

**Hybridisation in the brown alga *Carpophyllum*:
Investigating morphology, distribution and wave exposure**

By

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“There are known knowns. There are things we know that we know. There are known unknowns. That is to say, there are things that we now know we don’t know. But there are also unknown unknowns. There are things we do not know we don’t know.”

- Donald Rumsfeld

Abstract

Hybridisation can result in new hybrid lineages, parental species extinctions, the transfer of adaptations, or the merging of parental lineages. Subsequently hybridisation has important implications for the species involved. Hybridisation has recently been confirmed between the Fucalean brown algae *Carpophyllum angustifolium* and *Carpophyllum maschalocarpum* using the ITS2 marker. This study conducted a detailed morphometric analysis combined with molecular data to investigate morphology distribution and exposure at two sites on the East Cape. Hybridisation was also morphologically investigated at Leigh, where the previous work had been unable to resolve hybrids using the ITS2 marker.

Carpophyllum angustifolium, *C. maschalocarpum* and their hybrids had distinct and intermediate morphologies, and could be identified by stipe width alone. Individuals with hybrid genotypes with distinctive *C. angustifolium* morphotypes were also found, which suggests asymmetrical introgression is occurring. Some aspects of *C. angustifolium* and *C. maschalocarpum* morphology were found to be correlated with wave exposure. In the more exposed zones *C. angustifolium* individuals were longer, while *C. maschalocarpum* individuals were shorter, had thinner stipes and less frequent vesicle presence. There were also non-significant trends of *C. maschalocarpum* individuals having thinner lamina, and lower branch presence in higher wave exposures. The distributions of *C. angustifolium*, *C. maschalocarpum* and their hybrids were found to be correlated with exposure. *Carpophyllum angustifolium* was distributed only in the relatively exposed zones, while *C. maschalocarpum* was distributed mainly in the more sheltered zones. Hybrids were distributed in intermediate exposure zones where both parental species were present.

The hybrid distributions could be a reflection of environmental selection or of the parental contact zone. Morphological evidence was found for hybridisation at Leigh, although there were differences between the morphologies of East Cape and Leigh clusters of *C. angustifolium* and hybrids. These differences could be due to environmental differences, genetic differentiation or different levels of introgression between the two locations. The general findings in this study support the existing literature on hybridisation, which mainly comes from terrestrial plant and animal species complexes.

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Chapter 1: General Introduction

Hybridisation occurs when two species crossbreed to produce offspring. It is a common and important evolutionary process. Hybrids have been identified in a broad range of ecosystems and species, from irises in Louisiana (Emms & Arnold, 1997) to Cichlid fish in Lake Victoria (Crapon de Caprona & Fritzsch, 1984). It is estimated that 25% of plant species, and 10% of animal species are currently hybridising in the wild (Mallet, 2005).

Biodiversity can be significantly affected by hybridisation (Seehausen, 2004). Hybridisation can result in new hybrid lineages, parental species extinctions, or the merging of parental lineages. Introgression, the backcrossing of hybrids with parental species, can occur and can be common (Rieseberg, 1998). Introgression can affect parental genetic diversity by transferring genetic adaptations between parent species (Seehausen, 2004). Consequently hybridisation has important implications for the conservation and management of biodiversity. In New Zealand hybridisation and introgression affect the genetic integrity and conservation of the black stilt, *Himantopus novaezelandiae* (Greene, 1999), and the hen and chicken fern, *Asplenium bulbiferum*, (Perrie *et al.*, 2005). Hybridisation and introgression have also been hypothesised to stimulate invasiveness in exotic species (Ellstrand & Schierenbeck, 2000).

Hybridisation is interesting to study for a variety of reasons. Taxonomically it challenges existing species concepts, definitions, and boundaries. It also offers opportunities to investigate evolution and speciation processes in the wild. Hypotheses surrounding fitness and environmental selection can be explored with the

novel hybrid phenotypes and genotypes (Campbell & Waser, 2001; Fritz, 1999; Johansen-Morris & Latta, 2008). The mix of parental genes within hybrids allows investigation of co-adapted gene complexes (Baack & Rieseberg, 2007; Landry *et al.*, 2007; Landry *et al.*, 2005), and cytoplasm–nuclear DNA interactions (Fishman & Willis, 2006; Levin, 2002; Sambatti *et al.*, 2008).

The development of molecular markers has allowed more rigorous identification of hybridisation and introgression than traditional morphometric techniques (Rieseberg, 1998). Molecular markers have revealed hybrids between highly plastic or cryptic taxa (e.g. in fungi Kauserud *et al.*, 2007), as well as hybrids with parental morphologies (Lihova *et al.*, 2007). Introgression can also be identified and quantified with molecular markers (Chen *et al.*, 2004; Coyer *et al.*, 2007; Hardig *et al.*, 2000; Wallace *et al.*, 2004). Molecular techniques use species level variation in nuclear, chloroplast, or mitochondrial DNA markers with the assumption that species specific markers from both parental species will be found in the hybrids (Hegarty & Hiscock, 2005). The consensus in the literature is that combining morphological and molecular data is optimal for investigating hybrid systems (Lihova *et al.*, 2007; Repplinger *et al.*, 2007; Tiebre *et al.*, 2007).

Research into most hybrid systems now begins with identifying and confirming hybridisation using morphological and molecular tools (e.g. Coyer *et al.*, 2002a). Following this the phenotypes of hybrids and parental species are documented to generate hypotheses about the distribution and results of hybridised genomes (Thorsson *et al.*, 2007). A practical outcome of this documentation of phenotypes is the development of morphological traits for field identification. Hybrids usually have

intermediate morphologies to the parental species which reflect their mixed genotype (Coyer *et al.*, 2002a; Estabrook *et al.*, 1996; Wilson, 1992). However, the morphology of hybrids can be similar to, or even more extreme than, the parental species (Coyer *et al.*, 2002a; Rieseberg & Ellstrand, 1993; Seehausen, 2004). Morphologically distinguishing hybrids from parental species can be difficult due to intra-specific variation and phenotypic plasticity (Mathieson *et al.*, 1981; Rieseberg & Ellstrand, 1993; Scott & Hardy, 1994). Documenting the morphology of hybrids and parental species can be further complicated by introgression, which can result in a continuum of morphologies between the parental species (Albert *et al.*, 1997).

The distribution of hybrids is particularly intriguing. Hybrids tend to have unusual zoned distributions (Rieseberg, 1998). Hybrids are often found in intermediate environments from the parental species, but can also occur in marginal or novel environments (Arnold, 1997; Coyer *et al.*, 2006; Scott & Hardy, 1994). These distributions have been explained by a range of models that each place differing importance on the relative roles of endogenous hybrid fitness, environmentally dependent fitness, and dispersal limitations. These include the evolutionary novelty model (Arnold, 1997), the tension zone model (Barton & Hewett, 1985), the mosaic hybrid zone model (Harrison, 1986), and the bounded hybrid superiority model (Moore, 1977). Determining patterns of hybrid distribution relative to environmental parameters is an important first step to establishing the role of environmental selection (Rieseberg, 1998). There is generally support for the distribution of hybrids being environmentally dependent (Fritsche & Kaltz, 2000; Harrison, 1993; Wang *et al.*, 1997). This is a reflection of the relative fitness of hybrids in certain environments (Arnold & Hodges, 1995; Fritsche & Kaltz, 2000; Wang *et al.*, 1997).

Hybridisation research has disproportionately focused on vascular plants and animals. A recent literature review on hybridisation investigated plants and animals only (Mallet, 2005). The vast majority of research into hybridisation is done in terrestrial habitats (Gardner, 1997). Despite this the level of hybridisation in marine taxa is thought to be equivalent to terrestrial taxa (Gardner, 1997). Hybridisation has been identified in a range of marine taxa including mussels, and algae (Coyer *et al.*, 2007; Schneider *et al.*, 2005). Hybridisation occurs in the brown macro-algae, phylum Phaeophyta, kingdom Chromista (e.g. Coyer *et al.*, 2002a; Lewis & Neushul, 1995; Wallace *et al.*, 2004). Investigations of hybridisation in Phaeophyceae can provide insights from a taxonomically and ecologically distinct study system.

Phaeophyceae provide exciting, but challenging, study organisms for the investigation of hybrid morphology and distribution. Phaeophyceae are extremely plastic, which can make hybridisation morphologically difficult to identify (Scott & Hardy, 1994). Phaeophyceae are the dominant primary producers in temperate intertidal and subtidal ecosystems, and are often important ecological components of their local habitats (Graham & Wilcox, 2000). Intertidal and subtidal habitats are interesting to study environmental selection in as they have large environmental variation over small spatial scales (Nybakken & Bertness, 2004).

Exposure to water motion has been found to have particularly significant effects on the distribution and morphology of local macroalgae biota in the shallow subtidal (Engelen *et al.*, 2005; Johnson & Koehl, 1994; Tuya & Haroun, 2006). Water motion around macroalgae affects nutrient acquisition and creates mechanical fatigue (Hurd,

2000; Mach *et al.*, 2007). It can also result in the loss of biomass or dislodgement of entire individuals, which creates selection pressures (Hurd, 2000; Kawamata, 2001). The effects of wave exposure on different morphologies has been relatively well studied; flexible, streamlined and strong macroalgae fare best in high wave exposures (Puijalon & Bornette, 2004; Stewart, 2006; Thomsen *et al.*, 2004; Wernberg & Thomsen, 2005). Consequently interesting comparisons can be made on the effects of wave exposure between novel hybrid and parental phenotypes in Phaeophyceae.

The vast majority of work investigating hybridisation within Phaeophyceae has been done in the order Fucales (Billard *et al.*, 2005; Coyer *et al.*, 2002a; Lewis, 1996; Wallace *et al.*, 2004). Hybridisation between *Fucus* species has been observed and investigated morphologically since the 1850s (Thuret, 1854). Fucalean algae are useful organisms to study hybridisation in for a number of reasons. Firstly they are abundant and often ecologically dominant in easily accessible intertidal and high subtidal habitats (Graham & Wilcox, 2000). Secondly there is a good baseline of information on their life history, reproduction, and ecology (e.g. see review by Chapman, 1995). Thirdly Fucales have a diplontic life cycle with only one free living multicellular phase (Chapman, 1995). This simple life cycle makes considering fitness and the effects of selection much easier. Dispersal capacity in Fucales is limited, due to their large non-motile eggs, allowing study into hybridisation to occur at small spatial scales (Chapman, 1995; Schiel, 1980). Almost all of the study into hybridisation in Fucalean algae has been on the intertidal genus *Fucus* in the Northern Hemisphere (e.g. Billard *et al.*, 2005; Coyer *et al.*, 2002a; Wallace *et al.*, 2004). The dominance of Fucalean algae in the high subtidal is considered one of the

unusual aspects of New Zealand's algal flora; this provides a novel Fucalean study system (Schiel, 1990).

Carpophyllum angustifolium J. Agardh and *Carpophyllum maschalocarpum* Turner (Sargassaceae) are two Fucalean species which dominate high subtidal habitats in New Zealand (Schiel, 1990). The two species have different morphologies. *Carpophyllum angustifolium* is streamlined and flexible with strong aggregating holdfasts. *Carpophyllum maschalocarpum* is much less streamlined with large laminae, wide flat stipes, and often bearing vesicles (Adams, 1994; Dromgoole, 1965; Lindauer *et al.*, 1961). The parental species distribution are thought to be structured by wave exposure with *C. angustifolium* in more exposed areas, and *C. maschalocarpum* in more sheltered areas (Adams, 1994; Dromgoole, 1965; Lindauer *et al.*, 1961). Individuals with morphology intermediate to *C. angustifolium* and *C. maschalocarpum* have been observed since at least the early 1960s (Lindauer *et al.*, 1961). These individuals were long hypothesised to be hybrids (Dromgoole, 1973; Lindauer *et al.*, 1961), however no work was done to verify this hypothesis until recently (Buchanan, unpubl. data).

Recent molecular work confirmed several of these individuals with intermediate morphologies to be hybrids (Buchanan, unpubl. data). The internal transcribed spacer (ITS) sequence used to confirm the putative hybrids is one of the most widely used sequences in plant evolutionary studies (Feliner & Rossello, 2007). ITS is a biparentally inherited nuclear DNA marker; hybrids are the ITS heterozygotes where parental species have distinct ITS sequences (Coyer *et al.*, 2002b). *Carpophyllum angustifolium* and *C. maschalocarpum* in the East Cape populations each have a

single distinct ITS2 ribotype, which allows ITS2 based detection of hybrids (Buchanan, unpubl. data). However multiple ITS2 ribotypes were found in more northern *C. angustifolium* and *C. maschalocarpum* populations – some of which were shared (Buchanan, unpubl.). Consequently ITS2 alone cannot resolve hybrids in northern populations.

The presence of shared ribotypes in northern populations could be due to introgression. Vesicles, conceptacles, eggs and sperm have been found on *C. angustifolium* × *C. maschalocarpum* hybrids which suggests they could be fertile (Buchanan & Hodge, unpubl. data). These potentially fertile hybrids were found when both parental species were also fertile, which suggests the potential for introgression.

The aim of this thesis is to combine morphological and molecular tools to investigate *Carpophyllum angustifolium*, *Carpophyllum maschalocarpum*, and in particular their hybrids. In Chapter 2 the morphological differences between genetically identified *C. angustifolium*, *C. maschalocarpum*, and their hybrids will be explored. Are the three groups morphologically distinct? Do the hybrids have intermediate morphology? Is there evidence of introgression? Chapter 3 will investigate whether the morphology of *C. angustifolium* and *C. maschalocarpum* vary by wave exposure. Does the morphology of *C. angustifolium* and *C. maschalocarpum* individuals change in areas of different wave exposure? Is the relationship between wave exposure and morphology the same in both species? In Chapter 4 the distribution of parental species and hybrids with relation to each other and wave exposure will be analysed. Are the distributions of *C. angustifolium* and *C. maschalocarpum* correlated with

wave exposure? Do the hybrids occur in novel environments from the parental species? Are the hybrids in intermediate wave exposure environments relative to the parents? Finally in Chapter 5 *Carpophyllum* morphology will be compared between individuals from Leigh and the East Cape. Is hybridisation also occurring at Leigh? Is there evidence of extensive introgression?

It should be noted that the following chapters are written as independent scientific papers, and consequently there is some repetition of core ideas and methodology.

Chapter 2: Morphological differences between *Carpophyllum angustifolium* × *Carpophyllum maschalocarpum* hybrids and their parents

2.1 Abstract

Hybridisation is an important evolutionary process, which can have significant effects on biodiversity. Hybridisation tends to produce morphologies intermediate to the parent species. Most species of macroalgae in the class Phaeophyceae have highly plastic morphologies, which makes species identifications based on morphology difficult - particularly if hybridisation is occurring. Recent work using the molecular marker ITS2 has confirmed hybridisation between *Carpophyllum angustifolium* and *C. maschalocarpum* on the East Cape of New Zealand. In the present study, morphological differences were investigated between *C. angustifolium*, *C. maschalocarpum* and their hybrids from East Cape populations. It was found that the hybrids had an intermediate morphology to the parent species. Stipe width clearly differentiated hybrids from the parent species and was the best single character for identification. Some individuals with *C. angustifolium* morphology had hybrid ITS2 ribotypes suggesting backcrossing between the hybrids and *C. angustifolium*.

2.2 Introduction

Hybridisation is an important evolutionary process that brings together diverged genetic lineages, through the crossing of two genetically distinct species. Hybridisation challenges our species definitions and provides insight into speciation

processes (Seehausen, 2004). It also produces unique hybrid morphologies. Hybrids are generally expected to have intermediate morphologies reflecting their mixed genotype, but may also have morphologies similar to, novel from, or more extreme than the parental species (Coyer *et al.*, 2002a; Lihova *et al.*, 2007; Seehausen, 2004). Identification of hybrids based on morphology can be difficult due to the large degree of intra-specific variation and phenotypic plasticity present in many taxa (Rieseberg & Ellstrand, 1993; Scott & Hardy, 1994). Furthermore, morphological intermediacy does not always indicate hybrids and may reflect patterns of plasticity or environmental variation (Mathieson *et al.*, 1981).

Introgression, the backcrossing of hybrids with parental species, can result in a continuum of morphologies between the parental species (Albert *et al.*, 1997). Introgression has been described as the most common outcome of hybridisation (Rieseberg, 1998). Introgression is of great evolutionary significance as it can result in the transfer of adaptations between species (Seehausen, 2004). Other potential outcomes of introgression include the strengthening of reproductive barriers and further divergence of the parental species, or the development of new hybrid lineages (Grant *et al.*, 2004; Lihova *et al.*, 2007; Seehausen, 2004).

The Phaeophyceae, brown macroalgae, are a morphologically diverse group. Individuals range from microscopic filaments to the more familiar large conspicuous fleshy seaweeds (Graham & Wilcox, 2000; Phillips, 2007). Even within species there is great morphological variation, due to phenotypic plasticity and environmental selection (Mathieson *et al.*, 1981). Despite this variability, putative Fucalean hybrids have been observed in wild populations since the 1850s (and references within

Mathieson *et al.*, 1981; Thuret, 1854). More recently genetic methods have been used to confirm and identify hybrids (Coyer *et al.*, 2007; Wallace *et al.*, 2004).

New Zealand has a distinct brown algal flora, with high levels of endemism (Nelson, 1994). The dominance of Fucalean algae in the shallow subtidal is considered one of the more unusual aspects of the flora (Schiel, 1990). Fucalean algae in the Northern Hemisphere dominate the intertidal, and subsequently have been relatively well studied (Chapman, 1995). The vast majority of research into hybridisation in Phaeophyceae is on the Northern Hemisphere Fucalean genus *Fucus* (Billard *et al.*, 2005b; Chapman, 1995; Coyer *et al.*, 2006b; Mathieson *et al.*, 1981; Wallace *et al.*, 2004). Research into hybridisation has revealed hybrids with intermediate morphologies, and in some cases evidence of introgression has been reported (Billard *et al.*, 2005b; Coyer *et al.*, 2002a; Mathieson *et al.*, 2006; Wallace *et al.*, 2004). Little work has been done on hybridisation in Fucales outside of *Fucus*, despite the well documented abundance, diversity and ecological importance of other genera in the Southern Hemisphere (Clayton, 1984).

In north-eastern New Zealand two species of Fucales, Fam. Sargassaceae, form dominant stands in the high subtidal: *Carpophyllum maschalocarpum* Turner and *Carpophyllum angustifolium* J. Agardh (Dromgoole, 1973; Schiel, 1988; Schiel, 1990). Based on intermediate morphotypes it has been hypothesised that hybridisation is occurring between the two species (Dromgoole, 1973). Recent molecular work using the internal transcribed spacer 2 (ITS2) region supported this hypothesis (Buchanan, unpubl. data). The ITS2 sequence is part of the ribosomal region in the nuclear DNA, and it is one of the most widely used sequences in plant

evolutionary studies (Feliner & Rossello, 2007). ITS2 is a bi-parentally inherited marker, which is distinct between *C. angustifolium* and *C. maschalocarpum* in the East Cape populations of New Zealand (Buchanan, unpubl. data). Coyer *et al.* (2002b) showed that artificially produced F₁ hybrids of two *Fucus* species with distinct ITS ribotypes, were all heterozygous in ITS. The work on *Carpophyllum* by Buchanan (unpubl. data) found all putative hybrids, based on cursory identification of morphology, to be ITS2 heterozygotes.

ITS has low functional constraint, and highly constrained flanking genes which allow universal primers to be produced (Alvarez & Wendel, 2003). ITS is multi-copy, and homogenisation of the multiple ITS sequences can occur, meaning that only recent hybridisation is revealed (Alvarez & Wendel, 2003). Introgressed and F₂ individuals will not necessarily have heterozygous ITS2 ribotypes. Combining ITS and morphometric data could allow non-subjective identification of individuals, and help determine the presence of introgression and F₂ hybrids within a population.

This study aims to combine ITS2 identification and morphometric analysis to investigate the morphology of *C. angustifolium*, *C. maschalocarpum*, and their hybrids. Three questions will be addressed. Firstly are the parental species morphologically distinct? Secondly can hybrids be morphologically distinguished from the parental species? Finally are the hybrids morphologically intermediate to the parent species?

2.3 Methods

Sampling locations

Samples were collected from two sites on the East Cape of the North Island of New Zealand, where *C. angustifolium* × *C. maschalocarpum* hybrids were known to occur (Fig.1). The two sites are both within the greater Whanarua Bay, which faces north-west and contains a number of smaller sandy bays separated by large rocky islands. The first site is located at the south-eastern end of Maraehako Bay (S37° 40.4' E177° 47.8'). The second site was located at Uncles Bay (S37° 40.0' E177° 48.0'), which is the adjacent bay to the north-east. The two sites are approximately 500m apart, and are separated by Motu Kaimeanui Island and two deep inlets. Sampling was conducted during the 2007-2008 summer.

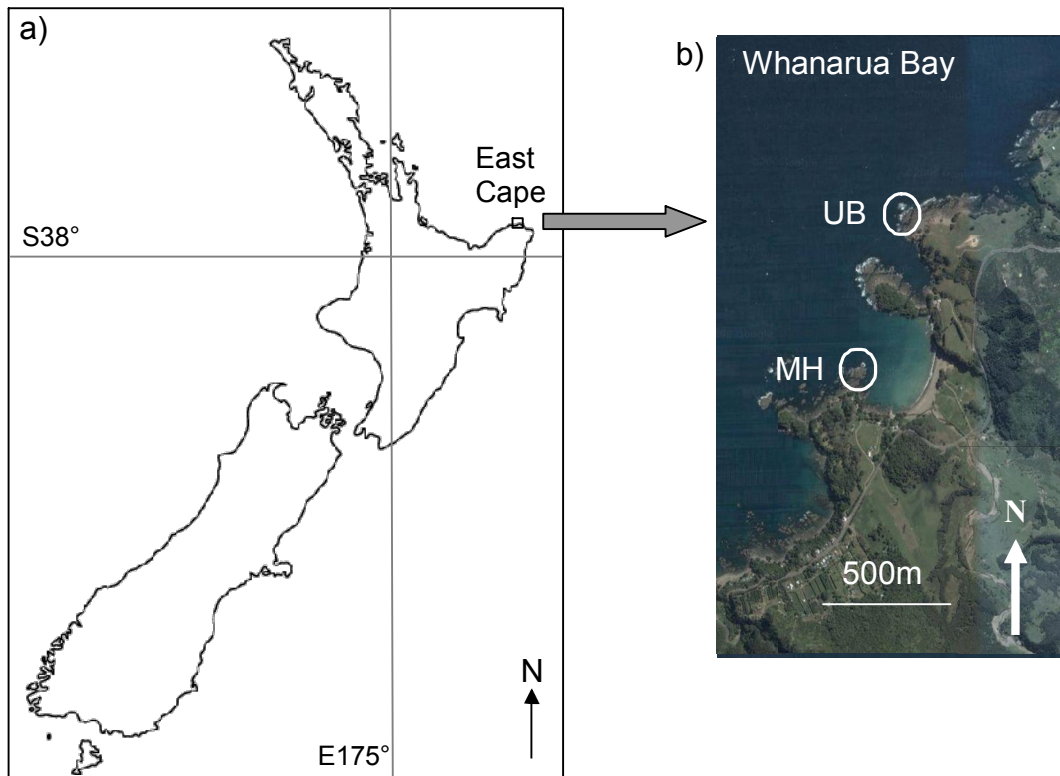


Figure 1: The location of sampling a) on the East Cape of the North Island of New Zealand, and b) the two sites in the greater Whanarua Bay: Maraehako Bay (MH) and Uncles Bay (UB) (Google Earth 5.0).

Sampling

Transect lines were laid along rock walls in the high subtidal, and quadrat locations were selected randomly from the top 0.5 m of *Carpophyllum* depth distribution. Quadrats were rejected if the rock face was not vertical, fewer than three individuals were present, or if the quadrat was less than 0.5 m from a previously sampled quadrat. All *Carpophyllum* individuals with holdfasts inside the 20 cm by 20 cm quadrats were collected. Sixty-nine quadrats were sampled in the two sites, with 280 *C. angustifolium*, 152 *C. maschalocarpum* and 32 hybrid adults collected in total. Collected material was frozen at -4°C within a week of collection, and thawed in seawater prior to morphometric analysis.

Morphometrics

Adults greater than 20 cm were morphologically identified and cleared of epiphytes, and measured using digital callipers. Only those individuals with complete haptera were used in morphometrics so as to avoid pseudo-replication (i.e. the doubling up of measurements from the same individual). Individuals were measured using digital callipers and a metre rule. A small section of lamina was removed from each individual after processing and placed into a labelled centrifuge tube with silica gel for later genetic analysis.

The morphometric characteristics selected for measurement were stipe width and thickness; and lamina length, width and thickness (Fig. 2). This study focused on the stipe and lamina characteristics of the primary axis (defined as the dominant axis which arises directly from the holdfast). The stipe and lamina measurements were taken from approximately 5, 10, 15, and 20 cm from the tip of the axis. Lamina length was measured from the edge of the stipe where the lamina forms to its tip.

Lamina and stipe width were measured at the widest point, perpendicular to an imagined mid-line. Stipe and lamina thickness was measured at the thickest point. In some places herbivory had altered the morphology of the stipe and lamina; these could be detected by sudden and distinct changes in lamina or stipe outline. Measurements of stipe and lamina that were affected by herbivory were excluded, and if possible replaced by measurements from the nearest intact equivalent within 3 cm. The total length of the individual, presence of vesicles longer than 8 mm, and presence of secondary branches longer than 20 cm were also recorded.

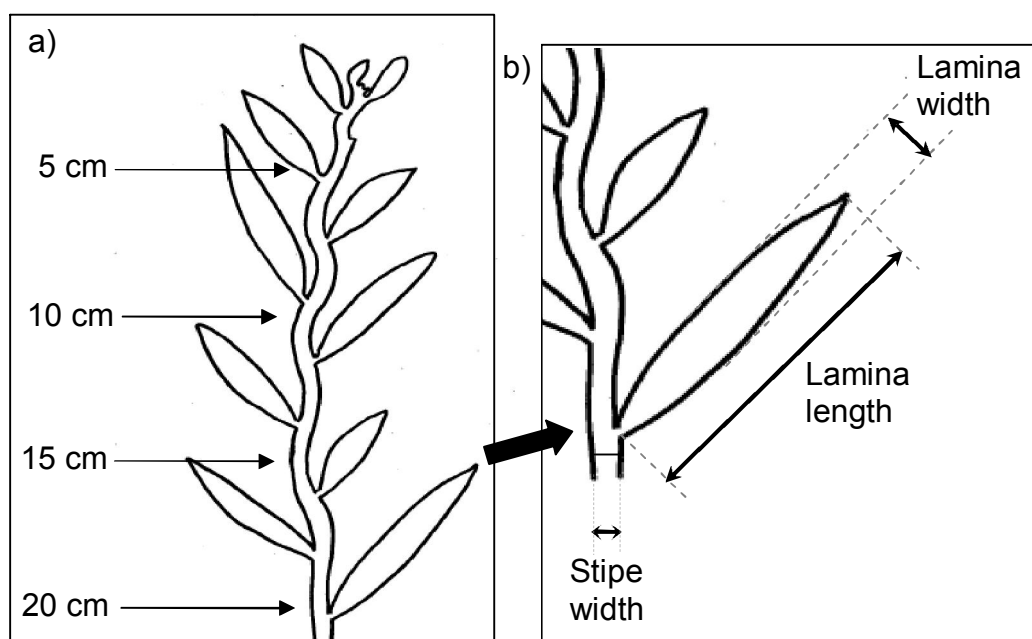


Figure 2: The location of the measurements taken from each individual are shown with arrows on a stylised axis. Stipe width, lamina length and width are illustrated at 20 cm from the apex.

Digital images for the *C. angustifolium* (#2572, Botanical Museum Lund) and *C. maschalocarpum* (#000562635, The Natural History Museum, London Department of Botany) type specimens were obtained and measurements of lamina length, lamina width and stipe width were taken where possible.

Genetic Analysis

Validity of the morphological identification for the hybrids and parental species were checked by genetically identifying a randomly selected sub-sample. Individuals with unusual morphological characteristics were also checked genetically.

DNA was extracted from approximately 3 mg of dry tissue using a CTAB extraction protocol (Zuccarello & Lokhorst, 2005). The tissue was ground in 500 μ L of CTAB extraction buffer (2% CTAB, 0.1 M Tris-HCl, 1.4M NaCl, 20 mM EDTA, 1% PEG 8000) with 2 μ L RNAase A (100mg/mL).

The ITS2 sequences were amplified using a PTC-100 (Programmable Thermal Controller, MJ Research Inc) PCR machine, and the primers KG4 (CTTTTCCTCCGCTTAGTTA TATG) and KP5 (ACAACGATGAAGAACGCAG) (Invitrogen). The PCR conditions were an initial 94°C for 4 minutes, followed by 5 cycles of [1 minute at 94°; 1 minute 59°; 1 minute 72°] with the annealing temperature decreased by 1°C each cycle. Then 30 cycles of [45 seconds 94°C; 45 seconds 53°C; 45 seconds 72°C] followed by ten minutes at 72°C. Sequencing was done commercially (Macrogen Inc, Korea). The chromatograms were visually examined and individuals identified as either a parental species or a heterozygous hybrid (where double peaks were present).

Morphometric Data Analysis

The morphometric data contained a large number of missing values. Scatterplots showed no difference between the character traits measurements at 5, 10, 15 and 20 cm down the axis, so the measurements on each individual were averaged. There

were some missing data for lamina characteristics after this averaging, as many laminae were excluded from measurement due to herbivory damage. The missing values were not randomly distributed, and *C. maschalocarpum* averages were based on fewer data points than the others due to greater levels of herbivory and exposure damage. Randomly removing data points to an equivalent level in *C. angustifolium* and hybrid data made little or no change to overall group averages (0.002% to 0.03% change). This suggests no bias in accuracy due to variation in the number of data points averaged between the species.

Cluster analysis finds natural groupings or clusters within the data (Fielding, 2007). These were conducted first with genetically confirmed data and type specimens, and then with all individuals, using the software NTSYSpc version 2.11 software (Rohlf, 2000). The unweighted pair-groups method average (UPGMA) clustering method was used (Fielding, 2007). Boxplots were used to identify morphological traits which showed divergence. These diverged characters were incorporated into a distance matrix using Gower's Index in Le Proiciel R 4.0 software (Casgrain & Legendre, 1999). This index was used as it can cope with missing values, count data and continuous data (Montanari & Mignani, 1994; Podani, 1999). It has also been used in other studies on macroalgae morphology (Fowler-Walker *et al.*, 2006). Cophenetic values were calculated to test how well the cluster tree represents the data (Rohlf, 2000; Rohlf & Sokal, 1981).

ANOVA was used to compare the morphological groups found in the cluster analysis. Non-normal data was log transformed prior to analysis with ANOVA. Log transformed data that still did not meet ANOVA assumptions was analysed using the

non-parametric Kruskal-Wallis test. Chi-squared tests were used to test for associations between wave exposure zones and the presence of vesicles and branches. Significance was determined at the 0.05 probability level.

2.4 Results

ITS2 sequences and morphological identification

Morphological identifications matched with genetic sequences in 93.8% of a random sub-sample of individuals (n=80). All *C. maschalocarpum* (n=25) and hybrid (n=27) morphological assignments were supported by the ITS2 sequences. 82.1% of the random sub-sample (n=28) of morphologically identified *C. angustifolium* had matching *C. angustifolium* ITS sequences. The remaining 17.9% were five individuals with hybrid sequences; these will be described from here on in as ‘putative backcrosses’.

Morphology of clusters

Stipe width, lamina length and lamina width were identified as divergent morphological traits with boxplots and used in the cluster analyses. The first cluster analysis was of the random sub-sample of genetically identified individuals and the type specimens. This had a cophenetic correlation of 0.84, indicating a ‘good fit’ (Rohlf, 2000). This cluster tree is shown in Figure 2.

Genetically identified individuals clustered into four main morphological groups. Group I contained all of the genetically confirmed *C. angustifolium* (n=23), two hybrids, and the putative backcrosses (n=5). Group II included all of the other hybrids (n=25), and a *C. maschalocarpum* individual. Closer examination of this *C. maschalocarpum* individual reveals it was only just long enough to be considered

‘adult’ (>20 cm long) and therefore included in this study. Groups III and IV were composed of *C. maschalocarpum* only (III n=19, IV n=6). Hereafter Group I will be referred to as *C. angustifolium*, Group II as the hybrids, Groups III and IV combined as *C. maschalocarpum*. A major subdivision between the morphology of *C. angustifolium* and hybrid individuals, and the *C. maschalocarpum* individuals, was revealed in the cluster analysis. The hybrid cluster had a higher similarity coefficient with the *C. angustifolium* cluster (0.75) than with the *C. maschalocarpum* clusters (0.49).

The second cluster analysis was of all morphologically identified individuals, including the genetically identified individuals and type specimens. This had a cophenetic correlation of greater than 0.90, indicating the cluster tree is a ‘very good fit’ of the data (Rohlf, 2000). This second cluster tree had the same group structure and membership of genetically identified individuals as the cluster tree of only genetically identified individuals (see Appendix 1).

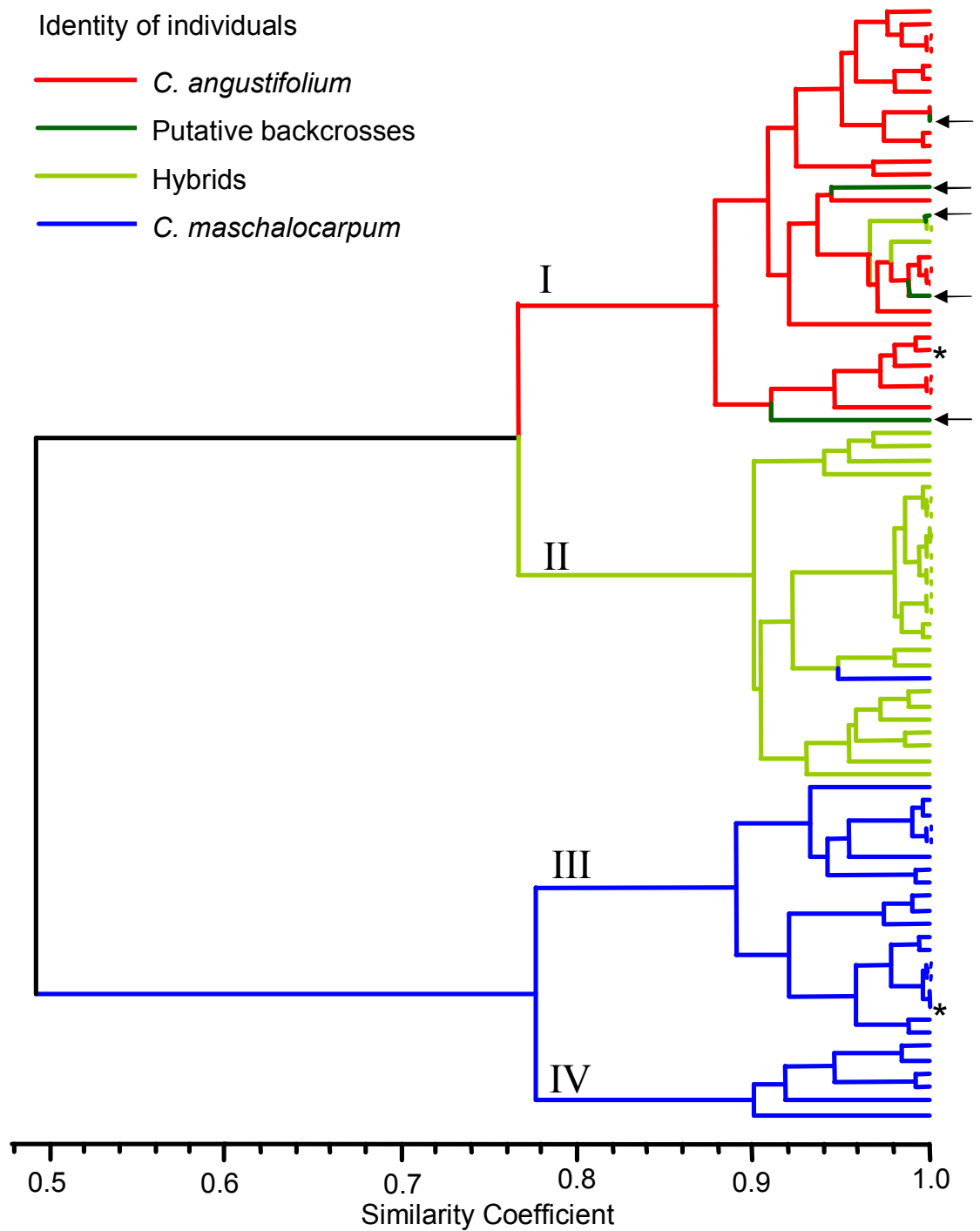


Figure 3: Cluster analysis showing the morphology of a random sub-sample of genetically identified individuals, and the type specimens for the parental species (shown by asterisks). Individuals cluster into four morphological groups as labelled I to IV. Colours represent the identity of the individuals (see legend). Putative backcrosses are those morphologically identified as *C. angustifolium* but with hybrid ITS sequences, as shown in with arrows. The coenphetic value of this tree is $r=0.84486$.

Comparing morphologies

Comparisons of the morphologies of the *C. angustifolium* group (I), the hybrid group (II) and the pooled *C. maschalocarpum* groups (III & IV) found a range of differences between them (Table 1). Log stipe width ($p<0.001$), lamina length ($p<0.001$), and lamina width ($p<0.001$) were different between the three groups (all pairwise Tukey Tests were $p<0.05$) (Fig. 3). The pooled *C. maschalocarpum* group had a wider stipe, and a shorter and wider lamina than the *C. angustifolium* group. The hybrid group exhibited traits intermediate to the parents for each of these traits. There was no overlap in logged stipe width for the three groups (Fig. 3a).

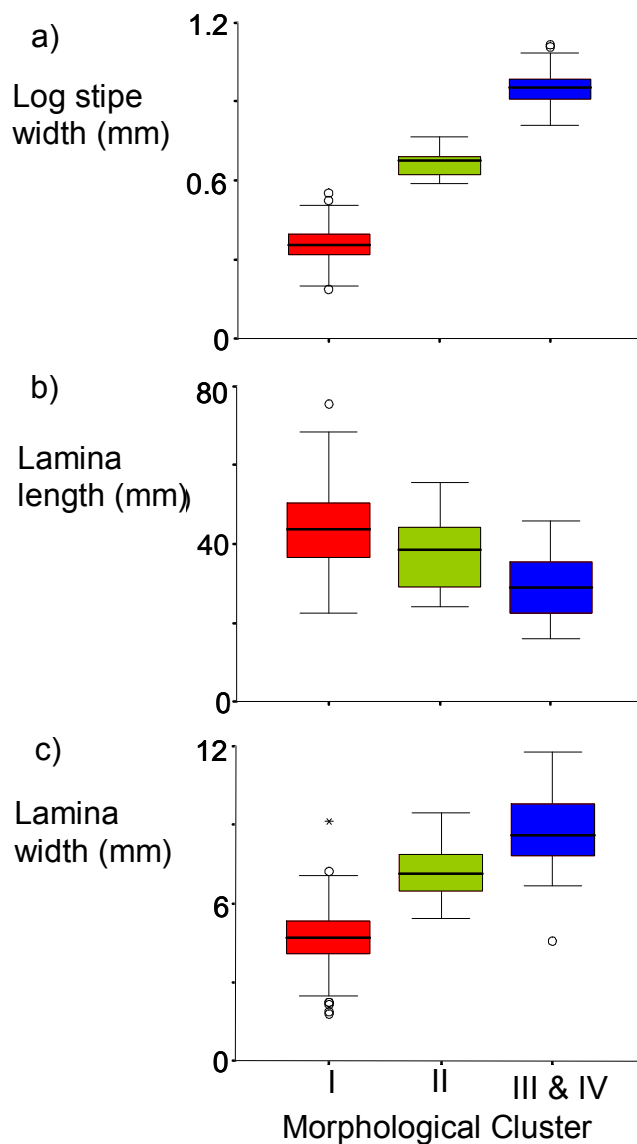


Figure 4: The three groups had significant different a) log stipe widths, b) lamina lengths and c) lamina widths. The boxplots show the spread of data with outliers marked by circles and extreme outliers marked with asterisks. Colours represent the general composition of groups (red for *C. angustifolium*, green for hybrids, and blue for *C. maschalocarpum*).

Stipe thickness was also different between the three groups ($p < 0.001$) with median stipe thickness being thickest for the *C. angustifolium* group (I), and thinnest for the hybrid group (II). Lamina thickness was also found to be non-uniform ($p < 0.00$). The *C. angustifolium* group (I) had significantly thicker lamina than the hybrids (II), and the pooled *C. maschalocarpum* group (III & IV) (Tukey tests: $p < 0.05$). The lamina thickness of the hybrid group (II) and the pooled *C. maschalocarpum* group (III & IV) were not significantly different (Tukey test: $p = 0.952$).

There were differences between the groups in the proportion of individuals with secondary axes ($X^2 = 13.529$, $df = 2$, $n = 461$, $p = 0.001$). The *C. angustifolium* group (I) had the lowest proportion of individuals with branches, while the hybrid group (II) and pooled *C. maschalocarpum* group (III & IV) had a similar proportion of individuals with secondary axes. The proportion of individuals with vesicles present also differed between the three groups ($p = 0.001$). No individuals in the *C. angustifolium* group (I) had vesicles, compared to 9.4% of the hybrid group (II), and 47.9% of the pooled *C. maschalocarpum* group (III & IV).

Table 1: Relative morphological differences between the three groups (tested with ANOVA and Kruskal-Wallis tests at the 5% significance level)

Character trait	Group I	Group II	Group III &	Statistics
Stipe width	Narrow	Intermediate	Wide	F=4843.291, df=2,
Lamina length	Long	Intermediate	Short	F=43.275, df=2,
Lamina width	Narrow	Intermediate	Wide	F=247.230, df=2,
Stipe thickness	Thick	Thin	Intermediate	$X_2=23.134$, df=2,
Lamina	Thick	Thin	Thin	F=21.22, df=2,
Proportion with secondary axes present	Low	High	High	$X_2=13.529$, df=2, n=461, p=0.001
Proportion with vesicles present	Absent	Low	High	$X_2=167.196$, df=2, n=461, p=0.001

There were significant differences in total length, stipe width, stipe thickness, presence of vesicles between the two groups (III and IV) of *C. maschalocarpum* (Table 2). Group IV was generally longer in total length, had a wider and thicker stipe, and had a greater proportion of individuals with vesicles present than Group III. There were also non-significant trends ($0.10 > p > 0.05$) of Group IV having wider and thicker lamina, and more frequently having secondary branches. There was no difference between the lamina length of the two groups. There was no difference in the distributions between the two *C. maschalocarpum* groups, either between sites ($X_2=3.146$, df=1, n=146, p=0.076) or exposure zones ($X_2=5.162$, df=3, n=146, p=0.160).

Table 2: Relative morphological differences between the *C. maschalocarpum* clusters III & IV.

Significance	Morphological trait	Group III	Group IV	Statistics
Statistically significant at 5% level	Total length	Shorter	Longer	F=6.803, df=1, n=146, p=0.010
	Stipe width	Narrower	Wider	F=250.906, df=1, n=146, p<0.001
	Stipe thickness	Thinner	Thicker	F=14.188, df=1, n=146, p<0.001
	Proportion with vesicles	Low	High	X ₂ =11.026, df=1, n=146, p=0.001
Trends in the data (significant at the 10% level)	Lamina width	Narrower	Wider	F=2.885, df=1, n=49, p=0.096
	Lamina thickness	Thinner	Thicker	F=3.837, df=1, n=49, p=0.056
	Proportion with secondary axes present	Low	High	X ₂ =3.161, df=1, n=146, p=0.075
Not significant and no trends	Lamina length	Same	Same	F=1.517, df=a, n=49, p=0.224

2.5 Discussion

Carpophyllum angustifolium, *C. maschalocarpum* and their hybrids have distinct and distinguishable morphologies. Identifiable hybrid and parental morphotypes have been found within many *Fucus* hybrid species complexes; however morphologically ambiguous individuals are often present as well (Coyer *et al.*, 2002a; Kucera & Saunders, 2008; Scott & Hardy, 1994). No other detailed morphometric analysis of randomly sampled and molecularly identified individuals has been done in a Fuclean hybrid system. Subsequently the range and degree of overlap of parental and hybrid morphologies are unknown in other Fuclean hybrid systems.

Stipe width alone can distinguish between *C. angustifolium*, *C. maschalocarpum* and their hybrids, and provides a useful taxonomic character for field identification. Stipe width meets three requirements described for a useful macroalgal taxonomic character in macro-algae; it is constant, easy to observe and non-destructive (Mathieson *et al.*, 1981). Stipe width of the parent species and hybrids was easy to measure and rarely affected by herbivory or exposure damage (pers. obs.). Stipe width was concluded to be a particularly valuable taxonomic trait for identification of *Fucus spp.* after an investigation into the spatial variability of *F. spiralis* and *F. vesiculosus* (Coleman & Muhlin, 2008).

Carpophyllum angustifolium × *C. maschalocarpum* hybrids generally had an intermediate morphology to the parent species. Hybrids had intermediate stipe width, lamina length, lamina width and vesicle presence. However, the thickness of hybrid lamina was novel, being thinner than both parents. Furthermore the proportion of hybrid individuals with secondary branching was indistinguishable from *C. maschalocarpum*. Intermediate hybrid morphologies are usually found in hybrid systems although not necessarily in all cases (Coyer *et al.*, 2006b; Rieseberg & Ellstrand, 1993; Seehausen, 2004). Intermediate morphology is used to identify putative hybrid individuals for further investigation in *Fucus*, a genus of Fucalean algae (Coyer *et al.*, 2002a; Scott & Hardy, 1994). Despite this there are examples of morphological traits in hybrids being aligned to one of the parental species. For example, hybridisation between the dioecious *Fucus vesiculosus* and the hermaphroditic *Fucus spiralis* results in hybrids with both maternal and paternal sexual phenotypes (Billard *et al.*, 2005b). In salt marsh habitat the

same *F. vesiculosus* × *F. spiralis* hybrids are morphologically indistinguishable from polyploid *F. vesiculosus* individuals also found in salt marshes (Coyer *et al.*, 2006b).

The presence of hybrid genotypes with *C. angustifolium* morphotypes suggests introgression is occurring. Hybrid ITS genotypes with parental morphology were found in the *F. vesiculosus* × *F. spiralis* species complex, and were also cautiously interpreted as possibly due to introgression (Kucera & Saunders, 2008). Introgression is an important evolutionary process that has the potential to integrate genetic material from one species into another (Coyer *et al.*, 2007). Introgression has been identified in *Fucus* hybrid complexes, and is considered a significant factor in the evolution of *Fucus* lineages (Coyer *et al.*, 2006a; Coyer *et al.*, 2007; Wallace *et al.*, 2004).

Fucus species seem to have maintained their genetic integrity despite extensive hybridisation. For example, hybrids made up 13% of *Fucus serratus* and *Fucus evanescens* in one population; however nuclear DNA introgression was only 1.5% in the two species, and both species maintain different distributions and morphologies (Coyer *et al.*, 2007; Coyer *et al.*, 2002a). Coyer (2006a) suggested different parental mating systems might act as a barrier to extensive introgression, and protect the parental species integrity. Hybridisation in *Fucus* appears to only occur between dioecious (having separate male and female individuals) and hermaphroditic species (e.g. Billard *et al.*, 2005a; Coyer *et al.*, 2007; Mathieson *et al.*, 2006; Wallace *et al.*, 2004). All members of the *Carpophyllum* genus are dioecious (Lindauer *et al.*, 1961). Extensive introgression, and the loss of parental species genetic integrity, could be more likely in *Carpophyllum* hybrid systems if Coyer *et al.* (2006a) hypothesis about mating systems is correct.

The absence of individuals with *C. maschalocarpum* morphology and hybrid genotypes suggests introgression may be asymmetrical, with backcrossing only between hybrids and *C. angustifolium*. Asymmetries in angiosperm reproduction are thought to be common in a broad range of taxa (Tiffin *et al.*, 2000). Asymmetrical hybridisation and introgression has been found in *Fucus spp.* (Coyer *et al.*, 2007; Coyer *et al.*, 2002a). Determining the symmetry of introgression is important for predicting its implications on parental species integrity (Coyer *et al.*, 2007).

The value of ITS for confirming and determining the extent of introgression is limited due to homogenisation of ITS (Alvarez & Wendel, 2003). Microsatellites have a high mutation rate and large variability, and have been used successfully to resolve relationships in a *Fucus* hybrid complex (Coyer *et al.*, 2006b; Coyer *et al.*, 2007; Engel *et al.*, 2005; Wallace *et al.*, 2004). Introgression can also be identified by comparing maternally inherited DNA, such as mtDNA or cpDNA, with nuclear DNA in the putative hybrids and introgressed individuals (Coyer *et al.*, 2007; Coyer *et al.*, 2002a).

The morphometric analysis distinguished variation within *C. maschalocarpum*, which could be correlated to recruitment. The differences between the two *C. maschalocarpum* clusters (i.e. total length, stipe width, stipe thickness, vesicles) were characteristics thought to be associated with maturity (Dromgoole, 1965). *Carpophyllum maschalocarpum* has pulsed peak recruitment, and has been shown to recruit in cohorts after bare rock is opened up by disturbance (Schiel, 1980; Schiel, 1988).

This work on *Carpophyllum* combined morphometric data with molecular ITS2 identifications found generally distinct and distinguishable parental and hybrid morphologies. Further investigation needs to be done using microsatellites and maternally inherited DNA in conjunction with morphology to confirm and determine the extent and symmetry of introgression, and identify backcrosses, F₂ and later hybrids.

Chapter 3: Wave exposure and morphology in *Carpophyllum angustifolium* and *Carpophyllum maschalocarpum*

3.1 Abstract

Environmental conditions can influence the morphology of local biota through phenotypic plasticity or local adaptation. Macroalgal morphologies are often associated with wave exposure conditions. This study investigated the relationship between morphology and wave exposure in two common endemic subtidal macroalgae, *Carpophyllum angustifolium* and *Carpophyllum maschalocarpum*, from the East Cape of New Zealand. Morphological comparisons were made between individuals from four different wave exposure zones, as defined by fetch and barnacle composition. *Carpophyllum angustifolium* individuals were longer in more wave exposed environments. *Carpophyllum maschalocarpum* were shorter with thinner stipes, and fewer individuals had vesicles, in more wave exposed zones. Morphological traits also varied between sites for both species suggesting that other influences are important in determining the species morphology. Further study is needed to investigate the role of phenotypic plasticity and genetic variability in driving morphological variation in *C. angustifolium* and *C. maschalocarpum*.

3.2 Introduction

Local environmental conditions can influence the morphology of organisms as a result of selection or phenotypic plasticity. This has been shown in a range of taxa and environments (Hochkirch *et al.*, 2008; Mboumba & Ward, 2008; Trussell, 2000). In coastal environments the morphology of local biota is significantly affected by wave

exposure (Denny, 2006; Stewart, 2006). For example Arsenault (2001) found the length of the legs of the barnacle *Balanus glandula* declined with increasing wave exposure.

Phaeophyceae, the brown macroalgae, have been found to be significantly affected by wave exposure (Engelen *et al.*, 2005; Fowler-Walker *et al.*, 2006; Tuya & Haroun, 2006). Water motion around macroalgae affects their nutrient acquisition, creates mechanical fatigue, and can result in the loss of biomass or dislodgement of entire individuals (Hurd, 2000; Kawamata, 2001). Phaeophyceae are highly plastic organisms, with numerous examples of environmentally induced forms (Mathieson *et al.*, 2006; Mathieson *et al.*, 1981). There are optimal morphologies for Phaeophyceae in different wave exposure regimes. Traits which reduce drag (i.e. small size, streamlined shape, flexibility) and increase strength (i.e. thickness, aggregation) are favoured in high wave exposed areas (Puijalon & Bornette, 2004; Ruuskanen & Nappu, 2005; Stewart, 2006; Stewart & Carpenter, 2003; Thomsen *et al.*, 2004; Wernberg, 2005). Johnson and Koehl (1994) found the blade morphology of the kelp *Nereocystis luetkeana* to be longer and thinner in more wave exposed areas, and to experience less drag than the sheltered blade form.

Carpophyllum angustifolium and *Carpophyllum maschalocarpum* dominate the high subtidal of north-eastern New Zealand (Schiel, 1990). The two species have different morphologies and wave exposure distributions. *Carpophyllum angustifolium* is streamlined, flexible, with strong aggregating holdfasts, and is distributed in exposed to moderately wave exposed areas (Chapters 2 & 4. Adams, 1994). *Carpophyllum maschalocarpum* has vesicles, is larger, has wide flat stipes and occurs in sheltered to

moderately exposed areas (Chapters 2 & 4. Adams, 1994). Determining how the morphology of these species is affected by wave exposure will provide insight into environmental selection and plasticity in *Carpophyllum*. It will also allow hypotheses to be generated about the fitness and distribution of their morphologically intermediate hybrids relative to wave exposure.

One of the major barriers in studying relationships between morphology and wave exposure is measuring the wave exposure component. Measuring wave exposure on rocky shores is difficult due to its spatial and temporal variability (Denny, 1988; Eckman *et al.*, 2003). Direct measurements of water motion can be done using a range of equipment including dynamometers and plaster of paris clod cards (Carrington Bell & Denny, 1994; Thompson & Glenn, 1994). All of these methods have limitations (Lindegarth & Gamfeldt, 2005; Porter *et al.*, 2000). One serious obstacle to deploying sensitive and expensive equipment is its attachment and survival in high wave exposure environments. Subsequently direct measurements often rely on short term data collected in calm water (Arsenault *et al.*, 2001; Stewart & Carpenter, 2003). For example Coleman *et al.* (2008) only measured water motion on days of 'low to moderate water motion'. Short term measurements can be misleading if extrapolated over long periods and varying environmental conditions (Gaylord, 1999).

The difficulties associated with direct measurement have led many researchers to infer water motion from fetch or biological information (Ballantine, 1961; Engelen *et al.*, 2005; McQuaid *et al.*, 2000). In two major journals approximately half of the papers on wave exposure over a recent ten year period developed categorical definitions of

exposure using fetch or local biota (Lindegarth & Gamfeldt, 2005). Fetch and local biota can provide information about wave exposure over a relatively long time frame. However, both have their limitations. Fetch data have low spatial resolution, while geographically specific information on the distribution or morphology of local biota might not be available (Arsenault *et al.*, 2001; Gaylord, 1999; Lindegarth & Gamfeldt, 2005). The distribution of barnacles by wave exposure in New Zealand has been well described (Foster, 1967; Foster, 1978). Barnacles were one of the first taxa to be considered for defining wave exposure, and are a useful guide due to their immobility and dominance on rocky shores (Ballantine, 1961; Heaven & Scrosati, 2008).

This study will compare the morphology of *C. angustifolium* and *C. maschalocarpum* between four wave exposure zones defined by fetch and barnacle distribution. Does the morphology of *C. angustifolium*, and *C. maschalocarpum* individuals change in areas of different wave exposure? Is the relationship between wave exposure and morphology the same in both species?

3.3 Methods

Sampling locations

Samples were collected from two sites on the East Cape of the North Island, New Zealand (Fig. 1). The two sites were both within the greater Whanarua Bay, which faces north-west and contains a number of smaller sandy bays divided by large rocky islands or outcrops. The first site is located at the south-eastern end of Maraehako Bay (S37° 40.4' E177° 47.8'). The second site was on the rock walls located at Uncles Bay (S37°

40.0' E177° 48.0'), which is the adjacent bay to the north-east. The two sites are approximately 500m apart, and are separated by Motu Kaimeanui Island and two deep inlets. Sampling was conducted during the 2007-2008 summer.

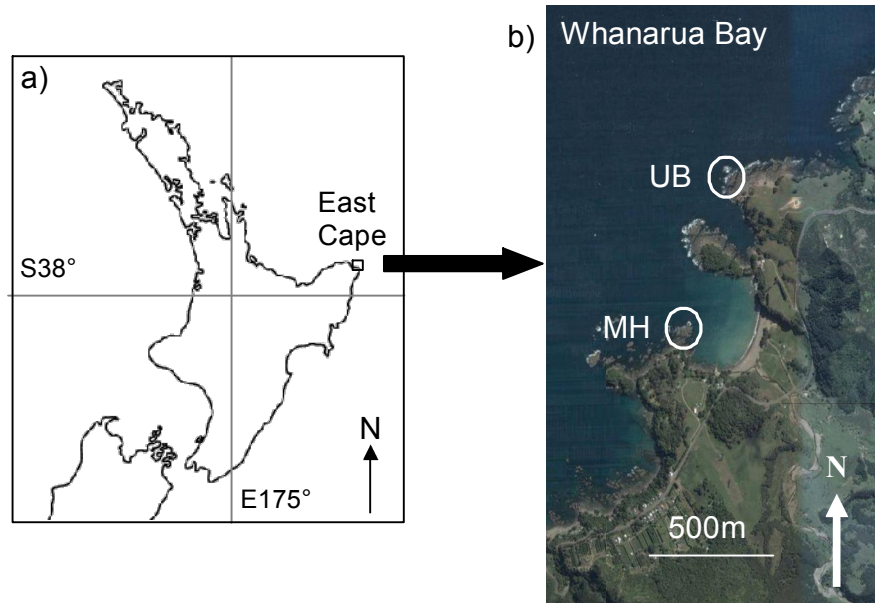


Figure 2: The location of sampling a) on the East Cape of the North Island of New Zealand, and b) the two sites in the greater Whanarua Bay: Maraehako Bay (MH) and Uncles Bay (UB) (Google Earth 5.0).

Assessing wave exposure

Four wave exposure zones were selected from each of the two sites. These zones were determined based on fetch and barnacle distributions. Firstly sites were divided using an estimate of fetch based on a modified Baardseth Index. The Baardseth Index is calculated by placing a transparent circular disc with a radius equivalent to 7.5 km on a point in a map and determining the number of 9° segments containing no land or islands (Baardseth, 1970). In this study the angle of open segments was summed *in situ* to incorporate small-scale disruptions to oncoming waves such as rocky outcrops. This sum was over 120° for the most exposed zone (zone 1), up to 60° in the second most exposed

zone (zone 2), and 0° in the two most sheltered zones. The two most sheltered zones were distinguished by the direction rock walls were facing. The rock walls in the moderately sheltered exposure zone (zone 3) faced the opposite side of the bay, whilst those in the most sheltered exposure zone faced the shore (zone 4) (Fig. 2).

Transect lines were laid in each zone, and quadrat locations were selected randomly from the top 0.5 m of the *Carpophyllum* depth distribution. The intertidal barnacle fauna was checked for consistency with wave exposure prior to sampling each quadrat. The expected barnacle fauna for the four exposure zones was determined based on work by Foster (1967; 1978), and is summarised in Figure 2. Quadrats were rejected if barnacle fauna did not match the expected fauna in the exposure zone, the rock face was not vertical, fewer than three individuals were present, or the quadrat was less than 0.5 m from a previously sampled quadrat. All *Carpophyllum* individuals with holdfasts inside the 20 by 20 cm quadrats were collected. Sixty nine quadrats were sampled in the two sites, with 280 *C. angustifolium* and 152 *C. maschalocarpum* adults collected in total. Collected material was frozen at -4°C within a week of collection, and thawed in seawater prior to morphometric analysis.

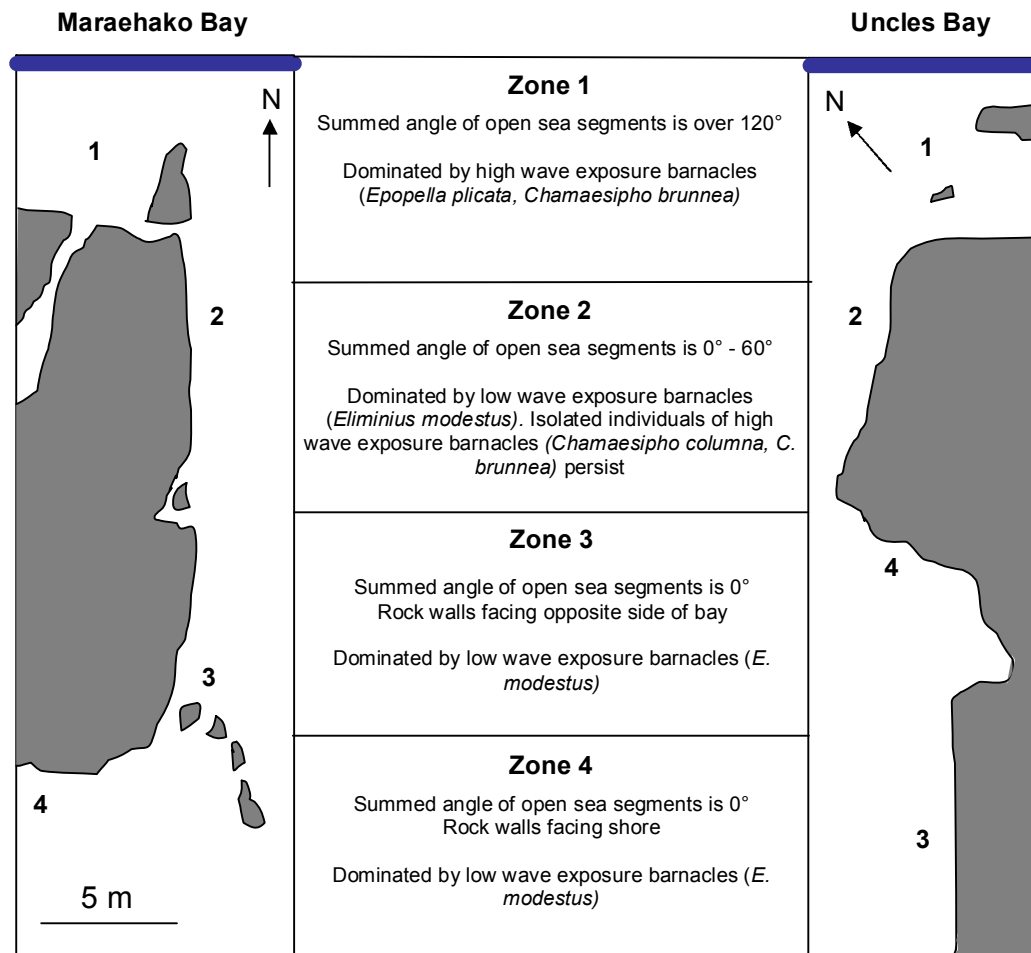


Figure 2: The wave exposure zones were defined by the degree of exposure to the open sea, orientation of the rock walls, and the barnacles in the intertidal portion of the rock wall. On the left and right simplified illustrations of the two study sites are shown with the general locations of the zones marked. The blue line represents the open sea.

Morphometrics

Adults were morphologically identified, and cleared of any epiphytes. Individuals longer than 20 cm were considered adults. Only those with complete haptera were used in morphometrics so as to avoid pseudo-replication (i.e. the doubling up of measurements from the same individual). Individuals were measured using digital callipers and a metre rule.

The morphometric characteristics selected for measurement were stipe width and thickness; and lamina length, width and thickness (Fig. 3). This study focused on the stipe and lamina characteristics of the primary axes (defined as the dominant axis which arises directly from the holdfast). The stipe and lamina measurements were taken from approximately 5, 10, 15, and 20 cm from the apex of the primary axis. Lamina length was measured from the edge of the stipe where the lamina forms to its tip. Lamina and stipe width were measured at the widest point, perpendicular to an imagined mid-line. Thicknesses were measured at the thickest point. In some places herbivory had altered morphology of the stipe and lamina; these could be detected by sudden and distinct changes in lamina or stipe outline. Measurements of stipe and lamina that were affected by herbivory were excluded, and if possible replaced by measurements by the nearest intact equivalent with 3 cm. The total length of the individual, presence of vesicles longer than 8 mm, and presence of secondary branches longer than 20 cm were also recorded.

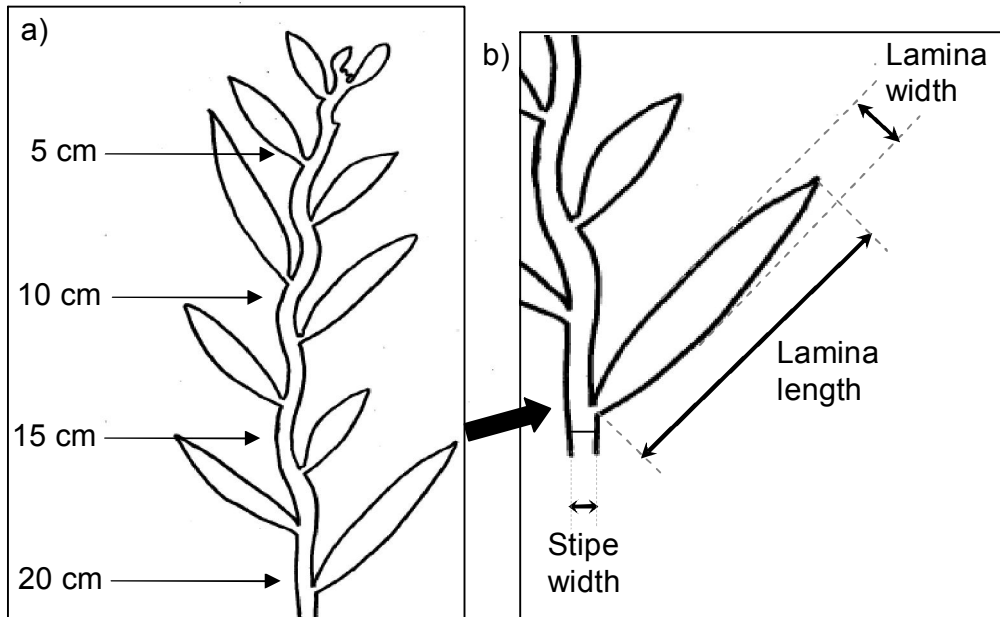


Figure 3: The location of the measurements taken at from each individual 5, 10, 15, 20 cm are shown with arrows on a stylised axis in a). Stipe width, lamina length and width are illustrated at 20 cm from the apex in b).

Data Analysis

The morphometric data contained missing values due to herbivory and wave exposure damage on some parts of individuals. There was no difference between the measurements taken at the different locations down the apex of individuals. Consequently these measurements were averaged for each individual. This resolved the missing values problem as most individuals had at least one measurement for each morphological trait. For example stipe width on an individual was an average from the measurements at 5, 10, 15, and 20 cm from the apex.

Morphological characters from individuals in different wave exposure zones were compared for each species. Data from the two sites were combined. ANOVA and Tukey

post-hoc comparisons were conducted where data were normally distributed and variances equal. Data were log transformed where ANOVA assumptions were not met. Log transformed data which still did not meet assumptions was analysed non-parametrically with Kruskal-Wallis tests. Chi-squared tests were used to investigate the presence data on vesicles and branching. The level of significance for all statistical tests conducted was determined using the value $\alpha=0.05$.

3.4 Results

Wave exposure and morphology

Carpophyllum angustifolium was found only in the two most wave exposed zones ($n_{\text{zone1}}=165$, $n_{\text{zone2}}=115$) whilst *C. maschalocarpum* was found in all four wave exposure zones ($n_{\text{zone1}}=7$, $n_{\text{zone2}}=31$, $n_{\text{zone3}}=62$, $n_{\text{zone4}}=52$). Some morphological traits were found to differ between exposure zones (Table 1). Total length for both species varied by wave exposure zone (Fig. 4). However the two species showed opposite trends for total length. *Carpophyllum angustifolium* individuals were shorter in the more sheltered zone of their distribution. By comparison *C. maschalocarpum* tended to be longer in the more sheltered zones, with individuals in exposure zones 3 and 4 significantly longer than those in zone 2 (Tukey Tests: $p<0.001$, $p=0.024$). There was no significant difference in *C. maschalocarpum* length between the two most sheltered zones (Tukey Test: $p=0.344$). Furthermore the seven *C. maschalocarpum* individuals in the most exposed zone had highly variable total lengths, and subsequently could not be differentiated from any other zone (Tukey Test: $p=0.807$, $p=0.452$, $p=0.902$).

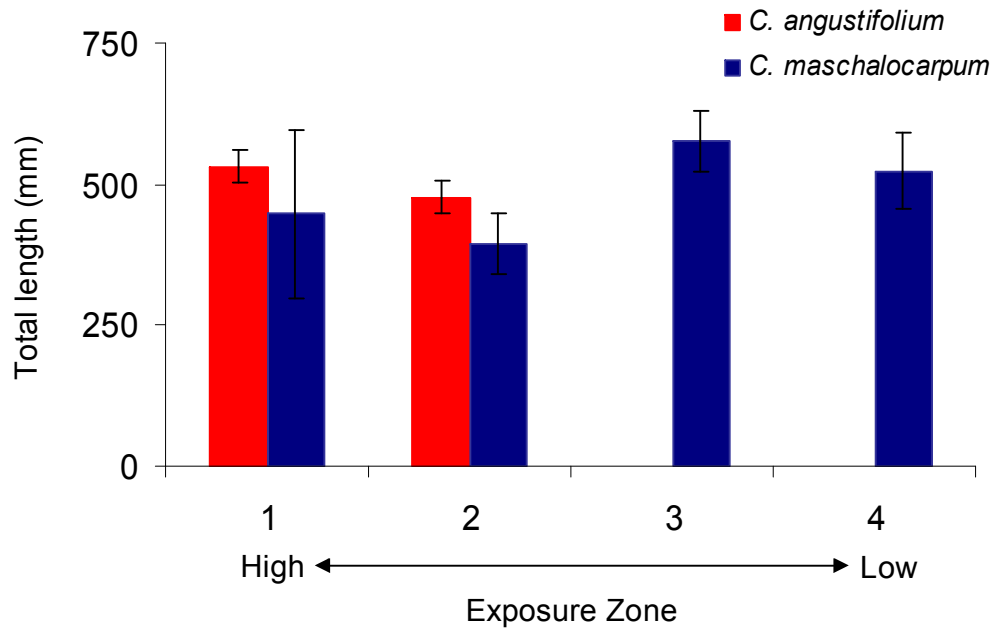


Figure 4: The average length of individuals in each sample differed between exposure zones in both species. The bars show average total length, with 95% confidence intervals as error bars.

None of the other morphological traits measured significantly varied, or showed non-significant trends of variation, by wave exposure zone in *C. angustifolium* (e.g. stipe width, stipe thickness, lamina length, lamina width, lamina thickness, presence of vesicles) (Table 1). Stipe thickness and vesicle presence varied in *C. maschalocarpum* from the different wave exposure zones. There were also non-significant trends of lamina thickness and the presence of branching varying between wave exposure zones.

The average *Carpophyllum maschalocarpum* stipe thickness increased as wave exposure decreased (Fig. 5a). Stipes were significantly thinner in exposure zone 2 than zones 3 and 4 at both sites (Tukey tests: $p=0.026$). The presence of vesicles in *C. maschalocarpum* individuals varied between the different exposure zones. The

proportion of individuals with vesicles was lowest in the most exposed zone, highest in the moderately exposed zone, and intermediate in the two most sheltered zones (Fig. 5b).

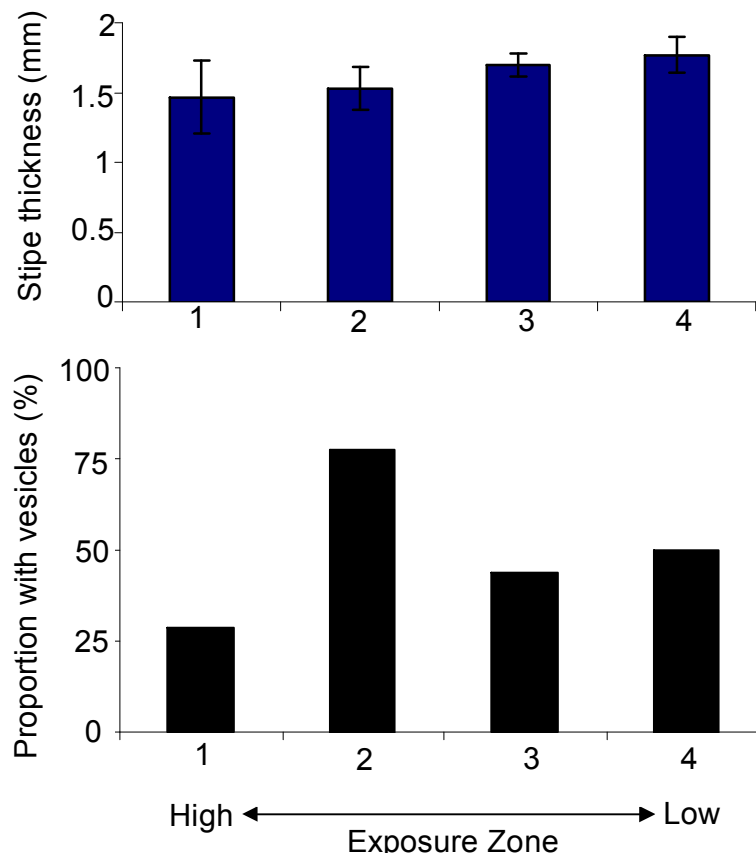


Figure 5: The variation in a) stipe thickness and b) vesicle presence between exposure zones for *C. maschalocarpum*. Error bars show 95% confidence intervals.

There was a non-significant trend of lamina thickness in *C. maschalocarpum* differing between exposure sites, being thinner in more exposed zones. Another non-significant trend in *C. maschalocarpum* was in branching; there appeared to be fewer individuals with major branches in more exposed zones (Table 1).

Table 1: Variation in morphological traits between exposure zones for *C. angustifolium* and *C. maschalocarpum*.

Species	Significance	Character trait	In more exposed zones	Statistics
<i>C. angustifolium</i>	Significant	Total length	Longer	$X_2=5.980$, $df=1$, $n=280$, $p=0.014$
	Not significant	Stipe width		$F=0.883$, $df=1$, $n=280$, $p=0.348$
		Stipe thickness		$F=1.944$, $df=1$, $n=280$, $p=0.164$
		Lamina length		$X_2=0.009$, $df=1$, $n=280$, $p=0.927$
		Lamina width		$F=1.137$, $df=1$, $n=173$, $p=0.288$
		Lamina thickness		$X_2=0.010$, $df=1$, $n=173$, $p=0.919$
		Proportion with vesicles		$X_2=0.529$, $df=1$, $n=280$, $p=0.467$
<i>C. maschalocarpum</i>	Significant	Total length	Shorter	$F=6.063$, $df=3$, $n=152$, $p=0.001$
		Stipe thickness	Thinner	$F=6.981$, $df=4$, $n=149$, $p<0.001$
		Proportion of individuals with vesicles	Lower	$X_2=11.491$, $df=3$, $n=152$, $p=0.009$
	Non-significant trends	Lamina thickness	Thinner	$F=2.689$, $df=2$, $n=49$, $p=0.079$
		Proportion with branches	Lower	$X_2=6.159$, $df=3$, $n=152$, $p=0.104$
	Not significant	Stipe width		$F=1.874$, $df=1$, $n=149$, $p=0.137$
		Lamina length		$F=1.461$, $df=3$, $n=49$, $p=0.289$
		Lamina width		$F=0.458$, $df=3$, $n=49$, $p=0.713$

Site effect

Carpophyllum morphological traits also varied between sites (Table 3). Stipe thickness and lamina thickness were significantly different between the two sites for *C. angustifolium* and *C. maschalocarpum*. *Carpophyllum angustifolium* stipe width and lamina width also varied between the two sites. *Carpophyllum maschalocarpum* lamina length varied between the two sites.

Table 1: Relative differences and statistics for character traits that significantly differed between the two sites (at the 5% significance level).

Species	Character trait	At Maraehako Bay	Statistics
<i>C. angustifolium</i>	Stipe width	Wider	F=23.917, df=1, n=280, p<0.000
	Stipe thickness	Thicker	F=6.848, df=1, n=280, p=0.009
	Lamina width	Wider	F=1.137, df=1, n=173, p<0.000
	Lamina thickness	Thinner	F=7.371, df=1, n=173, p=0.007
<i>C. maschalocarpum</i>	Stipe thickness	Thinner	F=42.163, df=1, n=149, p<0.000
	Lamina length	Thinner	F=4.782, df=1, n=49, p=0.034
	Lamina thickness	Longer	F=23.979, df=1, n=49, p<0.000

3.5 Discussion

Morphological traits differed between exposure zones, which suggests that *C. angustifolium* and *C. maschalocarpum* morphologies are affected by wave exposure. Previous studies have found algae in more wave exposed areas tend to be shorter with narrower and thicker lamina and stipes, and fewer or no vesicles (Fowler-Walker *et al.*, 2006; Johnson & Koehl, 1994; Kawamata, 2001; Stewart & Carpenter, 2003). These relationships have been detected in another species in the *Carpophyllum* genus: *Carpophyllum flexulosum* (Cole *et al.*, 2001). Some of these patterns were found in *C. maschalocarpum*; individuals were shorter, and a lower proportion had vesicles in the

more wave exposed zones. There was also a non-significant trend of *C. maschalocarpum* being less branched in the more wave exposed zones. However opposing patterns were also found, with *C. angustifolium* being longer, and *C. maschalocarpum* stipes being thinner in more wave exposed zones. A non-significant trend was also found for *C. maschalocarpum* lamina being thinner in more wave exposed zones.

Carpophyllum angustifolium individuals were marginally, but significantly, longer in the most wave exposed zone, going against the trends found in other macroalgae. High wave exposures can prevent macroalgae from reaching large sizes, and smaller sized macroalgae experience less drag and wave damage (Blanchette, 1997; Hurd, 2000). The results found in our study could reflect different strategies to dealing with mechanical stress from wave exposure. *Carpophyllum angustifolium* grows in higher densities than *C. maschalocarpum* (Hodge, pers. obs.). The wave exposure that individuals are subjected to can be modified and reduced in dense macroalgal stands (Eckman, 1983), potentially reducing the selection pressure on morphology. Also, *C. angustifolium* appears to be very flexible (Hodge, pers. obs.). Flexibility can allow morphological reconfiguration *in situ* which can reduce the drag forces a macroalga is exposed to (Carrington, 1990; Denny, 2006). Flexibility in some macroalgae can result in reduced drag in higher flows (Carrington, 1990; Gaylord *et al.*, 1994).

Another opposing trend to what has been found in other macroalgae was in the thickness of *C. maschalocarpum* stipes and lamina. Stipes were significantly thinner in *C. maschalocarpum* from more exposed zones, and *C. maschalocarpum* laminae showed a

similar non-significant trend. This pattern could be a reflection of the relationship between age and thickness. Laminae and stipes get thicker with age, and individuals and their components are often younger in more exposed areas due to wave damage (Dudgeon & Johnson, 1992). *Carpophyllum maschalocarpum* stipes and laminae might be thinner in more exposed areas because they are younger.

The relationships between wave exposure and morphology differed between the two species. The only trait that varied by wave exposure in both species was total length, which showed opposing trends in the two species. Also more morphological traits differed by wave exposure in *C. maschalocarpum* than in *C. angustifolium*. This could be due to their different wave exposure distributions. High levels of phenotypic plasticity has been hypothesised as being associated with generalist species, as opposed to specialist species (Sultan, 2000). *Carpophyllum maschalocarpum* had a much broader wave exposure distribution than *C. angustifolium*. *Carpophyllum angustifolium* was only found in the two most exposed zones, suggesting specialisation to high wave exposure. *Carpophyllum maschalocarpum* was found in all four wave exposure zones, which indicates that it is a generalist species (see also Dromgoole, 1973).

Differences in wave exposure - morphological relationships between the species could also be a reflection of the different strategies for dealing with wave exposure. The consequences of wave exposure on an individual are influenced by drag, and attachment strength (Kawamata, 2001). Changes in length, stipe and lamina dimensions can alter drag; but not attachment strength. Higher attachment strengths have been found in individuals with aggregating or larger holdfasts, and individuals from exposed locations

(Dudgeon & Johnson, 1992; Thomsen *et al.*, 2004; Wernberg, 2005). The relative importance of reducing drag, and increasing attachment strength could differ between the two species, with a greater emphasis on reducing drag in *C. maschalocarpum* and increasing attachment strength in *C. angustifolium*. Attachment strength, holdfast biomass, or holdfast aggregation, were not measured in this study but are hypothesised to vary between wave exposure zones and species. During sampling it was noted that in the most exposed zone individuals were strongly attached, often having to be cut off or prised off the rocks with scissors. Empirical testing of attachment strength could help in interpretation in the relationships between morphology and wave exposure found in this study. Differing emphasis on drag and attachment strength was concluded in a similar study which found a lack of correlation between the morphological changes of two aquatic angiosperm species, *Berula erecta* and *Mentha aquatica*, along a water flow gradient (Puijalon & Bornette, 2004).

The relationship between morphology and wave exposure was consistent between the two sites, but morphology differed between the sites. Morphological variation between sites has been found in a previous study on *Fucus* (Coleman & Muhlin, 2008). Morphological variation could be due to environmental variability between the two sites, although these were thought to be very similar and only 500 m apart. Alternatively variation could be due to genetic differences between the two populations. *Carpophyllum* has low dispersal capacity; the majority of recruits settle within metres of parental plants (Schiel, 1980). Such low dispersal capacity could allow genetic differentiation between the two populations. Previous studies have found fucoid algae to have genetic differentiation at very low spatial scales; populations just 10m apart

showed genetic differentiation in *Fucus vesiculosus* (Coyer *et al.*, 1997; Engelen *et al.*, 2001; Tatarenkov *et al.*, 2007; Williams & Di Fiori, 1996).

It should be noted that this study investigated wave exposure categorically, which has disadvantages. First relationships between wave exposure and morphology can be obscured or confused by small scale wave exposure variability. In a study aimed to compare algal morphology in exposed, semi-exposed and protected sites, Eckman (2003) found 30-50% of variation in water flow occurred between replicates at the same site. Secondly non-linear relationships between morphology and wave exposure can be distorted and misinterpreted when it is categorised (Kitzes & Denny, 2005, see Fig. 5).

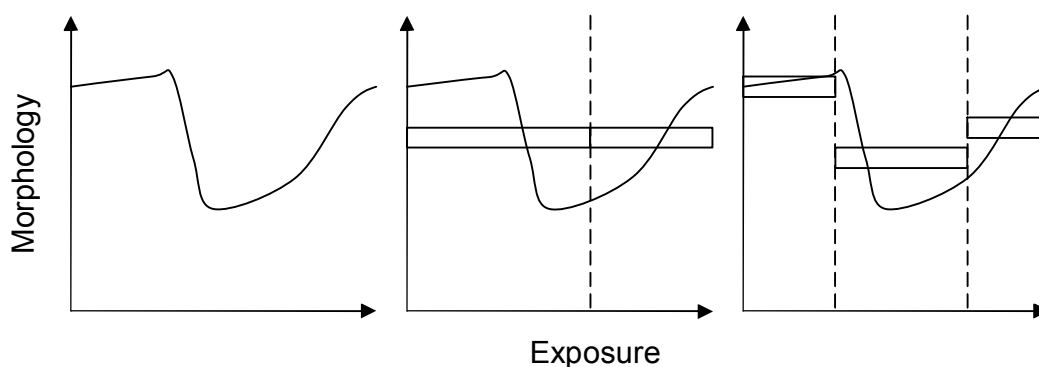


Figure 5: A hypothetical non-linear relationship between morphology and wave exposure, showing the different interpretations gained from a continuous measure, and two different categorical measures of wave exposure.

Further investigation of *C. angustifolium* and *C. maschalocarpum* is needed to understand the pattern of morphological variation found in this study. In particular analysis of morphology relative to a continuous measure of wave exposure could reveal finer scale trends, and non-linear relationships. Also investigation of attachment strength

and flexibility is required to determine the importance of morphology on the effects of wave exposure for the two species. The drivers of the morphological variations observed are unknown. Exploration of the role of the environment and genetic variation is required. Transplant experiments, and an understanding of the genetic structuring of *Carpophyllum* populations could provide further insight.

Chapter 4: Distribution of *C. angustifolium*, *C. maschalocarpum*, and their hybrids with regard to wave exposure

4.1 Abstract

An understanding of what shapes the distribution of species is one of the core questions in ecology. Wave exposure is one factor that affects the distribution of brown algae in the high subtidal. In north-east New Zealand *Carpophyllum angustifolium* and *Carpophyllum maschalocarpum* are major components of the shallow subtidal. The two species have different morphologies, which are each thought to be optimal in different wave exposure environments. *Carpophyllum* hybrids with intermediate morphology have been found in the wild. This study investigated the distributions of *C. angustifolium*, *C. maschalocarpum* and their hybrids relative to wave exposure in the high subtidal. Four wave exposure zones were defined within each site based on fetch and barnacle distribution. It was found that *C. angustifolium* was associated with high wave exposure while *Carpophyllum maschalocarpum* was associated with low wave exposure. Hybrids occurred in the intermediate wave exposure zones where parental distributions overlapped. Investigation of the distributions by depth is required to determine whether the hybrid distribution is environmentally selected or dispersal limited.

4.2 Introduction

Environmental conditions can structure species distributions locally. Factors such as temperature, desiccation stress, salinity and wave exposure have been found to influence species distributions in marine systems (Harley, 2003; Heaven & Scrosati, 2008; Pennings *et al.*, 2005). Exposure to water motion has been found to have significant effects on the distribution of macroalgae in the shallow subtidal (Engelen *et al.*, 2005) (Tuya & Haroun, 2006). Water motion around macroalgae affects nutrient acquisition, creates mechanical fatigue, and can result in the loss of biomass or dislodgement of entire individuals (Hurd, 2000; Kawamata, 2001).

Certain morphologies are more successful in avoiding damage in areas of high wave exposure. In macroalgae, traits which reduce drag (i.e. small size, more streamlined shape, increased flexibility) or increase strength (i.e. thickness, aggregation) are favoured in high wave exposed areas (Puijalon & Bornette, 2004; Ruuskanen & Nappu, 2005; Stewart, 2006; Thomsen *et al.*, 2004; Wernberg & Thomsen, 2005). For example the kelp *Nereocystis luetkeana* has narrower and longer blades in high wave exposed areas, which Johnson *et al.* (1994) found significantly reduced drag.

Water motion on rocky shores is difficult to measure due to its spatial and temporal variability (Denny, 1988; Eckman *et al.*, 2003). Direct measurements of water motion can be done using a range of equipment including dynamometers and plaster of paris clod cards (Carrington Bell & Denny, 1994; Thompson & Glenn, 1994). However, these methods have limitations (Lindegarth & Gamfeldt, 2005; Porter *et al.*, 2000). One major obstacle is the attachment and survival of equipment in high wave exposure

environments. Subsequently direct measurements often rely on short term data that is collected in calm water (Arsenault *et al.*, 2001; Stewart & Carpenter, 2003). For example, Coleman *et al.* (2008) only measured wave exposure on days of ‘low to moderate water motion’ to ‘gauge water motion’. Short term calm water measurements can be misleading if extrapolated over long periods, and varying sea conditions (Gaylord, 1999).

The difficulties associated with direct measurement have led many researchers to infer water motion from fetch or biological information (Ballantine, 1961; Engelen *et al.*, 2005; McQuaid *et al.*, 2000). In two major journals approximately half of the papers on wave exposure over a recent ten year period developed categorical definitions of exposure using fetch or local biota (Lindegarth & Gamfeldt, 2005). Fetch and local biota can provide information about water motion over a relatively long time frame. However both have their limitations. Fetch data has low spatial resolution, while geographically specific information on the distribution or morphology of local biota might not be available (Arsenault *et al.*, 2001; Gaylord, 1999; Lindegarth & Gamfeldt, 2005). The distribution of barnacles by wave exposure in New Zealand has been well described (Foster, 1967; Foster 1978). Barnacles were one of the first taxa to be considered for defining wave exposure, and are a useful guide due to their immobility and dominance on rocky shores (Ballantine, 1961; Heaven & Scrosati, 2008).

Carpophyllum angustifolium J. Agardh and *Carpophyllum maschalocarpum* Turner (Sargassaceae) are two species of macroalgae which dominate the high subtidal in New Zealand (Schiel, 1990). Their distributions are thought to be structured by wave

exposure with *C. angustifolium* described as occurring in more wave exposed areas, and *C. maschalocarpum* in less wave exposed areas (Dromgoole, 1965; Lindauer *et al.*, 1961). However, this is based solely on observations; their distributions have never been thoroughly investigated with regard to wave exposure. The two species have distinct morphology (Adams, 1994), with characters that appear suited to their hypothesised distribution. *Carpophyllum angustifolium* has been described as streamlined, flexible, with strong aggregating holdfasts, while *C. maschalocarpum* has been described as large, with vesicles and wide flat stipes (Dromgoole, 1965; Lindauer *et al.*, 1961). The two species hybridise in the wild, producing hybrids with intermediate morphologies (see Chapter 2). Based on the intermediate morphology of the hybrids it could be hypothesised that they might be distributed in areas of intermediate wave exposure relative to the parents.

There is support for the distribution of hybrids being dependent on environmental factors (Fritsche & Kaltz, 2000; Wang *et al.*, 1997). This is a reflection of the relative fitness of hybrids in certain environments (Arnold & Hodges, 1995). Hybrids are often found in intermediate or novel environments from the parental species (Arnold, 1997). The hybrids between the brown algae *Fucus spiralis* and *Fucus vesiculosus* have been identified as having both intermediate and novel distributions relative to the parental species (Coyer *et al.*, 2006; Scott & Hardy, 1994). *Fucus spiralis* and *Fucus vesiculosus* hybrids were distributed at intermediate shore heights to the parents on rocky shores (Scott & Hardy, 1994). *Fucus spiralis* × *F. vesiculosus* hybrids were also found with a dwarf morphology in a salt marsh which is a novel habitat from their diploid parents (Coyer *et al.*, 2006).

This study will investigate the distributions of *C. angustifolium*, *C. maschalocarpum* and their hybrids in the high subtidal with regard to wave exposure. Wave exposure zones will be defined within sites using fetch and barnacle distributions. Do the two parents occupy different wave exposure zones, with *C. angustifolium* in the more exposed and *C. maschalocarpum* in the more sheltered? Are the morphologically intermediate hybrids found in intermediate wave exposure zones?

4.3 Methods

Sampling locations

Samples were collected from two sites on the East Cape of the North Island of New Zealand, where *C. angustifolium*, *C. maschalocarpum* and their hybrids were known to be present (Fig. 1). The two sites were both in the greater Whanarua Bay, which faces north-west and contains a number of smaller sandy bays divided by large rocky islands. The first site was located at the south-eastern end of Maraehako Bay (S37° 40.4' E177° 47.8'). The second site was located at Uncles Bay (S37° 40.0' E177° 48.0'), which is the adjacent bay to the north-east. The two sites are approximately 500 m apart, separated by Motu Kaimeanui Island and two deep inlets (Fig. 1). Sampling was conducted during the 2007-2008 summer season.

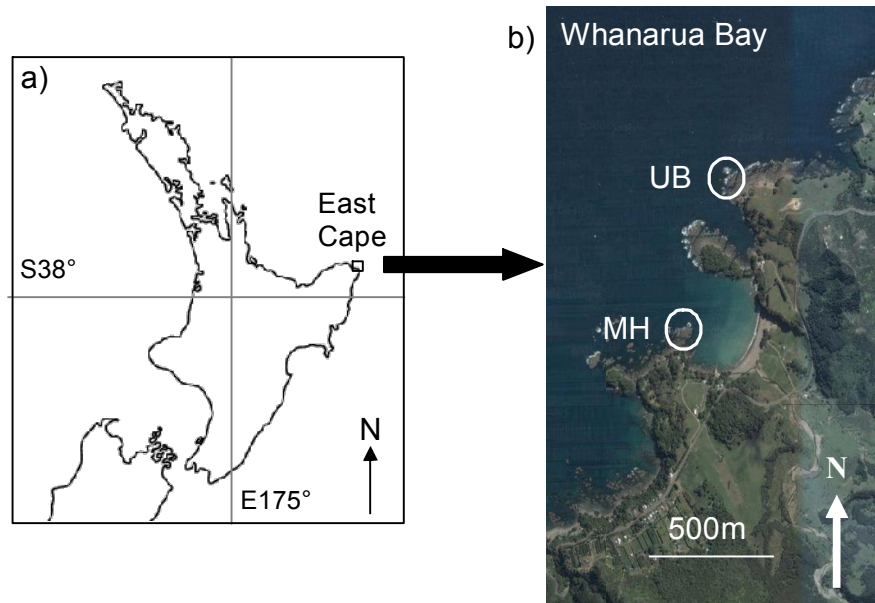


Figure 1: The location of the sampling sites a) on the East Cape of the North Island of New Zealand, and b) the two sites in the greater Whanarua Bay: Marachako Bay (MH), and Uncles Bay (UB).

Assessing wave exposure

Four wave exposure zones were selected from each of the two sites. These zones were determined based on fetch and barnacle distributions. Firstly sites were divided using an estimate of fetch based on a modified Baardseth Index. The Baardseth Index is calculated by placing a transparent circular disc with a radius of 7.5 km on a point in a map and determining the number of 9° segments containing no land, small islands, or rocky outcrops (Baardseth, 1970). In this study the angle of open segments was summed in-situ to incorporate small scale disruptions to oncoming waves such as rocky outcrops. This sum was over 120° for the most exposed zone, up to 60° in the second most exposed zone, and 0° in the two most sheltered zones. The two most sheltered zones were distinguished by the direction rock walls were facing. The rock walls in the

moderately sheltered exposure zone faced the opposite side of the bay, whilst those in the most sheltered exposure zone faced the shore.

Sampling

Transect lines were laid in each zone, and quadrat locations were selected randomly for the top 0.5 m of the *Carpophyllum* depth distribution. The intertidal barnacle fauna was checked for consistency with wave exposure prior to sampling each quadrat. The expected barnacle fauna for the four wave exposure zones was determined based on work by Foster (1967 and 1978), and is illustrated in Figure 2. Quadrats were rejected if barnacle fauna did not match the expected fauna in the wave exposure zone, the rock face was not vertical, less than three *Carpophyllum* individuals were present, or the quadrat was less than 0.5 m from a previously sampled quadrat. All *Carpophyllum* individuals with holdfasts inside the 20 cm by 20 cm quadrats were collected. Sixty nine quadrats were sampled in the two sites, with six to eight quadrats from each zone. Six additional quadrats were sampled from zones where hybrids were found in more than one of the original quadrats. Collected material was frozen at -4°C within a week of collection, and thawed in seawater prior to drying and weighing.

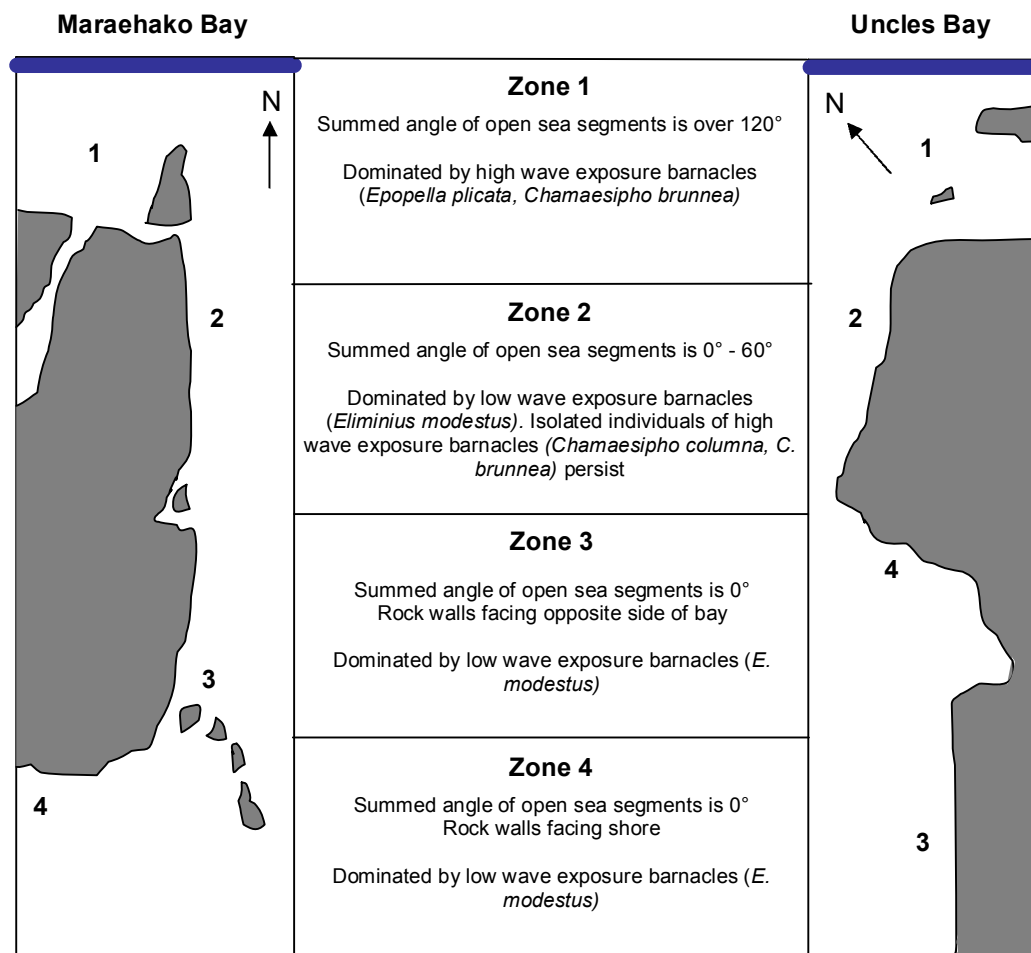


Figure 2: The wave exposure zones were defined by the degree of exposure to the open sea, orientation of the rock walls, and the barnacles in the intertidal portion of the rock wall. On the left and right simplified illustrations of the two study sites are shown with the general locations of the zones marked. The blue line represents the open sea.

Laboratory analysis

Adults were morphologically identified, cleared of epiphytes, dried and weighed. Individuals longer than 20 cm were considered adults. All holdfasts were removed as the amount of rock and non-algal biomass stuck in them was variable. Algae were dried at 50°C for 24 hrs or longer (in a Clayson Incubator) until thoroughly dry and then weighed using a balance accurate to 0.01g.

Data analysis

Carpophyllum distribution data were analysed in SPSS 17.0. The distributions of biomass were not normally distributed due to the large number of zeros from the absence data. Transformations were unable to normalise data, so data were analysed non-parametrically. The chi-squared test was used to test for distributional overlap between species, and non-random distribution across wave exposure zones and sites. Differences in biomass between the different zones were tested with the non-parametric Kruskal-Wallis and Mann Whitney tests. Statistical significance was inferred at the 5% level.

4.4 Results

Distribution of parent species

Carpophyllum angustifolium was distributed in different wave exposure zones from *C. maschalocarpum* (Chi squared test: $\chi^2=33.051$, $df=3$, $n=79$, $p<0.000$) (Fig. 3). *Carpophyllum angustifolium* was found *only* in the relatively high wave exposed zones 1 and 2. *Carpophyllum maschalocarpum* was found *mostly* in the relatively low wave exposed zones 3 and 4.

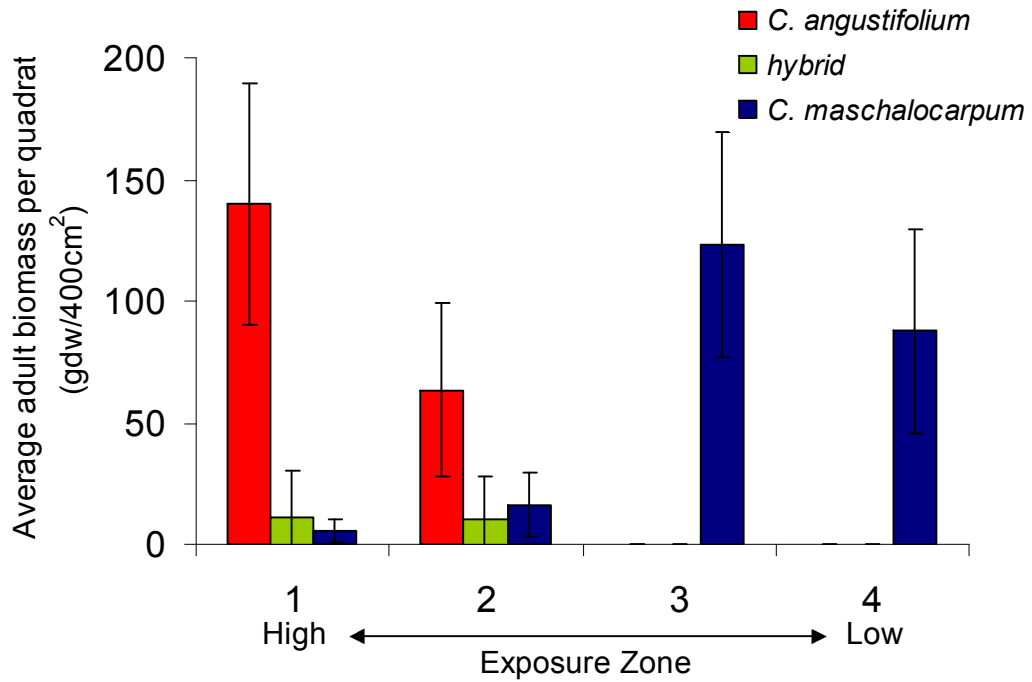


Figure 3: Average biomass of *C. angustifolium* and *C. maschalocarpum* and their hybrids in the 20 cm by 20 cm quadrats from each wave exposure zone. The error bars represent 95% confidence intervals.

Distribution of hybrids

The *C. angustifolium* × *C. maschalocarpum* hybrids were rare in comparison to the parent species (Fig. 3), and were found in only 9 out of the 69 quadrats. Wave exposure was a significant factor in adult hybrid distribution (Kruskal-Wallis $X^2=7.970$, $df=3$, $n=69$, $p=0.047$). Adult hybrids were found only in the relatively sheltered wave exposure zones 1 and 2, with no significant differences in hybrid adult biomass between these two zones (Mann-Whitney $U=187.000$, $Z=-0.794$, $n=41$, $p=0.427$). The wave exposure zones where hybrids were found were only those where both parental species were also present. Furthermore eight of the nine 400cm² quadrats containing adult

hybrids also contained one or both of the parental species. The other quadrat without adult parental species contained less than 2 gdw (grams dry weight) of adult hybrid. There was no significant difference in the total *Carpophyllum* biomass contained in the quadrats with and without hybrids ($F=0.444$, $df=1$, $n=69$ $p=0.507$).

Site differences

The composition of *C. angustifolium*, *C. maschalocarpum* and their hybrids in the high wave exposure zone 1 differed between the two sites. In this wave exposure zone both species and hybrids were found at Uncles Bay. In contrast only *C. angustifolium* was found in this zone at Maraehako Bay.

4.5 Discussion

The distributions of *C. angustifolium*, *C. maschalocarpum* and their hybrids were correlated with wave exposure. *Carpophyllum angustifolium* was distributed in the relatively exposed zones, while *C. maschalocarpum* was distributed mainly in the sheltered zones. This was consistent with the hypotheses and descriptions of Dromgoole (1973), Lindaeur (1961) and Adams (1994). Hybrids were distributed in the relatively exposed zones.

Hybrids are most commonly found in environmental conditions intermediate between the parental distributions (Arnold *et al.*, 2001; Campbell & Waser, 2007; Harrison, 1986; Wang *et al.*, 1998). Hybrids often have environmentally dependent fitness, and the importance of this forms the basis of two important hybrid distribution models: the

Bounded Hybrid Superiority model and the Evolutionary Novelty model (Arnold, 1997; Arnold & Hodges, 1995; Moore, 1977). However this pattern has not been confirmed for Fuclean hybrids due to a lack of study. In this literature review only two studies on Fuclean hybrid distribution were found, and both were based on only two plots or transects. In one of these studies the spatial distribution of hybrids could not be distinguished from the parental species (Coyer *et al.*, 2007). In the other study distributions were structured by shore height with hybrids having an intermediate distribution (Scott & Hardy, 1994). The intermediate wave exposure zones where *Carpophyllum* hybrids were found were also the zones where both parental species were present. Eight out of the nine quadrats where hybrids were found contained individuals of one or both the parental species. This pattern was also found in the two studies previously mentioned, where hybrid individuals were all within five metres of individuals from both parental species (Coyer *et al.*, 2007; Scott & Hardy, 1994).

The distribution pattern found could be a reflection of environmentally dependent selection in different wave exposures. This is concordant with what is known about their adult morphology. *Carpophyllum angustifolium* is more streamlined than *C. maschalocarpum* and hybrids are intermediate (Chapter 2; Adams 1994). More streamlined morphologies significantly reduce drag in high wave exposure, and as such are likely to reduce wave damage and confer fitness advantages in high wave exposure environments (Johnson & Koehl, 1994; Wernberg & Thomsen, 2005). Greater attachment strength and flexibility can also be selected for in high wave exposures, and could be more important than morphology in determining drag forces (Carrington, 1990; Denny, 2006). These characters have not been fully investigated in the two parental

species, although *C. angustifolium* is thought to have greater attachment strength (Dromgoole 1973; Hodge, pers. obs.).

Zygote attachment and settler survival in different wave exposures could also be structuring adult *Carpophyllum* distributions. The majority of mortality in Fucalean algae occurs in microscopic stages (Schiel & Foster, 2006). Only 2% of *C. maschalocarpum* settlers survive to reproductive age (Schiel, 1985). Distributions of *C. angustifolium*, *C. maschalocarpum* and their hybrids might be structured by selection acting on zygotes and settlers. Settlement of zygotes can be affected by wave exposure (Vadas *et al.*, 1992). Attachment of zygotes to the substrate has been described as one of the most important events in the life cycle of an intertidal alga (Hardy & Moss, 1979). One small wave is able to remove 90% of settlers in some Fucalean species (Vadas *et al.*, 1990). The effect of wave exposure on the attachment ability of zygotes to the substratum varies between species (Taylor & Schiel, 2003; Vadas *et al.*, 1992). Taylor and Schiel (2003) found that the variations in attachment ability of three Fucalean algae (*Hormosira banksii*, *Cystophora torulosa*, *Durvillaea antarctica*) under different water flow conditions correlated with their adult distribution. They suggest early environmentally dependent selection on zygotes structures adult distribution patterns (Taylor & Schiel, 2003). The attachment ability of *C. angustifolium*, *C. maschalocarpum* and hybrid zygotes is largely unknown (Schiel & Foster, 2006). No study has yet investigated the effects of wave exposure on zygote attachment in *Carpophyllum*, and the zygotes of *Carpophyllum* hybrids have never been observed at all.

Hybrid distribution could be a reflection of the contact zone - the area where parental species distributions overlap. The extent of the contact zone is determined by parental species distribution, as well as parental gamete and hybrid zygote dispersal capacities. Dispersal capacity in the order Fucales is limited, as their eggs are relatively large, non-motile and often coated in mucus. Consequently the settlement of zygotes tends to occur within metres of parents (Chapman 1995, Schiel & Foster 2006). Schiel (1980) found three quarters of *C. angustifolium* and *C. maschalocarpum* recruits settled within four metres of parent plants. Such a low dispersal capacity results in a narrow contact zone, and makes it likely hybrid zygotes will settle in areas close to an individual of both parental species.

This extent of the contact zone in this study is not known, as only the top 0.5 m of *Carpophyllum* distribution were investigated. Distributions of *C. angustifolium* and *C. maschalocarpum* extend to 7 m depth (Schiel, 1990). In high wave exposed areas *C. angustifolium* is distributed in a belt of varying thickness above *C. maschalocarpum* (Dromgoole, 1973; Hodge, unpubl. data). That no hybrids were found in the top 0.5 m in the most exposed zone at Maraehako Bay despite both *C. angustifolium* and *C. maschalocarpum* individuals being only one metre apart by depth (Hodge, unpubl. Data), suggests environmental factors and not dispersal are limiting hybrid distributions.

This study found a pattern of distributions being correlated with wave exposure. Further work is required to investigate if and how wave exposure selection pressures create the distributions of *C. angustifolium*, *C. maschalocarpum*, and their hybrids. Previous studies have used field and laboratory experiments and field transplants to determine the

drivers of different species distributions relative to wave exposure environments (Correa *et al.*, 2006; Kawamata, 2001; Ladah *et al.*, 2003). Determining whether attachment strength differs between the two species and their hybrid, and experimentally comparing zygote attachment and adult morphology in different flow regimes in the laboratory are important next steps (Taylor & Schiel, 2003). Field transplants in this system would be difficult and involve high loss rates; however if possible it is a good way of comparing fitness. Previous attempts transplanting the sister species *Carpophyllum flexulosum* were not particularly successful (Travers, 1996). Further work should be done investigating *C. angustifolium*, *C. maschalocarpum* and hybrid distributions by depth. Are the hybrids distributed at intermediate depths relative to the parents?

Chapter 5: Morphological evidence of hybridisation in *Carpophyllum angustifolium* and *Carpophyllum maschalocarpum* populations from Leigh

5.1 Abstract

Hybridisation is a common and important evolutionary process. The future and genetic diversity of the parent species involved can be significantly affected by hybridisation. A previous study revealed hybridisation between the subtidal brown algae *Carpophyllum angustifolium* and *Carpophyllum maschalocarpum* at the East Cape of the North Island, New Zealand. This previous research used the molecular marker ITS2 but was unable to resolve hybrids at Leigh in northern New Zealand as shared ITS2 ribotypes were present in the parental species. In the present study the morphologies of individuals at Leigh were compared with the genetically identified individuals from the East Cape to investigate whether hybridisation is also occurring at Leigh. Individuals at Leigh were found to form morphological clusters similar to those at the East Cape, which suggests that hybridisation is occurring. *Carpophyllum angustifolium* and hybrid morphology differed between the Leigh and East Cape populations. This could be due to environmental differences, a lack of genetic connectivity, or differences in the level of introgression between the sites.

5.2 Introduction

Recent reviews have concluded hybridisation is more important and prevalent than previously thought (Grant *et al.*, 2005; Mallet, 2005). Hybridisation can affect biodiversity through hybrid speciation, parental species extinctions, and transfers of

genetic information between parental species (Seehausen, 2004). Twenty-five percent of plant and ten percent of animal species are estimated to be currently hybridising (Mallet, 2005). Hybridisation also occurs in the kingdom Chromista, although the extent of hybridisation is unknown. This kingdom contains the diverse and highly plastic brown macroalgae in the phylum Phaeophyceae. Detecting hybridisation in the phylum Phaeophyceae is particularly challenging due to the high levels of plasticity exhibited (Scott & Hardy, 1994).

Morphological methods have been used to identify wild hybrids. The assumption of hybrid morphological intermediacy underpins most morphological analyses (Estabrook *et al.*, 1996). Hybrid indices, for example, create a morphological continuum between two parent species, and identify hybrids as those lying in the intermediate region of the continuum (Anderson, 1953; Estabrook *et al.*, 1996; Wilson, 1992). However, identifying hybrids based on intermediate morphologies has limitations. First hybrids can have morphologies similar to, novel from, or more extreme than the parental species (Coyer *et al.*, 2002a; Lihova *et al.*, 2007; Seehausen, 2004). Secondly, problems arise in highly plastic and variable taxa such as macroalgae (Rieseberg & Ellstrand, 1993; Scott & Hardy, 1994). Finally morphological differences might merely reflect patterns of plasticity or environmental variation (Mathieson *et al.*, 1981).

Molecular techniques using variation in nuclear DNA have also been used to identify hybrids. The assumption in molecular analyses is that species specific markers from both parent species will be found in hybrids (Hegarty & Hiscock, 2005). The internal transcribed spacer (ITS) sequence is part of the ribosomal region in the nuclear DNA,

and is one of the most widely used sequences in plant evolutionary studies (Feliner & Rossello, 2007). ITS has been used in a variety of taxa to identify hybrids (Casteleyn *et al.*, 2009; Coyer *et al.*, 2008; Kauserud *et al.*, 2007). It is biparentally inherited, so hybrids are ITS heterozygotes where parental species have distinct ITS sequences. The heterozygosity of hybrids has been confirmed with artificially produced F₁ hybrids in macroalgae (Coyer *et al.*, 2002b). However ITS has limitations. It is part of a multi-gene family that can undergo concerted evolution (Alvarez & Wendel, 2003). Concerted evolution has the effect of homogenising all ITS gene copies, converting heterozygotes to homozygotes. Subsequently ITS can only detect recent hybridisation (Alvarez & Wendel, 2003).

Hybridisation between two subtidal brown seaweeds, *Carpophyllum angustifolium* J. Agardh and *Carpophyllum maschalocarpum* Turner (Sargassaceae), is occurring in New Zealand (Buchanan, unpubl. data). Intermediate 'hybrid' morphologies have been recorded in the literature since the 1960s (Dromgoole, 1965; Lindauer *et al.*, 1961), and molecular work has confirmed these to be hybrids in East Cape populations (Chapter 2). Buchanan (unpubl) found in the East Cape populations the two parental species each had a single distinct ITS2 ribotype, which allowed the ITS2 heterozygotes to be identified as hybrids. In populations further north at Leigh both parental species had multiple ITS2 ribotypes, and some ITS2 ribotypes were shared between species (Buchanan, unpubl.). The presence of shared ITS2 ribotypes means ITS2 heterozygotes are not necessarily hybrids. Consequently ITS2 cannot be used to identify hybrids. The presence of shared ITS2 ribotypes might be due to introgression, or alternatively to incomplete lineage sorting of the ancestral ribotypes in this population.

Recent work investigating the morphology of genetically identified individuals in the East Cape populations found the morphologies of parents and hybrids to be distinct (see Chapter 2). This study used an identical morphometric analysis to investigate the morphologies in the northern Leigh populations of *C. angustifolium*, *C. maschalocarpum* and their hybrids. Do the morphologies of individuals in the *C. angustifolium* \times *C. maschalocarpum* hybrid system in Leigh populations form distinct morphological groups? How does the morphology of Leigh individuals compare with the genetically identified East Cape individuals?

5.3 Methods

Sampling locations

Samples were collected from two sites in Leigh on the North Island of New Zealand (Fig. 1). The first site was located around Surge Rock (S36° 16.3', E174° 48.1'), and the second site was found at the Ray Rock area (S 36° 16.3', E174° 48.2'). The two sites are approximately 500 m apart and are separated by rocky islands. Sampling was conducted in mid February 2008 in the summer season. The genetically identified individuals used for comparison were collected from the East Cape in Chapter 2.

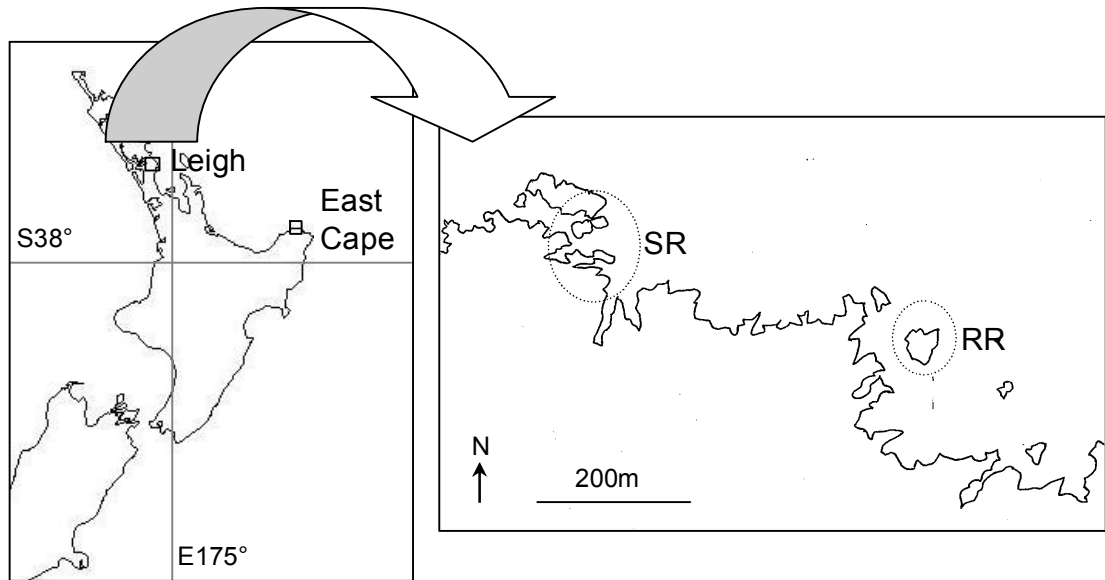


Figure 1: The location of sampling a) for the Leigh individuals and the East Cape reference individuals in New Zealand, and b) the two Leigh Sites: Surge Rock (SR), and Ray Reef (RR)

Sampling

Transect lines were laid along rock walls in the high subtidal, and quadrat locations were selected randomly for the top 0.5 m of *Carpophyllum* depth distribution. Quadrats were rejected if the rock face was not vertical, less than three individuals were present, or if the quadrat was less than 0.5 m from a previously sampled quadrat. All *Carpophyllum* individuals with holdfasts inside the 20cm by 20cm quadrats were collected. Thirty eight quadrats were sampled in the two sites, with 38 *C. angustifolium*, 41 *C. maschalocarpum* and 24 hybrid adults collected in total. Collected material was frozen at -4°C within a week of collection, and thawed in unfiltered seawater prior to morphometric analysis.

Morphometrics

Adults (longer than 20 cm) were morphologically identified, cleared of epiphytes, and measured using digital callipers. Only those individuals with complete haptera were used in morphometrics so as to avoid pseudo-replication (i.e. the doubling up of measurements from the same individual). Individuals were measured using digital callipers and a metre rule.

The morphometric characteristics selected for measurement were stipe width, lamina length and lamina width (Fig. 2). These were the characters which best distinguished between East Cape parent species and hybrids in Chapter 2. This study focused on the stipe and lamina characteristics of the primary axis (defined as the axis which arises directly from the holdfast). The stipe and lamina measurements were taken from approximately 5 cm from the tip of the axis. Lamina length was measured from the edge of the stipe to the tip of the lamina. Lamina and stipe width were measured at the widest point, perpendicular to an imagined mid-line. In some places herbivory had altered the morphology of the stipe and lamina; these could be detected by sudden and distinct changes in lamina or stipe outline. Measurements of stipe and lamina that were affected by herbivory were excluded, and if possible replaced by measurements from the nearest intact equivalent within 10 cm.

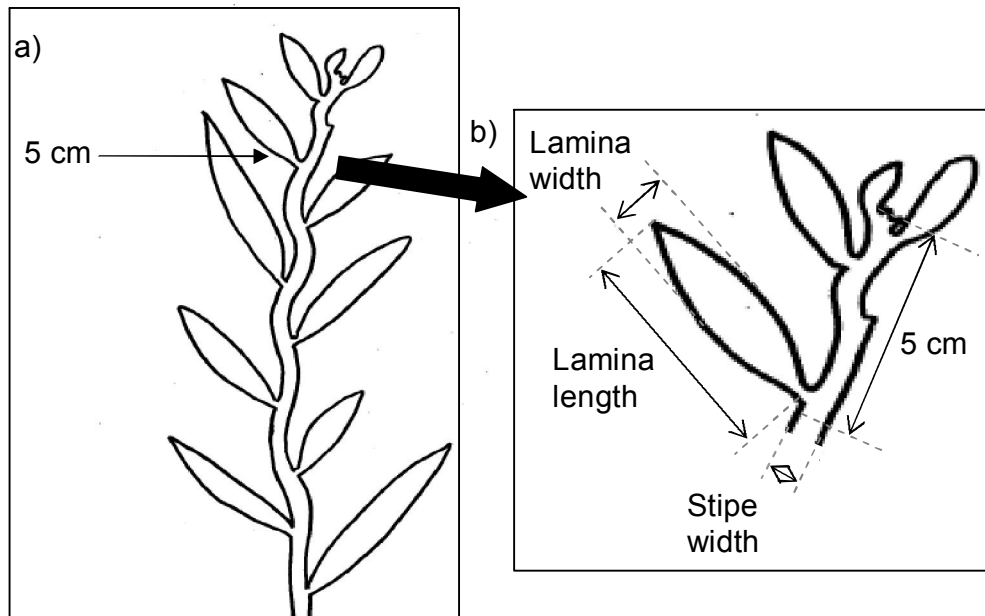


Figure 2: The location of the measurements taken from each individual a) on a stylised axis, and b) a close up of stipe width, lamina length and lamina width measurements.

Data Analysis

Cluster analysis finds natural groupings or clusters within the data (Fielding, 2007). These were done with the Leigh individuals using the software NTSYSpc, version 2.11(Rohlf, 2000). The unweighted pair-groups method average (UPGMA) clustering method was used, which is the most common and accepted method (Fielding, 2007), and also produced the highest cophenetic correlation. Boxplots were used to confirm divergence for the three morphological traits. The data were incorporated into a distance matrix using Gower's Index in Le Proiciel R 4.0 software (Casgrain & Legendre, 1999). This index was used as it can cope with missing values, and has been used in other studies on macroalgae morphology (Fowler-Walker *et al.*, 2006; Montanari & Mignani, 1994; Podani, 1999). Coenphetic values were calculated to test the fit of the cluster tree to the distance matrix (Rohlf, 2000; Rohlf & Sokal, 1981). The resulting

cluster tree was compared to the cluster analysis done on genetically identified individuals from the East Cape in Chapter 2.

The morphology of the Leigh clusters was compared with the genetically identified individuals from the East Cape. Stipe width, lamina length and lamina width were analysed with MANOVA where assumptions were met, and npMANOVA where assumptions were not met using SPSS 17.0 and PAST software (Hammer *et al.*, 2001). Assumptions of normality, equal variances and equality of covariance matrices were checked respectively with box plots, Levene's Test and Box's Test.

5.4 Results

Leigh morphologies

The cluster analysis of Leigh individuals revealed a tree with four distinct morphological clusters. This cluster tree was similar in structure to one produced for individuals genetically identified from the East Cape (Fig. 3). However, the clusters were more morphologically similar to each other at Leigh than at the East Cape, with an overall similarity coefficient of 0.60 compared to 0.49.

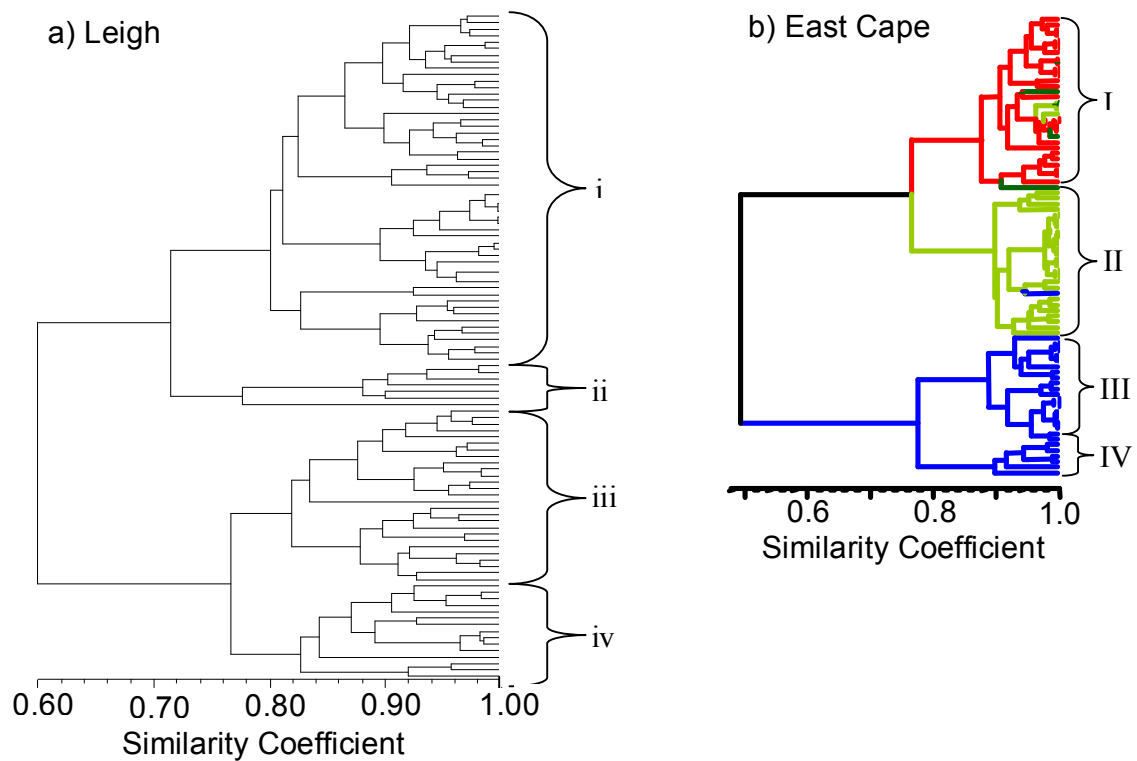


Figure 3: The morphological cluster trees of a) randomly sampled individuals from Leigh, and b) genetically identified individuals from the East Cape. Individuals cluster into four different groups on both trees as labelled i to iv at Leigh and I to IV at the East Cape. Colours on b) represent the identity of the individuals. *Carpophyllum angustifolium* is shown in red, *C. maschalocarpum* in blue, hybrids in light green, and putative backcrosses in dark green. The coenphetic value for the trees are 0.76 for Leigh, and 0.84 at the East Cape.

The four morphological clusters identified at Leigh were analogous to the clusters of genetically identified East Cape individuals, which allowed hypotheses of the identity of Leigh clusters. There were differences in the multivariate morphologies between Leigh clusters and their hypothesised taxa from the East Cape (Fig. 4). Leigh individuals in cluster I had different multivariate morphologies from the genetically identified *C. angustifolium* individuals from the East Cape ($n=64$, $F=8.531$, $p=0.0033$). The multivariate morphologies of Leigh individuals in cluster II differed from the confirmed

hybrids from the East Cape ($n=18$, $F=14.11$, $p=0.0019$). The multivariate morphology of clusters III and IV did not differ from that of *C. maschalocarpum* in the East Cape ($n=52$, $F=0.05$, $p=0.985$).

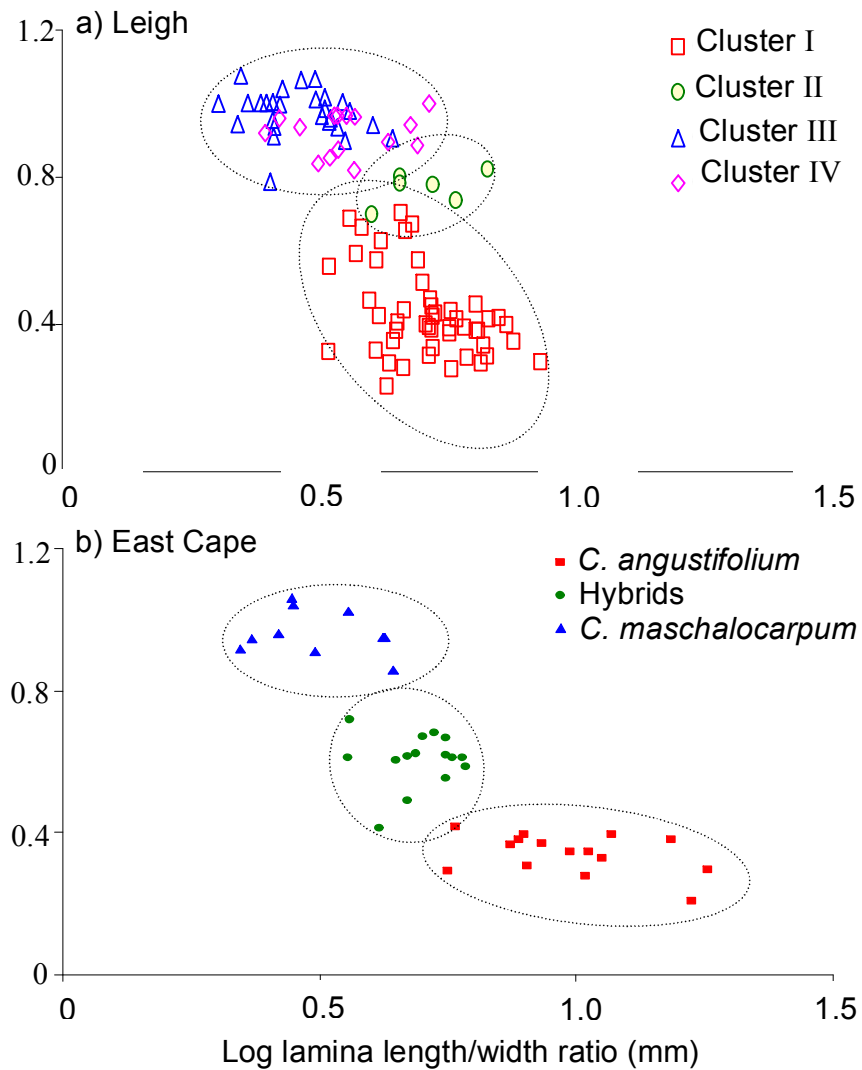


Figure 4: The multivariate morphology of a) the individuals from Leigh, and b) the genetically identified individuals from the East Cape. Circles with dashed borders show the general distributions of species and hybrids. Leigh individuals are plotted according to assigned cluster, and the dashed borders surrounds hypothesised identities based on the East Cape tree. NB clusters III and IV were both genetically *C. maschalocarpum* in East Cape cluster analysis.

5.6 Discussion

The individuals from Leigh formed a cluster tree with the same branching and shape as the cluster tree of individuals with known hybrids from the East Cape. *Carpophyllum angustifolium* and *C. maschalocarpum* produce hybrids with intermediate morphology at the East Cape, which form a morphological cluster related to *C. angustifolium* (Chapter 2). The presence of a similar shaped tree, and a comparable ‘hybrid’ morphological cluster in the Leigh cluster tree strongly suggests that hybridisation is also occurring at Leigh.

The *C. angustifolium* and hybrid morphological clusters at Leigh significantly differed from their genetically identified equivalents at the East Cape site 300 km away. Differences in *Carpophyllum* morphology have previously been found between sites less than a kilometre apart (Chapter 3). The variation found in between Leigh and East Cape morphological clusters could be due to several reasons. First environmental differences between the two sites could result in morphological differences through phenotypic plasticity. Secondly the morphological differences could reflect genetic divergence between the populations. Thirdly differing levels of introgression could be affecting *C. angustifolium* and hybrid morphologies at the two sites.

Macroalgae are highly plastic organisms whose morphologies are influenced by environmental conditions (Fowler-Walker *et al.*, 2006; Hurd, 2000; Johnson & Koehl, 1994; Kawamata, 2001). Leigh and the East Cape are located approximately 300 km apart, and are likely to experience different local environmental conditions. One major environmental difference between the two locations is their fetch, and consequently

wave exposure. Leigh is sheltered by the Coromandel Peninsula and a range of offshore islands (including Great Barrier Island). By comparison, the East Cape is completely open to the Pacific Ocean. The extent of wave exposure is likely to be much lower at Leigh. Wave exposure has been found to affect total length, stipe thickness and the presence of vesicles in *C. angustifolium*, *C. maschalocarpum* and their hybrids (Chapter 3). However, none of these traits were measured in this study. This study investigated only stipe width, lamina width and lamina length; none of which showed significant or non-significant trends in variation between wave exposure zones (Chapter 3). Reduced wave exposure would affect *C. angustifolium* and the hybrids more than *C. maschalocarpum* because of their relative wave exposure distributions. *Carpophyllum angustifolium* and the hybrids tend to be distributed in moderately exposed to exposed areas, while *C. maschalocarpum* is distributed in more sheltered areas (Chapter 4). The lack of difference between Leigh and East Cape *C. maschalocarpum* morphology could be explained by the presence of similarly sheltered areas at Leigh and the East Cape (see Chapter 4).

Morphological differences between Leigh and East Cape individuals could reflect the level of genetic connectivity between populations, and the different dispersal capacities of the two species. Leigh and the East Cape are separated by approximately 500 km of coastline. *Carpophyllum* have low dispersal capacity due to their large non-motile eggs, which in some cases are fertilised *in situ* on the maternal plant (Delf, 1939). Schiel (1980) found that three quarters of recruits settled within four metres of parent *C. angustifolium* and *C. maschalocarpum* plants. Rafting can allow long distance dispersal in some macroalgae. The potential for rafting differs between *C. angustifolium* and *C.*

maschalocarpum due to morphological differences. Vesicles can provide the buoyancy required for rafting, and are present in some *C. maschalocarpum* individuals but are completely absent in *C. angustifolium* (Chapter 2, Adams, 1994; Dromgoole, 1990). Rafting has the potential to move genetic material large distances, and could explain the morphological similarity between *C. maschalocarpum* individuals from Leigh and the East Cape.

Alternatively *C. angustifolium* and hybrid morphology could differ between Leigh and the East Cape because of differing levels of introgression, i.e. the backcrossing of hybrids with parental species. Introgression can facilitate the transfer of genetic information from one parental species to another (Seehausen, 2004), and therefore can affect the morphologies of parental species (Gammon *et al.*, 2007; Grant *et al.*, 2004; Lihova *et al.*, 2007). Eggs and sperm have been found in the hybrids, during the reproductive period of both parental species (Buchanan & Hodge, pers. obs.). East Cape individuals have been found with *C. angustifolium* morphology and hybrid ITS2 ribotype suggesting introgression (Chapter 2). That only *C. angustifolium* and not *C. maschalocarpum* backcrosses were found in the previous study suggests introgression could be one way, resulting in genetic modification of *C. angustifolium* only.

This study finds morphological support for hybridisation between *C. angustifolium* and *C. maschalocarpum* at Leigh. Further study needs to be done to investigate the morphological differences found in *C. angustifolium* and the hybrids between Leigh and the East Cape. Laboratory and field experiments need to be conducted to investigate the role of the environment on *Carpophyllum* morphology. Knowledge of the genetic

structure of populations at different spatial scales would add significantly to the existing information on egg and zygote dispersal. The presence and extent of introgression in this hybrid system needs to be explored with microsatellite data, or by comparing patterns in uniparental and biparental markers.

Chapter 6: General Discussion

The process of hybridisation has been identified as a common and important evolutionary force (Arnold, 1997; Morgan-Richards *et al.*, 2009; Seehausen, 2004). However, the vast majority of research into the topic is based on terrestrial plant and animal systems (Gardner, 1997). A New Zealand recent review made no mention of hybridisation outside the plant and animal kingdoms (Morgan-Richards *et al.*, 2009). The presence of hybridisation between the brown algae *Carpophyllum angustifolium* and *Carpophyllum maschalocarpum* provided an opportunity to investigate hybridisation in a marine system, and in species from the kingdom Chromista. Hybrids have been identified in other species of brown algae, in particular within the order Fucales (Billard *et al.*, 2005; Coyer *et al.*, 2006b; Wallace *et al.*, 2004). However, previous study on hybridisation in the order Fucales has not included a detailed morphometric analysis combined with molecular data, nor a detailed study of distribution (Coyer *et al.*, 2007; Scott & Hardy, 1994). In this case hybridisation resulted in morphologically intermediate hybrids which were distributed in intermediate wave exposure zones.

Morphology

Carpophyllum angustifolium, *C. maschalocarpum* and their hybrids had distinct and intermediate morphologies in this study. Hybrids have generally been found to have intermediate morphologies; although novel, similar and more extreme morphologies have also been found (Arnold & Hodges, 1995; Rieseberg & Ellstrand, 1993; Seehausen, 2004). Identifiable hybrid and parental morphotypes have been found within many *Fucus* hybrid species complexes but morphologically ambiguous individuals also

exist (Coyer *et al.*, 2002; Kucera & Saunders, 2008; Scott & Hardy, 1994). The presence of hybrid genotypes with distinctive *C. angustifolium* morphotypes suggests that asymmetrical introgression is occurring. Introgression has been identified in *Fucus* hybrid complexes, and determined as a significant factor in the evolution of *Fucus* lineages (Coyer *et al.*, 2006a; Coyer *et al.*, 2007; Wallace *et al.*, 2004).

Morphology and wave exposure

Some aspects of *Carpophyllum angustifolium* and *C. maschalocarpum* morphology were found to be correlated with wave exposure. In the more wave exposed zones *Carpophyllum angustifolium* individuals were longer, while *C. maschalocarpum* individuals were shorter, had thinner stipes and less frequent vesicle presence. There were also trends of *C. maschalocarpum* individuals having thinner lamina, and lower branch presence in higher wave exposures. Some of the patterns were as expected, namely that morphological traits which reduced drag would be present in high wave exposure zones. Some of these patterns, namely total length in *C. angustifolium* and stipe and lamina width in *C. maschalocarpum* were the opposite of those generally found in macroalgae with regard to wave exposure (Fowler-Walker *et al.*, 2006; Johnson & Koehl, 1994; Kawamata, 2001; Stewart & Carpenter, 2003). It is hypothesised that these patterns could be a result of flexibility reducing the importance of morphological traits in high wave exposures. Flexibility can allow morphological reconfiguration, which can reduce the drag forces macroalgae are exposed to (Carrington, 1990; Denny, 2006). Some flexible macroalgae can have reduced drag in higher flows (Carrington, 1990; Gaylord *et al.*, 1994). The patterns of thinner laminae and stipes in *C. maschalocarpum* could be a reflection of the age of these components in different wave

exposure zones. Laminae and stipes get thicker with age, and individuals and individuals and their components are often younger in more exposed areas due to wave damage (Dudgeon & Johnson, 1992).

That the relationships between wave exposure and morphology differed between the two species could be due to their different wave exposure distributions. High levels of phenotypic plasticity have been hypothesised as being associated with generalist species, as opposed to specialist species (Sultan, 2000). *Carpophyllum maschalocarpum* had a much broader wave exposure distribution than *C. angustifolium*. *Carpophyllum angustifolium* was only found in the two most exposed zones, which suggests specialisation to high wave exposure. *Carpophyllum maschalocarpum* was found in all four wave exposure zones, which indicates it is more of a generalist species.

Distribution

The distributions of *C. angustifolium*, *C. maschalocarpum* and their hybrids were found to be correlated with wave exposure. *Carpophyllum angustifolium* was only distributed in the relatively high wave exposed zones, while *C. maschalocarpum* was distributed mainly in the more sheltered zones, as hypothesised by Dromgoole (1973), Lindaeur (1961) and Adams (1994). Hybrids were distributed in intermediate wave exposure zones where both parental species were present. The presence of an intermediate hybrid distribution relative to environmental conditions in parental species' distributions has been found in other species, and is often a result of environmentally dependent hybrid fitness (Campbell & Waser, 2001; Campbell & Waser, 2007; Fritsche & Kaltz, 2000; Miglia *et al.*, 2005). *Carpophyllum* hybrids with their intermediate morphology could be

more successful in intermediate wave exposures. In the only other studies on Fucalean hybrid distribution the hybrid with extreme morphology occurred in extreme environments, and the hybrid with intermediate morphology was distributed in intermediate environments, (Coyer *et al.*, 2007; Scott & Hardy, 1994).

The intermediate wave exposure zones where *Carpophyllum* hybrids were found were also the zones with parental species overlap: the contact zone. The intermediate distribution could be a reflection of contact zones, parental gamete dispersal capacity and hybrid zygote dispersal. Difficulties arise in teasing apart dispersal limitation from environmental selection in this study, as the true extent of the contact zone was not determined. Parental species occur below the top 0.5 m depth investigated, and subsequently the contact zone could extend beyond this depth.

Hybridisation at Leigh

The individuals from Leigh formed a cluster tree with the same branching and shape as the cluster tree of individuals with known hybrids from the East Cape. This strongly suggests that hybridisation is also occurring at Leigh. That *C. angustifolium* and hybrid morphological clusters at Leigh significantly differed from their genetically identified equivalents at the East Cape site could be due to environmental differences, genetic divergence or differing levels of introgression between the two sites. Leigh and the East Cape are located approximately 300 km apart, and are likely to experience different local environmental conditions. One major environmental difference between the two locations is their fetch, and consequently wave exposure. The extent of wave exposure is likely to be much lower at Leigh. However, none of the traits measured on individuals at

Leigh were found to be affected by wave exposure in Chapter 3. Morphological differences between Leigh and East Cape individuals could reflect the level of genetic connectivity between populations, and the different dispersal capacities of the two species. Leigh and the East Cape are separated by approximately 500 km of coastline. *Carpophyllum* have low dispersal capacity, with recruits typically settling within metres of parent plants (Schiel, 1980). However, rafting can allow long distance dispersal in *C. maschalocarpum* due to the presence of vesicles (Chapter 2, Adams, 1994; Dromgoole, 1990). Rafting has the potential to move genetic material large distances, and could explain the presence of morphological differentiation between Leigh and the East Cape in *C. angustifolium* and not *C. maschalocarpum*. Alternatively *C. angustifolium* and hybrid morphology could differ between Leigh and the East Cape because of differing levels of introgression, i.e. the backcrossing of hybrids with parental species. Introgression facilitates transfer of genetic information from one parental species to another (Seehausen, 2004), which can affect the morphologies of parental species (Gammon *et al.*, 2007; Grant *et al.*, 2004; Lihova *et al.*, 2007). Eggs and sperm have been found in the hybrids during the reproductive period of both parental species (Buchanan & Hodge, pers. obs.). East Cape individuals have been found with *C. angustifolium* morphology and hybrid ITS2 ribotype suggesting introgression (Chapter 2). That only *C. angustifolium* and not *C. maschalocarpum* backcrosses were found in the previous study suggests introgression could be one way, resulting in genetic modification of *C. angustifolium* only.

Limitations of this study

This study investigated wave exposure categorically, which has several flaws. First non-linear relationships between morphology and wave exposure can be distorted and misinterpreted when exposure is categorised (Kitzes & Denny, 2005). Secondly relationships between wave exposure and morphology could be obscured or confused by small scale wave exposure variability. In a study aimed to compare algal morphology in exposed, semi-exposed and protected sites; Eckman (2003) found 30-50% of variation in water flow occurred between replicates at the same site. Attempts in this study to deploy dynamometers to measure maximum velocities were unsuccessful. Research with continuous measures of wave exposure could be limited by the lack of a comprehensive method for such measurements in the high wave exposed zones.

Future directions

The value of ITS for confirming and determining the extent of introgression is limited due to homogenisation of ITS (Alvarez & Wendel, 2003). Microsatellites have a high mutation rate and large variability, and have been used successfully to resolve relationships in a *Fucus* hybrid complex (Coyer *et al.*, 2006b; Coyer *et al.*, 2007; Engel *et al.*, 2005; Wallace *et al.*, 2004). Introgression can also be identified by comparing uniparentally inherited DNA, such as mtDNA or cpDNA, with nuclear DNA in the putative hybrids and introgressed individuals (Coyer *et al.*, 2007; Coyer *et al.*, 2002). Further investigation of this hybrid system using microsatellites and maternally inherited DNA in conjunction with morphology will have greater power to confirm and determine the extent and symmetry of introgression, and identify backcrosses, F₂ and later hybrids. Research investigating the attachment strength and tenacity of holdfasts for the parent

species and hybrids would provide insight into fitness in different wave exposure environments. Furthermore laboratory experiments investigating drag on different *Carpophyllum* morphologies in different flow regimes would also help resolve the importance of morphology in *Carpophyllum* distribution. Laboratory studies also need to be conducted to investigate how wave exposure affects zygote attachment, whether it differs between the two species and hybrids, and whether it could be structuring adult distributions. No study has yet fully investigated the reproductive potential of *Carpophyllum* hybrids; determining the quality and quantity of eggs and sperm produced will provide information on the potential for introgression in this species complex.

Conclusions

This study found *C. angustifolium* × *C. maschalocarpum* hybrids to have intermediate and distinct morphology from the parent species, as well as evidence which suggests introgression is occurring. Also some morphological traits in the parental species were found to be correlated with wave exposure. The distributions of the parental species and hybrids were also correlated with wave exposure, with hybrids occurring in intermediate areas. Hybrids were only distributed in wave exposure zones with parental species overlap. Finally the study found morphological evidence of hybridisation in northern populations where parental species have overlapping ITS2 sequences.

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Appendix 1: Cluster tree of all of the morphologically identified individuals with East Cape

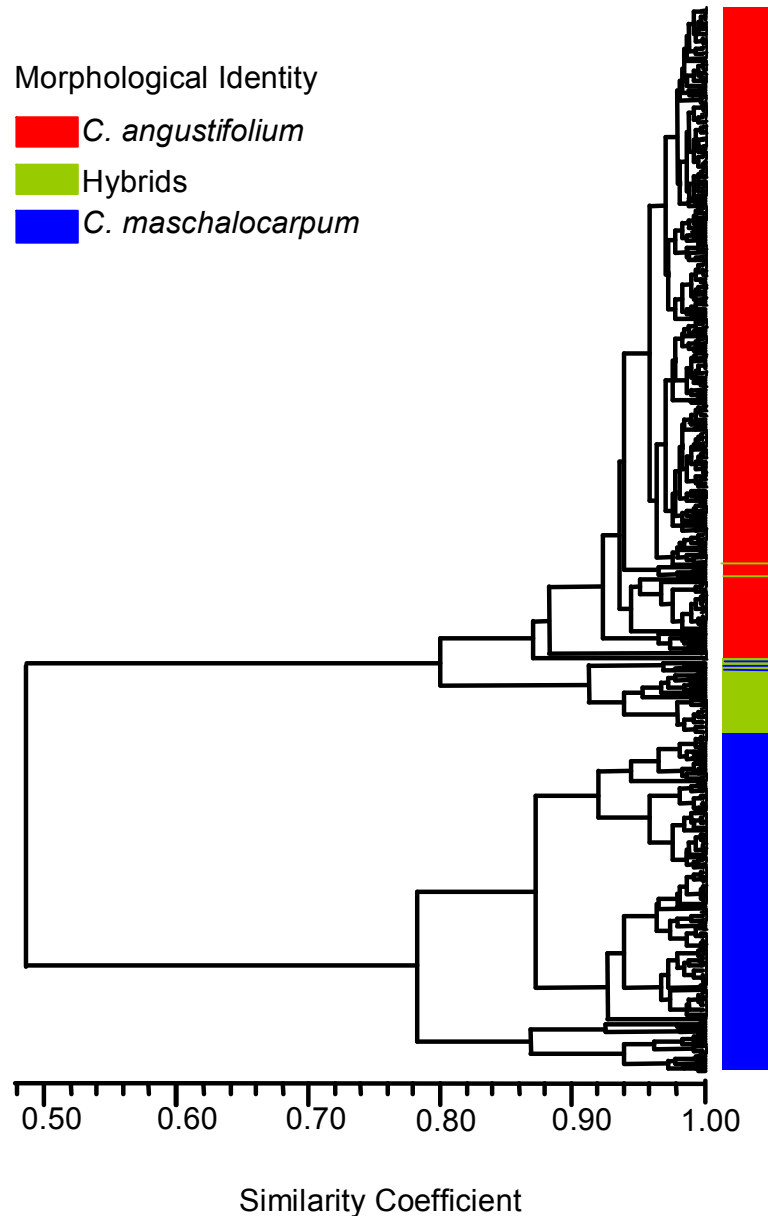


Figure 1: Cluster analysis of all the morphologically identified individuals from the East Cape. The coloured bars on the right of the tree show the morphological identity of individuals. The tree shows the same branching and structure as the tree of genetically identified individuals from the East Cape. The coenphenetic value of this tree is 0.90 indicating an excellent fit of the tree to the original data matrix.

Appendix 2: Herbarium specimens

Representative specimens from this study have been deposited in the Te Papa herbarium.

The WELT numbers of each specimens are listed below:

WELT No.	Identification Details	Collection location
A029610	<i>Carpophyllum angustifolium</i> J.Agardh	Leigh
A029611	<i>Carpophyllum angustifolium</i> J.Agardh	Leigh
A029612	<i>Carpophyllum angustifolium</i> J.Agardh	Leigh
A029613	<i>Carpophyllum angustifolium</i> J.Agardh	Leigh
A029614	<i>Carpophyllum angustifolium</i> J.Agardh	Leigh
A029615	<i>Carpophyllum x angustifolium x maschalocarpum</i>	Leigh
A029616	<i>Carpophyllum x angustifolium x maschalocarpum</i>	Leigh
A029617	<i>Carpophyllum x angustifolium x maschalocarpum</i>	Leigh
A029618	<i>Carpophyllum x angustifolium x maschalocarpum</i>	Leigh
A029619	<i>Carpophyllum x angustifolium x maschalocarpum</i>	Leigh
A029620	<i>Carpophyllum maschalocarpum</i> (Turner) Grev.	Leigh
A029621	<i>Carpophyllum maschalocarpum</i> (Turner) Grev.	Leigh
A029622	<i>Carpophyllum maschalocarpum</i> (Turner) Grev.	Leigh
A029623	<i>Carpophyllum maschalocarpum</i> (Turner) Grev.	Leigh
A029624	<i>Carpophyllum maschalocarpum</i> (Turner) Grev.	Leigh
A029625	<i>Carpophyllum angustifolium</i> J.Agardh	East Cape
A029626	<i>Carpophyllum angustifolium</i> J.Agardh	East Cape
A029627	<i>Carpophyllum angustifolium</i> J.Agardh	East Cape
A029628	<i>Carpophyllum angustifolium</i> J.Agardh	East Cape
A029629	<i>Carpophyllum angustifolium</i> J.Agardh	East Cape
A029630	<i>Carpophyllum x angustifolium x maschalocarpum</i>	East Cape
A029631	<i>Carpophyllum x angustifolium x maschalocarpum</i>	East Cape
A029632	<i>Carpophyllum x angustifolium x maschalocarpum</i>	East Cape
A029633	<i>Carpophyllum x angustifolium x maschalocarpum</i>	East Cape
A029634	<i>Carpophyllum x angustifolium x maschalocarpum</i>	East Cape
A029635	<i>Carpophyllum maschalocarpum</i> (Turner) Grev.	East Cape
A029636	<i>Carpophyllum maschalocarpum</i> (Turner) Grev.	East Cape
A029637/A	<i>Carpophyllum maschalocarpum</i> (Turner) Grev.	East Cape
A029637/B	<i>Carpophyllum maschalocarpum</i> (Turner) Grev.	East Cape
A029638/A	<i>Carpophyllum maschalocarpum</i> (Turner) Grev.	East Cape
A029638/B	<i>Carpophyllum maschalocarpum</i> (Turner) Grev.	East Cape