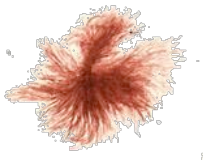

The coexistence of cryptic algal species

Temporal and spatial distribution of filamentous members of the red algal order Bangiales in the Wellington region

Thesis for Master of Science Part II

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September 2013

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Acknowledgments

I would like to thank Joe Zuccarello for supervising my thesis, and agreeing to take me on as a student, before he'd even arrived in New Zealand! Your unfailing enthusiasm for my topic and patience at teaching the molecular techniques were much appreciated. Shane Geange significantly improved this thesis by helping me with the statistical analyses in Chapter 3, and undertaking critical reviews of my other chapters. Thank you.

Thanks to Wendy Nelson for inspiring me to study algae when I worked as her assistant at Te Papa, and for supplying culture material and helping me locate known populations of *Bangia*. Peter Martin, thank-you for sequencing a number of my samples, an exercise that unexpectedly and interestingly revealed new sequences. Thanks to Judy Sutherland for providing her files of Bangiales sequence data. Most of all, thanks to my family and children who have supported, encouraged and tolerated me through this journey. At last Maia (my eldest), here is my "seaweed book".

Terminology

Lineage or species

In this thesis the following terms are used to refer to filamentous Bangiales entities: lineages, species, entities and taxa. Some researchers prefer the term lineage, as distinctions between taxa in this group are largely based on sequence data rather than morphological characters. Others prefer to use the term species. For the purposes of this thesis both terms are used interchangeably. When naming individual entities this thesis follows the convention of Sutherland et al. (2011) i.e., lineage BFK is referred to as *Bangia* sp. BFK.

Filamentous Bangiales

The term "filamentous Bangiales" is used throughout this thesis to include both *Minerva aenigmata* and filamentous *Bangia* spp. At study sites within Wellington where *M. aenigmata* has not been recorded, the term "*Bangia* spp." has occasionally been substituted.

Chapter 1. Introduction

Current research on marine cryptic species, through the use of molecular tools, is revealing unexpected diversity and relationships. Species, such as the red alga *Bangia fuscopupurea,* which were once thought to be a single globally distributed entity, are being revealed as a suite of species with localized distributions (Sutherland et al. 2011, Nelson et al. 2005). The human-mediated transport of species around the world and along coastlines, largely via hull-fouling and ballast-water (Ruiz & Carlton 2003), has further complicated patterns of distribution: potentially resulting in the coexistence of some cryptic species due to unnatural means.

Cryptic Species

There are many different concepts of what constitutes a species. "It may not be an exaggeration if I say that there are probably as many species concepts as there are thinking systematists and students of speciation" (Mayr 1942). Traditionally species, the basic category on which biology is based, were defined using morphological characters, such as length of blade or pattern of branching. The discovery, however, that some mosquito species of the genus *Anopheles* (known to vector the disease malaria), are actually a complex of morphologically identical species (e.g., Hackett 1934), dramatically advanced programmes to control malaria, and also raised the need for a new concept in species (Mayr 1942).

In 1942 Mayr developed the 'Biological Species Concept', which defines species based on the genetic isolation of populations, rather than on morphological characters. Mayr

proposed that species are “groups of actually or potentially interbreeding natural populations which are reproductively isolated from other such groups” (Mayr 1942).

Mayr’s biological species concept has some limitations as it cannot be applied to asexual species or to species which occasionally hybridise. In addition, breeding tests to define species limits are not always feasible. An alternative concept, which uses evolutionary history as revealed by genetic data, and does not require breeding tests, is the Phylogenetic Species Concept. The definition of a species under this concept is that a species is a “tip” on a phylogeny: a basal exclusive group of organisms all of whose genes coalesce more recently with each other than with those of any organisms outside the group, and that contains no exclusive group within it (Baum & Donoghue 1995).

Regardless of which species concept researchers subscribe to, cryptic species are a real biological phenomenon, and their existence invites further research. There are two scenarios covered by this definition of “morphologically indistinguishable”: either the different species all look the same (e.g., cryptic species of the genus *Bangia,* Farr et al. 2001;sponges, Nichols & Barnes 2005; marine clams *Lasaea australis*, Li et al. 2013), or, the morphology is variable but does not help in defining species limits (e.g., bonefish, Colborn et al. 2001; whalefish, Johnson et al. 2009;sea slugs of the genus *Pontohedyle*, Jörger et al. 2012).

Some authors use the term “pseudo-cryptic” for species for which a morphological identifier has been found, so that they can be distinguished from one another (Saez & Lozano 2005). The other use of the word “cryptic” or “crypsis” in the scientific literature refers to organisms which are hard to detect, such as those which use patterns

of colouration or decoration to camouflage themselves. For the purposes of this thesis "cryptic" means those species that are hard to distinguish from each other, not those which are camouflaged or secretive.

Records of cryptic species have increased dramatically over the last two decades, as more surveys of DNA variation are undertaken (see Fig 1.1 from Bickford et al. 2007, also Saez & Lozano 2005).

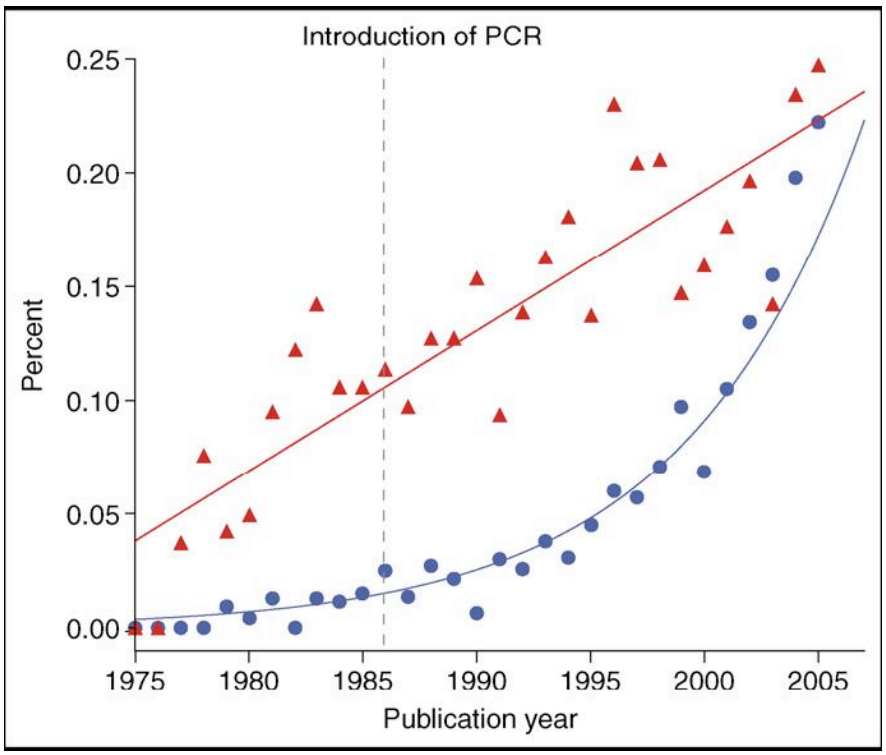


Fig 1.1. Study of cryptic species has increased exponentially over the past two decades. The percentage of peer-reviewed publications in Zoological Record Plus (CSA) that mention 'cryptic species' (circles) or 'sibling species' (triangles) in the title, abstract, or keywords has increased dramatically since the advent of PCR. Similar positive trends are observed in absolute number of publications per year, and in publications cited in other searchable databases of biological literature. Source: Bickford et al. 2007.

Cryptic species may represent optimal phenotypes, and so strong stabilising selection keeps their morphology similar despite the lack of gene flow between groups (Williamson 1987). Divergence could still have occurred in some respect (habitat, life history, chemical recognition system) but the morphology has been retained (Knowlton 1993). It is plausible that the nature of the environment itself encourages cryptic species

formation. For example, the stresses of the aquatic environment (and particularly the marine environment) are known to restrict the potential morphology and physiology of many plants, limiting the phenotypic differences between species (Niklas 1997, Knowlton 1993).

The simple filaments of the Bangiales, the subject of this thesis, may represent a highly successful structure for survival in the upper inter-tidal. *Bangia*-like organisms have persisted with little morphological change for some 1200 Myr (Butterfield 2000; Butterfield et al. 1990). In addition, *Bangia*-like fossils from deposits dated at 425 Myr (Campbell 1980) have also been found. The filament width and cell size of extant filamentous Bangiales in New Zealand show small variations in filament width and cell size between lineages, but the differences are so slight that a practical field method of identification is not possible (Bödeker et al. 2008), with one exception: *Dione arcuata* which has distinctly wider filaments (Nelson et al. 2005).

In the marine realm it is also proposed that the increasing discovery of cryptic species may be an indication of inadequate study ("pseudo-cryptic species"). Pfenninger & Schwenk (2007) reviewed the literature on cryptic species and found that once study intensity bias and species richness are accounted for, cryptic species (in animals anyway) are evenly distributed among major metazoan taxa and biogeographical regions.

Identifying cryptic species is important for conservation and for measures of species richness. Knowlton's review of cryptic species in the sea (1993), reported that cryptic species are common in all major marine groups and habitats, and that the number of

marine species would increase by an order of magnitude if cryptic species are considered. For example, a study of the planktonic groups (coccolithophores, diatoms and foraminifers) found 33 genetic species from 9 morpho-species (Thierstein & Young 2004), and a recent study of the sea slug genus *Pontohedyle* increased the species number from 2 to 12 (Jörger et al. 2012).

Discovery of cryptic species also has significant implications in the management of fisheries and monitoring of environmental quality. In fisheries cryptic species have been identified in *Crassostrea*, *Nototodarus, Penaeus, Menippe* and *Sphyrna* (e.g., Cordes et al. 2005, Quattro et al. 2005, Sekino & Yamashita 2013). Some taxa used to monitor environmental quality have been found to consist of several cryptic species: *Capitella, Montastraea, Mytilus* and *Baetis rhodani* (e.g., Grant et al. 1984, Williams et al. 2006).

Learning more about cryptic species can advance understanding of speciation processes, and the relationship between morphology and phylogeny. Their existence challenges an intuitively visual (morphological) concept of species, and requires researchers to think critically about what species are, why they look the way they do, and what evolutionary processes have shaped them.

Coexistence and niche partitioning

When cryptic species are found living in sympatry, as in the case of the Bangiales lineages in Wellington, and indeed throughout New Zealand (see Fig1.2), the question arises: how do cryptic species coexist? Hutchinson (1961) called this situation, where multiple species coexist and compete for the same resources, the "Paradox of the Plankton".

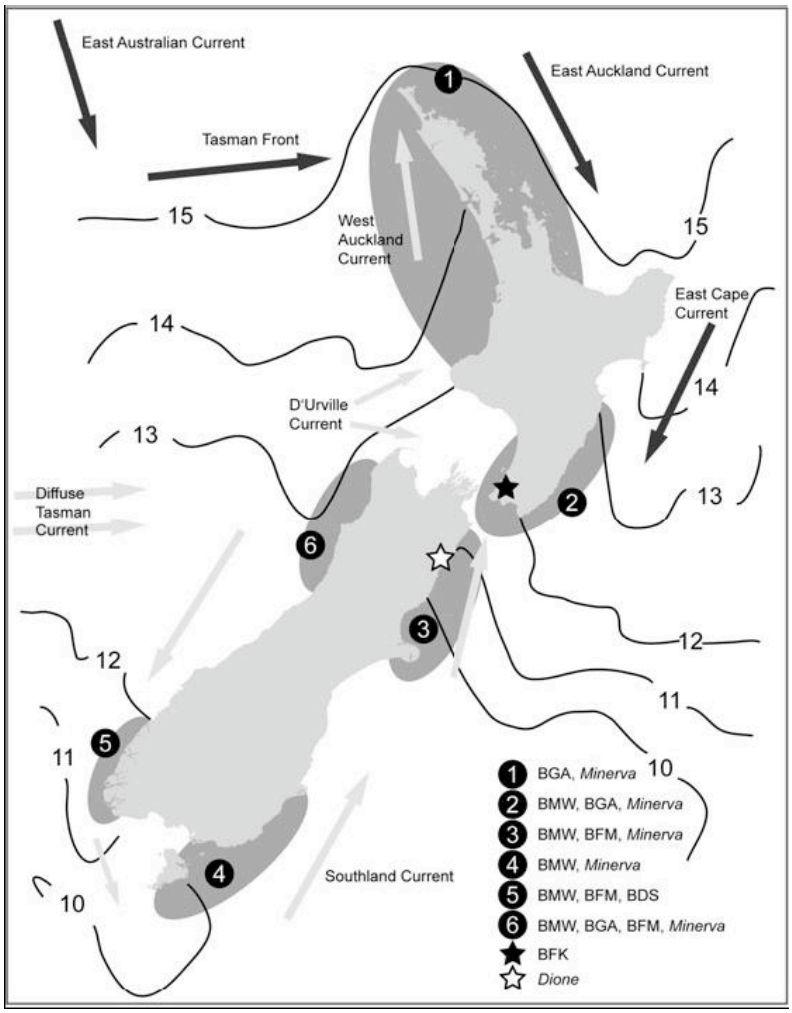


Fig 1.2. Map of New Zealand showing collection sites/putative distribution (gray ovals) of the taxa as indicated by numbers. The taxa identified from Wellington include those in group 2 (BMW, BGA and Minerva) as well as BFK. Single local collection regions of Bangia sp. BFK (Wellington) and Dione arcuata (near Kaikoura) are marked with a black and a white star, respectively. Thin black lines represent winter isotherms, numbers on lines are winter sea surface temperatures (°C) in August lighter arrows indicate sub-Antarctic currents, darker arrows subtropical currents. Oceanographic data from the National Institute for Water and Atmospheric Research, New Zealand (NIWA 2007). Source: Bödeker et al. (2008).

The classical model of competition developed independently by both Lotka (1925) and Volterra (1926), predicts that stable coexistence will occur between a pair of species if each inhibits its own population growth (through intraspecific competition) more than it inhibits that of the other species (through interspecific competition). Stable coexistence

is most likely to be reached if both species occupy separate niches. This is expressed in Gause's (1932) principle, which states that stable coexistence between competing species requires them to occupy different niches: if two species were to occupy the same niche one would eventually competitively exclude the other, driving it to extinction.

The niche concept was originally conceived by zoologists Joseph Grinnell (1917) and Charles Elton (1927), and emphasised differences in diet and foraging patterns to explain the coexistence of animals. This "trophic niche" as described for animals is not as applicable to the coexistence of plants which all require similar resources such as water, nutrients and UV light (Silvertown 2004). However, plants do vary in their resource requirements and tolerances e.g., UV levels, nutrients, moisture; and these differences can help to explain their coexistence.

Numerous theoretical models have been proposed to progress niche theory and explain how plant species coexist. Reviews by Wilson (2011, 1990) identify twelve theories that attempt to explain coexistence in plant communities: some depending on factors such as disturbance or succession or seed storage effects. Of the twelve theories reviewed, environmental fluctuation (including seed storage effects), allogenic disturbance, alpha-niche differentiation, and pest pressure were considered to be important; however this does not discount the other theories as more studies are required (Wilson 2011).The competition-colonisation trade-off model, originally proposed by Skellam (1951) and further developed by other researchers (e.g., Hastings 1980, Tilman 1994), suggests that the ability to colonise recruitment sites may compensate for the lack of interspecific competitive ability in some species, although researchers would argue that ultimately stable coexistence still requires a level of niche differentiation. The resource ratio model

proposes that spatial or temporal variation in just two resources can enable more than two competitors to coexist (Tilman 1985).

Niche theory, as proposed by Hutchinson (1957), distinguishes between a species' fundamental niche, its realised niche and its α niche. A fundamental niche is the region of its niche that a species can occupy in the absence of interspecific competition and natural enemies. The realised niche is that region it can occupy in the presence of interspecific competition and natural enemies. A species' α niche is community specific: in other words it is that region of the realised niche corresponding to species diversity at the local community scale at which interactions among species occur. Niche differences at the α scale are expected to facilitate coexistence.

Some researchers have called for an increased focus on niche partitioning and coexistence theory, arguing that the concept of the niche has been neglected, with few studies apparent in the literature (Chase & Leibold 2003, Silvertown 2004). More recent reviews have also emphasised the continued importance of the niche concept, and note that its application has grown much wider than the original concept, and now extends to modelling species distribution over time and adaptation to changing environments (Colwell 2009). Holt (2009) proposes that the niche concept is as relevant today as ever but needs to be more dynamic: rather than a static mapping of a species' abiotic and biotic tolerances onto an environment, niche theory needs to acknowledge the ability species have to influence their environment and moderate their own population growth and patterns, and to recognise that niches may be conserved over time or they may evolve.

There are also calls for an increased focus on positive feedback mechanisms in attempts to explain species' niches, plant community structure and coexistence. Callaway (2007) reviews twenty years of research on facilitation and other positive species interactions, suggesting they are a significant factor enabling the coexistence of species, and increasing performance of members of the same species: not by any altruistic intent but merely by the fact of their existence.

So how likely is it that identical species (i.e. cryptic species) could share the same niche and continue to coexist? Chesson (1991) reviewed models which propose exactly this scenario, and concludes that it would be unlikely to occur in reality, with the possible exception of social insects which deposit eggs via ovipositor following behavioural cues (Atkinson & Shorrocks 1981), and even then only under restricted conditions. In the case of cryptic species, one explanation for continued coexistence is that there must be some level of niche partitioning between species. Studies in niche partitioning look for the "niche axis": a dimension in the niche space along which species show segregated distributions. For example, Dudgeon et al. (1999) looked at factors which might explain the coexistence of two similar seaweeds *Chondrus crispus* and *Mastocarpus stellatus* in the lower intertidal of New England. Herbivory and storm damage were not found to be significant differentiators, but in the particular environment studied, differences in the productivity of each species explained their stable coexistence. Changing levels of productivity at tidal depth was found to be the axis along which niche separation occurs for these two species.

Silvertown (2004) suggests four tests for niche separation: competition, segregation, tradeoff and niche shift. Examples of evidence for niche segregation include rooting

depth in desert plants (Briones et al. 1996), nutrient and salinity gradients for mangrove trees (Lovelock 2003), salinity gradients in marsh herbs (Kenkel 1991), hydrological gradients for meadow herbs (Silvertown et al. 1999), and maximum height and light gradients in tropical forest trees (Kohyama et al. 2003).

In the inter-tidal environment, research suggests a number of factors and variables which help explain the coexistence of cryptic or similar species: adaptation to different temperatures (in algae: Bödeker et al. 2008, van der Strate et al. 2002; in barnacles: Wethey 1984), position in inter-tidal (Dudgeon et al. 1999, Nelson et al. 2005), salinity and differential dispersal ability (in marine nematodes: De Meester et al. 2011, De Meester et al. 2012), physical factors such as wave action or sand abrasion (Nelson et al. 2005). Changing climatic conditions and dispersal patterns over time can also explain overlaps in the distribution of cryptic species, and consequently their coexistence (Zuccarello et al. 2002, Van der Strate et al. 2002).

Upper limits of inter-tidal species' distributions may be attributed in part to air temperature, and partly to desiccation, while lower distribution limits are often determined by competitive ability rather than physical factors (Connell 1961). Studies of two barnacles *Chthamalus fragilis* and *Semibalanus balanoides* in the New England intertidal found that temperature tolerance was the major factor separating the niches of the two species. When *Semibalanus* was shaded to reduce the thermal stress, it was able to outcompete *Chthamalus* and overgrow it, but in the field it is limited to areas lower in the intertidal (Wethey 1984).

In the Bangiales, research has suggested that important factors determining distribution and stable coexistence are temperature and photoperiod (Bödeker et al. 2008); and tidal height and exposure to sand abrasion (Nelson et al. 2005).Culture experiments utilising different temperature and photoperiod regimes, have revealed differences in germination rates of spores, growth rates of filaments, timing and amount of spore release, and mortality of Bangiales filaments (Table 1.1, from Bödeker et al. 2008). Bödeker's research suggests that *Bangia* sp. BMW is adapted to cooler temperatures, a fact which correlates with its known distribution in New Zealand; whereas *Bangia* sp. BGA, recording highest growth rates at 15^{o}C and spore release only at a relatively warm 15 - 20°C in culture, is found only in the warmer waters of New Zealand (Fig 1.2). In culture *Minerva aenigmata* (previously known as *Bangia* sp. BTS) is able to survive a broad range of environmental conditions, explaining its wide distribution around New Zealand.

Bangia sp. BFK has only been recorded from one locality in New Zealand (Fig 1.2) yet culture experiments indicate it has the physiological capacity for a much wider distribution, suggesting that it may be an introduced species (Bödeker et al. 2008). Phylogenetic analysis may support this hypothesis, with BFK most closely related to lineages from the Northern hemisphere (Sutherland et al. 2011, Broom et al. 2004).

Table 1.1. Comparison of the morphology and physiology and inferred adaptation of four filamentous Bangiales lineages, as revealed by culture experiments. Source: selected data from table in Bödeker et al. 2008.

	Bangia sp. BFK	*Bangia* sp. BMW	*Bangia* sp. BGA	*Minerva aenigmata*
Filament width	Broad (25-29 µm)	Average (15-20 µm)	Average (17-22 µm)	Average (17-22 µm)
Growth rate	Average (low), no preference	High, especially at 12 and 15°C	Average (low), significantly higher at 15°C	Average (low), no preference
Filaments releasing spores	Some at 15 and 20°C	Very fast at all conditions	Some at 15 and 20°C	Never
Mortality at 20°C	No	100% (10 days)	No	No
Inferred adaptation	? generalist	Cold adapted	Warm adapted	? generalist

Adaptation to varying tidal heights and exposure appears to explain the distribution and habitat of some filamentous Bangiales lineages, e.g., unlike most lineages which grow in the upper inter-tidal, *Dione arcuata* grows in the upper sub-tidal (Nelson et al. 2005) only on exposed open coasts. Populations of *Minerva aenigmata* also appear in areas of moderate exposure: rapidly colonising bare rock surfaces exposed by the local erosion of sand (Nelson et al. 2005).

Systematics

Historically the family Bangiaceae has contained two genera distinguished by gross morphological differences, with filamentous specimens being classified as *Bangia*, and bladed specimens as *Porphyra* (Fig 1.3). Initially two species of *Bangia* were recognised: the freshwater species *Bangia atropurpurea* (Roth) C. Agardh and the marine *B. fuscopurpurea* (Dillwyn) Lynbye. Geesink (1973) proposed that these be united into one species; however, this taxonomic treatment was overturned by the advent of molecular phylogenetics.

Fig 1.3. An example of two morphologies present in the family Bangiaceae: a bladed or foliose form is shown on the left (website: www.botany.hawaii.edu), and a filamentous form on the right (website: dblab.rutgers.edu). Note that neither image is to scale.

Using molecular phylogenetic methods, researchers revealed that the genera of *Porphyra* and *Bangia* are not two divergent evolutionary paths, but are actually polyphyletic, with the filamentous form being the ancestral condition and the foliose form having arisen multiple times (Sutherland et al. 2011, Broom et al. 2004). Furthermore, sequence data reveals that, rather than one globally distributed *Bangia sp.*, there are at least seven separate genera of filamentous Bangiales (Sutherland et al. 2011).

The Rhodophyta (red algae) are divided into seven classes (Fig 1.4), including the class Bangiophyceae (Wettstein 1901) with a single order Bangiales (Nägeli 1847) and single family Bangiaceae (Engler 1892). A revision of red algal taxonomy by Yoon et al. (2006) identified six genera within the Bangiaceae: *Bangia, Bangiadulcis, Dione, Minerva, Pseudobangia* and *Porphyra*; however subsequent analysis of the Bangiales by Sutherland et al. (2011), based on detailed regional studies and molecular analyses, proposes at least fifteen genera: seven filamentous and eight foliose.

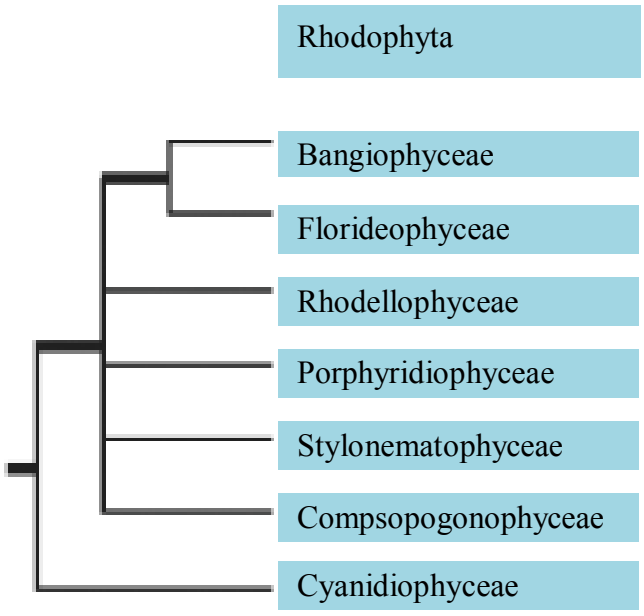


Fig 1.4. Red algal phylogeny of seven classes, proposed by Yoon et al. (2006) following analysis of 48 sequences of the PSI P700 chl a apoprotein A1 (psaA) and rbcL coding regions. Cyanidiophyceae shows the earliest divergence and is distinct from the remaining taxa. Source: Yoon et al. 2006.

The seven filamentous genera proposed by Sutherland et al. (2011) consist of four named and three unnamed genera (Fig 1.5). The four named genera; *Bangia, Dione, Minerva* and *Pseudobangia;* are monotypic (with the exception of *Bangia* which contains two species). In contrast the unnamed genera ("*Bangia*" 1, 2 and 3) each consist of multiple species, some with existing names; and the researchers expect that more detailed study would be able to identify taxonomically informative characters for each

new genus (clade); for example differences in cell wall polysaccharide chemistry (Hemmingson & Nelson 2002) or life histories (Cole & Conway 1980, Knight & Nelson 1999, Kikuchi et al. 2010).

Silva & Nelson (2008) propose that the genus *Bangia* (Lyngb. 1819: 82), once used to include all filamentous Bangiales, now be applied only to freshwater species, i.e. *B. atropurpurea*. The other named filamentous Bangiales genera are: *Minerva* W.A. Nelson (in Nelson et al. 2005: 141), endemic to the New Zealand region; *Dione* W.A. Nelson (in Nelson et al. 2005: 142), also endemic to New Zealand; and *Pseudobangia* K. M. Müll. et Sheath (in Müller et al. 2005), recorded from the Virgin Islands. The unnamed clades include "*Bangia*" 1, which may consist of a number of separate species. The "*Bangia*" 1 clade contains samples found in France, Korea, the Atlantic coast of North America, Australia and New Zealand (including *Bangia* sp. BFK, BMW and BRM). "*Bangia*" clade 2 contains at least 13 entities recorded from a number of regions including Alaska, the Pacific coast of Canada and the U.S.A, Japan, Taiwan, Korea and New Zealand. *Bangia* sp. BGA is found in "*Bangia*" clade 2. The third "*Bangia*" clade contains four entities: a northern cold-water species; an epiphytic species recorded in Japan, Korea and China; and two species from New Zealand (these are *Bangia* sp. BHH and BJB).

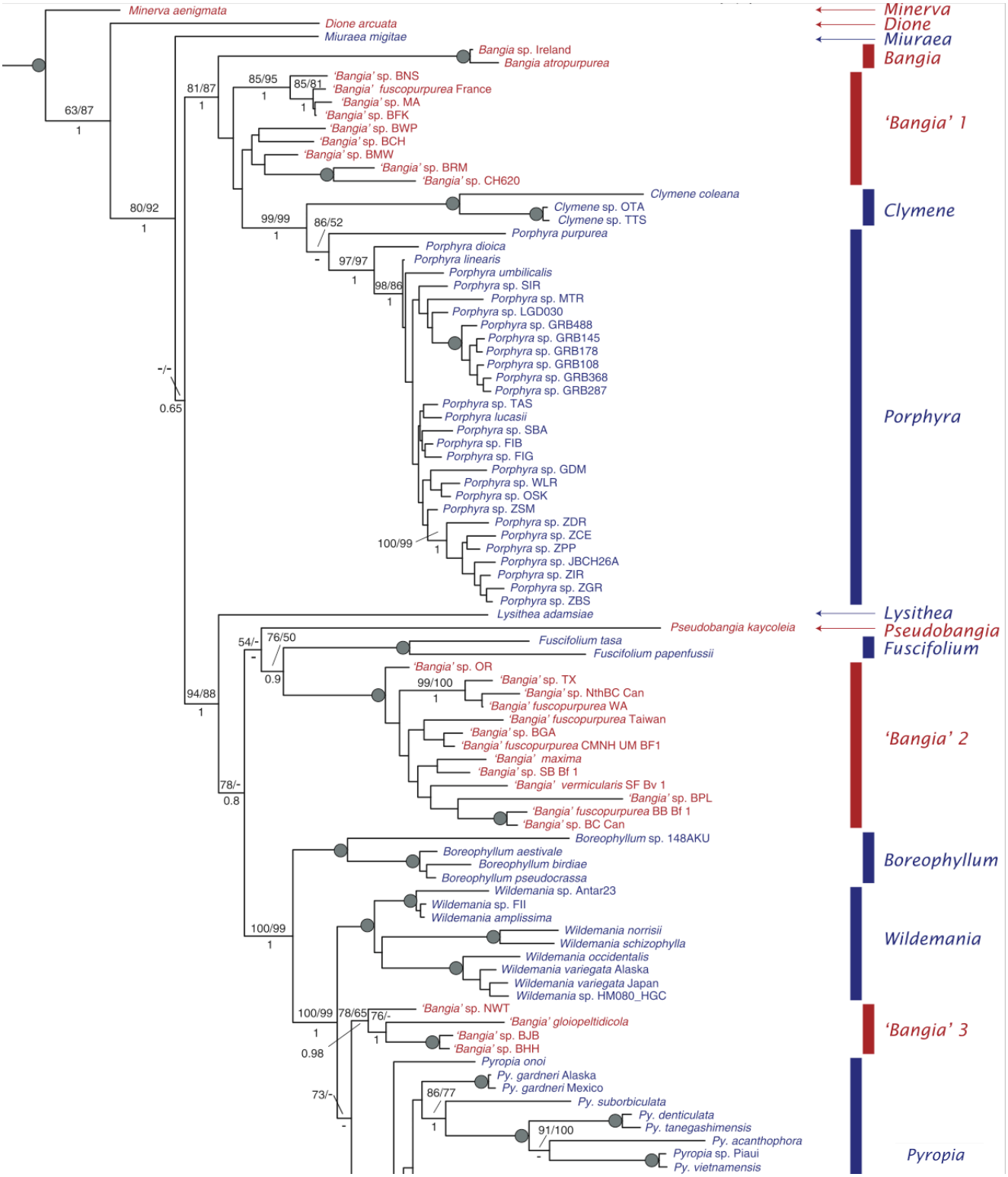


Fig 1.5. Bangiales phylogeny of fifteen genera, proposed by Sutherland et al. (2011) following analysis of 157 taxa using the nuclear SSU rRNA gene and the plastid RUBISCO LSU (rbcL) gene. Genera are indicated by lines and monotypic genera by arrows. Names of filamentous taxa are red and foliose taxa in blue. The phylogeny has been truncated from the original. Source: Sutherland et al. 2011.

Phylogenetic analyses of the Bangiales have found data from particular genes to be more informative than others; for example, a number of researchers have used nuclear small subunit (SSU) ribosomal ribonucleic acid (rRNA) 18S data, as the gene shows sequence variation between species but little or no variation within a species (e.g., Kunimoto et al. 1999, Broom et al. 1999). Nelson et al. (2005) used SSU data to support descriptions of the two new genera *Minerva* and *Dione*, from New Zealand, and identify *Minerva aenigmata*. Sutherland et al. (2011) found that combining data from the ribulose-1,5-bisphosphate carboxylase oxygenase large subunit (*rbc*L) gene and nuclear SSU rRNA gene produced more strongly supported clades than analysis based on a single gene alone.

Biology

Life cycle

Filamentous members of the Bangiales have a heteromorphic sexual life cycle that includes a filamentous gametophyte as well as a microscopic sporophyte (Fig 1.6). The sporophyte phase, known as conchocelis, was first thought to be a separate entity and was described as *Conchocelis rosea* Batters, until Drew (1949, 1954) identified *C. rosea* as the alternate life-stage of *Porphyra pupurea* (Roth) C. Ag. The filamentous form of Bangiales is ephemeral whereas the conchocelis phase may be perennial, as has been suggested in studies of *Porphyra* conchocelis phases (Dickson & Waaland 1985).

The filamentous gametophyte can reproduce sexually, releasing male and female gametes that fuse to form a zygotospore which then develops into the conchocelis

(Nelson et al. 1999) (Fig 1.6). The conchocelis or sporophyte stage produces conchosporangia that release conchospores. These conchospores then develop a short rhizoidal holdfast (Sommerfeld & Nichols 1973), attach to the substrate and grow into the simple unbranched filaments of the gametophyte stage.

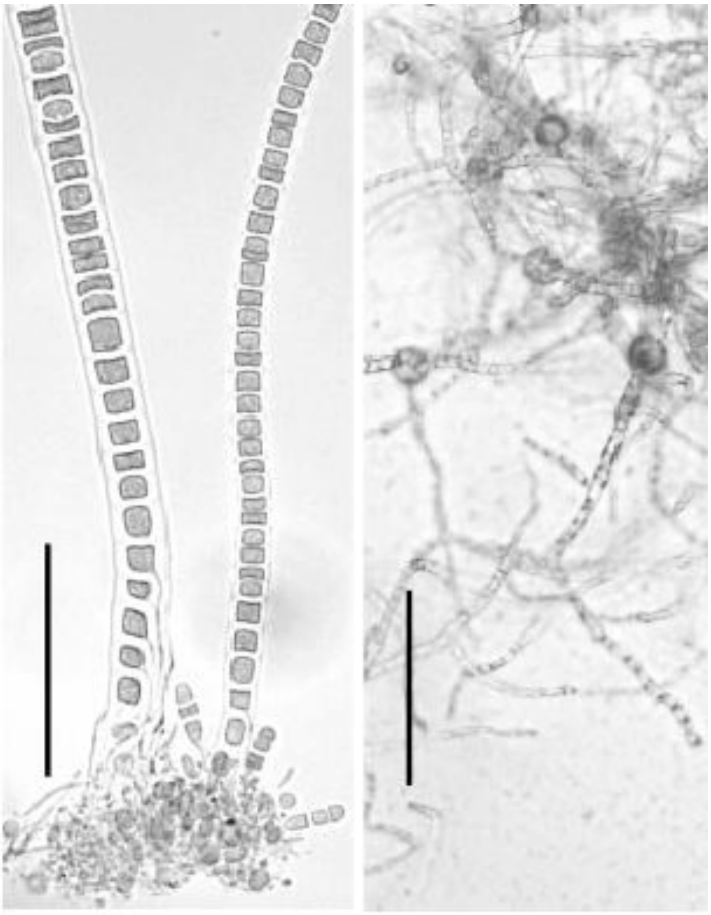


Fig 1.6. Minerva aenigmata from Nelson et al. 2005, showing the simple unbranched filamentous life-stage on the left (the gametophyte), and the branching conchocelis stage (sporophyte) on the right. Scale bar: 100 micrometres. Source: Nelson et al. 2005.

Both the gametophyte and sporophyte stages can also reproduce asexually by expelling the contents of a vegetative cell as a neutral (asexual) spore, also known as an archaeospore. The release of neutral spores can occur in large numbers, resulting in the rapid establishment of new populations (Sheath & Cole 1980, Sheath et al. 1985). Both temperature and photoperiod conditions have been found to trigger stages in the life-

cycle (Bödeker et al. 2008, Dixon & Richardson 1970, Sommerfeld & Nichols 1973, Waaland et al. 1990).

Spores of the red algae are non-flagellate so dispersal is largely passive, however settlement may be aided by their ability to glide or make amoeboid-type movements (Pickett-Heaps et al. 2001). Spores may also display phototactic or chemoattractive behaviours during settlement (Amsler 2008, Steinberg et al. 2002). The polysaccharide mucilage surrounding spores then provides the "stick" which enables them to attach to the substrate (Boney 1975, 1981; Ngan & Price 1979, Ouriques et al. 2012). Vegetative fragments of both filaments and conchocelis, created by wave action or grazers, may also be able to disperse and re-establish (Conway & Cole 1977): other small filamentous algae, such as filamentous *Ulva* species and *Ectocarpus*, are thought to be dispersed in this way (Fletcher & Callow 1992, Clokie & Boney 1980). In general, little is known about dispersal in the Bangiales, or life-cycle parameters such as which populations or lineages are sexual or asexual, and there are few records of conchocelis collected from its natural habitat.

Habitat

Filamentous members of the Bangiales are found in marine and freshwater habitats around the world (Lüning 1990). Marine populations are usually found in the upper intertidal or splash zone, exposed to direct sunlight and fluctuating salinity and temperature, where they produce sun-screen compounds and osmoregulators to survive in these habitats (Karsten & West 2000). The filamentous form grows on rock (Fig 1.7) but also manmade surfaces such as wooden palings or concrete slabs (Fig 1.8). The conchocelis phase bores into shells, barnacles or calcareous stones (Boney 1978). In *Porphyra* species the conchocelis phase has been recorded from depths of 78 m in the

Firth of Clyde, Scotland (Clokie & Boney 1980) and up to 1.4 m above mean lower low water at San Juan Island, Washington, USA (Martinez 1990); and in filamentous Bangiales one could speculate that a similar range is plausible.

Fig 1.7. Patches of filamentous Bangiales as they appear on natural rocky substrates.

Fig 1.8. Bangia sp. BFK, a possible invasive species, thrives on the textured concrete at Freyberg promontory, Wellington harbour.

Filamentous Bangiales populations consist of groups of individuals that appear as patches on the substrate, which, in the Wellington region, are generally in the order of a few cm^2, but range in size from < $1cm^2$ to > $1000cm^2$ in extreme cases. A patch of a few cm^2 contains hundreds of individual Bangiales gametophytes.

Research aims

A number of cryptic filamentous Bangiales have been recorded from Wellington, New Zealand; all morphologically indistinguishable and all apparently occupying the same niche within the upper inter-tidal. Researchers have reported finding more than one member of the filamentous Bangiales coexisting at a single location in Wellington's inner harbour (Farr et al. 2001, Wendy Nelson pers. comm.).

This raises the question: to what extent are these cryptic lineages coexisting? Are there differences in their temporal and spatial distribution? Does distribution at a small-scale, within sites in the Wellington region, reflect the physiological differences and ecological adaption reported from the culture studies of Bödeker et al. 2008?

This research sought to investigate the small-scale distribution of these cryptic lineages to test for temporal or spatial variation, and draw conclusions about the nature of their coexistence in the Wellington region. To achieve this, samples were taken from six sites in Wellington over a period of nine months. Molecular methods, as described in Chapter 2, were utilised to identify the various taxa. This identification data then provided the basis for the ecological analyses presented in Chapter 3.

Hypothesis: Filamentous Bangiales lineages in the Wellington region display different temporal and spatial distributions, reflecting their ecological adaptations and enabling their stable coexistence through a corresponding reduction in inter-specific competition.

Chapter 2. Molecular Identification

Introduction

To understand the ecology of a species it is crucial to be able to accurately identify that species. One of the challenges of working with cryptic species is that, by definition, they are difficult, or impossible, to identify morphologically. Consequently, samples of morphologically simple or phenotypically plastic species can only be reliably identified by molecular methods. For taxa such as the filamentous Bangiales, where gametophytes cluster in patches, each containing hundreds or even thousands of individuals, it is impractical to genetically identify every individual. It is therefore necessary to infer the identity of individuals from a sub-sample of the population that has been genetically identified. Once the identity of populations is ascertained with some confidence, only then is it possible to test hypotheses about the nature of spatial co-existence, population turnover, recruitment, patchiness and local diversity.

When only a single cryptic taxon is recorded from a particular site over time, subsequent population surveys may choose to assume genetic identity of that population. However, where a number of different cryptic taxa co-exist in close proximity at a site, more intensive sampling is essential for each survey, to establish seasonal patterns of abundance of each cryptic taxon. Up to five filamentous Bangiales entities have been recorded from a single location in New Zealand (Broom et al. 2004). In the Wellington region four entities have previously been recorded: *Bangia* sp. BGA, BMW, BFK, and *Minerva aenigmata* W.A. Nelson (Bödeker et al. 2008, Nelson et al. 2005, Broom et al.

2004); with two of these lineages (BGA and BFK) recorded from a single site (Wendy Nelson pers. comm.).

To gain insight into the genetic identity and diversity of filamentous Bangiales in the Wellington region, 167 filamentous Bangiales samples from six study sites were processed (Appendix A). Restriction digests were used to identify 82 samples, and nucleotide sequencing was used for the remaining 85 samples. Nucleotide sequencing of biological samples provides absolute confirmation of identity but it is also a relatively expensive technique, while restriction digests are much more cost effective.

This chapter (Chapter 2) describes only the molecular methods used and the new lineages and sequences discovered. Chapter 3 describes the methods and results relating to abundance and temporal and spatial variation, and discusses these findings.

Material and Methods

Mapping and sampling regime

Sampling was undertaken in the period Jul 04 to Jul 06 (Table 2.1). Six study sites were chosen, using the following criteria: a) that sufficient filamentous Bangiales be present for sampling and b) that different environmental conditions be represented, i.e. three of the six were inner harbour locations, and three were more exposed to wave action and sand abrasion. Samples from 2004 were used to trial the genetic techniques for identification; and also enabled assumptions to be tested about the genetic identity of Bangiales patches, i.e. whether patches of Bangiales consisted of filaments of the same lineage or were made up of different lineages.

The most comprehensive sampling dataset is from surveys in Oct 05, Dec 05, Feb 06 and Jun/Jul 06. Further sampling in Nov 05 and Jan 06 was undertaken.

Sites were surveyed at low tide. Each site was searched for between 5 and 10 minutes to identify all visible patches of Bangiales. Bangiales patches and adjacent organisms were traced onto A4 transparencies (21 x 29 cm) using a permanent marker. Samples were collected using needle-pointed tweezers, and their position marked on the corresponding transparency sheet. Each sample contained dozens of individual Bangiales filaments, and weighed less than 0.1g. Samples were placed in 1.5mL cryovials containing silica gel until genetic analysis was conducted.

The number of patches found at each site varied from zero to 68. All patches of $10mm^2$ or greater were sampled, but only when filaments were long enough (>10mm length). To test the assumption that each patch contains only individuals of a single Bangiales taxon, two or three samples were taken from individual patches that were larger than $50mm^2$.

Limitations of the sampling regimen

Sampling through Mar, Apr and May 06 was restricted to Frank Kitts. This was not driven by scientific considerations, but rather by personal ones. This researcher was finding that the time to process the volume of samples and molecularly identify each, was becoming overwhelming, and balanced against increasing family commitments it was perceived as unsustainable. To reduce the processing load the decision was made to reduce sampling to one site for a period of 3 months. The site chosen, Frank Kitts lagoon, was considered the most promising in terms establishing relationships between

the three lineages present. The change in sampling regimen was discussed with this researcher's supervisor, but not with a statistician, and unfortunately it was not appreciated that this change would seriously limit the statistical analysis possible. The effect on the statistical tests is discussed further in Chapter 3.

Table 2.1: Collection dates at the six study sites in the Wellington region, over the period Jul 04 to Jul 06. A "√" indicates a site was visited and filamentous Bangiales was present and sampled. A "0" indicates that a site was visited but no visible Bangiales was present. A "-" indicates that a site was not visited at that time. Columns with two months noted, e.g., Jul/Aug 04, indicate that data has been pooled, either for ease of presentation, or for the purposes of analysis.

Site	Lat	Long	Jul / Aug 04	Nov 04	Dec 04/ Jan 05	Mar / Apr 05	Jul / Aug 05	Oct 05	Nov 05	Dec 05	Jan 06	Feb 06	Mar 06	Apr 06	May 06	Jun / Jul 06
Frank Kitts	174.78	-41.2886	-	√	-	√	-	√	-	√	√	√	√	√	√	√
Freyberg	174.7908	-41.2902	-	-	√	-	-	√	0	√	√	√	-	-	-	√
Greta Point	174.8062	-41.3017	-	-	-	√	√	√	√	√	-	√	-	-	-	√
Owhiro Bay	174.7575	-41.3452	-	-	√	-	-	√	√	√	-	√	0	-	-	0
Seatoun	174.8293	-41.3183	√	-	-	√	√	0	-	-	-	√	-	-	-	-
Lyall Bay	174.804	-41.3288	√	√	-	-	0	0	-	√	-	0	-	-	-	0

Genetic identification

For genetic identification methods, an 880bp section of the SSU gene was chosen, covering the variable region V9 (Neefs et al.1993).

DNA extraction and PCR amplification

DNA was extracted by grinding samples with a 200µL solution of 5% Chelex® 100 sodium-form (Bio-Rad Laboratories, Hercules, California) in ultrapure H_20, boiling for 10 minutes, placing on ice, then centrifuging at 10,000g for 10 minutes and removing the supernatant to a new tube. The supernatant was stored at -20°C until required for PCR amplification.

The following PCR mix was used: 1X PCR buffer (Biotherm™, Genecraft GmbH, Lüdinghausen, Germany), 250nM dNTP, 2.5mM $MgCl_2$, 0.25% BSA, 5 pmoles of the primers J04 and G04(Saunders & Kraft 1997), which amplify an approximately 880bp section of the SSU gene, 1U of *Taq*DNA polymerase (Biotherm™) to a final volume of 30µL. The PCR reactions were performed on a PTC100 Thermocycler (MJ Research) and had the following conditions: an initial denaturing step at 94°C for 4 mins, 35 cycles of 94°C for 30 secs, 50°C for 1 min, 72°C for 1 min, and a final extension of 72°C for 5 mins, samples were then held at 4°C. Successful amplification was checked by electrophoresing PCR products in a 1% LE agarose gel at 100V for one hour, staining with ethidium bromide, and photographing under UV light.

Method 1: Restriction digest

Restriction digests enabled the rapid assignment of samples to the lineage level, at low-cost. A restriction enzyme that cuts the four lineages in a distinctive manner was found, using the

freeware Webcutter program (NEB cutter v2.0, New England Biolabs® Inc., http://www.neb.com) and sequence data supplied by Judy Sutherland, University of Otago (Genbank Accession numbers: *M. aenigmata* - AY184347, BFK - AY184338, BMW - AY184344, BCP AY184336 and BGA AY184341), and the following two-stage digest protocol was developed (Fig 2.1).

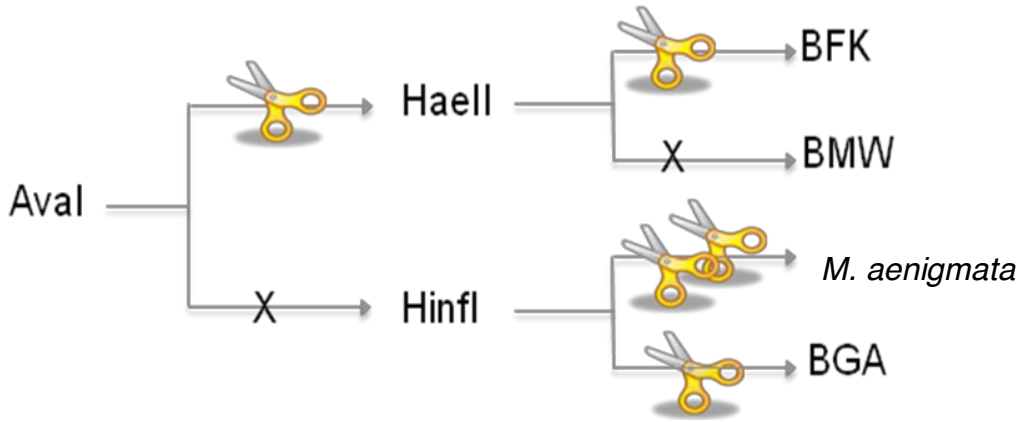


Fig 2.1. The restriction enzymes AvaI, HaeII and HinfI enabled each sample to be identified as one of the four lineages known to exist in the Wellington region, by a process of deduction. A single pair of scissors indicates a single cut by the enzyme, two pairs of scissors indicates two cuts, and an X indicates that the enzyme does not cut that lineage.

The first stage involved using the restriction enzyme *AvaI* (New England Biolabs® Inc., USA) to digest all samples and categorise them into two groups depending on the results: *AvaI* cuts BFK and BMW at position 625/629 of the 876 bp long 18S rDNA fragment, but not *M. aenigmata* or BGA. The second stage required the use of the enzymes *HaeII* and *HinfI* (both New England Biolabs® Inc., USA) to finally assign a lineage to each sample. *HaeII* will distinguish BFK (cuts at 829/825) from BMW (uncut), and *HinfI* distinguishes BGA (cuts once at 293/296) from *M aenigmata* (cuts twice at 296/293 and 486).

Samples were incubated with the restriction enzyme *AvaI* (5U) with 2µL of PCR product in a 10µL reaction at 37°C for 6 hours, then the resultant pattern of DNA fragments was used to decide which digest (either *HaeII* or *HinfI*) to run next. For *HaeII* digests 10U of enzyme were added to 5µL PCR product in a 10µL reaction, and for *HinfI*5U to 5µL PCR product in a 10µL reaction. Samples were incubated at 37°C for 6 hours. Digested samples were electrophoresed in 1.5% agarose at 120 volts, using 1 µL loading buffer (glucose and bromophenol blue) to 4 µL reaction product. Gels were electrophoresed for about one hour with a 100bp ladder to measure the size of the resultant DNA fragments. Gels were then stained in ethidium bromide for at least 10 minutes, then rinsed in water and photographed under UV light (Fig 2.2).

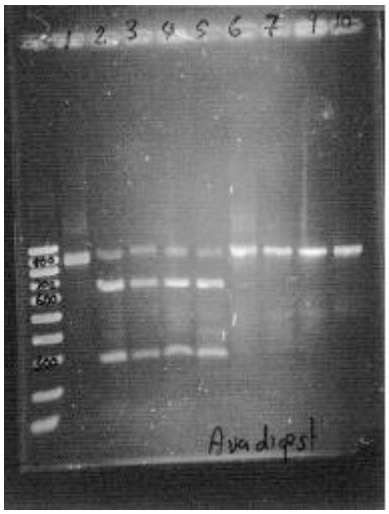


Fig 2.2. A gel with nine samples of Bangiales reaction product after an Ava1 digest and in lane 1 a 100bp ladder to enable measurement of DNA fragment lengths. The results show that products in wells 1 and 6-9 were not cut by the Ava1 digest, whereas products in wells 2-5 were cut. To identify the lineage of each product, further digests were run as follows: product 1 was digested with HinfI, and was cut once identifying it as Bangia sp. BGA; products 2 to 5 were digested with HaeII and did not cut, identifying them as Bangia sp. BMW; products 6 to 10 were digested with HinfI and were cut twice, identifying them as M. aenigmata (gel not shown).

Method 2: Nucleotide sequencing

An additional set of 85 samples was sequenced (Appendix A). The amplified products were cleaned using ExoSAP-IT (USB Corporation, Ohio, USA) following the manufacturer's protocol. Sequencing was done at Macrogen Inc. (Soeul, Korea). Generated sequences were

compared with previous vouchered sequences.

Phylogenetic analysis

During this study, new sequences were discovered: C8, BRMg, and BHH1. Sequences of C8, BHH1 and BRMg were deposited in GenBank and herbarium specimens lodged with Te Papa Tongarewa, Museum of New Zealand. Details are as follows: C8 (sample 181), GenBank ID HM014311, WELT A023241; BHH1: GenBank ID HM014313, WELT A023240; and BRMg: GenBank ID HM014312, WELT A023242.

For maximum-likelihood (ML) analysis the program Modeltest (version 3.7, Posada & Crandall 1998) was used to find the model of sequence evolution least rejected for the data set by a hierarchical likelihood ratio test ($\alpha = 0.01$). When the sequence evolution model had been determined, a maximum-likelihood analysis was performed in PAUP* (Swofford 2002) using the estimated parameters (substitution model, gamma distribution, proportion of invariable sites, transition-transversion ratio) (10 random additions).

Support for individual internal branches was determined by bootstrap analysis (Felsenstein 1985), as implemented in PAUP*. For ML bootstrap analysis, 100 bootstrap data sets were generated from resampled data (1 random sequence addition per replicate).A full bootstrap analysis was not practicable due to the size of dataset. The bootstrap values in this analysis may under-estimate “true” topology of branches, due to the fast method used (Felsenstein 1985).

Results

Molecular identification

This study found new sequences present in the Wellington region: C8, BHH1 and BRMg (Table 2.2). The most genetically distinct sequence was C8[1] (Appendix A: sample 181, Greta Point; GenBank ID HM014311; WELT A023241). Records of BHH1 (BHH1 - Appendix A: sample 169, from Owhiro Bay; GenBank ID HM014313; WELT A023240) and BRMg (Appendix A: sample 18, and 17 other samples; GenBank ID HM014312; WELT A023242) were also captured. This is the first record of these sequences.

A summary of the full sample set for the study, and genetic identity of samples, is presented in Table 2.2. *Bangia* sp. C8 was recorded only once, from Greta Point, in spring 2005. Likewise, the BHH1 sequence was recorded only once in spring 2005, this time from Owhiro Bay. More detail on the frequency of genotypes is included in Chapter 3 Ecology. Individual patches were found to consist of filaments of the same lineage, as tested by taking two samples from the same patch on thirteen occasions over the course of the study.

[1] C8 refers to the position of the sample in the 96-well plate used for sequencing.

Table 2.2: Summary of samples taken over the study period and their genetic identification. The total number of patches present at each site and the number actually sampled are included, where this information was recorded.

Location	Date collected	No of patches present	Patches sampled	Samples taken	Lineage(s) identified
Frank Kitts	Nov 04	Not recorded	18	18	13 BGA, 3 BFK, 2 BRMg
	Mar 05	Not recorded	10	10	7 BFK, 3 BGA
	Oct 05	37	7	9	5 BFK, 3 BGA, 1 BRMg
	Dec 05	59	8	8	4 BFK, 4 BRMg
	Jan 06	31	4	6	4 BRMg, 2 BFK
	Feb 06	6	2	2	2 BFK
	Mar 06	16	4	6	6 BFK
	Apr 06	19	5	7	7 BFK
	May 06	4	3	3	3 BFK
	Jun 06	11	3	3	3 BFK
Freyberg	Dec 04	Not recorded	6	6	6 BFK
	Oct 05	68	9	12	10 BFK, 2 BGA
	Dec 05	9	1	1	1 BFK
	Jan 06	30	5	5	5 BFK
	Feb 06	25	3	4	4 BFK
	Jul 06	7	4	5	5 BFK
Greta Point	Mar 05	Not recorded	11	11	9 BFK, 1 BMW, 1 BGA
	Jul 05	Not recorded	4	4	4 BFK
	Oct 05	34	4	4	2 BFK, 2 BRMg
	Nov 05	Not recorded	1	1	1 C8
	Dec 05	25	4	4	3 BRMg, 1 BFK
	Feb 06	30	3	3	2 BRMg, BFK
	Jun 06	20	1	1	1 BFK
Seatoun Boatshed	Aug 04	Not recorded	4	4	*Minerva aenigmata*
	Apr 05	Not recorded	2	2	*M. aenigmata*
	Aug 05	Not recorded	1	1	*M. aenigmata*
	Feb 06	16	2	2	*M. aenigmata*
Lyall Bay	Jul 04	24	4	4	4 BMW
	Nov 04	Not recorded	2	2	2 BMW
	Dec 05	54	3	3	2 BMW, 1 BFK
Owhiro Bay	Dec 04	Not recorded	4	4	1 BMW, 3 *M. aenigmata*
	Jan 05	Not recorded	4	4	1 *M. aenigmata*
	Nov 05	35	1	1	1 BHH1
	Dec 05	9	2	2	2 BMW

Phylogenetic analysis

The maximum-likelihood (ML) topology identifies *Bangia* sp. C8 as a distinct taxon, but its relationship with other clades is unresolved (Fig 2.3). The entity *Bangia* sp. BRMg groups strongly with the lineages *Bangia* sp. BRM and CH620 (95% bootstrap support). In the SSU sequence data only one base pair change was identified between *Bangia* sp. BRM and BRMg.

The topology also shows entity *Bangia* sp. BHH1 grouping with *Bangia* sp. BJB and BHH, with some support (76%). The sequence data identifies five base pair changes between *Bangia* sp. BHH and BHH1.

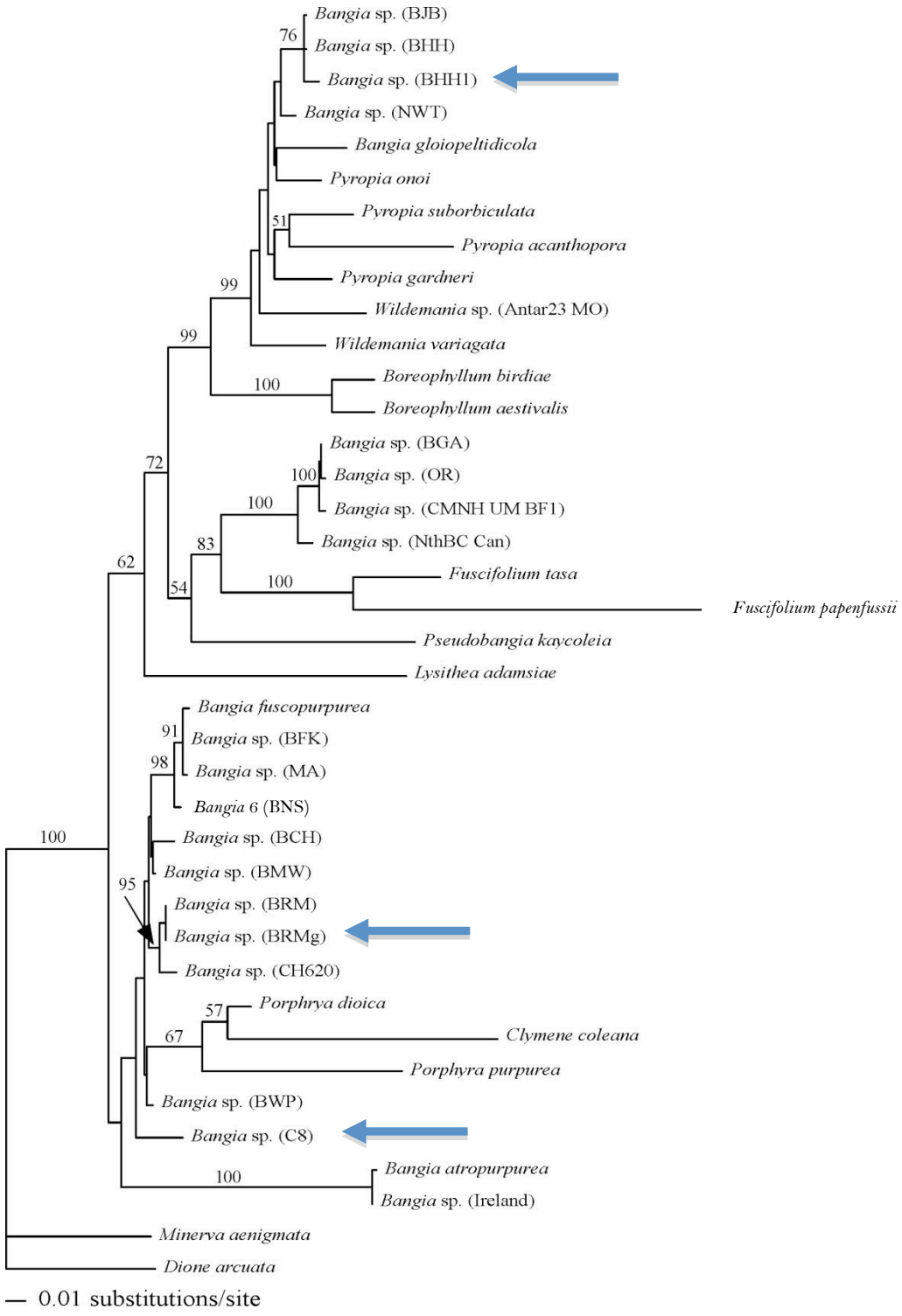


Fig 2.3. Maximum-likelihood (ML) topology of Bangiales samples (plus other closely related taxa) based on SSU sequence data. Sequences recorded as part of this study (BHH1, BRMg and C8) are indicated with arrows. Numbers above branches represent ML bootstrap values under the fast bootstrap method.

Discussion

Studies of marine cryptic species generally uncover more diversity than previously realised (Knowlton 1993), and in the Bangiales researchers have predicted that intensive regional collection will result in the recognition of new taxa or even genera (Sutherland et al. 2011). This study was no exception, with new and distinct genetic sequences identified in addition to the four taxa already known from the Wellington region.

One new entity, *Bangia* sp. C8, has a genetic sequence that differs markedly from other filamentous bangialean entities. Globally, this is the first known record of this taxon. However, the relationship with other *Bangia* and *Porphyra* clades remains unresolved (Fig 2.3). Further analysis, utilising more variable markers, would help to obtain better resolution of its phylogenetic position.

To date taxonomic treatments of New Zealand *Bangia* entities separate each by at least four pairwise sequence differences (Broom et al. 2004). However, there is no precedent in the literature for distinguishing bangialean taxa on the basis of one or two pairwise sequence differences; additional genetic markers and biological data would be required to determine whether such differences are simply intraspecific variation, or whether they indicate distinct entities.

In the present study new sequence, BHH1, resolves with *Bangia* sp. BHH and BJB in the phylogeny (Fig 2.3). BHH1 presumably falls within clade '*Bangia*' 3, in the recent taxonomy proposed by Sutherland et al. (2011). On the basis that the SSU sequence data of BHH1 differs from BHH by five base pairs, more than the four sequence differences which separate

other bangialean taxa (Broom et al. 2004), it could be argued that the record of BHH1 from this study presents another new taxon.

Another new sequence, BRMg, was recorded which differs by only one base pair from the previously known bangialean entity: *Bangia* sp. BRM. A single base pair difference would suggest that this entity is not distinct enough to warrant separate taxonomic status; i.e. BRMg may be considered the same entity as *Bangia* sp. BRM.

Historically, *Bangia* sp. BRM was recorded from elsewhere in New Zealand (Christchurch: GenBank Accession No. AY184346), but no records made in the Wellington region (Wendy Nelson pers. comm.). From the findings of this research, it is suggested that the known distribution of *Bangia* sp. BRM now include the Wellington region, on the basis that BRM and BRMg are the same species.

The new *Bangia* entity proposed here, *Bangia* sp. C8, in addition to another possible new entity BHH1, and the range extension for *Bangia* sp. BRM; bring the known diversity of filamentous Bangiales in the Wellington region to a possible seven taxa: *Bangia* sp. BFK, BGA, BMW, BRM, BHH1, C8 and *Minerva aenigmata*.

The findings of this study support high bangialean diversity in New Zealand. It has been suggested that high levels of bangialean diversity in New Zealand indicate that *Porphyra* and *Bangia* originated in the Southern Hemisphere (Broom et al. 2004); however, this may reflect the study efforts applied in this part of the world.

Hommersand (2007) suggests a bipolar distribution for the Bangiales, and the recent generic revision by Sutherland et al. (2011) supports this, with clades indicating both Northern and

Southern hemisphere radiations. It seems likely that further study, and intensive sampling, of *Bangia* populations globally, will not only continue to reveal greater diversity, but also help to resolve relationships within this phylogenetically-complex group.

The discovery of at least one new entity in this study may be surprising given the Bangiales research already conducted in this region (e.g., Nelson et al. 2005, Broom et al. 2004, Bödeker et al. 2008). However, *Bangia* sp. C8 was identified only once from a single sample, out of a total sample set of 167, suggesting that this genotype may be rare. Further sampling in the Wellington region would help to identify whether it is truly rare, or whether the results are an artifact of the survey methodology. This study confirms that intensive sampling and molecular identification are necessary in order to understand the diversity of Bangiales taxa in any given region, and supports the application of similarly intensive sampling methods for the study of other suites of cryptic species.

Recommendations for future research methods

The experience of this study was that rapid restriction digest methods should only be used as a means of identifying populations of cryptic species, when coupled with sufficient nucleotide sequencing of samples at the outset. The sampling design and restriction digest protocols for this study were based on the assumption that four filamentous Bangiales taxa were present in the region: *Bangia* sp. BGA, BFK, BMW and *Minerva aenigmata* (Bödeker 2003, Wendy Nelson pers. comm.). The discovery that there are at least six taxa present reduced the data captured for each entity and constrained statistical analysis of temporal and spatial distribution. It also opens up the possibility that some samples in this study have been misidentified, since under the digest protocols used, *Bangia* sp. C8 and BHH1 cut in the same pattern predicted for *Bangia* sp. BGA. For future studies, it is recommended that a number of samples from all study sites be molecularly sequenced at the outset, to provide a

better indication of the taxa present. Ideally one or two samples from each structure at a site (i.e., each rock, wooden paling, concrete slab) should be sequenced, and where possible, this effort should be continued over each season to identify rare taxa and seasonal differences. This information can then be used to inform sampling design, as well as confirming a suitable restriction digest protocol for those on a low budget.

It should be noted that the nucleotide section chosen for this research (an 880bp section of the SSU gene, covering the V9 region) may underestimate the diversity present as some specimens with identical V9 sequences have been shown to differ in other regions of the SSU gene (Jones et al. 2004). In the future, sequencing of other sections of the DNA of the filamentous Bangiales, such as the *rbc*L gene (Sutherland et al. 2011), would help to clarify phylogenetic relationships between these taxa.

Chapter 3. Ecology

Introduction

Culture studies suggest that filamentous Bangiales have different temperature and photoperiod adaptations, and that their distribution throughout New Zealand reflects these physiological adaptations (Bödeker et al. 2008). Reported distribution of Bangiales lineages in New Zealand also identifies tidal height and exposure to sand abrasion as important (Nelson et al. 2005). A number of filamentous Bangiales have been recorded from the Wellington region, and are reported as coexisting in some locations (Farr et al. 2001, Wendy Nelson pers. comm.). However, little is known about the small-scale distribution of Bangiales or which factors might enable coexistence at particular locations.

Having established the genetic identity of filamentous Bangiales in the Wellington region (including a level of diversity higher than previously suspected) (Chapter 2), it is possible to use this information alongside the distribution data to examine possible environmental adaptations between lineages. The present study examined a number of environmental variables for effects on filamentous Bangiales in Wellington: 1) abundance over time irrespective of lineage, 2) seasonal differences in lineage richness, 3) seasonal abundance of individual lineages irrespective of site, 4) the effect of each site on the seasonal abundance of lineages, 5) effect of harbour or coastal conditions on seasonal abundance of lineages, 6) the use of substrate by each lineage, and 7) differences in vertical distribution of taxa in the inter-tidal zone.

Methods

Study sites

As described in Chapter 2, the intensive study period ran from Oct 05 to Jul 06, and six sites were surveyed over this time: two on the South coast (Owhiro Bay and Lyall Bay), one at the harbour entrance (Seatoun Boatshed) and three in the inner harbour (Freyberg Promontory, Greta Point and Frank Kitts lagoon) (Fig 3.1).

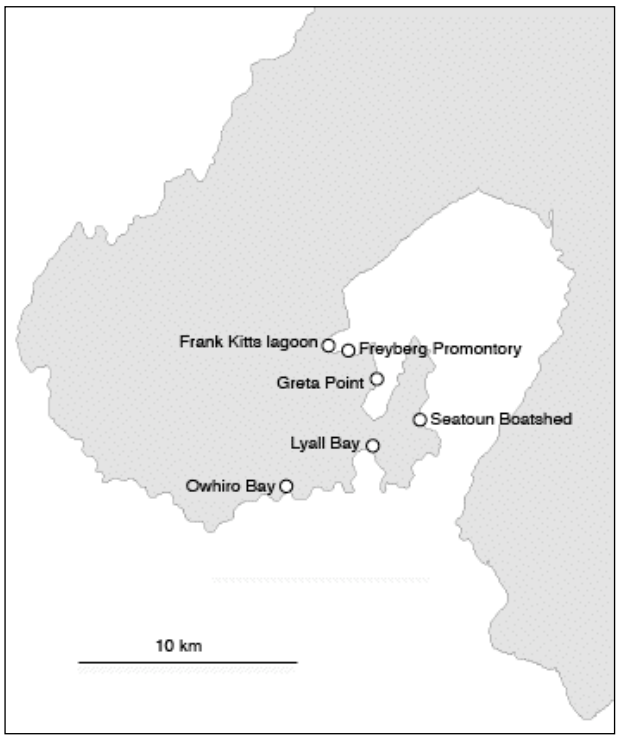


Fig 3.1. Map of the Wellington region, showing the six study sites. Three locations are inside the harbour, one close to the harbour entrance, and two on the South Coast.

All of the sites, except Owhiro Bay, are modified habitats (Table 3.1). Exposure varies among sites: from the relatively sheltered inner harbour sites of Frank Kitts lagoon, Freyberg Promontory and Greta Point; to the strong wave action, sand-scour, and intermittent sand/gravel burial experienced at the Seatoun, Owhiro Bay and Lyall Bay sites.

Table 3.1. Characteristics of the six study sites in the Wellington region.

	Lat	Long	Description	Exposure	Modified habitat?	Substrate	Human influence
Frank Kitts	174.78	-41.2886	Artificial lagoon, inner harbour	Enclosed / very sheltered	Yes	Rock, wood, iron	Heavy foot traffic in parts
Freyberg	174.7908	-41.2902	Concrete promontory with boulder rip-rap. Inner harbour	Moderately sheltered	Yes	Rock, concrete	Heavy foot traffic in parts
Greta Point	174.8062	-41.3017	Riprap	Moderately sheltered	Yes	Rock	Some foot traffic, fishing spot
Seatoun Boatshed	174.8293	-41.3183	Near harbour entrance, concrete boat-ramp, naturally occurring rock projecting from sand	Moderately exposed	Partially	Rock, concrete	Cars and trailers, some foot traffic
Lyall Bay	174.804	-41.3288	Riprap boulders constructed on sandy beach	Exposed	Yes	Rock	Occasional foot traffic
Owhiro Bay	174.7575	-41.3452	Natural rock projecting from sand / gravel	Exposed	No	Rock	Occasional foot traffic

Each of the six sites differs in its composition of inter-tidal flora and fauna. Frank Kitts lagoon shows the clearest example of upper inter-tidal zonation (see Fig 3.2) with the crustose black lichen *Verrucaria maura*, above the filamentous Bangiales zone, and green *Ulva* species below. At other sites, such as Greta Point, *Littorina* (periwinkles) and barnacles co-exist with filamentous Bangiales (see Fig 3.3). Limpets, bladed Bangiales and *Mytilus sp.* (blue mussels) also grow on the same structures at various sites. On occasion, filamentous Bangiales grow directly over barnacles and limpets, or interspersed amongst lichen or *Ulva* species, however most frequently Bangiales patches are surrounded by apparently bare rock (biofilms may well be present but were not discernible to the naked eye).

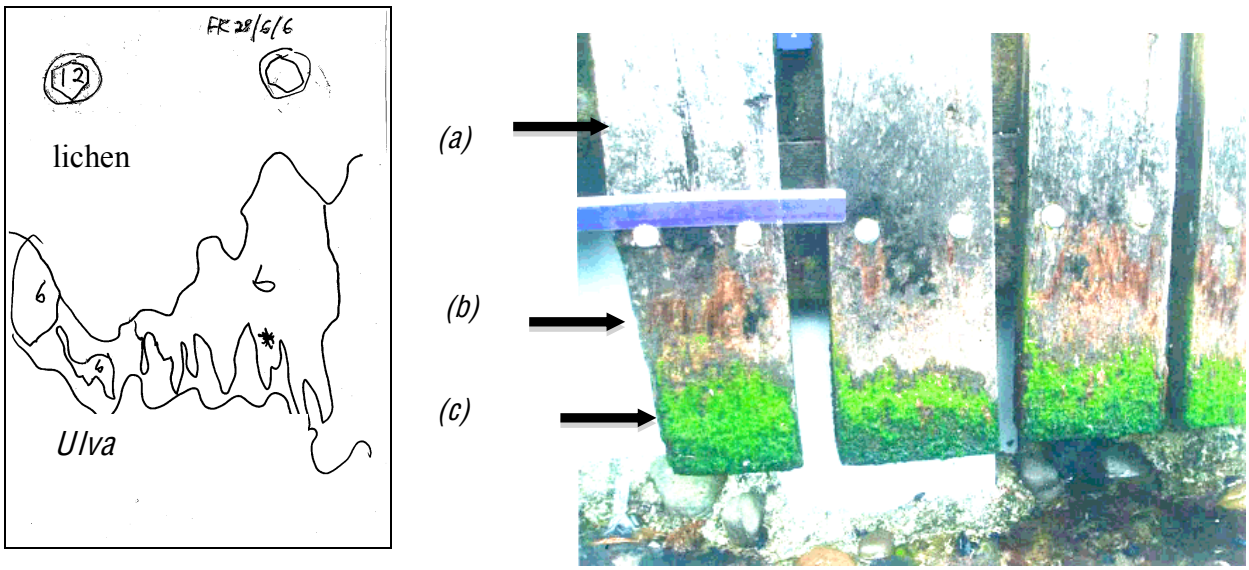


Fig 3.2. Frank Kitts lagoon: tracing of structure 12 and photo of structures 12, 13 and 14, showing lichen (a), filamentous Bangiales patches (b) and Ulva species (c).

Sampling methods

Transects or random sampling methods would not be effective when applied to taxa with such small, patchy populations; therefore, a single 5x5m^2 quadrat was placed at each site. Expocrete™, a rapid-setting two-part concrete designed for marine environments, was used to mark site boundaries (Fig 3.3). Sites were surveyed at low tide, and all visible patches of Bangiales, and adjacent organisms, were traced onto A4 transparencies 21x29cm (609cm^2) using a permanent marker (Fig 3.2). The exact location of each Bangiales patch within the 5x5m^{2}quadrat, and the nature of the substrate (rock, wood, iron or concrete), was recorded.

Molecular identification and calculation of abundance

Identity of individual patches was confirmed or inferred from genetic analysis, as described in Chapter 2 and Appendix B. Abundance of each taxa was calculated retrospectively once the results of the molecular analyses were known, using tracings from the field and the assistance of a 1cm^2 grid.

Fig 3.3. Use of Expocrete™, indicated by the white arrow, to mark the boundary of the 5x5m study area at each site. The example shown is the Greta Point site. A 30cm ruler is shown for scale. The brownish-red filamentous Bangiales patches on the right of the photograph were identified molecularly as Bangia sp. BFK.

Climate data

Data on climatic conditions during the intensive study period, Oct 05 to Dec06, including mean daily air temperatures, sunshine hours and daily rainfall, were sourced from New Zealand's National Climate Database, administered by NIWA (Website: http//Cliflo.niwa.co.nz). Data are captured at monitoring stations in Wellington city, Kelburn and Wellington airport.

Field sampling limitations on statistical analysis

As described in Chapter 2, in this study, a decision was made, for personal reasons, to reduce data collection in the period March to May 06 to one site only (Frank Kitts lagoon). This unfortunately constrained statistical analysis of these data. In the final statistical analyses each site was treated as one 5x5m^2 quadrat, so the decision to narrow data collection to a single site for a period of a few months, meant that site-to-site comparisons could not be

made. Possible patterns were observed within the Frank Kitts site, such as apparent seasonal changes and substrate preferences of each lineage, but these observations could not be statistically tested due to the lack of other sites against which to compare the data. For future studies it is recommended that a statistician be consulted early in the design stage of the study, so that the sampling design takes into consideration the statistical tests that are likely to be applied after the data are collected.

Tests for differences in distribution

Two way Analysis of Variance (ANOVA) was used to test for differences in abundance of Bangiales, irrespective of lineage, over the four sampling periods and also to test for differences between the inner harbour ($n = 3$: Frank Kitts, Freyberg and Greta Point) and the south coast sites ($n = 2$: Lyall Bay, Owhiro Bay). The two factors in the model were: location (2 levels; south coast, harbour) and month (4 levels: Oct 05, Dec 05, Feb 06 and Jun 06). An analysis of deviance was conducted to determine the statistical significance of including an interaction between location and time in the model. The interaction did not contribute significantly to the model ($P = 0.631$); therefore the interaction term was removed in order to concentrate on the main effects of time and location.

A relaxed variance ANOVA was used to determine if lineage richness of Bangiales varied through time. Only months for which two or more sites were visited were included (data for Nov 05, Apr 06 and May 06 were excluded).

For six lineages (*Bangia* sp. BFK, BGA, BRMg, BMW, C8 and BHH1), a relaxed variance ANOVA was conducted to determine if abundance varied through time (*Minerva aenigmata* was not included due to insufficient data). Only months for which two or more sites were visited were included (data for Nov 05, Apr 06 and May 06 were excluded).

For four sites (Freyberg Pool, Frank Kitts, Greta Point, Owhiro Bay), the association between Bangiales lineages (*Bangia* sp. BFK, BGA, BRMg, BMW, C8, BHH1 and *M. aenigmata*) and seasonality was quantified using a Euclidean dissimilarity matrix to examine differences in the abundance of Bangiales. Sites and/or seasons with similar Bangiales abundances are characterised by small Euclidean distances. For all four sites, data from Oct 05, Dec 05, Jan 06 and Jun 06 were included in the analysis. For three sites, additional sampling dates were also included as follows: for the Frank Kitts site data from Feb, Mar, Apr and May 06; for the Freyberg site data from Feb 06; and for Owhiro Bay data collected in Mar 06. From the resulting dissimilarity matrix, a Principle Component plot was constructed using the vegan package in R 2.10.1 (R Core Development Team 2009) to graphically display the relationships between lineages and seasonality.

To determine the relationship between the abundance of each Bangiales lineage (*Bangia* sp. BFK, BGA, BRMg, BMW, C8, BHH1 and *M. aenigmata*) and site (Freyberg Pool, Frank Kitts, Greta Point, Lyall Bay, Owhiro Bay) for each of three sampling dates (Oct 05, Dec 05 and Feb 06), the association between lineages and seasonality was quantified using a Euclidean dissimilarity matrix. Note that sufficient data was available for site comparisons in October, December and February, but the absence of many lineages from the five sites visited in June meant that there was insufficient data available for a relationship plot. From the resulting dissimilarity matrix, a Principle Component plot was constructed using the vegan package in R 2.10.1 to graphically display the relationships between lineages and seasonality. An Analysis of Similarity (ANOSIM) was then conducted to determine if south coast sites (Lyall Bay and Owhiro Bay) were more similar to one another than they were to harbour sites (Freyberg Pool, Frank Kitts and Greta Point).

The use of substrate by each lineage was recorded during each site survey; however the data set did not contain sufficient replicates to enable a test for habitat use such as a Manly's Alpha calculation (Manly 1972, Chesson 1978), as most study sites contained only a single substrate: rock.

Vertical distribution of taxa at Frank Kitts lagoon was measured using a spirit level and one metre rulers.

Results

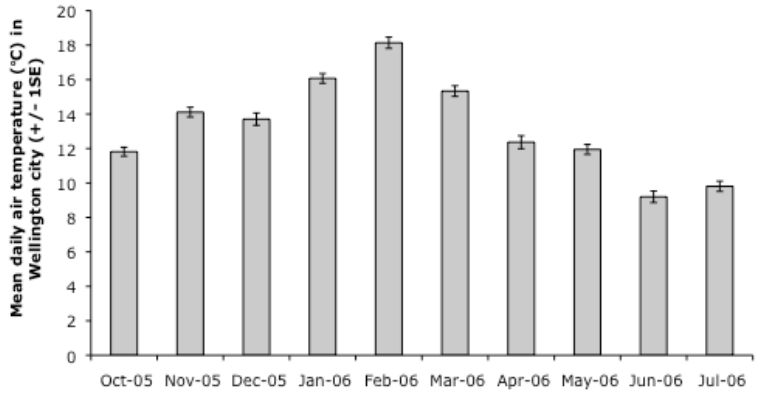
Climate data

During the summer months of the study period (Dec 05 - Feb 06), mean daily air temperature ranged between 21.1°C and 21.3°C, and in winter between 12.1°C and 12.5° (Jun - Jul 06) (Fig 3.4a). Daily sunshine hours ranged between zero and 13.7 hrs in summer, and zero to 9.1 hrs in winter (Fig 3.4b). In summer daily rainfall varied between zero and 25.4mm, and in winter between zero and 40.8mm. July was the wettest month with a daily mean of 6.96mm, and November the driest month with 1.09mm the daily mean (Fig 3.4c).

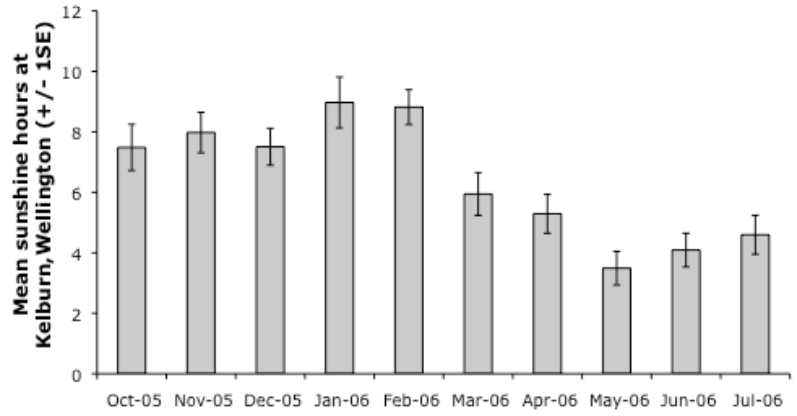
Compared to climate data from previous years, as held in the National Climate Database (http//Cliflo.niwa.co.nz), the intensive study period (Oct 05 to Jul 06) was characterised by a hotter, drier spring than usual, followed by a hot and sunny summer, average autumn and a wet but sunny winter. (NIWA 2005-06 Climate Summary www.niwa.co.nz).

Sea surface temperature data were not recorded during this study, however the Greater Wellington Regional Council holds data from two years later (Oct 07 – Jul 08), for four of the study sites: Freyberg beach, Seatoun beach at the wharf, Owhiro Bay and Lyall Bay (Fig 3.5). During the period Oct 07 – Jul 08, sea-surface temperatures at all four sites peaked in Jan 08: Freyberg 21.5°C, Seatoun 20.5°C, Owhiro Bay 18.6°C and Lyall Bay 19.6°C; whilst the lowest temperature at each site was recorded in May: Freyberg 10.6°C, Seatoun 10.4°C, Owhiro Bay 10.7°C and Lyall Bay 10.4°C. These data give some indication of the range and pattern of sea surface temperatures over the study period (Oct 05 – Jul 06), although it should be noted that compared to other years the 07/08 summer experienced more sunlight hours, and that May 08 was colder than the same month in previous years (NIWA 2007-08 Climate Summary www.niwa.co.nz).

a)



b)



c)

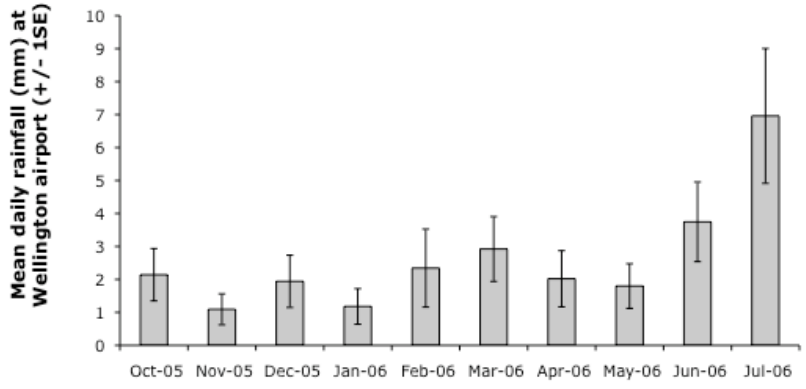


Fig 3.4. Mean daily air temperatures, sunshine hours and daily rainfall, as recorded by monitoring stations in Wellington city, Kelburn and Wellington airport respectively, over the intensive study period, Oct 05 to Dec 06. Source for data: New Zealand's National Climate Database, administered by NIWA. Website: http//Cliflo.niwa.co.nz.

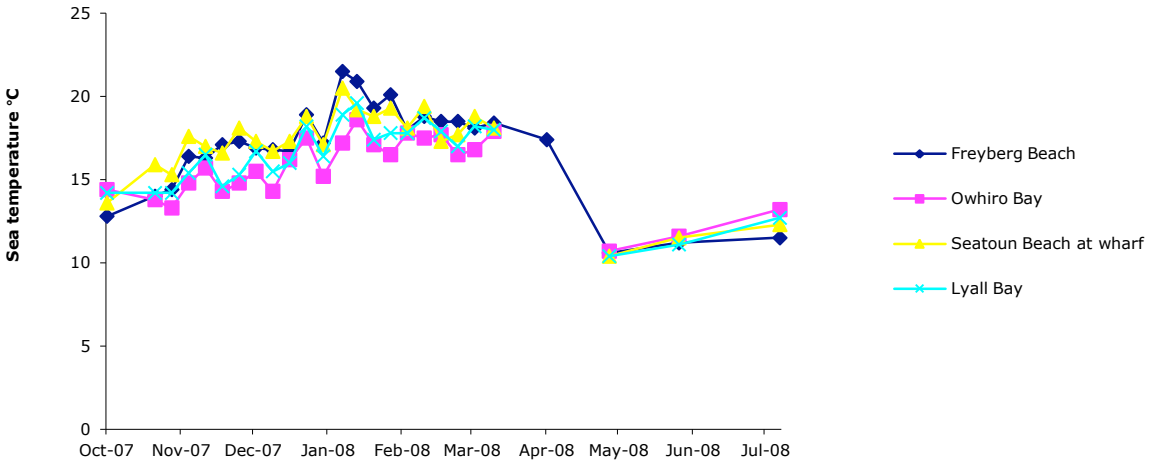


Fig 3.5. Spot near-surface sea temperatures taken at four of the study sites over the period Oct 07 – Jul 08. Note that these data were recorded two years after the study period, as data was not collected by the council until this time. Source: Greater Wellington Regional Council data collected as part of the bathing water quality monitoring programme. Note that the 07/08 period was characterised by a cold October, warm summer with higher-than-average sunshine hours for Wellington, and a colder-than-average May. Source: NIWA 2007 - 8 Climate Summary www.niwa.co.nz.

Abundance of Bangiales over time (irrespective of lineage)

ANOVA analysis showed that abundance of Bangiales (irrespective of lineage) did not vary significantly over the four sampling periods of the study (Fig 3.6: $F_{3,135} = 0.826$, $P = 0.482$); although there was a marginally significant difference in abundance between sites on the south coast and those in the harbour (Fig 3.6: $F_{1, 138} = 2.989$, $P = 0.086$). On average, abundance was 18.34 times greater at the harbour sites relative to the south coast sites (Fig 3.6).

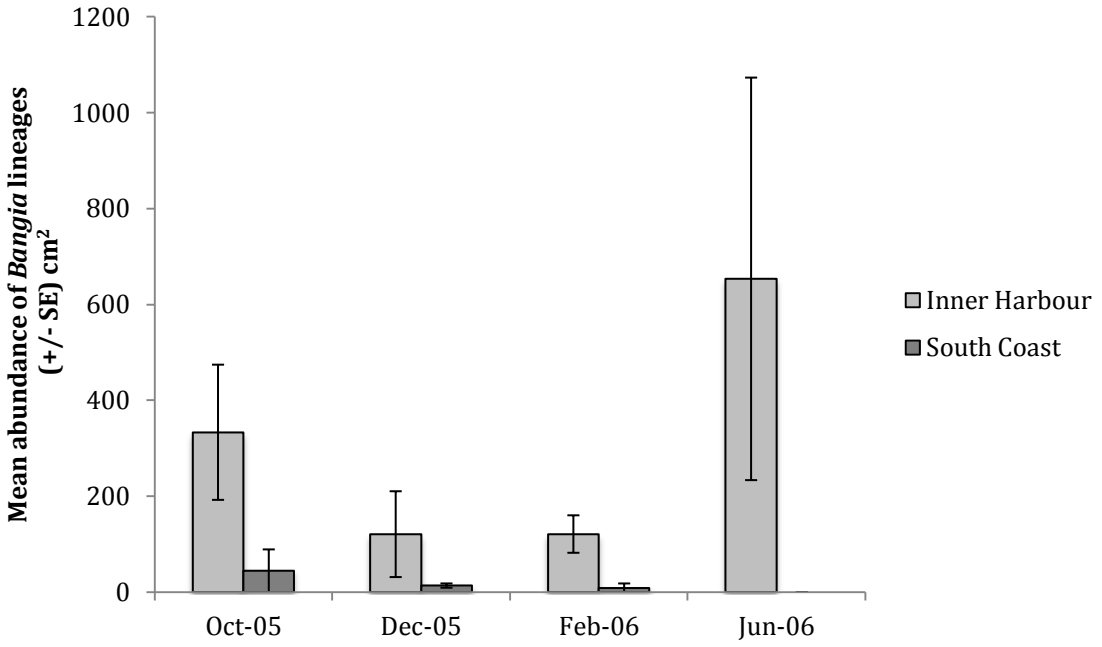


Fig 3.6. Average abundance of Bangiales (irrespective of lineage) at inner harbour sites (n=3: Frank Kitts, Freyberg and Greta Point) compared to South coast sites (n=2: Lyall Bay, Owhiro Bay), between Oct 05 and Jun 06. All five sites were visited in the months indicated.

Lineage richness (number of lineages) of Bangiales through time

Filamentous Bangiales populations were present year-round in the Wellington region (Table 3.2). Some lineages, e.g., *Bangia* sp. BGA and BRMg, were recorded only in the warmer months (Table 3.2), while others were present year-round (i.e., *Bangia* sp. BFK, BMW and *M. aenigmata*). *Bangia* sp. C8 and BHH1, were both recorded only once, in spring.

Table 3.2. Presence of filamentous Bangiales in the Wellington region pooled from all study sites (Frank Kitts, Freyberg, Greta Point, Seatoun boatshed, Lyall Bay and Owhiro Bay) during the period Jul 04 – Jul 06. Note that there is sampling bias, with reduced number of surveys during winter, as indicated in Chapter 2, Table 2.1.

	Spring			Summer			Autumn			Winter		
	Sept	Oct	Nov	Dec	Jan	Feb	Mar	April	May	June	July	Aug
BFK		●		●	●	●	●	●	●	●	●	
BRMg		●		●	●	●						
BGA		●	●	●	●							
BMW		●	●	●		●					●	●
M. aenigmata					●	●		●				●
BHH1			●									
C8			●									

During the intensive study period, Oct 05 to Jun 06, lineage richness was greatest between October and January (Fig 3.7); although richness did not vary significantly through time ($F_{5,5} = 2.577$, $P = 0.160$).

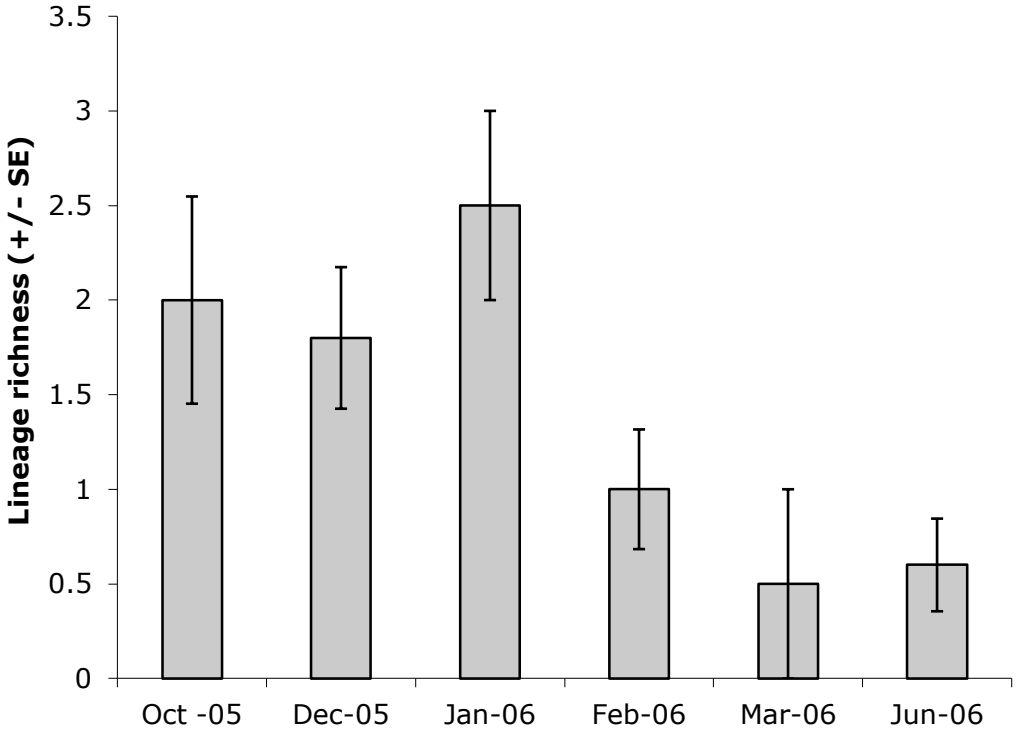


Fig 3.7. Average lineage richness of Bangiales in the Wellington region for six sampling periods between Oct 05 and Jun 06. Only months for which two or more sites were visited were included. Six sites were visited in October and February (n=6: Frank Kitts, Freyberg, Greta Point, Seatoun, Lyall Bay, Owhiro Bay). Seatoun was not visited in December or June (n=5 for both months). January data is from Frank Kitts and Freyberg (n=2), March data from Frank Kitts and Owhiro Bay (n=2).

Lineage richness (number of lineages) of Bangiales at each site

Not all filamentous Bangiales lineages were found at each site (Table 3-3). Greta Point had the greatest number of lineages (5), followed by Frank Kitts and Owhiro Bay (both 3), Freyberg and Lyall Bay (both 2) and Seatoun Boatshed (1).

Bangia sp. BRMg, BGA and C8 were only found in the inner harbour. BHH1 was only found on the south coast. *Bangia* sp. BFK and BMW were recorded from inner harbour and south coast sites. *M. aenigmata* was recorded from the harbour entrance and the south coast.

Table 3.3. Presence of filamentous Bangiales at each study site in the Wellington region during the period Jul 04 – Jul 06.

	Inner harbour sites			Harbour entrance	South coast sites	
	Frank Kitts	Freyberg	Greta Point	Seatoun Boatshed	Lyall Bay	Owhiro Bay
BFK	●	●	●		●	
BRMg	●		●			
BGA	●	●	●			
BMW			●		●	●
M. aenigmata				●		●
BHH1						●
C8			●			

Abundance of individual Bangiales lineages over time

The abundance of *Bangia* sp. BFK had bimodal peaks in Jan 06 and Jun 06 (Fig 3.8a), but abundance did not vary significantly through time ($F_{5,18} = 2.182$, $P = 0.108$). *Bangia* sp. BGA also showed a bimodal distribution with peaks in Oct 05 and Jan 06 (Fig. 3.8b), but again changes in abundance through time were not statistically significant ($F_{5,18} = 1.509$, $P = 0.236$). *Bangia* sp. BMW and BRMg both showed a unimodal distribution over time, with peaks in Oct 05and Dec 05, respectively (Fig. 3.8d and c). These changes in abundance were also not statistically significant (BMW: $F_{5,18} = 0.484$, $P = 0.784$; BRMg: $F_{5,18} = 0.636$, $P = 0.675$). *Bangia* sp. C8 and BHH1 were both only recorded in October 2005 (Fig 3.8e and f), so changes in abundance could not be statistically tested.

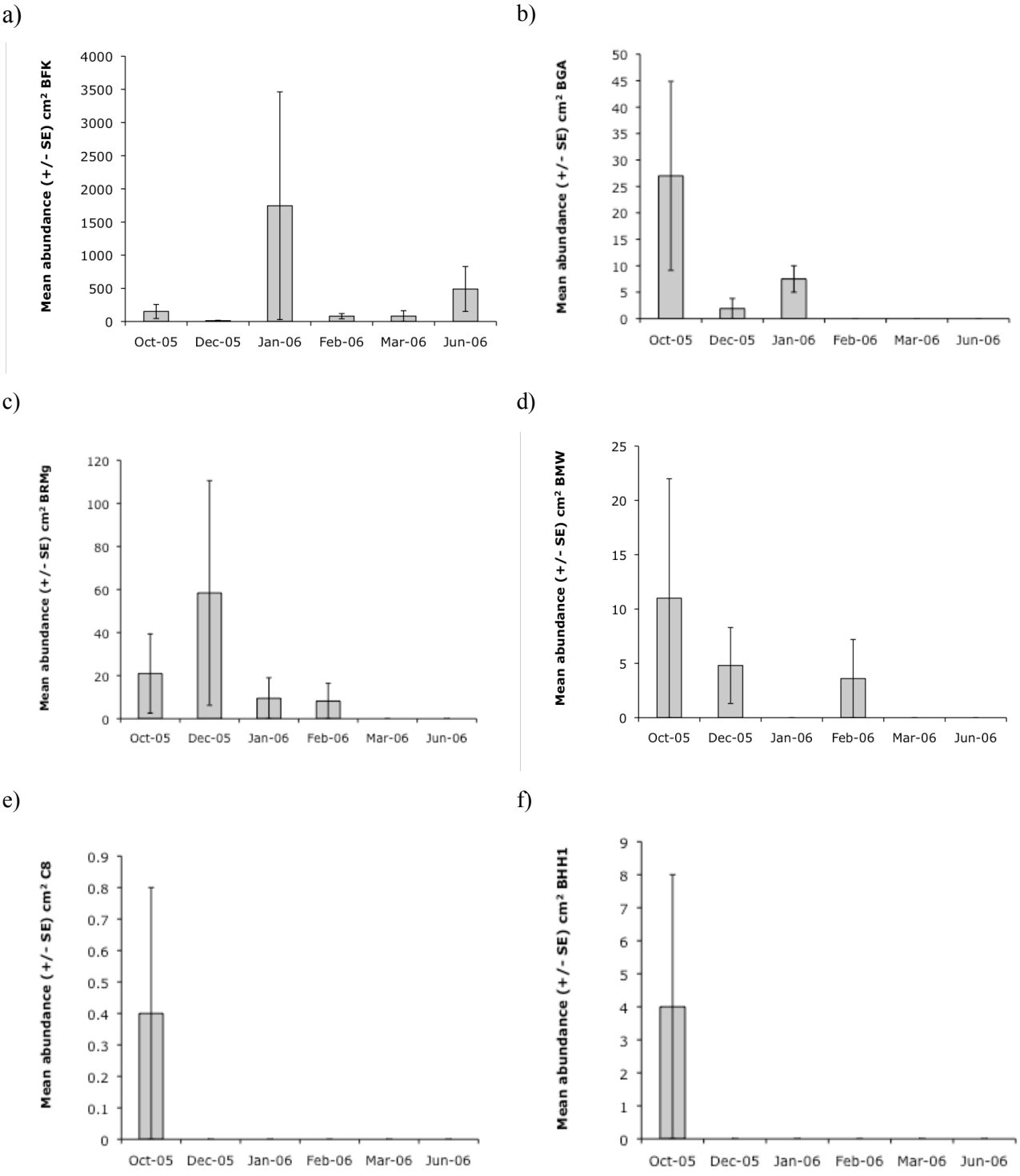


Fig 3.8. Mean abundance of six lineages of filamentous Bangiales in the Wellington region for six sampling periods between Oct 05 and Jun 06. Only months for which two or more sites were visited were included. Five sites were visited in October, December, February and June (n=5: Frank Kitts, Freyberg, Greta Point, Lyall Bay, Owhiro Bay). January data is from Frank Kitts and Freyberg (n=2), March data from Frank Kitts and Owhiro Bay (n=2).

Site-specific effects on seasonal abundance of individual Bangiales lineages

The seasonality of individual Bangiales lineages varied substantially between sites. At the Freyberg site, the entity *Bangia* sp. BFK revealed strong seasonality, with a strong presence in January, and a weak relationship with the other sampling dates (Fig 3.9a). The largest two patches of filamentous Bangiales recorded for any site during the study period, were those of *Bangia* sp. BFK at Freyberg in January: with patch sizes of 2500cm^2 and 900cm^2 respectively. There was no strong seasonal component to the abundance of any of the other lineages (as indicated by the clustering of the other lineages about 0). The separation of *Bangia* sp. BFK from the other lineages on PC1 results from its abundance being orders of magnitude greater than the other lineages.

At the Franks Kitts site, *Bangia* sp. BFK was most strongly related with the autumn and winter months compared with other sampling dates (Fig 3.9b), indicating higher abundance during these seasons. The only other seasonal pattern at Frank Kitts was the strong relationship of *Bangia* sp. BGA with Oct 05.

At Greta Point, the entity BRMg strongly related with Dec 05 and weakly with other sampling dates (Fig 3.9c), indicating a strong seasonal component in the abundance of BRMg. The data show that it was also the only lineage recorded during that month. *Bangia* sp. BFK was also distinct at this site, and strongly related with Feb and Jun 06. There were no strong seasonal components to the abundances of the other lineages.

At Owhiro Bay, two lineages, *Bangia* sp. BMW and *M. aenigmata*, showed a seasonal component to their abundance (Fig 3.9d), peaking in Oct 05.

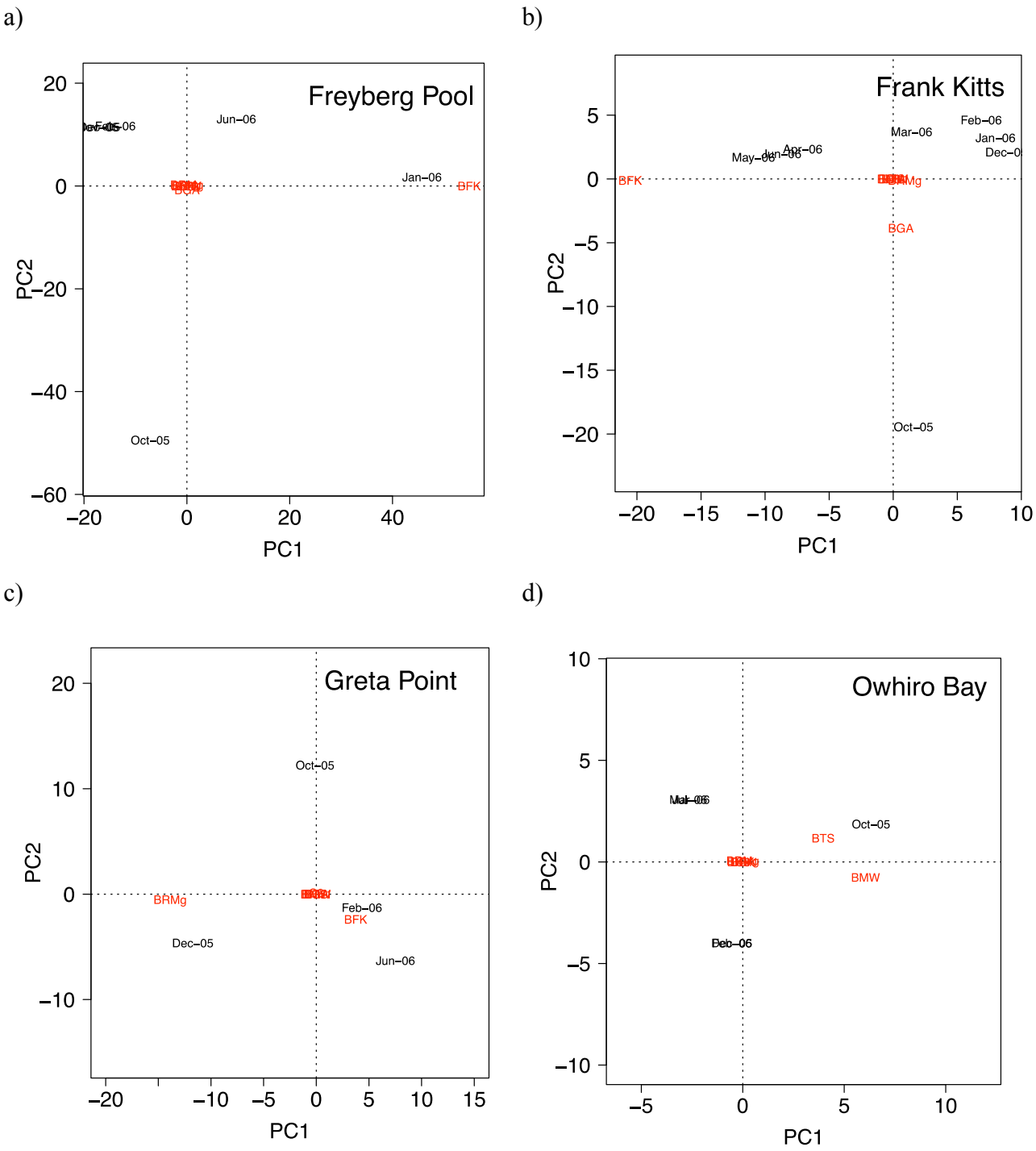


Fig 3.9. PCA plots of the relationship between season and the abundance of filamentous Bangiales lineages at four sites in the Wellington region over the period Oct 05 to Jun 06. Freyberg was surveyed in Oct, Dec, Jan, Feb and Jun; Frank Kitts in Oct, Dec and then every month till Jun; Greta Point in Oct, Dec, Jan and Feb; Owhiro Bay in Oct, Dec, Feb, Mar and Jun. Note: the data-point BTS refers to M. aenigmata.

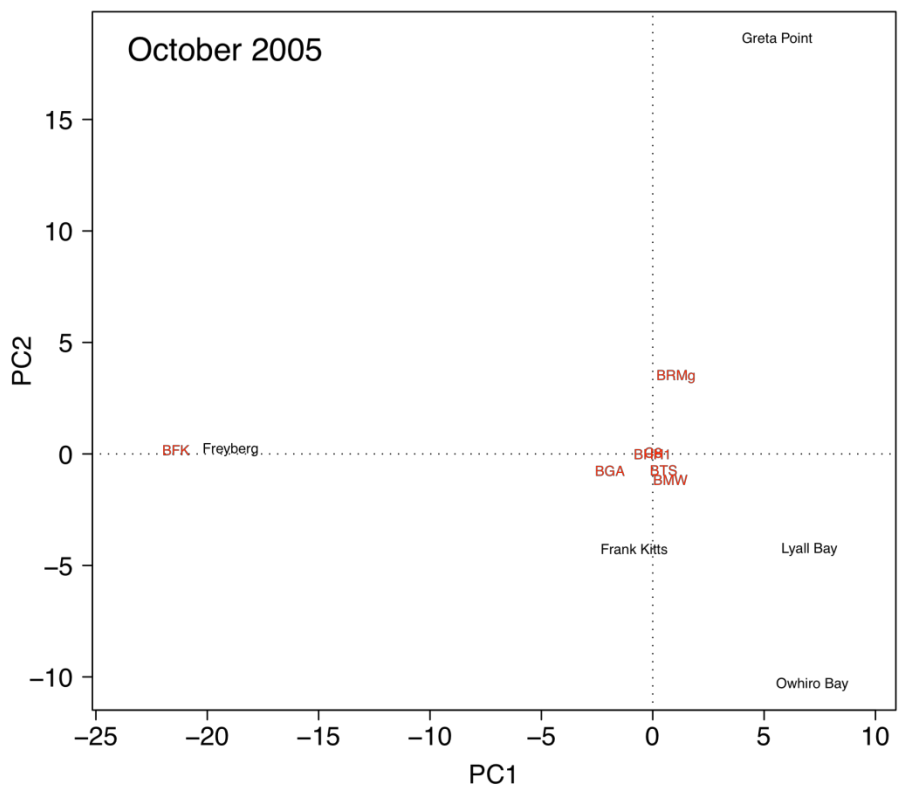
Effect of harbour or coastal conditions on seasonal abundance of individual Bangiales lineages

For the month of Oct 05, Freyberg was strongly related with *Bangia* sp. BFK (Fig 3.10a). Greta Point, an inner harbour site, was strongly separated from the other sites, and Franks Kitts, Lyall Bay and Owhiro Bay formed a loose group (Fig 3.10a); consequently, results of the ANOSIM indicated that the Bangiales flora of the south coast sites were not more similar to one another than they were to the harbour sites ($R = 0.167$, $P = 0.302$).

For the month of Dec 05, Greta Point strongly related with entity BRMg (Fig 3.10b). Frank Kitts and Freyberg grouped together, and Owhiro Bay and Lyall Bay also formed a loose group (Fig 3.10b). However, results from the ANOSIM show that the Bangiales flora of the south coast sites were not more similar to one another than they were to the harbour sites ($R = 0.167$, $P = 0.286$).

For the month of Feb 06, there was no strong site to lineage relationship (Fig 3.10c). Lyall Bay and Owhiro Bay grouped closely together, but there were not any relationships between any of the other sites. Results from the ANOSIM show that the Bangiales flora of the south coast sites were not more similar to one another than they were to the harbour sites ($R = 0.25$, $P = 0.168$).

a)



b)

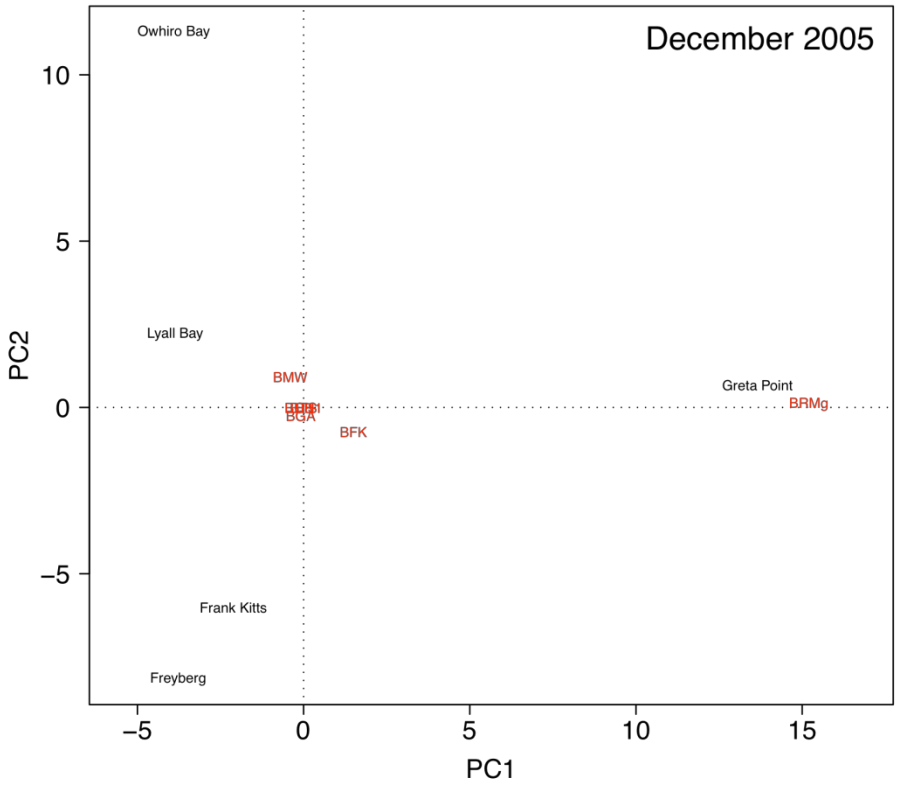


Fig 3.10 (this page and the following). PCA plots of the relationship between site and the abundance of Bangiales lineages and three sampling dates in the Wellington region. All six sites (Freyberg, Frank Kitts, Greta Point, Seatoun, Lyall Bay, Ohwiro Bay) were surveyed in Oct 05 and Feb 06. Five sites were surveyed in February: all except Seatoun. Note: the data-point BTS refers to M. aenigmata.

c)

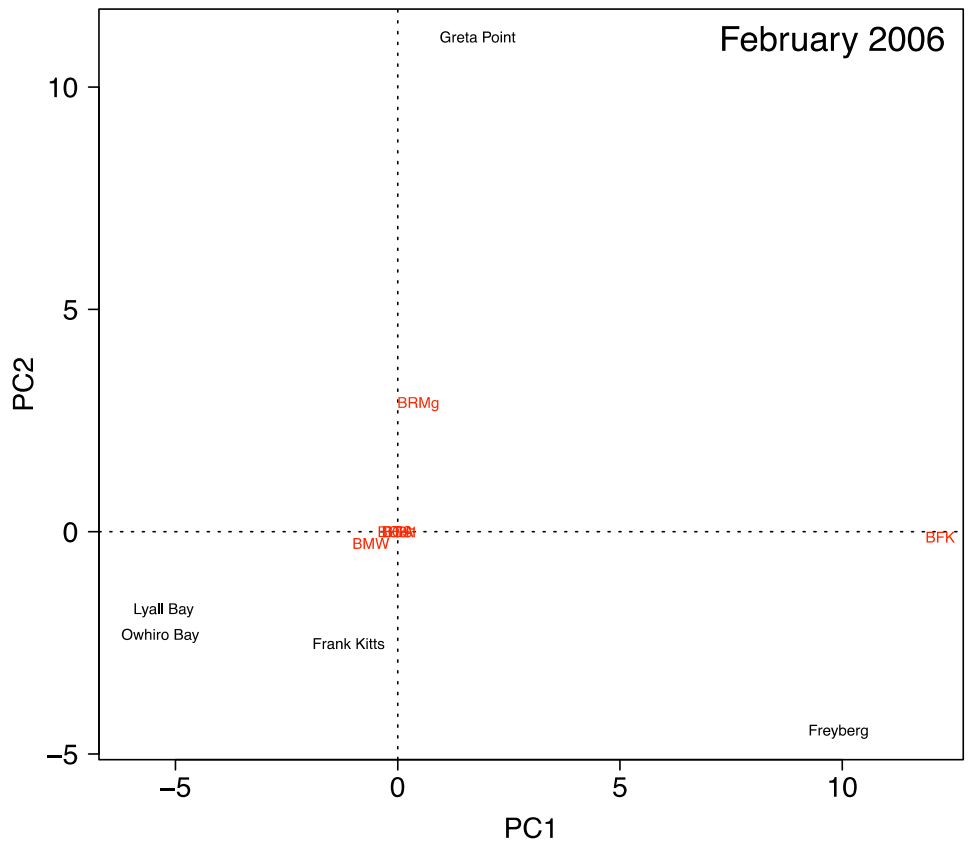


Fig 3.10 continued.

Use of substrate by individual Bangiales lineages

Filamentous Bangiales lineages in the Wellington region were found on five different substrate types (Table 3.4). All lineages were recorded from rocky substrate. Only two (*Bangia* sp. BFK and *M. aenigmata*) were recorded on concrete, two on wood (*Bangia* sp. BFK and BGA), one on iron (*Bangia* sp. BGA) and one growing on a limpet's shell (*M. aenigmata*). *Bangia* sp. BFK, BGA and *M. aenigmata* were recorded from more substrates than the other lineages: each found on three different substrate types.

Table 3.4. Substrate types associated with filamentous Bangiales lineages in the Wellington region.

	Iron palings	Wooden palings	Concrete	Rock	Limpet shell
BFK		•	•	•	
BRMg				•	
BGA	•	•		•	
BMW				•	
M. aenigmata			•	•	•
BHH1				•	
C8				•	

Quantification of habitat use relative to habitat availability, e.g., through a Manly's Alpha calculation (Manly 1972, Chesson 1978), was not possible due to the low number of replicates in the data set. As there were only two sites that contained both multiple substrates and lineages (Frank Kitts and Freyberg), the sample size was too low to conduct a meaningful test.

Anecdotally however, survey results may indicate possible relationships between lineages and substrates. Over the nine-month survey period at Frank Kitts lagoon *Bangia* sp. BFK was recorded exclusively from wooden palings, despite the apparent availability of three other

substrates (rock, iron and concrete), whereas *Bangia* sp. BRMg at the same site was found exclusively on rock. None of the three lineages recorded from the Frank Kitts site (BFK, BRMg and BGA) established on the smooth concrete steps. In contrast, the rough-textured concrete slabs of the Freyberg site provided a substrate for the largest discrete patch of filamentous Bangiales recorded during this study: a single patch measuring 2500cm^2 identified as *Bangia* sp. BFK.

Differences in vertical distribution of Bangiales lineages in the inter-tidal zone

At Frank Kitts lagoon and Freyberg in Nov 05 populations of *Bangia* sp. BFK and BRMg grew within a vertical range of 0.5m. At Frank Kitts, *Bangia* sp. BFK was recorded on wooden palings, growing in a narrow 0.2m vertical range, with a mean tidal height of 1m (Fig 3.11), while BRMg grew on rocks below the palings within a vertical range of 0.3m with a mean tidal height of 0.72m (Fig 3.11). At the Freyberg promontory site, *Bangia* sp. BFK was recorded growing within a greater vertical range than the population at Frank Kitts lagoon (0.5 m at Freyberg compared to 0.2 at Frank Kitts), with a mean height of 0.87m (Fig 3.11).

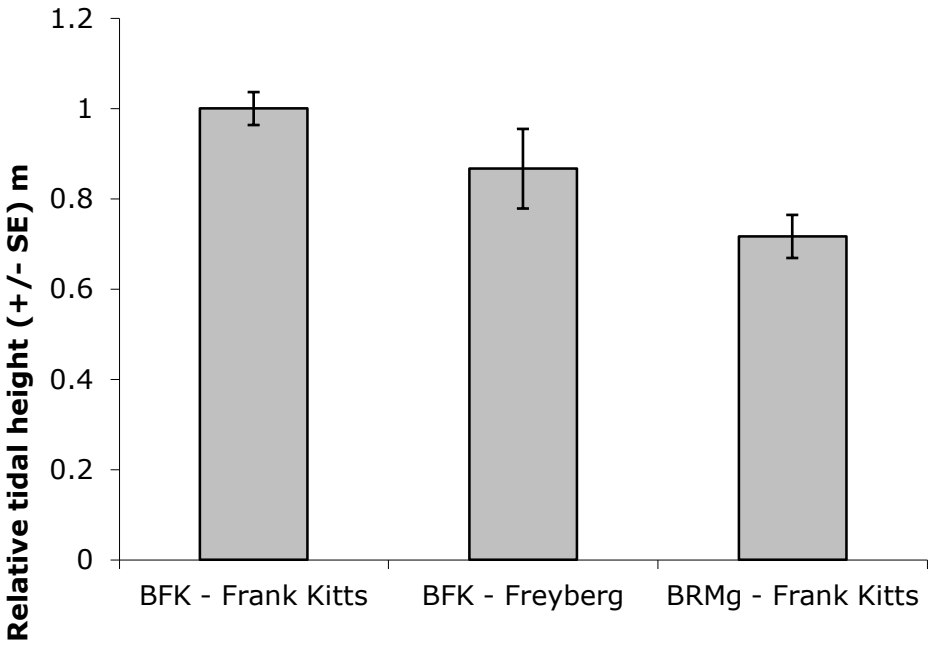


Fig 3.11. Mean tidal height of Bangia patches at Frank Kitts lagoon as recorded in Nov 05, with error bars showing +/- standard error of the mean height. The vertical mid-point of patches within each 5x5m^2 study site (BFK and BRMg) was recorded (n=6 for each lineage/site combination).

Small-scale patch dynamics of filamentous Bangiales lineages

Patches of filamentous Bangiales were found, through repeated molecular identification of multiple samples, to consist of individual plants of the same lineage. Patches were highly ephemeral, with populations present on one sampling date usually absent from the same structure (i.e., an individual rock, wooden paling or section of concrete slab) on the following sampling date, one month later.

When *Bangia* was present on the same structure in consecutive sampling dates, it tended to be the same entity: 19 times out of 23 (Appendix B). For example, *Bangia* sp. BFK was identified from wooden paling 13 at Freyberg in Oct and Dec 05 and then every month through to Jun 06 (Appendix B).

Occasionally another lineage had replaced the other when the structure was sampled the following month, e.g., at Greta Point *Bangia* sp. C8 was identified on rock 1A in Nov 05 and had been replaced by entity BRMg in Dec 05 (Appendix B).

Discussion

For the Bangiales, research has suggested that important factors determining distribution and stable co-existence are temperature and photoperiod (Bödeker et al. 2008); and tidal height and exposure to sand abrasion (Nelson et al. 2005).This study found a marginally significant difference in abundance of filamentous Bangiales between the exposed South coast compared to the inner harbour, suggesting that sand scour and wave action may be factors limiting abundance. Abundance of filamentous Bangiales, whether that of individual lineages or collectively, was not shown to differ significantly over time in the Wellington region; however, the data do suggest relationships between lineages and sites in some seasons, indicating possible seasonal patterns for some lineages. Stochastic events and site-specific conditions also appear to influence the abundance of individual lineages over time at each site. The distribution of the conchocelis phase of filamentous Bangiales in Wellington may also influence distribution of the gametophyte generation, and would make for an interesting future study.

Bangiales populations were found to be highly ephemeral, with gametophytes generally persisting a matter of weeks. The abundance of populations at the study sites varied greatly over the study period: ranging from zero (at all sites except Frank Kitts and Greta Point which recorded continuous populations) up to 3472cm^2 for the Freyberg site. The largest individual patch recorded was *Bangia* sp. BFK: 2500cm^2, suggesting that intra-specific competition may not be a limiting factor. On several occasions one Bangiales lineage would replace another on the same rock, and two lineages were recorded from the same rock on more than one occasion (Appendix B) indicating that they are in close proximity at times, both temporally and spatially, and, if suitable substrate is a limiting factor, it could be that a level of inter-specific competition may be occurring. However, at most sites filamentous Bangiales are surrounded by bare rock,

suggesting that inter-specific competition is not a key factor determining their distribution. Desiccation, wave action and herbivory may limit these populations more than intra- or inter-specific competition.

Proximity to the conchocelis phase may also be an important determinant of distribution and seasonal abundance of filamentous Bangiales taxa. Little is known about the conchocelis phase of these taxa, but it could speculated that it is perennial and also growing in very close proximity (or in fact directly in the same location) as the filamentous stage. A future study which maps conchocelis as well as gametophyte distribution could reveal important information about the relationship between the distributions of the two life stages, and determine whether proximity to conchocelis is a factor which may explain the consistent re-appearance of the ephemeral filamentous stage in similar locations over time. The filamentous stage can also reproduce asexually (Dixon & Richardson 1970, Conway & Cole 1977, Nelson et al. 1999, Bödeker et al. 2008), so is not exclusively dependant on the conchocelis for persistence, but the availability of conchospores may nevertheless be an important factor in explaining temporal and spatial distribution.

The benefit of the survey technique, using Expocrete permanent markers and detailed tracings, enabled observations of small-scale patch distribution over time: these observations revealed that between one month's survey and the next, previously recorded patches were often no longer visible and "new" patches had established in close proximity, often without overlapping where the previous patches had been. Filaments cut due to grazing pressure or wave action, could be invisible to the naked eye; but could then potentially regrow from the remaining cells, through intercalary cell divisions (Coomans & Hommersand 1990). Growth rates in culture of most lineages have been shown to be in the order of less than 1mm / month (Bödeker 2003), so it

would take some months before patches were visible again; the exception is the fast-growing *Bangia* sp. BMW which is discussed later.

In some cases "new" patches may actually be regrowth, but they may also be newly established filaments generated either from a conchospore released from the sporophyte phase, or from a neutral or asexual spore generated directly from another gametophyte filament (Dixon & Richardson 1970, Conway & Cole 1977, Nelson et al. 1999, Bödeker et al. 2008). In culture investigations, lineages vary in the time recorded to complete an entire life cycle, e.g., 8 weeks for *M. aenigmata* and 16 weeks for *Bangia* sp. BFK (Bödeker 2003). Presumably regrowth or asexual reproduction from the gametophyte would provide a more rapid means of patch establishment.

All of the methods of regeneration mentioned above may be occurring in populations in Wellington, but it is not known which is the predominant means. Environmental triggers, temperature and photoperiod, are known to trigger changes in the life cycle (Sommerfeld & Nichols 1973; Waaland et al. 1987), and in the present study a pollution event may have triggered a decline followed by an unusual surge in abundance at one site (discussed later). Microscopic examination of filaments from the field could have provided information about the reproductive state of populations, however this was not conducted as part of this study.

The exception to this pattern of dynamic small-scale distribution, was the apparent persistence for at least nine months (Oct 05 to Jun 06) of *Bangia* sp. BFK patches on wooden palings at Frank Kitts lagoon. This persistence of sizable patches (greater than $10cm^2$) in the exact same location over time, was rarely seen for other lineages at any site, or for *Bangia* sp. BFK populations existing on other substrates such as rock. Algae are a food source for many marine

species (Levinton 2009, Karleskint et al. 2009, Duarte & Cebrian 1996), and grazers, such as limpets, may be less abundant on the vertical wooden palings than on the rocks nearby: where, in contrast, small-scale distribution of entities BRMg and BGA changed frequently, possibly as a result of herbivory. As the study sites were surveyed at low tide there was little opportunity for observation of marine herbivores, but herbivory was observed in the form of sparrows: seen at Frank Kitts lagoon with Bangiales filaments in their beaks. Herbivore-exclusion experiments would reveal more about the influence of herbivory on filamentous Bangiales distribution.

Another factor in the stable distribution of *Bangia* sp. BFK at Frank Kitts may be the sheltered, shaded environment of the site: reduced wave action and lower UV exposure may enable longevity of this taxon. The wood substrate here is weathered, and appears to retain moisture after the tide has withdrawn, protecting filaments from desiccation; further, the texture of the wood may provide ample catchment for Bangiales spores, facilitating constant regeneration of the population. Although not measured and subjected to statistical tests, field observation indicated that *Bangia* sp. BFK filaments may grow to a greater length on these palings than populations at other sites. The persistence over time of *Bangia* sp. BFK on the Frank Kitts palings supports the suggestion that it is an introduced species (as proposed by Broom et al. 2004), as the gametophyte may be able to survive the journey to New Zealand on slow-moving wooden vessels such as sea barges. This lineage is known to tolerate high temperatures, and so could survive the journey across the equator (Bödeker et al. 2008); and phylogenetic analyses reveal *Bangia* sp. BFK is most closely related to northern hemisphere and Australian Bangiales entities, indicating it may be a recent arrival to New Zealand (Broom et al. 2004).

Red algae have been shown experimentally to survive sand-burial in some cases, but they also rely on crustose or microscopic forms for regeneration (Anderson et al. 2008). The effects of

sand abrasion and wave action may explain why the overall abundance of filamentous Bangiales was found to be, on average, 18.34 times greater in the inner harbour compared to the South coast; and the size of patches was observed to be generally much smaller on the South Coast. Wellington's South coast experiences greater wave action and changing sand levels than the inner harbour, for example, site markers at Lyall Bay (Wellington's most popular surf beach) disappeared repeatedly, most likely due to sand burial, sand scour, or possibly movement of the boulders which had been marked. At Owhiro Bay, a storm on the 2nd of March 2006 caused the sand level within the study site to drop by over a metre; and the population of *Bangia* sp. BMW which had been present in February had gone, also possibly due to sand scour.

Five entities were recorded in the inner harbour (*Bangia* sp. BGA, BFK and BRMg, with single records of BMW and C8), while the South coast has only four (*Bangia* sp. BMW, *M. aenigmata*, and single records of *Bangia* sp. BFK and BHH1). *Bangia* sp. BRMg, BGA and C8 and were found exclusively in the inner harbour, one (*Bangia* sp. BHH1) was found only at a South coast site, two (*Bangia* sp. BMW and BFK) were collected from both inner harbour and South coast sites, and one (*Minerva aenigmata*) was found at a South coast site as well as in the harbour entrance. The differences in abundance between inner harbour and South coast, and the distribution of individual lineages in the Wellington region, support suggestions that exposure to sand abrasion is a factor determining distribution of filamentous Bangiales (Nelson et al. 2005).

The only lineage that was found to persist through all seasons on the South coast was *Bangia* sp. BMW. *Bangia* sp. BMW was recorded in spring, summer and winter at Lyall Bay and Owhiro Bay, with one inner harbour presence recorded at Greta Point in autumn. The only other lineages recorded on the south coast were *M. aenigmata* in summer, and one instance of *Bangia* sp. BHH1 in spring, and one of *Bangia* sp. BFK in summer. *Bangia* sp. BMW's ability to persist on

the exposed south coast even through the winter months, suggests some unique adaptation to this environment; perhaps its ability to grow significantly faster than other lineages, up to 204 μm per day compared to 7 - 30 μm per day for *Bangia* sp. BFK, BGA and *M. aenigmata* (Bödeker 2003, Table 1.1), is an adaptation to sand-scour. It is not known how quickly *Bangia* sp. BMW regrew after the storm in March 2006, as unfortunately the site was not visited again until July of that year.

Distribution records and culture studies suggest that *Bangia* sp. BMW is a cold-adapted lineage, with greatest growth under culture conditions of 12°C, which in the field is the spring sea-surface temperature on Wellington's South coast (Fig 3.5), and 100% mortality after 10 days at 20°C (Bödeker 2003, Table 1.1, Fig 1.2). Sea temperatures in Wellington's inner harbour and even the harbour entrance can climb over 20°C in mid-summer (Fig 3.5), and in Owhiro Bay and Lyall Bay fall just short of 20°C (2008 data record temperature peaks of 18.6°C and 19.6°C, respectively, Fig 3.5), suggesting that Wellington may be close to the northern limit of *Bangia* sp. BMW's distribution within New Zealand, as it would be likely to experience high mortality at these temperatures.

Although no statistically significant seasonal differences were found for individual lineages, this study only recorded the presence of *Bangia* sp. BGA, BHH1, C8 and BRMg in the warmer months (Table 3.2), suggesting they may be adapted to warmer environmental conditions, while others (*Bangia* sp. BFK, BMW and *M. aenigmata*) were present year-round, suggesting a more generalist or cooler adaptation. Although not conclusive, field data from the present study, compared with the experimental findings of Bödeker et al. (2008), suggests that both datasets support a generalist-adaptation for *Bangia* sp. BFK and *M. aenigmata* (Table 3.2 and 1.1). One of the strongest site to lineage relationships revealed by this study was that of entity BRMg at

Greta Point in December, supporting suggestions that it is warm-adapted. It should be noted that *Bangia* sp. C8 and BHH1 appear to be rare lineages, each only recorded once, in Oct 05, so any conclusions about seasonality are only speculative.

For some lineages, the data suggests different patterns of abundance over time at each site, which may reflect site-specific local conditions and stochastic events. For example, at Frank Kitts, *Bangia* sp. BFK was most strongly related with the autumn and winter months (Fig 3.9b), indicating higher abundance during these seasons; however at the Freyberg site, *Bangia* sp. BFK showed a strong relationship with Jan 06 (Fig 3.9a), and at Greta Point it showed bimodal seasonal surges in both February and June. Influencing factors at each site may be the substrate available at Frank Kitts (wooden palings), perhaps enabling greater persistence through winter, and the possible influence of a pollution event at Freyberg. Both these factors are discussed below.

If Wellington has four warm-adapted lineages as well as several generalist lineages, as suggested, then lineage richness would be expected to be greater over the warmer months. This study found that although lineage richness in the Wellington region was greatest in the warmer months: between October and January, there was no statistically significant seasonal variation in lineage richness. More data may help to determine patterns of richness over time. In the present study there was also a high degree of variability in the data-set: some sites, such as Frank Kitts and Greta Point, recorded as many as three taxa each in spring and dropped to one in winter; however other sites, such as Freyberg and Owhiro Bay, dropped to one lineage each in early summer and again in late summer. Further research would also help to identify any lineages potentially missed due to the sampling and identification methodology: e.g., there may be more rare lineages, such as *Bangia* sp. C8 and BHH1. It seems likely that there are further

undiscovered lineages present in Wellington, given the number of new entities uncovered by the present study.

The lack of statistical significance in the analysis of seasonal patterns may be due partly to the small number of study sites, but also to the large natural variance in the abundance of lineages between and within sites. Data was also constrained to comparison of abundance for the four months (Oct, Dec, Feb and Jun) which had the greatest data-set (5 sites); meaning that some data such as the extreme peak of *Bangia* sp. BFK abundance in January were not included in the statistical analyses.

In some cases observed variations in abundance may have been caused by stochastic events, such as storms, unusually hot dry weather, or even pollution events, all of which may mask underlying seasonal patterns. For example, a pollution event at Freyberg promontory may have stressed the *Bangia* sp. BFK population within the study area, leaving only a mass of white (presumably dead) filaments observed in Nov 05. Such an event could have been triggered by the unusually hot dry weather that characterised the spring of 05 (Fig 3.4, http://cliflo.niwa.co.nz), as *Bangia* sp. BFK is known to release spores most readily at temperatures of 15°C and greater (Bödeker et al. 2008), and the Freyberg site may experience more sunlight than other sites as it is the most northerly exposed; however, this whiteness of all filaments was not observed at any other time over the study period, even through a hot summer, which suggests that possibly the mass shock to the population was a result of human activity, i.e., release of chlorinated water from maintenance activities, or detergent or oil from boat traffic. Nearby Freyberg pool underwent its 5 year maintenance programme around this time, but according to the council management, the chlorinated water is discharged into the wastewater system, not directly into the harbour so would not be the cause in this case (Cordwell pers.

comm., Wellington City Council). Boat ramps close to the site are known to be cleaned with chlorine on occasion, which may provide an explanation.

Previous research has found that under conditions of stress, *Bangia* spp. produce asexual spores(Cole & Conway 1980), and it is possible that the white appearance of *Bangia* sp. BFK in November resulted from a mass reproductive event in which all filaments expelled their cell contents as spores (Bödeker pers. comm.). Unfortunately the white filaments were not examined under the microscope, which might have revealed whether spore release had occurred. Two months later, an unusually high spike of *Bangia* sp. BFK abundance was recorded at Freyberg; the two events may or may not be related. In culture experiments *Bangia* sp. BFK takes 16 weeks to complete its full life cycle (Bödeker 2003), so it is perhaps too soon for large patches in January to have been the result of sexual reproduction in November. Even asexual reproduction directly from the filaments would take several months before the filaments would be visible, assuming a growth rate of less than 1mm per month as indicated by culture experiments (Bödeker 2003).

Substrate may be a limiting factor for some taxa, while for others, such as *Bangia* sp. BFK, it may explain high abundance or continuous persistence at particular sites. All lineages were found on rocky substrate, but only two were recorded on concrete and two on wood, and one on iron. *Bangia* sp. BFK and BGA most found to be most versatile: recorded from three different substrate types. The high abundance of *Bangia* sp. BFK on both concrete and wood, supports suggestions that it may be an invasive species (Broom et al. 2004).

Quantifying habitat preference was made difficult by the fact that of the six study sites, three only have rock substrate, hence only one habitat available for use. The dataset is also constrained

by the fact that most entities only occur on one or two sites, making it difficult to calculate means for the Manly's alpha value for habitat preference across the locations. For future research into habitat preference in the filamentous Bangiales, it is recommended that researchers identify three or four sites at which the same Bangiales entities are present, and which provide a mix of the same habitat types.

Texture of concrete may be important, for example, none of the three taxa at Frank Kitts established on concrete, compared to abundant *Bangia* sp. BFK on textured concrete at Freyberg. At Frank Kitts *Bangia* sp. BFK was only found on wooden palings, but not on rock, whereas at Freyberg it was found on both substrates. It is possible that the presence of *Bangia* sp. BGA and BRMg on the limited rocky substrates available precludes establishment of *Bangia* sp. BFK but this would not explain winter patterns when *Bangia* sp. BGA and BRMg are absent.

It is possible that more sampling would find that *Bangia* sp. BFK is also present on rock at Frank Kitts, and that its apparent success on highly textured surfaces (Freyberg's concrete Fig 3.10a, and Frank Kitts wooden palings Fig 3.9b) explain more about its distribution than vertical (tidal) height (Fig 3.11) or season, in the Wellington region.

This study did not reveal statistically significant seasonal patterns of abundance in Wellington. However, the findings indicate that exposure is an important factor influencing abundance and distribution of filamentous Bangiales, supporting suggestions of other researchers (Nelson et al. 2005). Stochastic factors appeared to play a part in the seasonal abundance of lineages, and may be a major factor influencing both abundance and distribution of these algae.

Recommendations for future research

Given the short-lived ephemeral nature of these populations, the occasional brief presence of rare taxa (such as *Bangia* sp. C8 and BHH1), and the assumptions which working with cryptic species necessitates (i.e. reliance on molecular identification of a subset of individuals); it is suggested that intensive sampling over a number of years would be required to build a suitable dataset from which to identify any seasonal patterns in the distribution of filamentous Bangiales in the Wellington region. It is also recommended that a larger number of study sites be included, to provide a stronger dataset for analysis. However, it should be noted that even with a larger dataset, the influence of stochastic processes and the ephemeral nature of these organisms, may still provide a level of variation which masks underlying adaptation to particular environmental conditions.

Distribution of the conchocelis phase may be an important factor in the distribution of the gametophyte generation. Future studies could attempt to collect data on both conchocelis and gametophyte distributions, and test whether there is a statistically significant relationship between the distributions of the two life stages. A study using settlement plates could also look at the seasonal and temporal availability of Bangiales spores in Wellington, and analyse to what extent this is a factor in determining patterns of distribution and abundance in the filamentous generation.

Chapter 4. Discussion

The coexistence of cryptic species challenges competition theory, yet new records of these morphologically indistinguishable entities have increased dramatically over the last two decades (Bickford et al. 2007) and studies into marine cryptic species particularly have revealed more diversity than previously realised (Knowlton 1993). The more researchers look the more they find, and in the case of the red algal order Bangiales it is predicted that intensive sampling will reveal not only new taxa but even new genera (Sutherland et al. 2011).

The present study supports such predictions, with a new entity, *Bangia* sp. C8, recorded, characterised by a genetic sequence which differs markedly from other filamentous bangialean entities. Another new sequence was also recorded, BHH1, which may represent a second new entity. The presence of BRM in Wellington, not previously recorded from this location, brings the number of filamentous Bangiales in Wellington to a possible seven taxa: *Minerva aenigmata*, Bangia sp. BFK, BGA, BMW, BRM, BHH1 and C8. Previously only the first four had been recorded in Wellington (Bödeker et al. 2008).

Given the work undertaken by previous researchers in the Wellington region it is perhaps surprising that so many new records were found in the present study. However, when it is considered that of the samples processed for this study (167 in total) only a single sample identified as C8 and a single sample as BHH1, it seems that rarity has made it difficult for researchers to uncover the true diversity.

Is the apparent rarity of two lineages, out of the seven lineages recorded, important? Researchers examining the role of rare species in ecosystems have found that often the functionally-important species are in fact rare, e.g., in coral reef ecosystems 98% of fish species that are likely to support highly vulnerable functions are regionally rare (Mouillot et al. 2013). Filamentous Bangiales provide a food source for some marine herbivores, and while the various lineages are morphologically very similar and appear to fulfil a similar role in the ecosystem, it is perhaps possible that their chemistry and consequential palatability to herbivores may vary, and they are fulfilling different functions.

The present study did not sample for distribution of the conchocelis phase of these Bangiales taxa, and future studies could test hypotheses about the distribution and persistence of the conchocelis phase compared to the ephemeral filamentous phase. Does the niche occupied by the conchocelis phase determine the distribution of the filamentous phase? Do filamentous patches establish in close proximity to conchocelis? Does conchocelis persist in the same rock on which filamentous patches appear, explaining observed patterns of distributions?

Environmental factors are also important in the distribution of filamentous Bangiales, with exposure to sand abrasion important for some lineages (Nelson et al. 2005). In this study, wave action and sand scour were found to be in factor affecting the abundance of filamentous Bangiales, with lower abundance on the exposed South coast compared to the inner harbour. The findings also support suggestions that *Bangia* sp. BMW is a cold-adapted lineage (Bödeker 2003), being the only lineage which persisted through all seasons on Wellington's South coast, and only one inner harbour presence recorded at Greta Point in autumn. In the laboratory BMW has been found to grow up to seven times faster than other lineages (Bödeker 2003), which may be an adaptation to sand-scour.

Stochastic factors such as storms and pollution events may also be important factors, possibly masking underlying seasonal patterns. During this study the filamentous Bangiales population at one South coast site disappeared entirely following a storm, and at Freyberg the population was reduced to zero following a possible pollution event, with uniformly bleached filaments observed, followed by the population's disappearance.

Neither intra- nor inter-specific competition appears to limit the distribution of filamentous Bangiales. Availability of substrate does not appear to be a limiting factor at most sites, where patches are surrounded by bare rock or concrete; nor does the population appear to limit itself, with patches of 2500cm^2 recorded in sheltered conditions.

Further study is needed to examine what effect the distribution of the conchocelis has on the temporal and spatial distribution of the filamentous phase. The influence of environmental factors should also be more fully tested in future research.

Appendix A. Collection and Identification Data

Sample no.	DNA / PCR no.	Location	Location details	Substrate	Date collected	Ava (cut?)	Hinf I (cut?)	HaeII (cut?)	lineage- from digests	lineage - from sequence
2	2	Lyall Bay	LB1 - N	Rock	12/07/04	Yes		No	BMW	
3	3	Lyall Bay	LB1 - S	Rock	12/07/04	Yes		No	BMW	
4	4	Lyall Bay	LB2 - W	Rock	12/07/04	Yes		No	BMW	
5	5	Lyall Bay	LB2 - E	Rock	12/07/04	Yes		No	BMW	
6	6	Seatoun Boatshed	SBS1 - N	Rock	12/08/04	No	Twice		*Minerva aenigmata*	
7	7	Seatoun Boatshed	SBS1 - NW	Rock	12/08/04	No	Twice		*M. aenigmata*	
8	8	Seatoun Boatshed	SBS2 - NW	Rock	12/08/04	No	Twice		*M. aenigmata*	
9	9	Seatoun Boatshed	SBS2 - N	Rock	12/08/04	No	Twice		*M. aenigmata*	
10	10	Frank Kitts	FK1a (formerly labelled 1- 1)	Iron (painted)	12/11/04					BGA
43	12	BFK entity	Tracey's sample	Culture	n/a	Yes		Yes	BFK	
58	15	BFK entity	Tracey's sample	Culture	n/a	Yes		Yes	BFK	
59	16	BMW entity	Tracey's sample	Culture	n/a	Yes		No	BMW or BCP	
60	17	BMW entity TAC2289	Tracey's sample	Culture	n/a	Yes		No	BMW or BCP	
13	18	Frank Kitts	FK2 - S (formerly labelled FK3)	Rock	12/11/04	No	Once		BGA	
15	19	Frank Kitts	FK4 - top	Rock	12/11/04	No	Once		BGA	
16	20	Frank Kitts	FK5 - paling 21 from south	Wood	12/11/04	Yes		Yes	BFK	
61	21	Frank Kitts	Jo's sample	Unknown	15/11/04	No	Once		BGA	
62	22	Frank Kitts	Jo's sample	unknown	15/11/04	No	Once		BGA	
65	23	Frank Kitts	Taranaki Wharf - Jo's sample	Unknown	15/11/04	No	Once		BGA	
27	24	Freyberg	3 - east	Concrete	28/12/04	Yes		Yes	BFK	
29	25	Freyberg	4 - north	Concrete	28/12/04	Yes		Yes	BFK	
66	29	Owhiro Bay	Jo's sample	Unknown	27/12/04	Yes		No	BMW or BCP	
67	30	Owhiro Bay	Jo's sample	Unknown	27/12/04	No	Twice		*M. aenigmata*	
12	32	Frank Kitts	FK1 - top	Rock	12/11/04	No	Once		BGA	
14	33	Frank Kitts	FK2 - N top (formerly labelled 3)	Rock	12/11/04	No	Once		BGA	
17	34	Frank Kitts	FK13 (formerly labelled 5-21)	Wood	12/11/04	Yes		Yes	BFK	
18	35	Frank Kitts	FK6-W (boulder - N edge of pond)	Rock	12/11/04					BRMg
19	36	Frank Kitts	FK6-E (boulder next to N end of pond)	Rock	12/11/04	No				BRMg

Sample no.	DNA / PCR no.	Location	Location details	Substrate	Date collected	Ava (cut?)	Hinf I (cut?)	HaeII (cut?)	lineage- from digests	lineage - from sequence
20	37	Frank Kitts	FK7-E (alongside steps on N side of pond)	Rock	12/11/04	No	Once		BGA	
21	38	Frank Kitts	FK8-S (boulder down from anchor)	Rock	12/11/04	No	Once		BGA	
22	39	Frank Kitts	FK8 - NE (boulder down from anchor)	Rock	12/11/04	No	Once		BGA	
23	40	Frank Kitts	FK9 - N (nr anchor, E side of opening)	Iron (painted)	12/11/04	Yes		Yes	BFK	
24	41	Freyberg	FB1 - N	Concrete	28/12/04	Yes		Yes	BFK	
25	42	Freyberg	FB1 - E	Concrete	28/12/04	Yes		Yes	BFK	
26	43	Freyberg	FB2-E	Concrete	28/12/04	Yes		Yes	BFK	
28	44	Freyberg	FB3 - E(2)	Concrete	28/12/04	Yes		Yes	BFK	
45	54	Owhiro Bay	OWB1	Rock	25/01/05	No	Twice		*M. aenigmata*	
46	55	Owhiro Bay	OWB2	Rock	25/01/05	No	Twice		*M. aenigmata*	
47	56	Owhiro Bay	OWB3	Rock	25/01/05	No	Twice		*M. aenigmata*	
48	57	Owhiro Bay	OWB4	Rock	25/01/05	No	Twice?		*M. aenigmata*	
50	59	Frank Kitts	FK1a	Iron (painted)	13/03/05	No	Once		BGA	
51	60	Frank Kitts	FK 1a (formerly labelled FK 2)	Iron (painted)	13/03/05	No	Once		BGA	
52	61	Frank Kitts	FK12	Wood	13/03/05					BFK
53	62	Frank Kitts	FK11	Wood	13/03/05	Yes		Yes	BFK	
54	63	Frank Kitts	FK13	Wood	13/03/05	Yes		Yes	BFK	
55	64	Frank Kitts	FK9 (Nr anchor - formerly labelled FK6)	Iron (painted)	13/03/05	Yes		Yes	BFK	
56	65	Frank Kitts	FK10 Taranaki wharf (formerly labelled FK7)	Wood	13/03/05	No	Once		BGA	
63	66	Frank Kitts	Jo's sample	Unknown	15/11/04	No	Once		BGA	
64	67	Frank Kitts	Jo's sample	Unknown	15/11/04	No	Once		BGA	
68	68	Owhiro Bay	Jo's sample (1)	Unknown	27/12/04	No	Twice		*M. aenigmata*	
71	71	Owhiro Bay	Jo's sample (6)	Unknown	27/12/04	No	Twice		*M. aenigmata*	
72	72	Lyall Bay	Jo's sample (1)	Unknown	15/11/04	Yes		No	BMW or BCP	BMW
73	73	Lyall Bay	Jo's sample (2)	Unknown	15/11/04	Yes		No	BMW or BCP	BMW
74	74	Greta Point	1-1	Rock	13/03/05	Yes		Yes	BFK	
76	76	Greta Point	2-2E	Rock	13/03/05	yes		Yes	BFK	
77	77	Greta Point	2-2N	Rock	13/03/05	yes		Yes	BFK	
78	78	Greta Point	3-1N	Rock	13/03/05	yes		Yes	BFK	
79	79	Greta Point	3-2	Rock	13/03/05	yes		Yes	BFK	
80	80	Greta Point	3-3	Rock	13/03/05	Yes		No	BMW	

Sample no.	DNA / PCR no.	Location	Location details	Substrate	Date collected	Ava (cut?)	Hinf I (cut?)	HaeII (cut?)	lineage- from digests	lineage - from sequence
81	81	Greta Point	4-1	Rock	13/03/05	Yes		Yes	BFK	
82	82	Greta Point	4-2	Rock	13/03/05	Yes		Yes	BFK	
83	83	Greta Point	5-1	Rock	13/03/05	Yes		Yes	BFK	
84	84	Greta Point	5-2	Rock	13/03/05	No	Once		BGA	
85	85	Greta Point	5-3	Rock	13/03/05	Yes		Yes	BFK	
86	86	Frank Kitts	Hikitia Lift 1	Iron	14/03/05	Yes		Yes	BFK	
87	87	Frank Kitts	Hikitia Lift 2	Iron	14/03/05	Yes		Yes	BFK	
88	88	Frank Kitts	Hikitia Lift 3	Iron	14/03/05	Yes		Yes	BFK	
96	96	Seatoun Boatshed	Ramp nr shed 1	Concrete	22/04/05	No	Twice		*M. aenigmata*	
97	97	Seatoun Boatshed	Ramp nr shed 2	Concrete	22/04/05	No				*M. aenigmata*
109	109	Greta Point	1-1	Rock	8/07/05					BFK
111	111	Greta Point	2-1	Rock	8/07/05					BFK
113	113	Greta Point	3-1	Rock	8/07/05					BFK
117	117	Greta Point	5-1	Rock	8/07/05					BFK
124	124	Seatoun Boat Ramp	Boat ramp	Concrete	10/08/05					*M. aenigmata*
125	125	Frank Kitts	FK1a-a	Iron (painted)	24/10/05					BGA
127	127	Frank Kitts	FK5-a	Rock	24/10/05					BRMg
129	129	Frank Kitts	FK1b (Paling 9) - a	Wood	24/10/05					BGA
130	130	Frank Kitts	FK1b (Paling 9) - b	Wood	24/10/05					BGA
131	131	Frank Kitts	FK11a	Wood	24/10/05					BFK
132	132	Frank Kitts	FK11b	Wood	24/10/05					BFK
133	133	Frank Kitts	FK12a	Wood	24/10/05	Yes		Yes	BFK	
134	134	Frank Kitts	FK12b	Wood	24/10/05	Yes		Yes	BFK	
135	135	Frank Kitts	FK13a	Wood	24/10/05					BFK
136	136	Freyberg	FB1a	Concrete	27/10/05	Yes		Yes	BFK	
137	137	Freyberg	FB1b	Concrete	27/10/05	Yes		Yes	BFK	
138	138	Freyberg	FB2a	Concrete	27/10/05	Yes		Yes	BFK	
139	139	Freyberg	FB2b	Concrete	27/10/05	Yes		Yes	BFK	
140	140	Freyberg	FB3a	Concrete	27/10/05	Yes		Yes	BFK	
141	141	Freyberg	FB3b	Concrete	27/10/05	Yes		Yes	BFK	
142	142	Freyberg	FB4a	Concrete	27/10/05	Yes		Yes	BFK	
143	143	Freyberg	FB5a	Concrete	27/10/05	Yes		Yes	BFK	
148	148	Greta Point	NIWA2a	Rock	29/10/05	Yes				BFK

Sample no.	DNA / PCR no.	Location	Location details	Substrate	Date collected	Ava (cut?)	Hinf I (cut?)	HaeII (cut?)	lineage- from digests	lineage - from sequence
152	152	Freyberg	FB14a	Rock	31/10/05	No (?)				BGA 1
153	153	Freyberg	FB12a	Rock	31/10/05	No (?)				BGA 2
161	161	Freyberg	FB13b	Rock	31/10/05	Yes				BFK
162	162	Freyberg	FB10b	Rock	31/10/05	Yes				BFK
164	164	Greta Point	NIWA3a	Rock	29/10/05	No				BRMg
165	165	Greta Point	NIWA1b	Rock	29/10/05	No				BRMg
166	166	Greta Point	NIWA2b	Rock	29/10/05					BFK
169	169	Owhiro Bay	OB1a	Rock	1/11/05					BHH1
181	181	Greta Point	1a-a	Rock	15/11/05					C8
184	184	Freyberg	W - nr jetty	Rock	15/11/05	Yes		Yes	BFK	
185	185	Frank Kitts	1	Rock	18/12/05	No				BRMg
187	187	Frank Kitts	5a	Rock	18/12/05	No				BRMg
188	188	Frank Kitts	5b	Rock	18/12/05	No				BRMg
189	189	Freyberg	8a	Concrete	18/12/05	Yes		Yes	BFK	
190	190	Freyberg	13a	Rock	18/12/05	Yes		Yes	BFK	
191	191	Freyberg	Y (W of plot 14)	Rock	18/12/05	Yes		Yes	BFK	
192	192	Freyberg	Q (N of plot 1)	Concrete	18/12/05	Yes		Yes	BFK	
193	193	Lyall Bay	1a	Rock	22/12/05					BMW
194	194	Lyall Bay	1b	Rock	22/12/05					BMW
195	195	Lyall Bay	2	Rock	22/12/05	Yes		Yes	BFK	
201	201	Greta Point	1a	Rock	26/12/05					BRMg
205	205	Greta Point	1 site a (a)	Rock	26/12/05					BRMg
207	207	Greta Point	2a	Rock	26/12/05					BFK
211	211	Greta Point	3a	Rock	26/12/05					BRMg
220	220	Owhiro Bay	5b	Rock	26/12/05					BMW
222	222	Owhiro Bay	6b	Rock	26/12/05					BMW
223	223	Frank Kitts	1	Rock	26/12/05	No				BRMg
224	224	Frank Kitts	3a	Rock	29/01/06	No				BRMg
225	225	Frank Kitts	3b	Rock	29/01/06	No				BRMg
226	226	Frank Kitts	5a	Rock	29/01/06	No				BRMg
227	227	Frank Kitts	5b	Rock	29/01/06	No				BRMg
228	228	Frank Kitts	11a	Wood	29/01/06	Yes		Yes	BFK	
230	230	Frank Kitts	13a	Wood	29/01/06	Yes		Yes	BFK	
232	232	Freyberg	1a	Concrete	29/01/06	Yes		Yes	BFK	

Sample no.	DNA / PCR no.	Location	Location details	Substrate	Date collected	Ava (cut?)	Hinf I (cut?)	HaeII (cut?)	lineage- from digests	lineage - from sequence
233	233	Freyberg	1b	Concrete	29/01/06					BFK
235	235	Freyberg	2b	Concrete	29/01/06					BFK
240	240	Freyberg	X	Concrete	29/01/06					BFK
242	242	Freyberg	Z	Rock	29/01/06					BFK
243	243	Seatoun Boatshed	1a (S)	Rock	11/02/06					*M. aenigmata*
249	249	Seatoun Boatshed	4b (limpet!)	Rock	11/02/06					*M. aenigmata*
253	253	Greta Point	2b	Rock	28/02/06					BFK
254	254	Greta Point	1	Rock	28/02/06					BRMg
257	257	Greta Point	3b	Rock	28/02/06					BRMg
258	258	Freyberg	2a	Concrete	28/02/06					BFK
259	259	Freyberg	2b	Concrete	28/02/06					BFK
260	260	Freyberg	15b	Concrete	28/02/06					BFK
261	261	Freyberg	16	Concrete	28/02/06					BFK
262	262	Frank Kitts	13a	Wood	28/02/06					BFK
263	263	Frank Kitts	13b	Wood	28/02/06					BFK
264	264	Frank Kitts	13c	Wood	28/02/06					BFK
268	268	Frank Kitts	11a	Wood	28/03/06					BFK
270	270	Frank Kitts	12	Wood	28/03/06					BFK
271	271	Frank Kitts	13a	Wood	28/03/06					BFK
272	272	Frank Kitts	13b	Wood	28/03/06					BFK
273	273	Frank Kitts	13c	Wood	28/03/06					BFK
274	274	Frank Kitts	11a	Wood	27/04/06					BFK
275	275	Frank Kitts	11b	Wood	27/04/06					BFK
276	276	Frank Kitts	11c	Wood	27/04/06					BFK
277	277	Frank Kitts	12a	Wood	27/04/06					BFK
278	278	Frank Kitts	12b	Wood	27/04/06					BFK
279	279	Frank Kitts	13a	Wood	27/04/06					BFK
280	280	Frank Kitts	13b	Wood	27/04/06					BFK
281	281	Frank Kitts	11	Wood	30/05/06					BFK
282	282	Frank Kitts	12	Wood	30/05/06					BFK
283	283	Frank Kitts	13	Wood	30/05/06					BFK
285	285	Frank Kitts	11	Wood	27/06/06					BFK
286	286	Frank Kitts	12	Wood	27/06/06					BFK

Sample no.	DNA / PCR no.	Location	Location details	Substrate	Date collected	Ava (cut?)	Hinf I (cut?)	HaeII (cut?)	lineage- from digests	lineage - from sequence
287	287	Frank Kitts	13	Wood	27/06/06					BFK
288	288	Greta Point	2a	Rock	29/06/06					BFK
292	292	Freyberg	1	Concrete	1/07/06					BFK
293	293	Freyberg	2	Concrete	1/07/06					BFK
294	294	Freyberg	16a	Concrete	1/07/06					BFK
296	296	Freyberg	17	Concrete	1/07/06					BFK
297	297	Freyberg	18	Concrete	1/07/06					BFK

Appendix B. Identification Assumptions

To provide useful data for statistical analysis of spatial and temporal distribution data (see Chapter 3 Ecology), the genetic identity of as many samples as possible was required. However it was impractical to confirm the identity of all individuals molecularly, therefore assumptions about the identity of Bangiales patches needed to be made. The following table shows where actual genetic identifications were made, and also where assumptions about identity were made.

Note that where two or three Bangiales samples were taken from a single structure (rock, wooden paling or concrete slab section) at that field visit and each confirmed genetically, the entity is marked with a superscript "2" or "3" as appropriate. The superscript "p" indicates presumed identity; this is where Bangiales was present at the field visit however genetic identity was not confirmed via a sample, and therefore an assumption is made about the identity, based on genetic confirmation of other samples from the same structure and/or other structures in close proximity. A "√" indicates where Bangiales was recorded as present, however the lineage was not genetically confirmed, and there was insufficient information to make an assumption about the identity of that population. A "0" indicates no visible Bangiales was present on that structure at the time of the field visit. A "-" indicates that a site was not visited at that time.

Bangia sp. BMW	*Minerva aenigmata* (BTS)
Bangia sp. BFK	*Bangia* sp. C8
Bangia sp. BRMg	*Bangia* sp. BHH1
Bangia sp. BGA	

Site	Struct-ure	Substrate	July/ Aug 04	Nov-04	Dec 04/ Jan 05	Mar / Apr 05	July / Aug 05	Oct-05	Nov-05	Dec-05	Jan-06	Feb-06	Mar-06	Apr-06	May-06	Jun / July 06
Frank Kitts	1A	Iron	-	BGA	-	BGA^2	-	BGA	-	BGA^p	BGA^p	0	0	0	0	0
Frank Kitts	1B	Wood	-	0	-	0	-	BGA	-	BGA^p	0	0	0	0	0	0
Frank Kitts	1	Rock	-	BGA	-	-	-	0	-	BRMg	$BRMg^p$	0	0	0	0	0
Frank Kitts	5	Rock	-	-	-	-	-	BRMg	-	BRMg	$BRMg^2$	0	0	0	0	0
Frank Kitts	11	Wood	-	-	-	BFK	-	BFK^2	-	BFK^p	BFK	BFK^p	BFK	BFK^3	BFK	BFK
Frank Kitts	12	Wood	-	-	-	BFK	-	BFK^2	-	BFK^p	BFK^p	BFK^p	BFK	BFK^2	BFK	BFK
Frank Kitts	13	Wood	-	BFK	-	BFK	-	BFK	-	BFK	BFK	BFK^3	BFK^3	BFK^2	BFK	BFK
Freyberg	1	Concrete	-	-	BFK^2	-	-	BFK^2	0	0	BFK^2	BFK^p	-	-	-	BFK
Freyberg	2	Concrete	-	-	BFK	-	-	BFK^2	0	0	BFK^2	BFK^2	-	-	-	BFK
Freyberg	3	Concrete	-	-	BFK^2	-	-	BFK^2	0	0	0	BFK^p	-	-	-	0
Freyberg	4	Concrete	-	-	BFK	-	-	BFK	0	0	0	0	-	-	-	0
Freyberg	5	Concrete	-	-	-	-	-	BFK	0	0	0	0	-	-	-	0
Freyberg	8	Concrete	-	-	-	-	-	BFK^p	0	BFK	0	0	-	-	-	0
Freyberg	10	Rock	-	-	-	-	-	BFK	0	0	0	0	-	-	-	0
Freyberg	11	Rock	-	-	-	-	-	BFK	0	0	0	0	-	-	-	0
Freyberg	13	Rock	-	-	-	-	-	BFK	0	BFK	0	0	-	-	-	0
Freyberg	14	Rock	-	-	-	-	-	BGA	0	0	BGA^p	0	-	-	-	0
Freyberg	15	Concrete	-	-	-	-	-	0	0	0	0	BFK	-	-	-	BFK^p
Freyberg	16	Concrete	-	-	-	-	-	0	0	0	0	BFK	-	-	-	BFK
Freyberg	X	Concrete	-	-	-	-	-	0	0	0	BFK	0	-	-	-	0
Greta Point	A	Rock	-	-	-	BFK	BFK	-	-	-	-	-	-	-	-	-
Greta Point	B	Rock	-	-	-	BFK^2	BFK	-	-	-	-	-	-	-	-	-
Greta Point	C	Rock	-	-	-	BFK^2 BMW	BFK	-	-	-	-	-	-	-	-	-
Greta Point	1A	Rock	-	-	-	-	-	0	C8	BRMg	-	$BRMg^p$	-	-	-	0
Greta Point	1	Rock	-	-	-	-	-	BRMg	-	BRMg	-	BRMg	-	-	-	0

Site	Struct -ure	Substrate	July/ Aug 04	Nov-04	Dec 04/ Jan 05	Mar / Apr 05	July / Aug 05	Oct- 05	Nov- 05	Dec- 05	Jan- 06	Feb- 06	Mar- 06	Apr- 06	May- 06	Jun / July 06
Greta Point	2	Rock	-	-	-	-	-	BFK[2]	BFK[p]	BFK	-	BFK	-	-	-	BFK
Greta Point	3	Rock	-	-	-	-	-	BRMg	-	BRMg	-	BRMg	-	-	-	BFK[p]
Greta Point	4	Rock	-	-	-	BFK[2]	-	√	-	√	-	0	-	-	-	0
Greta Point	5	Rock	-	-	-	BFK[2] BGA	BFK	0	-	0	-	0	-	-	-	0
Owhiro Bay	X	Rock	-	-	BTS	-	-	-	-	-	-	-	-	-	-	0
Owhiro Bay	A	Rock	-	-	0	-	-	-	0	0	-	-	-	-	-	0
Owhiro Bay	1	Rock	-	-	0	-	-	-	BHH1	0	-	-	-	-	-	0
Owhiro Bay	2	Rock	-	-	BTS	-	-	-	√	0	-	-	-	-	-	0
Owhiro Bay	3	Rock	-	-	BTS	-	-	-	√	0	-	-	-	-	-	0
Owhiro Bay	4	Rock	-	-	BTS	-	-	-	√	0	-	-	-	-	-	0
Owhiro Bay	5	Rock	-	-	√	-	-	-	√	BMW	-	-	-	-	-	0
Owhiro Bay	6	Rock	-	-	0	-	-	-	BMW[p]	BMW	-	-	-	-	-	0
Seatoun	1	Rock	BTS[2]	-	-	0	0	-	-	-	-	BTS	-	-	-	-
Seatoun	2	Rock	BTS[2]	-	-	0	0	-	-	-	-	0	-	-	-	-
Seatoun	Ramp	Concrete	0	-	-	BTS[2]	BTS	-	-	-	-	0	-	-	-	-
Seatoun	4	Limpet	0	-	-	0	0	-	-	-	-	BTS	-	-	-	-
Lyall Bay	1	Rock	BMW[2]	-	-	-	0	0	-	-	-	-	-	-	-	-
Lyall Bay	2	Rock	BMW[2]	-	-	-	0	0	-	-	-	-	-	-	-	-
Lyall Bay	X	Rock	-	BMW	-	-	0	0	-	-	-	-	-	-	-	-
Lyall Bay	3	Rock	-	-	-	-	0	0	-	BMW[2]	-	-	-	-	-	-
Lyall Bay	4	Rock	-	-	-	-	0	0	-	BFK	-	-	-	-	-	-

Appendix C. References

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