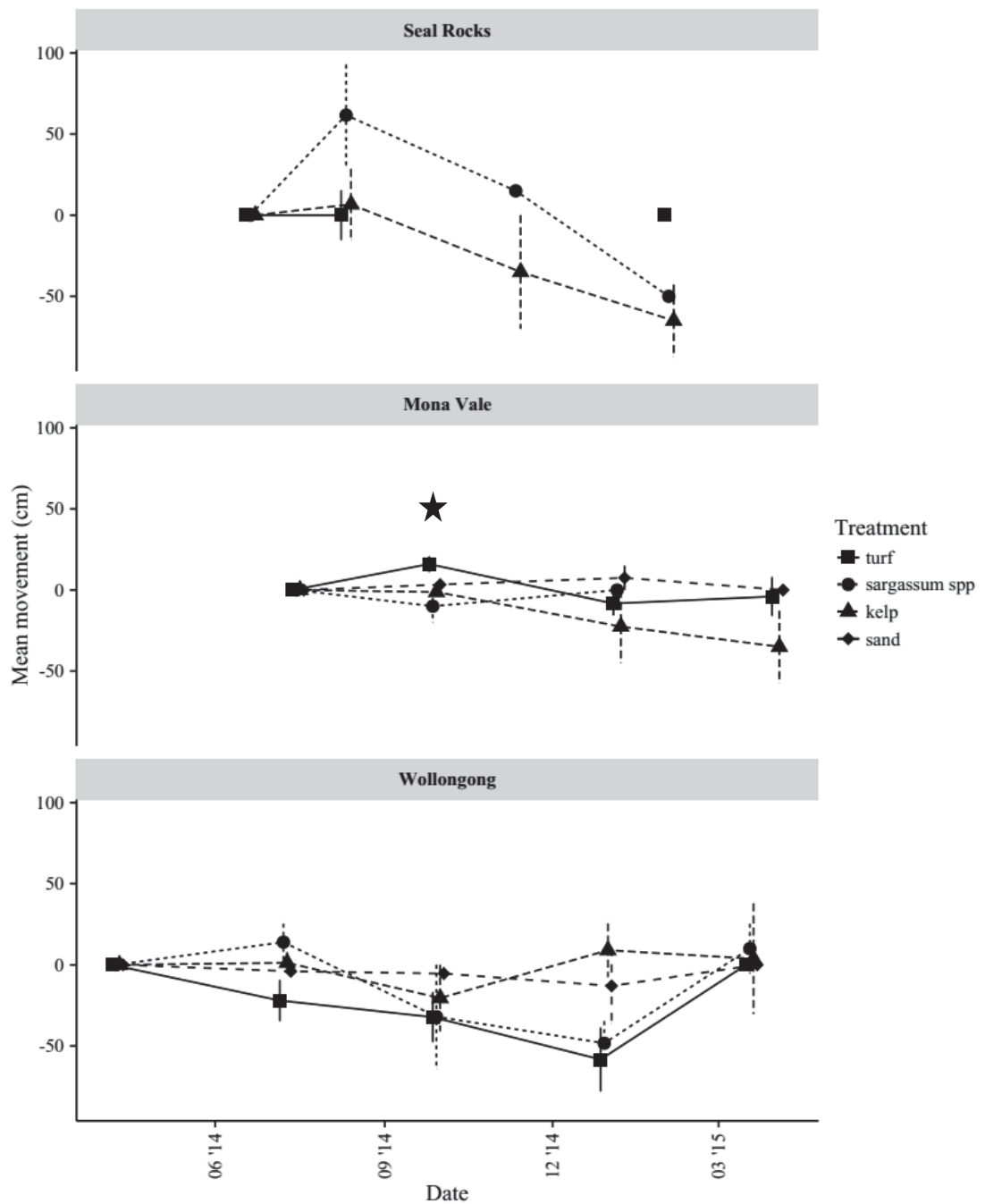


Fig. 5. 5. Horizontal movement ( $\pm$  SE) of *C. filiformis* from marked borders into neighbouring habitats. Positive values indicate expansion, negative values indicate retreat since the start of the experiment. The star symbol indicates a significant difference among treatments ( $p < 0.05$ ). Data is spread out horizontally ( $\pm 3$  d) for better visualization.

## 5.5 Discussion

Nutrient disturbance is a key mechanism promoting the growth of rapidly spreading algae, and their horizontal spread may change as a function of neighbouring habitats. This study investigated the potential role of nutrient disturbance on *C. filiformis*' growth, tested two competing models regarding the negative association between *C. filiformis*' abundance and turf, and additionally investigated the role of intact canopies and sand as neighbouring habitats in affecting the horizontal spread of *C. filiformis*.

The historical spread of *C. filiformis* was thought to be caused by high levels of raw sewage being pumped into the nearshore coastal environment around the Sydney metropolitan region (May, 1976). The porewater N concentration of the high nutrient treatment in this study was comparable to concentrations from direct sewage outfall, that can reach ~44 mg/L N and ~ 8 mg/L P (median value of undiluted effluent from Sydney ocean outfalls 1996-97) (Pritchard et al., 2001). However, these experiments showed that the highest levels of nutrients had negative effects on *C. filiformis*. Thalli in the high nutrient treatment showed large tissue necrosis (browning), while thalli at all other treatments looked healthy. Although elevated nutrient concentrations may benefit certain opportunistic or tolerant species, when nutrient concentrations surpass a certain threshold it can also negatively affect those species (Schramm, 1999). However, mixing happens rapidly once effluents reaches the ocean in surrounding waters by e.g. wave action (Borowitzka, 1972, Pritchard et al., 2001). Diluted effluent near coastal outfalls may have reached levels of 0.15 mg/L of only nitrate and nitrite (Borowitzka, 1972).



This study showed, that at these levels these nutrients can directly benefit *C. filiformis* (also discussed below). Alternatively, the initial spread of *C. filiformis* was made possible by the simultaneous loss of algal competitors that were also negatively affected by sewage outflows (Coleman et al., 2008). Although both hypotheses are likely explanations for the alga's historical expansion was indeed associated to sewage outlets, due to the lack of historical data this can't be determined with certainty. While historically ocean outfalls outlets were only 3 m away from the low tide level and thus could affect seaweed communities (Borowitzka, 1972), the commission of deep ocean outfalls in the early 1990s far away offshore removed the influence of nutrient elevation on shallow coastal shores, and thus are unlikely to benefit *C. filiformis* these days (Pritchard et al., 2001).

The low levels of nutrient used in this experiment (low nutrient treatment) enhanced growth rates. As water column nutrient concentrations did not differ between treatments (excluding the high nutrient treatment), and were low in comparison to pore water concentrations, it is likely that differences in the alga's growth rates and tissue concentrations among those treatments followed uptake of sediment porewater via its rhizome structure. The experimental N levels of the low nutrient treatment are very similar to concentrations observed in the runoff from urban areas after light to heavy rainfall or agricultural areas after heavy rain fall, ranging between 0.1 and 0.8 mg/L (Gorman et al., 2009). Sediment runoff from terrestrial sources, associated with high nutrient concentrations, is common (Bouwman et al., 2005), and may also benefit *C. filiformis* because of its high tolerance to sedimentation (Chapter 4) and its greater

growth under increased sediment nutrient availability. Although the large-scale distribution and spread of *C. filiformis* has not been associated with nutrient concentrations of nearshore coastal waters (Glasby et al., 2015), this study suggests that *C. filiformis* may be able to utilise nutrient pulses associated with local sediment and nutrient runoff. Similarly, Gennaro and Piazzì (2011) showed increased growth and competitiveness of the invasive *Caulerpa cylindracea* in algal assemblages under sediment nutrient addition during a ~4-month field experiment. Ceccherelli and Cinelli (1997) also showed a similar pattern for *Caulerpa taxifolia* in competition with seagrass under nutrient addition to the sediment, however, this was only a short term (4 month) response (Ceccherelli and Sechi, 2002). An ability to uptake nutrients may also have longer term consequences for *C. filiformis* via carry-over effects. For example, *Caulerpa* experience seasonal dieback and pulses of nutrients supplied prior to die-back may add recovery during periods of growth (Velasco et al., 2006). We did not observe any strong trends in dieback with season in this study, however this may occur (Glasby, personal observations).

The different responses (positive vs negative) between the low nutrient and the high nutrient treatment on *C. filiformis* growth may have resulted from indirect effects of the high nutrient concentrations. Namely, during the experiment plankton seemed to have proliferated by the green colour of the water, possibly leading to reduced oxygen and light levels (Schramm, 1999). Although increased nutrient concentrations often increase abundance of opportunistic species such as *Caulerpa* spp. (Lapointe and Bedford, 2010,

Malta et al., 2005), hypertrophic conditions can result in mortality of those species (Schramm, 1999).

Although it was hypothesised that turf sediment would have increased nutrient availability with positive effects on *C. filiformis* growth, this was not observed. The experimental work showed that turf did not promote *C. filiformis* growth directly. Firstly, *C. filiformis* did not grow any better on natural turf than its structural mimic artificial turf, indicating no positive association between the algae species themselves. *C. filiformis* did not grow any better when sediment (control or turf sediment) was present either, indicating no direct benefit of the sediment trapped by the turf algae. Indeed, although increased nutrient concentrations could enhance *C. filiformis* growth, nutrient availability was not enhanced in the turf sediment. Crossman et al. (2001) demonstrated that organic content of turf sediment is negatively associated with wave energy. As *C. filiformis* predominantly occurs on exposed shorelines (Glasby et al., 2015), nutrient concentrations in the turf sediment may be low and potential benefits of turf sediment may thus be limiting.

Alternatively, turf may facilitate expansion in other ways than nutrient availability. Turf may enhance horizontal spread through its complex structure which may enhance anchoring of rhizomes and stolons (Bulleri and Benedetti-Cecchi, 2008). Additionally, sediment trapped inside the turf may protect the alga from grazing (D'Antonio, 1986). However, our field observation did not show a strong expansion of *C. filiformis* into turf

habitat, and contrary to our predictions we did not find any evidence for turf being rapidly outcompeted by *C. filiformis*.

The limited spread of *C. filiformis* into turfing habitat was surprising. Our other research showed a highly positive relationship between turfing habitat and *C. filiformis* recruits (Chapter 3) and other studies have described positive effects on the spread of other species of *Caulerpa* from the conversion of macroalgal stands to turfing habitat (e.g. (Bulleri and Benedetti-Cecchi, 2008, Gennaro and Piazzzi, 2014). It is possible that turf habitat only benefits recruitment and that further disturbances are needed to encourage its horizontal spread (e.g. pulses of nutrients from sediments as discussed above). It is also possible that overgrowth of this and other habitats takes longer than the time span of this study's surveys. Alternatively, its spread could be mediated by the differential susceptibility of algal competitors to disturbance, such as sedimentation (Chapter 4), which did not happen during the course of these surveys at the sites investigated.

No *C. filiformis* encroachment was found into intact kelp or *Sargassum* habitats. Intact stands of kelps and other large brown algae commonly resist competition from competing invasive alga (Thompson and Schiel, 2012b, Valentine and Johnson, 2003), so the lack of expansion of *C. filiformis* in *E. radiata* and *Sargassum* spp. beds is not surprising. This study found no evidence that once established, *C. filiformis* strongly outcompetes those native canopies. The alga's horizontal expansion may require further disturbances to algal canopies (see also Zhang et al., 2014). Stafford and Bell (2006) also demonstrated rapid expansion rates of *C. prolifera* following experimental

disturbance to native seagrass stands. However, the photosynthetic health of *Sargassum* spp. can be reduced when in contact with *C. filiformis* (Zhang et al., 2014). Thus *C. filiformis* may indirectly increase the susceptibility of native species to removal via sublethal effects on their condition.

Finally, in contrast to earlier observations in laboratory settings (Chapter 4), *C. filiformis* was observed encroaching onto rock with a sediment veneer present. However, the sediment border edging the rocky reef was highly variable through time, and was sometimes observed away from the border. The high temporal variability in sediment cover on rocky reefs (e.g. Harley et al., 2011a) may allow for expansion during a temporary retreat of the sediment layer. Further experimental work is needed to fully understand the impacts of sediment disturbance on *C. filiformis* horizontal spread.

A recent focus in ecology is whether invasive species, including marine macrophytes, are passengers or drivers of ecological change (South and Thomsen, 2016, Bulleri et al., 2010, Ceccherelli et al., 2014, Didham et al., 2005, Tamburello et al., 2015). While disturbance may be necessary in the early stages of an invader's spread, suggesting the passenger model, established population may elicit change without the requirement of further disturbance, suggesting a driver model. This suggests that invasive macrophytes may transition from passengers to drivers of change without the requirement of further disturbance to promote their spread. For example, Bulleri et al. (2010) showed that although *C. cylindracea* benefitted from disturbance to otherwise resisting canopies, once established their presence further drove species interactions likely resulting from

its ability to trap sediment. The results of this study showed that the post-recruitment spread of *C. filiformis* is inhibited by competing algae, possibly depending on disturbance benefitting the alga itself or removing or reducing the fitness of competitors, thus *C. filiformis* appears a passenger of change.

In conclusion, *C. filiformis* does not appear a dominant competitor. Its horizontal spread may benefit from disturbance (e.g. nutrient addition) affecting the fitness of the alga as well as disturbances that remove competitors (e.g. *Sargassum* and kelp). Turf habitat did not enhance *C. filiformis* growth and was not rapidly outcompeted in the field, thus *C. filiformis* expansion onto this habitat may also depend on disturbances altering competitive outcomes of this biotic relationship. Thus, *C. filiformis* appears to be a passenger of change.

## **Chapter 6**

### **General discussion**





## **6.1 Native invaders and *C. filiformis***

The impacts of native invaders can challenge those of their famous non-native counterparts (Simberloff and Rejmánek, 2010, Valery et al., 2009, Carey et al., 2012) and their role in invading communities is expected to increase with continuing climatic change and increasing anthropogenic pressures on ecosystems worldwide (Davis et al., 2011). Yet when, why and how native species start to behave like invaders are still little understood (Carey et al., 2012). This thesis examined the spread of the native alga *Caulerpa filiformis* and the different mechanisms that may promote its success. First, the habitat associations of established populations and newly established recruits were examined throughout the species' distribution. Secondly, hypotheses about the mechanisms underpinning these relationships were tested using field and laboratory experiments, with a focus on the role of disturbance (i.e. sedimentation and nutrient pollution) in promoting the spread of *C. filiformis*.

## **6.2 Historical and contemporary drivers of *C. filiformis* spread**

### **6.2.1 Historic proliferation and the role of nutrient disturbance**

A comparison of the current distribution of *C. filiformis* with historical records and anecdotal reports provides strong support for the suggestion that the distribution of the alga has increased over at least the past two decades and potentially longer (Glasby et

al., 2015). The same period coincided with large point-source pollution from several ocean outfalls in and around the major metropolitan areas along the NSW coastline (Wollongong, Sydney, the Central Coast, Newcastle, Port Stephens and Coffs Harbour) that functioned from the turn of the 19th century to accompany the increasing population density (Borowitzka, 1972, Roberts et al., 1998, Smith, 1996, Roberts and Scanes, 1999). Outfalls discharged up to 70 million gallons primary treated sewage, stormwater, and urban runoff into the shallow subtidal reefs per day, which had drastic implications for the macrophyte composition in the intertidal (Borowitzka, 1972, Smith, 1996, Cosser, 1997) and in the subtidal (BurrIDGE et al., 1996, Coleman et al., 2008, Smith, 1996, Roberts et al., 1998). May (1976) suggested that *C. filiformis* may have benefitted from these sources of nutrients, causing its abundance to increase in and around Sydney, although no quantitative data on the abundance or distribution of the species were available. In this study, we found that high nutrient concentrations of ~25 mg/L N and ~ 4 mg/L P, similar to those found in effluent from sewage outfalls (~44 mg/L N and ~ 8 mg/L P) (Pritchard et al., 2001), reduced the alga's health drastically (Chapter 5). However, effluent is rapidly diluted by water mixing (within meters) once it reaches the ocean and further away from the outlets (Borowitzka, 1972, Pritchard et al., 2001, Thrush et al., 2004). In diluted form sewage effluent may have benefitted *C. filiformis* as increased growth rates under moderately increased nutrient availability (~0.2 mg/L N and ~ 0.08 mg/L P) were found (Chapter 5). Additionally, *C. filiformis* may benefit from disturbance indirectly. The loss of competing algae associated to urban pollution around the Sydney metropolitan area (e.g. Coleman et al., 2008) may

have facilitated *C. filiformis* expansion, although experimental work is needed to fully tease apart the role of nutrient disturbance on those biotic relationships.

Nutrient pollution in aquatic systems typically results in the loss of long-lived species, and the spread of highly opportunistic macroalgae that can quickly outgrow long lived and typically slow growing species (Diaz-Pulido and McCook, 2008, Nixon and Fulweiler, 2009, Lapointe and Bedford, 2011, Bertocci et al., 2015, McCook et al., 2001, Cosser, 1997). This commonly includes fast growing green alga species that are able to utilize increased nutrients and proliferate rapidly, such as *Ulva* spp. (Borowitzka, 1972, Cosser, 1997), and also *Caulerpa* spp. (Gennaro and Piazzzi, 2011, Ceccherelli and Cinelli, 1997, Lapointe et al., 2005, Lapointe et al., 2010). Those studies represent the influence of nutrient pollution near estuaries or sewage outfall. Similar observations have been made in NSW in association ocean outfalls. Namely, although nutrient plumes from ocean outfalls can be transported for several 100s of meters along the coast (Cosser, 1997), Smith (1996) showed that impacts were often not observed > 200 m away from the outfalls (but see Coleman et al., 2008). The study by May (1976) suggested the increase in *C. filiformis* abundance both in and around the Sydney metropolitan area following nutrient disturbance may therefore be limited to areas in close proximity to the sewage outfalls, and does not support the alga's proliferation along wider stretches of coastline as suggested by May (1976). Moreover, the commission of offshore outfalls in the early 1990's in Sydney resulted in a drastic improvement of coastal water quality (Pritchard et al., 2001), thus unlikely explains the

contemporary dominance of the alga over large stretches of coastline (see also Glasby et al., 2015).

### **6.2.3 Contemporary distribution and the role of sediment disturbance**

Although the historic proliferation of *C. filiformis* may have been aided by point source nutrient pollution (although quantitative support is missing), there is no indication that the current distribution of *C. filiformis* is linked to large scale variation in nutrient concentrations (using chl a concentration as a proxy), although local scale pollution benefits cannot be excluded (Glasby et al., 2015). Sedimentation appears to be an important factor underlying its current distribution, with surveys demonstrating sediment associations at multiple spatial scales (Chapter 2). At large spatial scales, *C. filiformis* is more abundant along stretches of coastline with a high ratio of sand:reef (Glasby et al., 2015). More locally, narrower reefs are associated with a large abundance of the alga than larger reefs (Chapter 2). Within a reef, the alga is more abundant on rocky reef with a sediment veneer than areas without and positively associated with reduced slope and reduced distance from shore possibly linked to increased sediment accumulation (Chapter 2).

Although this study found no indication that sand positively affects *C. filiformis* directly as shown by the laboratory experiment (Chapter 3), sand likely benefits *C. filiformis* indirectly. Many species are negatively affected by sand (Airoldi, 2003, Watanabe et al., 2016, Díaz-Tapia and Bárbara, 2013), including competitors of *C. filiformis* (Phillips

and Blackshaw, 2011, Wernberg et al., 2005). Conversely, *C. filiformis* has traits that make it highly tolerant of sediment (Chapter 4). The distribution of *C. filiformis* in South Africa is also affiliated with sand (Anderson et al., 2016), and is similarly seen as a general trait of many of the species' congeners from around the globe (Coppejans and Beeckman, 1990, Cribb and Cribb, 1954, Svedelius, 1906, Weber-van Bosse, 1901).

Sediment – reef interactions are common along coasts worldwide (Gallop 2013), and the influence of natural or anthropogenic sediment disturbance has been observed on rocky reefs in the Mediterranean, Atlantic coastline in southern Europe, southern Australia and New Zealand and the west coast of the United States (e.g. Vaselli et al., 2008, Irving and Connell, 2002, Hurley, 2009, Balata et al., 2007, Díaz-Tapia et al., 2013, Littler et al., 1983, Airoidi and Cinelli, 1997, Araujo et al., 2012, Airoidi, 1998). Sediment disturbance often allows for dominance of the most tolerant and adaptive species, similar to the situation described in this study. Temporal or spatial patterns of altered disturbance regimes (such as sedimentation), in combination with differing susceptibility or tolerance to disturbances of species, is well known to shape local and large-scale distribution patterns of macroalgal communities (Sousa 1983).

Data on how sediment movement and deposition rates may have changed over the past few decades in NSW is extremely limited, with the possible exception of a study by Harley et al. (2011a) looking at long term shoreline variability of a sandy beach near Sydney. This study showed that patterns in beach erosion and replenishment were predominantly shaped by periods of high wave action (i.e. storms) shaping onshore-

offshore sediment exchange. Those wave-driven currents are the primary mechanism for sand transport in the coastal zone, and the strength and direction of these currents are dependent on the height and direction of the waves off the continental shelf (km away) (Komar, 1998). Hemer et al. (2010) showed that between 1999 and 2006 the mean wave height increased in the southern hemisphere, which may have corresponded with a change in sediment movement over the same period. The observed increased wave action and associated sediment movement may be linked to increased dominance of *C. filiformis* over the past few decades.

### **6.3 Linking recruitment to established beds**

Successful spread of a species depends on the success of several invasion stages. Importantly, different mechanisms may affect each of those stages (Blackburn et al., 2011, Colautti and MacIsaac, 2004). Large scale observations and experimental work conducted in this study identified different mechanisms at various life stages of *C. filiformis* that may be important in influencing its spread. Those associations were often opposing (Chapters 2, 3, 5), and reflect the different requirements of each stage, although the magnitude of influence of each stage on the overall distribution of the alga may vary (see e.g. Wright and Davis, 2006).

### 6.3.1 What is it about turf?

Field studies revealed that recruits of *C. filiformis* showed a high affinity with turf habitat, particularly geniculate corallines. Watanabe et al. (2009) demonstrated the potential benefits of turf alga in enhancing *Codium* spp. recruitment success by entangling fragments. Increased fragment trapment may have similarly contributed to the observed positive association between *C. filiformis* and turf habitat observed. Moreover, experimental work in this study showed that turf offers more suitable substrate for rapid and strong attachment in comparison to bare rock or sand substrate (Chapter 3). This is in line with the findings of Herren et al. (2006) who found increased attachment of *Dictyota* spp. fragments to the calcifying green alga *Halimeda tuna*. Conversely, the positive association between *C. filiformis* recruits and turf was absent for adult beds observed throughout the distribution of *C. filiformis* (Chapter 2). It was hypothesized that this may be caused by 1) *C. filiformis* quickly outcompeting (overgrowing) turf or 2) turf not being a good habitat for the growth and expansion of *C. filiformis*. Experimental work found no direct benefits of turf substrate, nor of the trapped sediment inside the turf (Chapter 5) on *C. filiformis* growth rates. Furthermore, long term monitoring of *C. filiformis* bordering turf showed that turf was not rapidly outcompeted by *C. filiformis* (Chapter 4).

This study identified an interesting relationship between *C. filiformis* stands and turf assemblages: turf promotes *C. filiformis* recruitment (Chapter 3). Vice versa, *C. filiformis* retreat may be rapidly filled by opportunistic turf (Chapter 5). This turf cover

can in turn limit the recruitment of large brown alga that otherwise resist *C. filiformis* spread (Kennelly, 1987b, Vadas et al., 1992), while not (fully) inhibiting the horizontal spread of *C. filiformis* and promoting its recruitment (Chapter 5).

### **6.3.2 What is it about sediment?**

Rapid horizontal spread into turf may depend on disturbance (e.g. sedimentation) affecting the competitive outcome not observed over the course of the border study (Chapter 5). Chapter 2 showed a high abundance of *C. filiformis* on narrow reefs suggested by increased sediment movement, while the reefs selected for the long-term monitoring of *C. filiformis* borders were all wide reef sites. Mona Vale has a reef width of ~80m, and falls in the wide reef range similar to Wollongong and Seal Rocks (Chapter 2), the other sites used in the border study. Thus, those wide reef sites may represent low sediment disturbance sites in comparison to narrow reefs, limiting competitive dominance of the alga.

Similar processes may be responsible for the observed relationship between *C. filiformis*' adult bed size and recruit abundance. Adult abundance and recruitment abundance at a site are connected by propagule abundance. For example, Wright and Davis (2006) demonstrated increased recruitment success with increasing propagule pressure. It was slightly surprising that this study did not detect a positive association between adult beds and recruit abundance, while a negative association might be present (Chapter 2). Rather than this observation reflecting recruitment success, observations



may be confounded by the ability of recruits to transfer to the adult stage. Namely, variables that are observed associated with increased adult abundance (e.g. narrow reefs) may similarly be the ones that show reduced recruitment abundance, resulting in the negative relationship between adult and recruit abundance observed.

While the abundance of *C. filiformis* was positively associated with sand presence on the rocky reef (Chapter 2), the opposite was observed for recruit abundance within a site (Chapter 3). Our experimental work showed that the lack of recruits on soft sediment is likely due to the slow fragment attachment and low attachment strength on sand substratum, limiting recruitment success in a highly turbulent environment. This has also been observed for other algal recruits and adult forms (Thomsen et al., 2004, Vadas et al., 1992). This indicates that a sediment veneer associated to *Caulerpa* beds may be a result rather than a cause of the alga's adult distribution within a site, which was also supported by the observation that stolons were always attached to the rocky substrate underneath the sediment veneer (Chapter 2). Sand may be trapped and retained by a dense network of stolons and closely distributed fronds, to which the alga was shown to be highly tolerant (Chapter 4). Similar observations of increased sediment retention have been made for seagrasses and several other species of *Caulerpa* (Hendriks et al., 2010). For example, communities in the presence of *C. cylindracea* contained seven times more sediment than uninvaded communities (Piazzi et al., 2007). Although chapter 4 showed reduced horizontal expansion potentially as a short-term response to experimentally applied sediment disturbance, horizontal spread onto rock with a

sediment veneer was observed (Chapter 5). However, this expansion have followed a temporary retreat of the sediment layer.

#### **6.4 *C. filiformis* – a passenger or driver of ecological change?**

Observed impacts associated with the presence of invasive species have recently been challenged, and many have questioned whether invasive species are “drivers” or “passengers” of ecosystem change (for a summary see MacDougall and Turkington, 2005). Namely, impacts such as changes in abundance or composition of recipient communities may be distorted by environmental factors and habitat degradation which have allowed for species invasion in the first place (i.e. invaders as passenger of change). In certain cases, invasive macroalgae may depend on disturbance to otherwise resisting canopies, but may continue as drivers of further change once successfully established (Bulleri et al., 2010). To fully understand the impacts of invaders and their potential for further disruption, or the reasons for their success, it is important to distinguish between the different models. Although not tested explicitly in this study, some inferences can be made by the observed patterns of other algal abundances associated with *C. filiformis* presence and rates and potential for *C. filiformis* spread into neighbouring habitats.

*Caulerpa filiformis* presence is associated with a change in the nearby seaweed community, specifically a reduction in diversity and abundance of other macroalgae (Chapter 2). The observed impacts on biodiversity increased with increasing *C.*

*filiformis* abundance, as the abundance of other species decreases with increasing *C. filiformis* abundance (i.e. limitation of space). Chapter 2 showed that recruitment success may be limited in intact (kelp and *Sargassum*) habitats, but is aided by turf habitat that typically represents a post-disturbance state (Connell, 2007). Moreover, Chapter 5 showed that *C. filiformis* is not a competitively superior species that rapidly overtakes communities, but horizontal spread appears to depend on some sort of disturbance that removes or reduces competitors or alters competitive success (e.g. complete removal by storms or reduced competitors' fitness by sediment disturbance). Thus, the observed high abundance of *C. filiformis* has likely followed disturbance to otherwise resisting canopies, indicating *C. filiformis* is a passenger of change.

However, species dominance can trigger shifts to alternative ecosystem states that may be difficult to reverse (see Connell, 2007 for a review). For example, the dominance of turfing algae can be stable for years and inhibit recovery of other algae by trapping sediments and creating an environment that is unsuitable for many algae (Airoldi, 1998, Toohey et al., 2007, Birrell et al., 2005, Kennelly, 1987b). Trapped sediment may similarly play an important role in the continuing of competitive dominance of *C. filiformis*, as also suggested to benefit the competitiveness of *Caulerpa cylindracea* (Piazzi et al., 2007). That is, once established, *C. filiformis* may create alternate conditions (r+s substrate) that may fully exclude other species (Chapter 2). Zhang et al. (2014) showed the lack of recovery of most alga species after patches inside *C. filiformis* beds were cleared. The authors suggested this was due to the sediment trapped by surrounding *C. filiformis*, while the recovery of *C. filiformis* by predominantly

horizontal expansion was high. Although highly variable, the overall change in *C. filiformis* abundance was minimal over the course of one year (Chapter 4) or over a 5-year time period (Glasby et al., 2015). Thus, although the initial spread of *C. filiformis* may depend on disturbance removing competitors, once established it may form a highly stable alternate state.

## 6.5 Conclusion

In conclusion, habitat suitability was an important factor in describing the distribution and abundance of both the adult and the recruitment stage, although habitat associations differed among those stages. Disturbance appeared to be an important determinant in *C. filiformis* abundance and distribution (For an overview see Fig. 6.1). These disturbances may act either directly by increasing *C. filiformis* fitness, i.e. nutrient disturbance, or indirectly by reducing that of others, i.e. sediment or nutrient disturbance. Sediment disturbance is associated to *C. filiformis*' distribution across multiple spatial scales (Glasby et al., 2015; Chapter 2). *C. filiformis* is highly tolerant to sedimentation, aided by morphological plasticity, which likely gives the alga a competitive advantage. Loss of erect and canopy forming alga that may resist recruitment and horizontal spread of *C. filiformis* can have additive positive impacts when cleared space is colonized by turfing algae, as turf habitat promotes *C. filiformis* recruitment success. Once established, *C. filiformis* was not found to be a dominant competitor and may depend on further disturbance to expand. However, once established, sediment trapped by *C. filiformis* can inhibit the recovery of other algae and may drive further change.

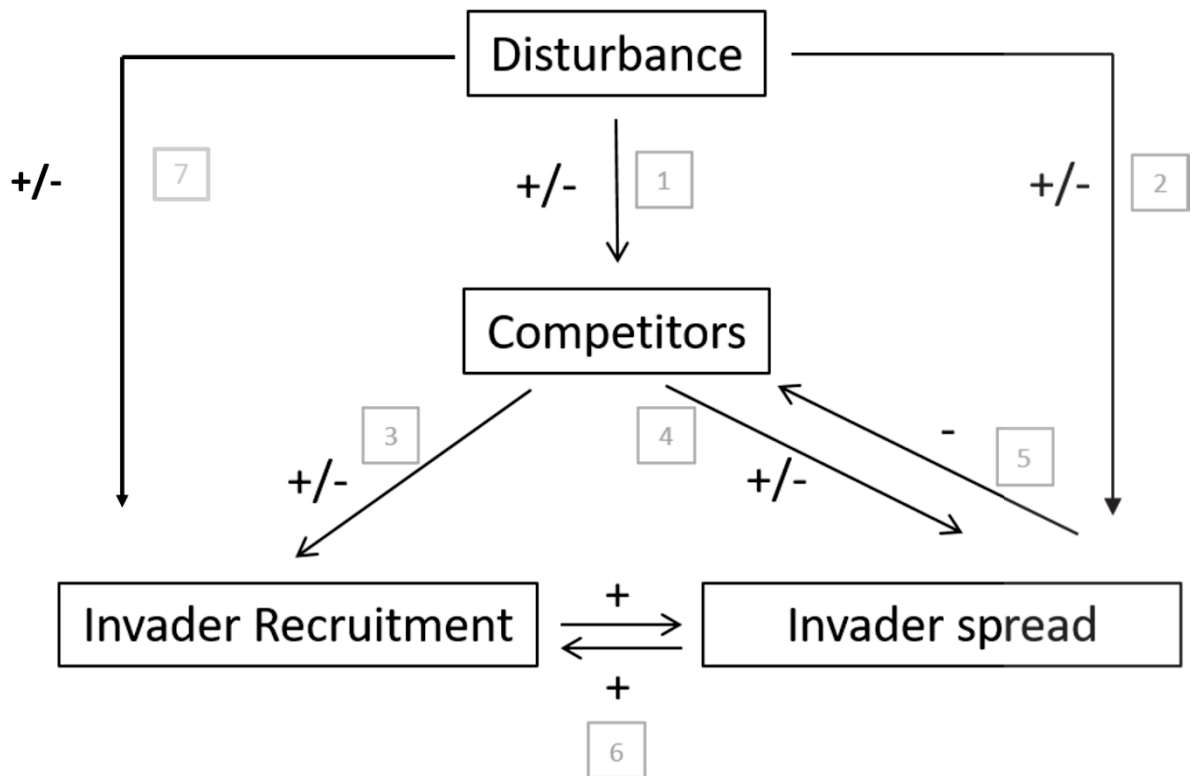


Fig. 6. 1. Schematic overview of the processes affecting *C. filiformis* abundance found in this study. Positive interactions are indicated with +, negative interactions with -. Different numbers indicate following processes in the case of *C. filiformis*:

- 1) Disturbance such as sediment may negatively affect competitors, while not negatively affecting *C. filiformis* (Chapter 4);
- 2) High nutrient loads negatively affect *C. filiformis*;  
Low nutrient loads (further away from storm water effluent, or direct effluent from agriculture) promote its growth (Chapter 5);  
Sediment disturbance did not positively nor negatively alter growth rates (Chapter 4).
- 3) Turf algae (coralline species) promote recruitment success, aided by increased structural complexity that allow for rapid and strong attachment;  
*Sargassum* spp. and kelp may inhibit successful recruitment, although results are not conclusive.  
Under increased swell *Sargassum* habitat may benefit fragment retention (Chapter 3).
- 4) *Sargassum* spp. and kelp may inhibit *C. filiformis* horizontal spread (Chapter 5);  
There was no support that turf habitat promotes horizontal spread (Chapter 5).
- 5) Results suggest that *C. filiformis* is not a dominant competitor that rapidly outcompetes its neighbours (Chapter 5);

However, there are indications that *C. filiformis* can trap sediment, possibly negatively affecting its competitors (Chapter 2, 3).

- 6) There was no indication that increased adult abundance increased recruit abundance, but rather the opposite pattern was observed. This may represent the ability of recruits to transition to the adult stage.
- 7) Wave action resulted in a low fragment retention, successful recruitment appears to depend on a calm weather window (Chapter 3).

## **6.6 Conservation issues: problems and solutions and further research**

### **6.6.1 Climate change, coastal development and potential *C. filiformis* spread**

*C. filiformis* will likely benefit from any future increased sedimentation rates. Altered sediment disturbance may arrive from marine (e.g. via long shore sediment movement) or terrestrial (e.g. via runoff) sources. Sediment movement on reefs along the NSW coastline is highly variable through time and space, and relates to long term oscillating weather patterns, the proximity of reefs to beaches, and their aspect (Harley et al., 2011b, Hemer et al., 2010, Short and Trembanis, 2004), but are in particular defined by large wave action (i.e. storms) (Harley et al., 2011a). Predicted future climate scenarios of increasing storm intensity and frequency and changes in wave direction (Hemer et al., 2010, Poloczanska et al., 2007 and ref therein) can translate into increased longshore sediment fluxes in shallow coastal systems (e.g. Hemer et al., 2010, Miles et al., 2013, Roleda and Dethleff, 2011). Altered sediment patterns will be spatially variable along the shoreline (Hemer et al., 2010, Harley et al., 2011a), but will undoubtedly continue to shape macroalgal composition along the NSW coastline, potentially favouring sediment tolerant species (Airoldi, 2003). Sedimentation rates may also alter with changes in terrestrial runoff (Presto et al., 2006, Thrush et al., 2004). The Australian population is expected to continue to grow, with much of this growth going towards increased population density of coastal regions (Hugo, 2012) and nearshore habitats predicted to be under increasing pressures (Raupach et al., 2012, Bouwman et al., 2005). Increasing coastal development and agriculture affects sediment and nutrient runoff (Bouwman et

al., 2005), which this study has shown may both positively (directly or indirectly) affect *C. filiformis* (Chapter 2, 5). Impacts may be concentrated around in estuaries and in a lesser extend storm water outfalls which export most of the terrestrial runoff (Bouwman et al., 2005, Thrush et al., 2004, Scanes), so this may be where *C. filiformis* will expand.

Additionally, *C. filiformis* will likely benefit from other kinds of disturbances that negatively affect competitors (for a review see Wernberg et al., 2011a). For example, increased storminess may alter sediment patterns but will also create more available space by removing competitors (Thomsen et al., 2004, Wernberg and Connell, 2008, Kennelly, 1987a). However, how storminess may affect *C. filiformis* stands itself is hard to predict. *C. filiformis*' patches may be similarly negatively affected by storms by physical removal of part of the alga stands or tissue breakage. However, this may simultaneously lead to increased propagule supply. After storms, there are large amounts of fragments washed up on the beach (authors personal observations). However, recruitment success may again depend on weather patterns as observed in this study (i.e. a calm weather window) (Chapter 3).

Irrespective of the disturbance in question, it is likely that species interactions may change by increasing environmental change at large and local scales. Often, this benefits the most tolerant and opportunistic species. *C. filiformis* has both of those traits.



### **6.5.2 Management suggestions**

If *C. filiformis* behaves as a passenger of change, relying on disturbance to otherwise competitively superior species, then its future distribution and abundance are likely to depend on the condition of algal communities along the NSW coast. Sediment and nutrient runoff from terrestrial sources may in part be mitigated by increasing water quality before release to the ocean and improvement of sustainable methods of land fertilization and reduction of soil erosion (Bouwman et al., 2005, Syvitski et al., 2005). However, climate related changes in weather patterns, storminess and sediment movement cannot be tackled at local scales (Raupach et al., 2012). Species resilience to large scale disturbances such as climate depends on the health of local ecosystems (Bertocci et al., 2015), arguing for increasing importance of local scale management (see e.g. Levin and Lubchenco, 2008) to improve ecosystem health under increasing global pressures.

### **6.5.3 What is known & recommendations for further study**

The following table outlines the major publications and the answers they provided regarding *C. filiformis* spread.

Table 6. 1. Overview of publications and major findings regarding the spread of *Caulerpa filiformis* to date.

(Khou, 2007)	<i>C. filiformis</i> fragments found in the field consist of variable morphologies (from small fronds to entire thalli) and sizes (0.1–60 cm in length). All fragments of <i>C. filiformis</i> are viable propagules. Growth rates of fragments differ with different sizes. Attachment force is related to the number of rhizoid clusters.
(Zhang, 2014)	Horizontal spread of <i>C. filiformis</i> is higher than expansion by <i>Sargassum</i> spp. in cleared plots on common boundaries. Recruitment of other species is reduced in cleared plots within <i>C. filiformis</i> ' beds. <i>C. filiformis</i> has a very high recruitment into cleared plots within its on beds and within beds of <i>Sargassum</i> spp. Photosynthetic fitness of <i>Sargassum</i> spp. was reduced when neighbouring to <i>C. filiformis</i> .
(Glasby, 2015)	Anecdotal evidence for the spread of <i>C. filiformis</i> within its historical distribution (Wollongong ~ Newcastle region). Evidence for a potentially novel population 500 km North of its historical distribution. No obvious association found between population size and temperature rise, nutrient availability and anthropogenic density. Association found between population distribution and Reef: Beach index and <i>C. filiformis</i> occurrence with reef width
(Lanham, 2015)	<i>C. filiformis</i> associated macrofauna is different from that associated to neighbouring <i>Sargassum</i> spp., with a potential spill over effect to neighbouring habitat.

The following sections outline major research gaps and recommendations for future study are made:

### ***1) Sediment movement at local scales and its impacts***

Although changes in SST and nutrient pollution are relatively well studied, the influence of climate-related change related storminess on sediment movement has received much less attention (Hemer et al., 2010, Halpern et al., 2007, Worm and Lenihan, 2013, Wernberg et al., 2011a). How sediment movement may change in the

future under continuing climatic change and increasing coastal development remains largely unknown, and altered sediment movement may be hard to predict at a local scale (Hemer et al., 2010). Increased knowledge on sedimentation patterns at local scales, and how they may change over time, will be highly valuable to understand past and predict future change of rocky reef communities. Moreover, with increased understanding on current and future sediment movement and species responses, models could be built to predict future change in seaweed assemblages (see e.g. Shackelford et al., 2013).

Additionally, the impacts of altered sediment movement, especially associated with deep water sources, have not received much attention. In a review on the impact of climate change on temperate marine communities in Australia, Wernberg et al. (2011a) argued that the lack of observed impact is likely due to a lack of data, rather than the absence of change happening. The continued study of changes in abundance of indicator species, such as *C. filiformis*, will aid in the understanding of altered sedimentation regimes. This study has provided the most comprehensive baseline of *C. filiformis* distribution and abundance in NSW and can be used to assess future changes that might occur in response to changing climatic conditions or specific anthropogenic disturbances.

## ***2) Impacts of sediment on C. filiformis and competitors***

Although there is a clear association with sediment movement in the distribution and abundance of *C. filiformis*, the role of sediment in benefitting *C. filiformis* abundance

still has to be demonstrated. No direct benefit of sediment on *C. filiformis* growth was found in this study. However, there are other potential effects of sediment presence, not tested for in this thesis, that may benefit the species. For example, sediment may deter grazers such as urchins and may help anchoring stolons (see e.g. Bulleri et al., 2009). Both hypotheses require further experimental work.

Additionally, in this study inferences were made about *C. filiformis*' competitive dominance under increased sedimentation disturbance due to its high tolerance of smothering. While the literature is clear on the negative effects of sediment on many algal species, as also referred to in this thesis, no actual tests were performed on how competitive outcomes between *C. filiformis* and co-occurring algae may change under increased sedimentation rates. This important avenue of further study will benefit from experimental work under field conditions where the role of multiple effects of sedimentation (e.g. direct and indirect effects) can be tested.

### ***3) Passenger vs driver of environmental change***

Experimental work is needed to fully tease apart the biotic relationships between *C. filiformis* and competing algae observed (MacDougall and Turkington, 2005, Didham et al., 2005). Following the framework suggested by MacDougall and Turkington (2005), understanding the inhibiting traits of other species (i.e. passenger model) will benefit from experimental tests by removing competitors and investigating altered spread rates (see for example Ceccherelli et al., 2014). Ideally, competing species fitness and

invader-recipient community interactions should be investigated under increased sediment disturbance (e.g. Piazzì et al., 2005). Additionally, the potential impacts of *C. filiformis* (i.e. driver model) should be further investigated by removing the invader and testing for community responses. I.e. a “recovery” to similar to uninvaded sites would indicate a driver model, and potential legacies of its invasion (e.g. sediment layer) should be removed as well (e.g. Bulleri et al., 2010).

#### ***4) Alternative mechanisms that can initiate native invasion***

Carey et al. (2012) identified several other anthropogenic impacts that can initiate native species to become invasive that have not been investigated in this study. For example, a change in grazing pressure can result in a native species to become to dominate communities. Grazing pressure along the NSW coastline has been changing with warming waters and tropical fishes moving southwards, altering grazing patterns on macroalgal communities (Vergés et al., 2014). This may negatively affect competing alga or reduce grazing on *C. filiformis*, and will be an interesting avenue of further study. Carey et al. (2012) noted the increasing influence of climate change on species composition. SST rise can affect seaweed fitness in multiple ways and has already shown retreat of several seaweed species along the NSW coastline (for a review see Wernberg et al., 2011a). This trend is predicted to continue to alter biotic relationships (Wernberg et al., 2011b, Bennett et al., 2015). However, how changing temperatures will affect *C. filiformis*' fitness remains to be determined.

### **5) *Hard data on C. filiformis increased abundance***

Although there is ample anecdotal evidence on the increased abundance of *C. filiformis* over the past decades (May, 1976, Glasby et al., 2015), hard data is missing to support this. Old field surveys that cover areas now dominated by *C. filiformis* have not been found yet. A contributing factor may be *C. filiformis* association to very exposed rocky reefs often not chosen for field studies. However, a lack of hard data does not mean no change has occurred. The lack of baseline data will remain a challenge for many changing habitats around the world, especially in the often-invisible marine environment (Pauly, 1995). This often asks for more creative ways to demonstrate a change has occurred, for example with the use of anecdotal evidence (e.g. Chapter 2) or the use of data on indicator species (e.g. Voerman et al., 2013).

### **6) *Linkage between C. filiformis populations***

State-of-art population genetics would contribute to the understanding on how current populations are inter-related, and to test if the proposed novel population 500km North of its main distribution (Chapter 2) is a relict or a new introduction.

*“Plant success in the world today is less a function of geography of origin but more basically, one of which species have the good fortune to have the suite of traits that will enable them to exploit the increasingly disturbed and eutrophic 21st-century landscape” (Davis et al., 2011)*





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