Harvest ecology and biodiversity of South African *Porphyra*

Neil John Griffin

Thesis presented for the Degree of
DOCTOR OF PHILOSOPHY
in the Department of Botany
UNIVERSITY OF CAPE TOWN
October 2003

Abstract

Harvest ecology and biodiversity of South African *Porphyra*

Neil John Griffin

October 2003

Porphyra (Bangiales, Rhodophyta) is the world's most valuable maricultured seaweed, due to its high value as a food crop. The vast majority of *Porphyra* in South Africa belongs to *P. capensis*, a morphologically and ecologically plastic taxon apparently endemic to the region. There is no demand for *P. capensis* as a food crop, as it is unsuitable for the market, and there are no records of its customary use locally. *Porphyra capensis* is however a potentially highly valuable fodder for the mariculture of abalone (*Haliotis midae*), and pressure to harvest it has recently increased.

This study aims to assess the potential for harvest of *Porphyra* on the south-western shores of South Africa. There are two main thrusts to this work.

- The first thrust examines *Porphyra* as an ecological entity in the region. Seasonal *Porphyra* populations are quantified, and the role of *Porphyra* in the rocky shore community is examined in order to predict likely impacts of wide-scale harvesting on *Porphyra* as well as the eulittoral community. The effect of small-scale harvesting of *Porphyra* in the light of the above is assessed. Management proposals for *Porphyra* harvesting are presented.
- The second thrust reassesses the taxonomy of *Porphyra* species in the region. As morphological data alone has proved unreliable in delimiting *Porphyra* species, measures of underlying genetic variation are used to provide extra data in order to assess the biodiversity present within South African *Porphyra*.

Seasonal *Porphyra* biomass was determined at 40 sites, using biomass data from quadrats combined with measurements of population size. These data were extrapolated to adjacent rocky shores to provide an estimate of the total seasonal biomass in the region. Shorelines were assessed in the light of existing protected areas and reserves to determine the extent of *Porphyra* populations protected from potential harvesting. *Porphyra* varies seasonally,

but is present across the shore in summer and winter. More than half the biomass is within reserves or areas protected from harvesting.

Population biology of *Porphyra* gametophytes was assessed in the light of rocky shore community ecology at one site, using data from a combination of transects, random quadrats and fixed quadrats. *Porphyra* gametophytes were seasonal, recruiting in spring and autumn to form large summer and winter biomasses. Mortality among recruits was high, but decreased with age. Growth rates were initially very rapid, but stabilized with time. Growth within dense patches was better than when thalli were isolated. Distinct seasonal patterns were present as winter populations grew epilithically high in the eulittoral, while summer populations grew lower on the shore, frequently on other taxa and in particular on limpets and *Aeodes orbitosa*. Despite the application of a number of analyses, no clear association of *Porphyra* with other eulittoral taxa, beyond *Porphyra*'s growth on certain mid-low eulittoral taxa, was detected.

Harvesting *Porphyra* in fixed quadrats had the effect of eliminating *Porphyra* populations for the remainder of the season, as no regrowth of *Porphyra* from holdfasts occurred and recruitment into harvested quadrats was low. The analysis of the effect of harvesting on shore fauna was complicated by natural *Porphyra* die-back in control populations. Nevertheless, the primary impact of harvesting on *Porphyra* was to reduce populations in advance of natural seasonal population collapse. Harvesting had a detectable impact on shore fauna, and a noteworthy decrease in the frequency of *Nodilittorina africana* after harvesting was detected. Faunal taxa identified as most likely to be affected by harvesting were amphipods, isopods and *Nodilittorina africana*. There was no recruitment of other eulittoral macrophytes into harvested quadrats.

A new species of *Porphyra*, *P. aeodis*, is described. *Porphyra aeodis* is morphologically very similar to the endemic *P. saldanhae*, but clearly distinct using data from isozyme electrophoresis. *Porphyra aeodis* is a summer annual that grows epiphytically on *Aeodes orbitosa*.

A survey of variation within nSSU rDNA revealed a high level of variation within South African *Porphyra* species.. Of the eleven haplotypes detected, ten appear endemic to South Africa, and eight are part of the *P. capensis* species complex. *Porphyra aeodis* and *P.*

saldanhae seem to be indistinguishable using this locus. The number of species in the *P. capensis* complex was estimated by comparing variation and phylogeny within the complex with that of other *Porphyra* species. Results suggest that there are at least ten to fourteen *Porphyra* species present in South Africa, of which four to eight are members of the *P. capensis* complex. As samples for the biodiversity study were only taken during the summer, the number of species in the region will be higher.

Porphyra in South Africa seems likely to be resilient to harvesting, and the impact on the eulittoral community of harvesting *Porphyra* should be low. Conservative guidelines for harvesting are presented (see below), with the caveat that harvesting should be accompanied by a monitoring programme to ensure that localized impacts are minor. Wider scale impacts will be mediated as a large proportion of the shoreline is protected from harvesting. The impacts of harvesting on individual species of *Porphyra* cannot be predicted from this study as many cryptic, undescribed species are present, and a review of the taxonomy of South African *Porphyra* is critical to future management of the genus.

Recommendations for harvesting:

1. No more than 80 % of the harvestable biomass of *Porphyra* present in any 50m stretch of shore should be removed by harvesters.
2. Between 50-75 % of the *Porphyra* left unharvested at harvest sites should be in dense patches, representative of all components of the original *Porphyra* population.
3. Once harvested, a site should remain undisturbed thereafter for a minimum of six months.
4. As far as is possible, harvesting should take place in late summer or winter.
5. Harvesters may collect *Porphyra* by hand plucking or using shears or knives or similar instruments.
6. Harvesters should minimise removal of or damage to substrate fauna or flora.
7. Harvesters should shake or rinse thalli to avoid the removal of fauna associated with *Porphyra*.
8. Selected sites under regular or frequent harvesting regimes must be continuously monitored.

Acknowledgements

I would like to thank my supervisors, John Bolton and Rob Anderson, for their support of this thesis throughout its long gestation.

A large number of people helped with my attempts to find out just what *Porphyra capensis* was, either through input and advice, or through help in tracking the tortuous path of Kützing's original specimens through collections and herbaria. Thanks in this regard to Willem Prud'homme van Reine, Richard Moe, Jenny Moore, Pierre Compère, Olivier de Clerck, Eric Coppejans, Inke Bartsch, and Herre Stegenga. Maria Eliana Ramírez and Wendy Nelson helped with recommendations on the validity of *P. capensis* records from waters beyond Southern Africa.

I am particularly indebted to those who contributed to the work presented in the biodiversity chapter. Without their considerable and selfless assistance, in collecting samples, undertaking all of the sequencing and preliminary analysis leading to the production of 11 unique, near-complete nSSU sequences, and in arranging funding for the above, my chapter on the biodiversity of South African *Porphyra* would not have been possible. I am deeply indebted. Specifically, Wyn and David Jones, with some help from C. Sapsford, surveyed 17 sites along the coast from St Helena Bay on the west coast to Sheffield Beach on the east coast, and collected 57 specimens between St Helena Bay and Port Alfred. Suzanne Fredericq supplied one more specimen from Oudekraal. Wyn Jones extracted DNA from 45 of these specimens at the University of Otago, and, together with Judy Broom, amplified and sequenced DNA from these samples. Preliminary sequence comparisons were undertaken by Wyn Jones and Judy Broom. Wyn Jones supplied details and photographs of specimen morphologies and habitat. The images of specimens in Figure 6-3 were supplied by Tracy Farr from voucher specimens housed in WELT, Museum of New Zealand Te Papa Tongarewa. Wendy Nelson supplied data for Table 6-2, and comments on putative species morphologies. Vernon Coyne and Elke Burkhardt helped me with initial DNA extractions and amplification attempts at the University of Cape Town. Frans Weitz generously made facilities available at the University of the Western Cape for phylogenetic analyses, and along with Judy Broom, Cathal Seoighe and Enrico Tronchin offered advice on analyses.

Sue Janse van Rensburg assisted with setting up of facilities for isozyme electrophoresis, and took charge of all testing of and modifications to methods and stain recipes. William Bond and Janet Thomas made facilities available for the work that made led to the description of *Porphyra aeodis*.

Thanks to those intrepid souls who were suffered being jammed into one tiny vehicle and rushed from site to site in the fearfully early morning to survey *Porphyra* populations, before heading home to weigh bags and bags of *Porphyra* throughout the afternoon and into the night: Graham Levitt, Frederik Kemp, Chris Boothroyd, Geoff Fridjon, Alison Pudney, Ronnie Brereton-Stiles, Allan Ellis, Rob Dunn, David Stoloff, Johnny Kingston, Donovan Kirkwood, Claire Lewis, Emma Horrell and Sean Privett.

I am grateful to all who tempted and distracted me from writing up this thesis. In particular, my appreciation goes to Susi Vetter, who offered much support and much distraction during the writing and wrapping up of this thesis.

Finally, I owe a great deal to my parents, Ruth and Mike Griffin, for supporting and encouraging me throughout my biological career. This thesis is dedicated to them both.

I wish to thank the Foundation for Research Development (now National Research Foundation), Department of Sea Fisheries (now Marine and Coastal Management), the University of Cape Town, and the New Zealand Foundation for Research, Science and Technology (Contract MNZX0202) for supporting the work presented in this thesis.

Contents

1 Introduction

1.1 Economic seaweeds and South Africa

The economic importance of seaweeds is very well documented. Seaweeds have commercial value predominantly as food crops (e.g. *Porphyra*, *Undaria*, *Laminaria*, *Hizikia*, *Enteromorpha*) and as a source of phycocolloids (mostly agar, carrageenan, and alginate). Food species have the greatest value per unit mass, and are usually cultivated, rather than harvested from the wild. In many cases, specific strains have been selected over time, and these cultivars are preferred for mariculture. Although several seaweeds are cultivated for phycocolloid production and valuable strains have been selected, most phycocolloids derive from seaweeds harvested from the wild.

In contrast to many other regions of the world, there is not a long history of seaweed use in South Africa. Interest in the commercial potential of local seaweeds was sparked by a shortage of Japanese seaweed products due to the Second World War, and a number of species were identified as being potentially of value (Isaac, 1942; Isaac & Molteno, 1953). Currently, the local seaweed industry uses two kelp species, *Gracilaria*/*Gracilariopsis* and three species of *Gelidium* (Anderson *et al.*, 2003). These are mainly used for phycocolloid extraction. No phycocolloid processing currently occurs within South Africa, although it has in the past. The only locally processed seaweed product is the liquid plant growth stimulant Kelpak, derived, as the name suggests, from kelp. Other uses of seaweed include fish-feed additives and abalone fodder. Although a considerable body of research exists on *Gracilaria* mariculture in South Africa (Anderson *et al.*, 2003, and references therein), and less on *Gelidium* mariculture (Aken *et al.*, 1993), no seaweed mariculture for colloid production currently occurs. There is some small-scale mariculture of *Gracilaria* and *Ulva* for abalone fodder (Anderson *et al.*, 2003).

1.2 *Porphyra* as an economic seaweed

Porphyra species have been traditionally harvested for food in many, if not most, of the areas where they are found (Chapman, 1970). Places where harvesting of *Porphyra* species for local use is documented include South East Asia (Kang, 1971; Miura, 1975; Tseng, 1981; Lewmanomont, 1998; Nang & Dinh, 1998), Indonesia (Istini *et al.*, 1998, and refs.

therein), the Philippines (Trono, 1998), Israel (Lipkin & Friedlander, 1998), Azores (Sousa-Pinto, 1998), Great Britain (Landsborough, 1857; Jones & Holt, 1998), Ireland (Guiry & Hession, 1998), North America (Hus, 1902; Turner & Bell, 1971; Turner, 1973; Turner & Bell, 1973; Conway *et al.*, 1975; Roland & Coon, 1984, Lindstrom, 1998; Merrill & Waaland, 1998; Stekoll, 1998; Turner, 2003), Hawaii (Isaac, 1942; Cannon, 1984), South America (Acleto, 1998; Alveal, 1998; de Zaixso *et al.*, 1998; Santelices, 1996) and New Zealand (Nelson & Conroy, 1989; Schiel & Nelson, 1990). The harvesting of wild populations of *Porphyra* continues, particularly to satisfy local demands; however, natural populations have often proved insufficient to meet the demand for *Porphyra*, and most *Porphyra* consumed today is produced by commercial cultivators.

In Japan, *Porphyra* has been cultivated for nori since between 1624 and 1680 near the Sumida river estuary in Tokyo Bay (Ueda *et al.*, 1963; Miura, 1975). Early cultivation involved little more than placing additional substrates, often bundles of bamboo or twigs, where they might be colonised by naturally produced *Porphyra* spores (Miura, 1975; Akatsuka, 1992). Later, nets of palm fibre or horizontal curtains made from woven bamboo strips were used as artificial substrates. In China, for more than 200 years, substrates were made available by clearing rocky shores prior to seasonal spore release (Wu, 1998). In Korea, records of *Porphyra* processing date from before 1425 (Bae, 1991), and collection of spores on bamboo twigs is reputed to date from between 1623 and 1649 (Kang & Koh, 1977).

After the discovery by Drew (1949) that *Porphyra* had a biphasic life-history, cycling between the leafy gametophyte stage and the '*conchocelis*' sporophyte stage, the *Porphyra* industry was revolutionised, as, for the first time, artificial, controlled seeding of nets or ropes with conchospores became feasible. Since then, yields have increased greatly, to the extent that markets for *Porphyra* in Japan have become saturated, and farmers need to produce a better quality of *Porphyra* to compete (Oohusa, 1993; Ohno & Largo, 1998). Japanese *Porphyra* strains and farming technology have been exported to China and Korea, which are now both large-scale *Porphyra* producers. *Porphyra* is currently the most valuable seaweed produced by mariculture, with an annual value of over US$ 1.8 billion (Jensen, 1993; Sohn, 1998; Ohno & Largo, 1998; Wu, 1998).

The market for cultivated *Porphyra* is essentially an Asian one, and in the west *Porphyra* is sold largely through Asian specialty food stores, Asian restaurants, and natural and health food stores (Merrill, 1993). There is no indication that the west will ever form as large a market as Asia for cultivated *Porphyra*, despite the increasing popularity of oriental food (in particular sushi) and the traditional consumption of *Porphyra* in many parts of the world. A few *Porphyra* farms have recently been established in the west (e.g. Bergdahl, 1990; Mumford, 1990). At the time of writing, however, none of the farms mentioned by these authors are operational (S. Lindstrom, pers. comm.).

Despite the vast majority of *Porphyra* production deriving from cultivated thalli, often from a small number of strains carefully selected for taste and growth properties, commercial harvesting of wild populations continues in some areas. Harvests of *P. columbina* Montagne in Chile were relatively large at 1119 tons in 1994, but varied considerably from year to year (Alveal, 1998). Chile has developed the technology to farm *P. columbina*, but has not started commercial scale farming (Santelices, 1996). Harvests of wild populations continue in Korea, despite cultured *Porphyra* having long been produced there in quantities that dwarf wild harvests (Sohn, 1998). Harvests in other areas where *Porphyra* is traditionally collected are often relatively small (e.g. Schiel & Nelson, 1990), and data on them is not easily available.

Porphyra has also been proposed as a candidate for bioremediation of eutrophic waters, owing to its rapid growth and above average nutrient accumulation, and potential value on harvesting (Cuomo *et al.*, 1993; Chopin, 1998; Chopin *et al.*, 1999; Kraemer & Yarish, 1999). Potentially, this application could generate income from the sale of *Porphyra* while scrubbing nutrients from water. However, no known commercial-scale use of *Porphyra* in this capacity is known.

1.3 Economic value of South African *Porphyra*

There have in the past been some small-scale exports of South African *Porphyra* to Japan (18.5 dry tonnes from 1965 to 1978; Anderson *et al.*, 1989). These exports have not been resumed. Many, if not most South African *Porphyra* species, particularly those found in the eulittoral, are very thick (150 µm or more), and have been rejected as too tough for sale as nori by Japanese buyers. As a result, there is little incentive to harvest and export South

African *Porphyra* for sale for human consumption on international markets. *Porphyra* has been harvested locally and marketed in South Africa as a locally produced 'nori', usually sold in health food outlets in competition with the more expensive imported products. The quantity harvested is not documented; not surprisingly perhaps, as no permits for harvesting *Porphyra* have been issued for some time, and these harvests have therefore been illegal (R.J. Anderson, pers. comm.). The market for *Porphyra* in South Africa, as a food crop, appears small.

1.4 Taxonomy of South African *Porphyra*

The genus *Porphyra* C. Agardh was first validly described by C. Agardh in 1824. The type of the genus is *P. purpurea* (Roth) C. Agardh, described initially as *Ulva purpurea* Roth from a specimen collected near Eckwarden, Niedersachsen, in Germany (Roth, 1797). The generic name *Porphyra* was first published, invalidly, by C. Agardh (1823), where he described *Porphyra* as being *tribus Ulvae purpureae*. The *Porphyra* species that had been described prior to C. Agardh's (1824) circumscription of *Porphyra* had mostly been assigned to *Ulva* Linnaeus [e.g. *P. laciniata* (Lightfoot) C. Agardh and *P. umbilicalis* (Linnaeus) Kützing].

Yoshida *et al.* (1997) catalogued 133 species of *Porphyra*, and studies since then have both added to and subtracted from that estimate (Coll & Oliveira, 2001; Nelson *et al.*, 2001; Broom *et al.*, 2002; Neefus *et al.*, 2002; Lindstrom & Fredericq, 2003; Nelson *et al.*, 2003). *Porphyra* species occur around the world, and are particularly common in the temperate zones (Yoshida *et al.*, 1997). In regions where *Porphyra* has received considerable attention, the local diversity of species is generally high (e.g. Tseng, 1984; Kornmann & Sahling, 1991; Lindstrom & Cole, 1992b; Broom *et al.*, 2002). Where *Porphyra* has received less attention, it is possible that the application of early species concepts based on the limited European *Porphyra* flora may have been too widely applied, concealing localized species diversity (Bird & van der Meer, 1993). The status of the genus is currently not clear, as *Porphyra* seems to be polyphyletic within *Bangia* (Müller *et al.*, 1998; Broom *et al.*, 1999; Oliveira & Bhattacharya, 2000; Müller *et al.*, 2001). No formal proposals have been made to address this problem.

Porphyra from South Africa was first formally described by Kützing (1843) from samples collected from '*Cap.*' (*Caput Bona Spei).* Although this translates as the Cape of Good Hope, this name has been applied to sites from an area stretching from Cape Town to at least Port Natal (Durban) (Stegenga *et al.*, 1997). The precise type locality for Kützing's samples is therefore not known. Kützing described two species, and named one *P. capensis* Kützing and the other *P. augustinae* (nom. illeg.) (Figure 1-1). As he cited *Iridaea augustinae* Bory in synonymy with the latter, the name is illegitimate. His later treatment (Kützing, 1849) of *P. augustinae* suggests that he was not referring to the original Bory specimens of *I. augustinae* [which are now considered conspecific with *Sarcothalia crispata* (Bory) Leister (Leister, 1977; Hommersand *et al.*, 1993)], but to specimens from the Cape that had been misidentified. His later treatment also listed *P. vulgaris* (nom. illeg.) as being found in Atlantic African bays, and *P. laciniata* from *Caput Bona Spei* (Kützing, 1849).

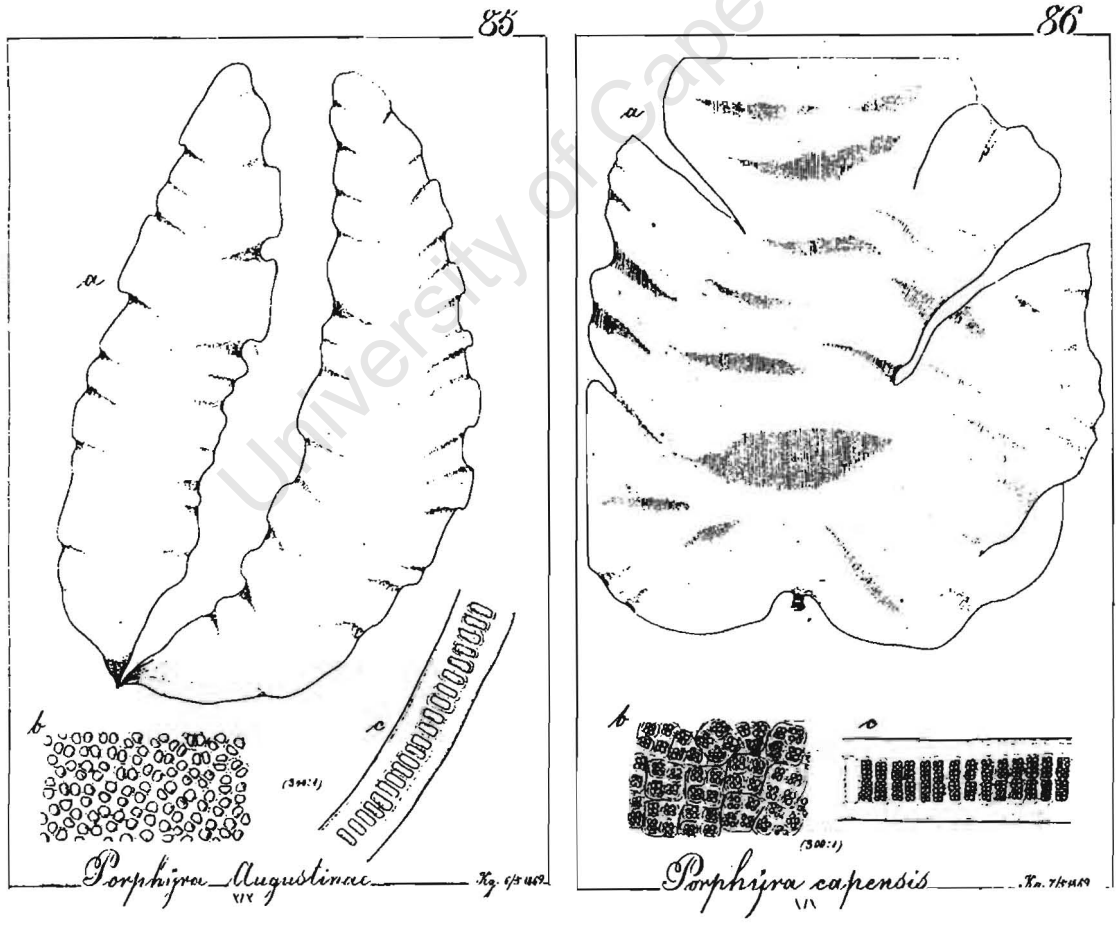

Figure 1-1 Line drawings of *Porphyra capensis* and *Porphyra augustinae* from Kützing (1869).

J. Agardh (1890), in proposing that *P. augustinae* was conspecific with *P. capensis*, explicitly excluded the Bory synonyms. He stated that *P. capensis* and *P. augustinae* were one species in different stages of development, and as a result, placed them in synonymy under *P. capensis*. De Toni (1897), who concurred with J. Agardh (1890) as regards the synonymy of *P. capensis* and *P. augustinae*, also explicitly excluded *I. augustinae* from *P. augustinae*.

J. Agardh's (1890) revision of southern African *Porphyra* species was widely accepted, and the majority of records published since then refer to *P. capensis*. Earlier records vary: for example, Drège (1843) reports *P. vulgaris*, and Areschoug (1851) reports *P. laciniata* and *P. vulgaris*, stating that *P. capensis* is a morphologically variable form of *P. laciniata*. Barton (1893) refers to collections and published records of *P. capensis*, *P. augustinae*, *P. laciniata* and *P. vulgaris* from various sites along the South African coast from Robben Island to Port Natal (now Durban, and past the generally accepted northernmost limit of *Porphyra* on the east coast). More recently, Delf & Michell (1921) cited *P. vulgaris* and *P. laciniata*. Citations of *P. vulgaris* and *P. laciniata* are thought to be based on *P. capensis* (Silva in Seagrief, 1984; Silva *et al.*, 1996).

Isaac (1957) and Graves (1969) reviewed the taxonomy of *Porphyra* in South Africa. Both listed three main morphological variants of *P. capensis*, but did not consider them to be different species, and both described *P. capensis* as showing considerable morphological plasticity. Both felt that *P. capensis* encompassed all forms that they examined, and, as such, neither recorded any other species. Although the morphological variants that Isaac and Graves report are considered by them to all be *P. capensis*, morphological variation between their forms is often greater than that between different species of *Porphyra* growing sympatrically in regions where *Porphyra* has been more closely studied (e.g. see Tseng, 1981; Lindstrom & Cole, 1992*b*; Brodie *et al.*, 1998). Even within the forms they describe, variation is high. This suggests that historical *Porphyra* taxonomy in South Africa may be in common with that in other localities [for example, New Zealand (Nelson & Adams, 1990) and South America (Oliveira Filho & Coll, 1975; Coll & Oliveira Filho, 1976)] where the application of early species concepts gave rise to widely distributed form species, encompassing a number of similar taxa (Bird & van der Meer, 1993).

A major change in the taxonomy of South African *Porphyra* occurred when Stegenga *et al.* (1997) recorded four species of *Porphyra* from the South African coast: *P. capensis*, *P. suborbiculata* Kjellman (as *P. carolinensis* Coll *et* Cox), *P. gardneri* (Smith *et* Hollenberg) Hawkes, and the new species *P. saldanhae* Stegenga, Bolton *et* Anderson. They also included a description of an unnamed species.

The forms that Isaac (1957) and Graves (1969) described overlap relatively little with the species recorded by Stegenga *et al.* (1997). All of the forms of Isaac and Graves are epilithic (though they mention that *Porphyra* may grow epiphytically), but only two of species recorded by Stegenga *et al.* are epilithic. The epiphytic species reported by Stegenga *et al.* are much more delicate that the fairly robust epilithic forms described by Isaac and Graves, and differ in several characters. The new epilithic species *P. saldanhae* cannot be clearly identified as any of the forms of Isaac and Graves, though Graves (1969) mentions thalli that correspond to the description of *P. saldanhae*, and seems to include them in her typical west coast form.

Isaac (1957) recorded a typical west coast form (monostromatic, dull purple, reniform to cordate, and relatively large), a small, pale-coloured form (monostromatic, pale, and smaller than the west coast form with twisted or curled thalli), and a linear or lanceolate form (monostromatic, and linear to lanceolate). He noted that pale thalli could be found high in the eulittoral on the colder west coast, and attributed the growth form to exposure [he drew this conclusion after transferring rocks with typical west coast thalli from low in the eulittoral to higher on the shore. After a period of exposure the transplanted thalli were pale and twisted], but mentioned that similar thalli can be found low in the eulittoral in warmer False Bay waters. Isaac (1957) felt that, despite the differences between the forms he described, there were insufficient consistent differences in structural features between the forms to justify the separation of *P. capensis* into different species. Graves (1969) expanded on Isaac's (1957) description of *Porphyra* in South Africa, but still maintained that only three main growth forms were present. She noted that thalli might be monoecious or dioecious, and that monoecious thalli either had zygotosporangia and spermatangia in wide sectors of the thallus or intermingled in small patches (Graves, 1969). Though she recorded a range of division patterns of zygotosporangia and spermatangia, she stated that the patterns were not constant (Graves, 1969). She also described dwarf plants (*sensu*

Conway, 1965) that often had serrated margins and appeared to reproduce only asexually (Graves, 1969).

Molloy (1990) recorded *P. capensis* from all rocky shores examined in Namibia, and noted that it was variable in form and that populations probably accounted for more than one species.

Three of the species recorded by Stegenga *et al.* (1997) appear, on morphological grounds, to be valid species. However, the species described as *P. capensis* seems to encompass at least two species. A comparison of transverse sections of zygotosporangia of *P. capensis* presented by Stegenga *et al.* (1997) with those of an isotype of *P. capensis* [coll. Drège, C.B.S. (*Caput Bona Spei*). Rijksherbarium Leiden L4318 No 133] suggests that the umbilicate plant(s) that Stegenga *et al.* (1997) studied may not in fact be *P. capensis* (the holotype could not be located-this isotype is one of five samples from Kützing's collection originally collected from the Cape of Good Hope by Drège and identified as *P. capensis* by Kützing). Alhough there are many similarities between the isotype and the *P. capensis* of Stegenga *et al.* (1997), the presence of prototrichogynes with surface bumps on the thallus in the isotype, and the absence of surface bumps and prototrichogynes in the drawings of Stegenga *et al.* (1997) suggests that they may have examined different species (prototrichogynes are easily located in fertile female material, and surface bumps remain over the zygotosporangia after fertilisation). Neither Isaac (1957), Graves (1969) nor Stegenga *et al.* (1997) appear to have examined the type specimens of *P. capensis* (or *P. augustinae*), and this reliance on the descriptions and drawings of Kützing (1843, 1849, 1869) and the failure to carefully compare collected material with types may well have hampered the taxonomy of *Porphyra* in South Africa.

Porphyra capensis has been reported from locations other than southern Africa. In general, these records are old, and more recent evidence of the presence of *P. capensis* from shores beyond southern Africa is lacking (see Ramírez & Santelices, 1991; Silva *et al.*, 1996). Many of the records are referrable to Harvey, Hooker and Kützing: Harvey & Hooker (1844) refer to *P capensis* from Campbell and Auckland Islands, though they state elsewhere that *P. capensis* is probably conspecific with *P. laciniata* (Harvey & Hooker, 1847); Kützing (1849) reported that *P. capensis* can be found at Cape Horn and Kerguelen's Land (from specimens collected by Hooker). Ardissone (1888) also reported

on *P. capensis* collected from Tierra del Fuego by Spegazzini. Papenfuss (1964) noted that Antarctic and sub-Antarctic records of *P. capensis* required verification; Chapman (1969) held Harvey's records to be misidentifications of *P. columbina* var. *laingii* Levring, and Hay *et al.* (1985) stated that records of *P. capensis* from Auckland and Campbell Islands are referable to *P. columbina*. M.E. Ramírez (pers. comm.) and W.A. Nelson (pers. comm.) maintain that *P. capensis* does not occur in South America or New Zealand, respectively. *Porphyra columbina* has been frequently recorded from South America and New Zealand (Ramírez & Santelices, 1991; Adams, 1994). Harvey and Hooker either made no mention of *P. columbina*, or referred records of *P. columbina* to *P. capensis*; Kützing recorded both species. Chamberlain (1965) recorded *P. tristanensis* Baardseth from Gough Island, which she suggested is conspecific with *P. capensis*; however, she maintained the name *P. tristanensis* until formal taxonomic revision. It seems likely from the above that reports of *P. capensis* from non-southern African shores can be referred to *P. columbina* or other *Porphyra* species. Nevertheless, *P. capensis* continues to be recorded from outside the region (González & Santelices, 2003).

The taxonomy of *Porphyra* worldwide has been greatly improved through the use of techniques that reveal characters derived from the genetic code, such as isozyme electrophoresis and gene sequencing (Lindstrom & Cole, 1990*a*, 1990*b* 1992*a*, 1992*b*, 1992*c*, 1993; Stiller & Waaland, 1993, 1996; Brodie *et al.*, 1996, 1998; Woolcott & King, 1998; Kunimoto *et al.*, 1999*a*, 1999*b*; Broom *et al.*, 1999, 2002; Nelson *et al.*, 2001, 2003; Neefus *et al.*, 2002; Klein *et al.*, 2003; Lindstrom & Fredericq, 2003). All studies of South African *Porphyra* have only used morphological characters, which are highly conserved (Stiller & Waaland, 1993), and South African *Porphyra* taxonomy would probably benefit from the application of these methodologies, particularly given the apparent variability within *P. capensis*.

1.5 Background to this study

In feeding trials, *Porphyra* was found to enhance the growth in mariculture of the South African abalone (*Haliotis midae* Linnaeus) when supplied as fodder either together with the kelp *Ecklonia maxima* (Osbeck) Papenfuss, or in rotation with *E. maxima* (Simpson, 1994; Stepto & Cook, 1996). *Haliotis midae* preferred *Porphyra* to all other seaweeds it was offered during these trials. It also grew faster with improved shell elongation, and

showed most efficient biomass conversion when fed on *Porphyra*. South African cultured abalone are fed almost entirely on seaweeds, in particular kelp, as it improves flesh taste and the value of the product (Anderson *et al.*, 2003). Farmers are looking for a natural fodder that will give growth rates comparable to those obtained using food pellets but that will maintain the distinctive taste of wild or seaweed-fed abalone. The abalone farming industry in South Africa is expanding rapidly, and the demand for kelp by this industry is increasing almost exponentially (Anderson *et al.*, 2003; Rotmann *et al.*, 2003). Several farmers are experimenting with other seaweed as supplementary feed, and the demand for *Porphyra* by this industry is increasing.

No data are available on the size and distribution of *Porphyra* populations present in South Africa. In addition, no assessment has been made of the impact of harvesting *Porphyra*, either on *Porphyra* itself, or on the associated eulittoral community. It is therefore not possible for managers to make anything but crude recommendations regards harvesting of *Porphyra*.

1.6 Objectives of this study

The Department of Sea Fisheries (now Marine and Coastal Management) received several queries regarding the harvesting of wild *Porphyra* for mariculture of *H. midae*. This study was initiated to assess the potential of South African *Porphyra* for harvest. When the study commenced, only *P. capensis* was reported from South Africa. Specific objectives of the study are listed below.

1. *Porphyra* populations in the Western Cape are surveyed to determine the seasonal biomass of *Porphyra* available for harvest, and the extent to which populations are protected from harvesting due to existing harvesting refuges (e.g. nature reserves).
2. *Porphyra* population biology is studied at one site in order to identify potential impacts of harvesting on *Porphyra*.
3. The position of *Porphyra* within the eulittoral community is examined, to identify possible impacts of harvesting of *Porphyra* on the broader eulittoral community.
4. Predictions from (2) and (3) are tested by small-scale harvesting of *Porphyra* gametophytes at the same site. Potential impacts not identified above are described.

5. Data from biomass surveys, and ecological and harvesting assessments are used to draw up a management plan for *Porphyra* in the region.

During the course of the study, more species were reported, and it became apparent that an assessment of the taxonomic status of South African *Porphyra* was necessary for effective management of *Porphyra* stocks in the country. The following objective was therefore added to those from the start of the project.

6. The biodiversity within *Porphyra* in South Africa is assessed in order to guide future research regarding management of the taxon.

1.7 Conventions

When any results are described as significant or statistically significant, this refers, unless otherwise indicated, to the rejection of the null hypothesis with the probability of a type I error (rejection of a true null hypothesis) being 0.05 or less. Error values presented are standard error unless otherwise indicated.

The eulittoral, as used in this thesis, is that area of the shore directly affected by the tide. It therefore extends from the level of the lowest low tide to that of the highest high tide, and also includes any shore immediately above the level of the highest high tide that is splashed by waves at high tide.

I have generally following Nelson *et al.* (1999) regards terminology used to describe reproduction and life history stages in *Porphyra*. I have maintained gametophyte to describe the foliose phase, assuming this to be haploid. Likewise, I have frequently used sporophyte to describe the conchocelis phase of the life history. I have also used archeospores to describe mitotically derived spores produced by the foliose phase.

2 Biomass survey

2.1 Introduction

Porphyra species have been recorded around the majority of the coastline of the Northern, Western and Eastern Cape, South Africa (Graves, 1969; Anderson *et al.*, 1989; Stegenga *et al.*, 1997), although potentially harvestable quantities are restricted to more temperate waters, especially south western shores influenced by the cold, nutrient-rich waters of the Benguela upwelling system (Isaac, 1957; Graves, 1969). No *Porphyra* has been recorded north of Port Edward (S31°03′ E 30°13′) on the east coast, where the algal flora has many tropical elements. Most abalone farms are on the south western coast, where *Porphyra* is relatively common. Demand for *Porphyra* is likely to be centered around abalone farms.

Previous authors have differed regards *Porphyra* seasonality in South Africa. Some have indicated that *Porphyra* is generally not present or is rare in the eulittoral during summer (Day, 1969; Branch & Branch, 1981). McQuaid (1985), on the other hand, found *Porphyra* to be present year-round at the site he studied, with greater populations present in summer. Isaac (1942) stated that *Porphyra* in South Africa showed seasonality in warmer water, but was present year-round in cooler water. The same author also noted that standing biomass decreased to the east of False Bay, and that it occurred in 'great profusion' on western shores of the Cape Peninsula.

This chapter reports on a survey of the biomass of *Porphyra* available to harvesters on the south-western coast of South Africa. It does not differentiate between different species, as, at the time that this study commenced, *P. capensis* was the only potentially harvestable species of *Porphyra* known from South Africa. In the view of harvesters and coastal managers, this largely remains the case.

2.2 Methods

A survey of *Porphyra* biomass was undertaken at 40 sites between St Helena Bay and Cape Agulhas. The survey aimed to quantify standing biomass of *Porphyra* species in the study area. Sites were surveyed during spring low tides during the periods 21 June 1993 until 21 August 1993 (winter) and 10 February 1994 until 17 April 1994 (summer). The

location of the surveyed sites is indicated in Figure 2-1. Table 2-1 gives accurate site locations and the shore length sampled in winter and summer sampling sessions.

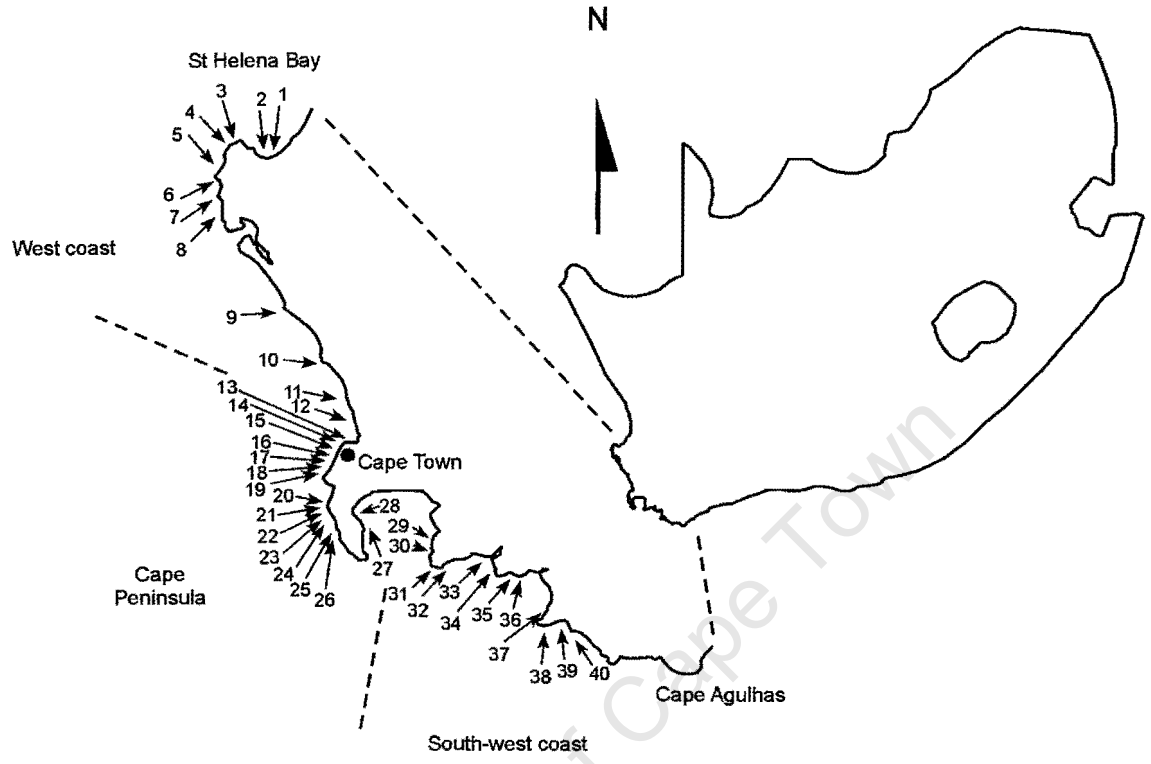

Figure 2-1 Forty sites along the South African coastline between Cape Agulhas and St. Helena Bay were selected for assessment of standing stocks of harvestable *Porphyra* species. See Table 2-1 for details of sample sites.

Sites selected for survey were evenly spread across rocky shores through the survey area. As sites were chosen as being representative of shores where harvesting might take place, all were accessible by road, and none were located in areas where harvesting might not be feasible, viz. nature reserves and areas controlled by the military.

Table 2-1 Sites chosen for assessment of *Porphyra* biomass giving the coordinates and lengths of rocky shores sampled. See Figure 2-1 for numbered site location.

Site name and number		Coordinates	Sampled shore length (m) Winter	Summer
West coast				
1	Hannasbaai	S32°45'31" E18°01'55"	190	370

Site name and number		Coordinates	Sampled shore length (m) Winter	Summer
2	Middelbaai	S32°43'50" E17°59'45"	234	237
3	Britannia Point	S32°42'55" E17°56'25"	200	230
4	Groot Paternosterpunt	S32°44'08" E17°54'37"	230	235
5	Abdolsbaai	S32°48'52" E17°52'02"	460	560
6	Tietiesbaai	S32°50'28" E17°51'37"	225	247
7	Rooisteen	S32°53'54" E17°51'55"	860	840
8	Jacobsbaai	S32°58'09" E17°52'10"	218	205
9	Yzerfontein	S33°20'47" E18°09'04"	145	145
10	Wintersteen	S33°35'28" E18°21'37"	395	460
11	Melkbosstrand	S33°43'16" E18°26'36"	232	224
12	Bloubergstrand	S33°48'18" E18°27'48"	400	387
Cape Peninsula				
13	Mouille Point	S33°53'54" E18°24'32"	680	660
14	Three-anchor Bay	S33°54'18" E18°23'52"	410	210
15	Rocklands	S33°54'28" E18°23'25"	600	450
16	Graaf's Pool	S33°54'36" E18°23'17"	450	500
17	Sunset Beach	S33°55'11" E18°22'49"	410	410
18	Camp's Bay	S33°57'21" E18°22'31"	500	500
19	Oudekraal	S33°58'51" E18°21'47"	850	850
20	Kommetjie (north)	S34°08'31" E18°19'16"	252	260
21	Kommetjie (Kom)	S34°08'40" E18°19'10"	890	740
22	Slangkoppunt	S34°09'06" E18°19'22"	810	810
23	Soetwater (pool)	S34°09'18" E18°19'33"	360	730
24	Soetwater (south)	S34°09'47" E18°19'50"	490	585
25	Misty Cliffs	S34°10'50" E18°21'38"	630	900
26	Scarborough	S34°11'49" E18°22'10"	1150	445
27	Miller's Point	S34°13'36" E18°28'12"	4000	2256
28	Glencairn	S34°10'00" E18°25'57"	1100	698
South-west coast				
29	Rooi Els	S34°17'55" E18°49'04"	788	665
30	Pringle Bay	S34°20'53" E18°49'10"	470	590
31	Hangklip	S34°22'13" E18°49'51"	278	500
32	Silver Sands	S34°22'25" E18°52'53"	400	465
33	Kleinmond	S34°20'38" E19°00'42"	450	489
34	Harry's Bay	S34°24'02" E19°07'16"	515	683
35	Sandbaai	S34°25'50" E19°11'21"	470	560
36	Hermanus	S34°26'03" E19°13'45"	459	694
37	Stanford's Cove	S34°34'03" E19°21'10"	658	410
38	Danger Point	S34°37'28" E19°18'57"	400	447
39	Franskraal	S34°36'30" E19°24'02"	277	277
40	Pearly Beach	S34°40'22" E19°30'38"	570	700

A stratified sampling technique at each site was adopted, as *Porphyra* gametophyte thalli were not evenly distributed throughout the eulittoral, but often grew in clumps. At each site, surveyors assessed the distribution of gametophyte thalli present, and established three subjective biomass density classes (low, medium and high biomass per unit area). The quantity of *Porphyra* within any biomass density class was determined as the product of the biomass density within that class and the area covered by that class. The mass of *Porphyra* within each of the three biomass density classes was then pooled to give the biomass of *Porphyra* present at that site.

The biomass density within each biomass density class at each site was determined by sampling the wet mass of *Porphyra* in four quadrats placed haphazardly within that biomass density class. The size of the quadrats used varied, being either 0.25×0.25 m, 0.25×0.50 m, 0.25×0.75 m or 0.25×1.00 m. Larger quadrats were used where biomass was low and/or *Porphyra* patchily distributed, and smaller quadrats were chosen where biomass was high and the distribution of thalli was uniform. Thalli in quadrats were hand picked by surveyors (thalli smaller than *ca.* 3 cm in length were not collected in an attempt to simulate the actions of commercial harvesters).

The dimensions of patches covered by each biomass density class were measured, and the approximate area covered by each biomass density class estimated from the measured dimensions.

Shorelines examined were as great as was feasible in the time allowed by tidal dynamics and *Porphyra* distributions, and varied from 145 m of rocky shore to 4000 m. The position of each site was recorded on 1:50000 topocadastral maps, and fine details were noted on a hand-drawn map. Each site was assessed by a minimum of two surveyors to ensure that decisions on area and length estimates and the classification of areas containing *Porphyra* were a consensus.

*S*amples collected for biomass density class calibration were immersed in seawater for 15 minutes at ambient temperature to completely rehydrate collected thalli. After soaking, superficial water was removed by spinning the thalli in a salad spinner until no more water was collected, then blotting the thalli with paper towels. Following this standardisation, the wet mass of *Porphyra* in each quadrat was determined. Once the biomass density of

Porphyra in each biomass class was known, the estimated total wet mass of *Porphyra* in each biomass class and in each sampled shorelength was calculated. Biomass per unit length of shore was calculated for each sample site to facilitate comparison between sites.

The data from sample sites were extrapolated to give an estimate of the standing crop of *Porphyra* between St. Helena Bay and Cape Agulhas. The sample sites were taken as representing surrounding areas of rocky shore as follows: Hannasbaai (Slippers Bay to Sandy Point); Middelbaai (Sandy Point to Stompneusbaai); Britannia Point (Stompneusbaai to Klippiesbaai); Groot Paternosterpunt (Klippiesbaai to Tweede Mosselbank); Abdolsbaai (Tweede Mosselbank to Ossebaai); Tietiesbaai (Ossebaai to Hoebank); Rooisteen (Hoebank to Die Witsand); Jacobsbaai (Die Witsand to Sestienmylstrand); Yzerfontein (Sestienmylstrand to Waaisand); Wintersteen (Waaisand to Waaisand); Melkbosstrand (Waaisand to Kreeftebaai); Bloubergstrand (Kreeftebaai to Cape Town Harbour); Mouille Point (Cape Town Harbour to Green Point); Three-Anchor Bay (Green Point to rocky point between Rocklands Bay and Three-Anchor Bay); Rocklands (rocky point between Rocklands Bay and Three-Anchor Bay to narrow shoreline midway between Rocklands Bay and Graaf's Pool); Graaf's Pool (narrow shoreline midway between Rocklands Bay and Graaf's Pool to Seapoint Pavilion); Sunset Beach (Seapoint Pavilion to Clifton Beach); Camps Bay (Clifton to Klein Koeëlbaai); Oudekraal (Klein Koeëlbaai to Chapman's Bay); Kommetjie north (Chapman's Bay to The Kom); Kommetjie Kom (The Kom to Slangkoppunt lighthouse); Slangkoppunt (Slangkoppunt lighthouse to The Anchor); Soetwater pool (The Anchor to Soetwater change-rooms); Soetwater south (Soetwater change rooms to Die Eiland); Misty Cliffs (Die Eiland to Mosselbaai); Scarborough (Mosselbaai to Cape Point); Miller's Point (Cape Point to Simonstown harbour); Glencairn (Simonstown harbour to Muizenberg); Rooi Els (Muizenberg to Roman Rock); Pringle Bay (Roman Rock to Grootbaai); Hangklip (Grootbaai to Aasbank); Silver Sands (Aasbank to Dewetsbaai); Kleinmond (Dewetsbaai to Rooisand); Harry's Bay (Rooisand to Hoek van den Berg); Sandbaai (Hoek van den Berg to Swartdam); Hermanus (Swartdam to Sophiesklip); Stanford's Cove (Sophiesklip to Danger Point); Danger Point (Danger Point to Rooikrans); Franskraal (Rooikrans to Uilenkraalsmond); and Pearly Beach (Uilenkraalsmond to Pearly Beach).

The extent of rocky shores was established from 1:50000 topocadastral maps, Jackson & Lipschitz (1984) and personal observation. The location and extent of marine reserves

were determined from 1:50000 topocadastral maps, Jackson and Lipschitz (1984), Sea Fisheries (1996) and personal observation. For the purpose of this study, two classes of reserve are considered.

The first, from Sea Fisheries (1996), is an area where seaweed collection is explicitly proscribed by national law, and which I refer to as a reserve. The second, here termed a restricted area, is a region where commercial collection of eulittoral seaweed would be in contravention of local regulations, or an area where, although the collection of eulittoral seaweed might itself be lawful, access to the shore, unless by boat, is only through reserves or areas with restricted access (for example, military land, or municipal or private reserves). The reserves and restricted areas are listed in Table 2-2.

Table 2-2 Extent of rocky shores, reserves and restricted areas used for extrapolation of biomass data. Reserves and restricted areas are listed. Reserves and restricted areas present at the time of sampling only are listed.

		Shore length (km)			
	Site	Full area	Restricted	Reserves	Reserves and restricted areas
West coast					
1	Hannasbaai	5.85	0	0	
2	Middelbaai	2.3	0.5	0	Shell Bay Point
3	Britannia Point	2.05	1.05	0	Shell Bay Point
4	Groot Paternosterpunt	5.75	5.1	0	Groot Paternosterpunt
5	Abdolsbaai	7	4.95	0	Cape Columbine Nature Reserve
6	Tietiesbaai	9.3	6.9	0	Cape Columbine Nature Reserve
7	Rooisteen	7.65	6.3	0	Duminy Point
8	Jacobsbaai	57.45	15.35	9.85	SAS Saldanha, West Coast National Park
9	Yzerfontein	13.25	0	0	
10	Wintersteen	24.9	0	0	
11	Melkbosstrand	1.5	0	0	
12	Bloubergstrand	12.15	0	0	
Cape Peninsula					
13	Mouille Point	2.45	0	0	
14	Three-anchor Bay	1.4	0	0	
15	Rocklands	0.75	0	0	
16	Graaf's Pool	1.25	0	0	
17	Sunset Beach	3.25	0	0	

		Shore length (km)			
	Site	Full area	Restricted	Reserves	Reserves and restricted areas
18	Camp's Bay	5.8	0	0	
19	Oudekraal	28.55	0	0	
20	Kommetjie (north)	2.35	0	0	
21	Kommetjie (Kom)	1.2	0	0	
22	Slangkoppunt	1.25	0	0	
23	Soetwater (pool)	0.7	0	0	
24	Soetwater (south)	3.2	0	0	
25	Misty Cliffs	2.35	0	0	
26	Scarborough	25.6	0	24.6	Cape Point
27	Miller's Point	27.65	0	15.9	Cape Point, Castle Rock
28	Glencairn	8.35	0	5.4	Glencairn, Kalk Bay, St James
South-west coast					
29	Rooi Els	28.2	0	4.2	Strand
30	Pringle Bay	12.2	0	0	
31	Hangklip	10.1	0	0	
32	Silver Sands	11.3	0	5.1	HF Verwoerd
33	Kleinmond	7.8	2.3	0	Kleinmond Coastal Nature Reserve
34	Harry's Bay	5.9	5.9	0	Mudge Point Marine Conservation Area
35	Sandbaai	9	1.5	1.3	Mudge Point Marine Conservation Area, Harder Bay/Onrus
36	Hermanus	8.85	0	4.5	Hermanus
37	Stanford's Cove	17.65	0	0	
38	Danger Point	9.25	0	0	
39	Franskraal	5.55	0	0	
40	Pearly Beach	15.9	8.55	0	Dyer Island

2.3 Results

The *Porphyra* biomass per running metre of shore was greatest at sites on the Cape Peninsula, most notably on the relatively short (*ca.* 8 km) stretch of Atlantic coast from Kommetjie (20) to Scarborough (26) (Figure 2-2). Biomass measurements within this stretch were, in places, orders of magnitude greater than those from sites nearer the outer extremes of the sampled shoreline. The greatest biomass recorded for a biomass class (3.45 $kg.m^{-2}$) was also from this area.

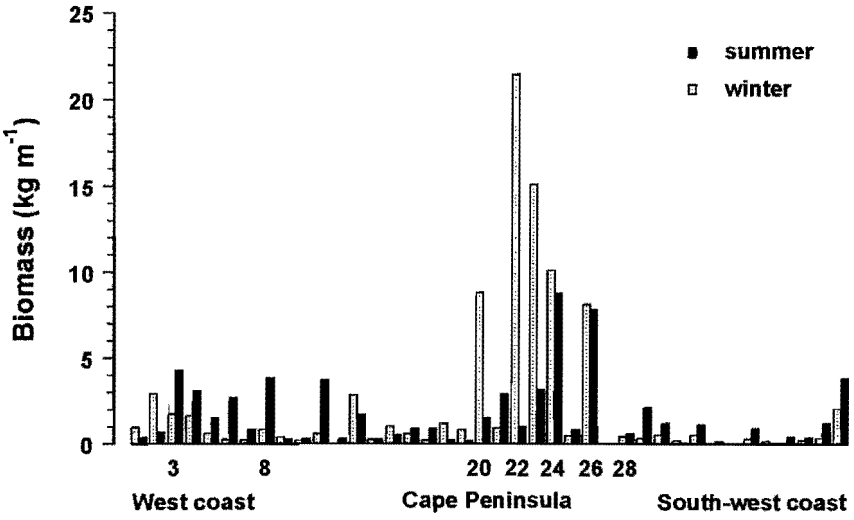

Figure 2-2 Extrapolated wet biomass of *Porphyra* per metre of shore at each of forty sample sites during winter (June—August 1993) and summer (February—April 1994). Numbered sites are those referred to in the text (3-Britannia Point; 8-Jacobsbaai; 20-Kommetjie north; 22-Slangkoppunt; 24-Soetwater south; 26-Scarborough; 28-Glencairn). See Appendix A for tabular data.

No clear overall seasonality of *Porphyra* populations is apparent from the results of this biomass survey, though biomass at all sites varied to some extent from summer to winter. At some sites this variation was dramatic: this was most notable at Slangkoppunt, where the summer running biomass was 1.01 $kg.m^{-1}$ of shore and the winter running biomass was 21.44 $kg.m^{-1}$ of shore. In two stretches of shore (Britannia Point–3 to Jacobsbaai–8, and Kommetjie north–20 to Soetwater south–24) contiguous sites showed similar population dynamics, suggesting that those stretches of coast may contain the same mix of *Porphyra* species in similar environments. In the case of the Kommetjie north (20) to Soetwater south (24) stretch, the sites are immediately adjacent and so can be expected to be reasonably homogenous with respect to both species complement and environment. However, the sites from Britannia Point (3) to Jacobsbaai (8) are spaced along a largely rocky coastline of approximately 47 km. Notably high biomass was recorded in winter on the west coast of the Cape Peninsula in the Kommetjie north (20) to Soetwater south (24) area. West coast sites generally had a higher summer biomass, although there were several exceptions. Sites on the south west coast, and those on the False Bay side of the Cape Peninsula, usually had a greater biomass in summer.

When measured biomass data were extrapolated to adjacent rocky shores, more than half of the projected biomass of *Porphyra* in the study area was located within reserves or areas with restricted access (Figure 2-3). This was especially so on the Cape Peninsula, where two-thirds of the total annual *Porphyra* biomass was located in areas where seaweed collection is explicitly prohibited. The Cape Peninsula, though having a generally high biomass of *Porphyra* per unit shore length, has a relatively short rocky shore, 40 % of which is within reserves. A large proportion of west coast rocky shore is also protected by reserves and restricted areas; however, many of these reserves are municipal or private reserves, and access to the shore in these reserves may be feasible.

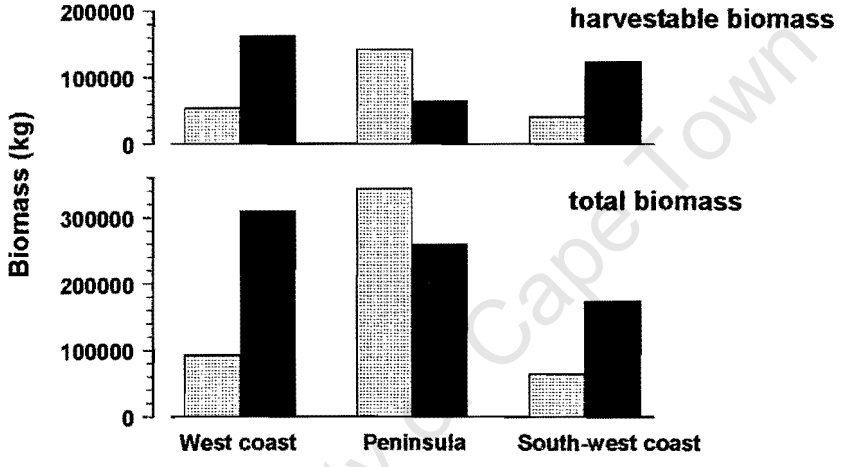

Figure 2-3 Extrapolated wet biomass of *Porphyra* in the eulittoral in the study area as calculated from samples taken during winter (June—August 1993) (shaded bar) and summer (February—April 1994) (solid bar). Total biomass and harvestable biomass (that available outside reserves and areas with restricted access) are shown. See Appendix A for full data.

The high biomass per unit shore length of *Porphyra* noted at some of the sample sites on the Cape Peninsula does not result in a particularly high projected biomass of *Porphyra* on the peninsula, as the sites with high biomass are generally representative of relatively short shores. Sites on the peninsula are easily accessible compared to sites further from major centers. However, there are no abalone farms on the Cape Peninsula, and a high harvesting pressure in this region seems unlikely.

2.4 Discussion

Porphyra was found at all sites examined during the course of this survey, with much spatial and temporal variation in *Porphyra* biomass distribution. Estimated *Porphyra* biomass was generally greatest in the summer at west and south-west coast sites, and in the winter at sites on the Cape Peninsula. Many other macrophytes besides *Porphyra* have a high biomass on the Cape Peninsula (Isaac, 1942; Isaac & Molteno, 1953; Levitt *et al.*, 1995; Anderson *et al.*, 1989), making this area a potentially worthwhile one for harvesting of several seaweeds having commercial potential.

Generally, the predicted biomass of *Porphyra* on the west coast and Cape Peninsula was greater than elsewhere. Most rocky shores between St Helena Bay and Cape Agulhas have potential for the collection of *Porphyra*, and harvest site selection will likely be dictated more by operational factors, for example, site access (by road or by boat) and transport distance versus potential return, than by the biomass present.

Yields of *Porphyra* from this study, in terms of wet biomass per unit area, are comparable to those reported for harvests of mixed *Porphyra* species from several sites in New Zealand (Nelson & Conroy, 1989), and far exceed those reported from British Columbia (Roland & Coon, 1984). Yields from the west coast of the Cape Peninsula are high compared with both these studies. Nelson & Conroy (1989) indicated they chose sites known to have abundant *Porphyra* growth for their study, and average biomass along the coast may have been lower. Both studies examined the impact of harvesting on *Porphyra* populations, and data on harvestable biomass were not presented.

McQuaid (1985) reported large high-shore populations from Dalebrook in False Bay (approx. 4 km north of Glencairn) that corresponded with summer and with increased tidal height (the two could not be distinguished) (McQuaid, 1985). Data from Glencairn (this study) show only slightly higher summer biomass. The observations of Isaac (1942) on seasonality of *Porphyra* are largely corroborated by this study. Generally, the results of this study show *Porphyra* populations in the study area to often be greater in summer than in winter, although this tendency is reversed in a number of sites along the west coast, and in particular on the west coast of the Cape Peninsula.

Seasonality in *Porphyra* populations is to be expected, as most species of *Porphyra* show a seasonal alternation of generations. Annual gametophyte recruitment often occurs in spring-summer or autumn-winter, resulting in annual summer or winter populations (Dickson & Waaland, 1985; Avila *et al.*, 1986; Waaland *et al.*, 1990). Though the density and distribution of *Porphyra* on any shore is responsive to environmental factors and biotic interactions, the variation from site to site in the seasonality of populations suggests the presence of a number of *Porphyra* species.

This survey only examined winter and summer biomass distribution on the south-western coast of South Africa. The data cannot be used to estimate interannual variation in biomass; however, between-site and between-season variation in the extrapolated biomass per metre of shore suggest that interannual variation may be high.

3 Seasonality and population dynamics

3.1 Introduction

The role of *Porphyra* in the ecology of intertidal zones has been described before, most notably from the Pacific and Atlantic coasts of the United States (Dayton, 1975; Lubchenco & Menge, 1978; Lubchenco, 1983; Cubit, 1984; Sousa, 1984; Harley, 2002), and from the coast of Chile (Jara & Moreno, 1984; Santelices *et al.*, 1981; Santelices & Martínez, 1988; Santelices, 1990*a*). *Porphyra* has been described as an ephemeral or fugitive taxon, that rapidly invades cleared patches in the eulittoral, but soon dies back or is replaced by later successional macroalgae (Dayton, 1975). As such, *Porphyra* is often grouped with *Ulva* and *Enteromorpha* as an opportunist, according to the scheme of Littler and Littler (1980). Seasonality in *Porphyra* propagule availability, and consequent recruitment rates, is often not observed or commented on, although there are exceptions (Santelices *et al.*, 1981; Santelices & Martínez, 1988).

Herbivores are important in mediating interactions between *Porphyra* and other macroalgae. Herbivores may act to considerably accelerate succession by grazing preferentially on *Porphyra*, thereby favoring the growth of later successional algae (Lubchenco, 1983). In the latter study, *Porphyra* and other ephemeral algae maintained dominance over *Fucus vesiculosus* Linnaeus for at least one year when herbivores were excluded. Macroalgal competition may also decrease *Porphyra* populations, and herbivore grazing favor *Porphyra*: Jara & Moreno (1984) found that *P. columbina* was outcompeted by *Iridaea boryana* Setchell & Gardner, but survived due to preferential grazing on *I. boryana*.

The presence of patches or bands of *Porphyra* in the upper eulittoral, and its capacity for growth under desiccation stress (Santelices, 1990*a*; Levitt & Bolton, 1991; Lipkin *et al.*, 1993) was generally not addressed in the above studies. Cubit (1984) found that the growth of macroalgae, including *Porphyra*, in the upper intertidal was controlled by a combination of grazer pressure and the environment. Blooms of macroalgae in the upper intertidal were present during the cooler, wetter winter, and were absent during the drier summer. Decreased algal production during the summer was unable to support high intertidal herbivore populations, with consequent overgrazing and late summer decreases in

herbivore numbers. This resulted in decreased herbivore pressure when growth conditions became more favorable, and macroalgae were able to establish and grow during the winter. In this scenario, cycles of macroalgal and herbivore populations in the upper eulittoral are a function of varying algal production rates in seasonal conditions.

Classically, the upper limits of eulittoral organisms have been considered to be physical, and the lower limits defined by biological interactions (Connell, 1972). The effects of biological interactions may be underestimated in this model, as biological interactions may also establish the upper limits of a species in the intertidal (Underwood & Jernakoff, 1984; Hawkins & Hartnoll, 1985; Chapman & Johnson, 1990). However, algae growing low or high in the intertidal have been found to have their upper limits set by physical factors (Schonbeck & Norton, 1978; Davison & Pearson, 1996) and may be close to the limits of the fundamental niche (Chapman, 1986). The classical model may seem intuitively correct for *Porphyra* growing in the upper eulittoral, as stress levels are high and *Porphyra* is capable of growth lower on the shore. However, the work of Cubit (1984) indicates that upper limits of *Porphyra* may be controlled by biological interactions. Chapman (1986) criticized the application of the classical model, pointing out that factors controlling the distribution of macroalgae can only be determined using a careful experimental approach. Even when a shore has been thoroughly studied, the results cannot necessarily be extrapolated over wider distances on the same coastline (Foster, 1990), or between seasons at the same site (Jara & Moreno, 1984).

Despite the potential value of *Porphyra* in South Africa, few data are available on *Porphyra* populations in South Africa. There are a number of publications that consider South African *Porphyra*, but these are largely taxonomic, and little attention has been paid to *Porphyra* as an ecological entity. Isaac (1942, 1957) and Graves (1969) provide descriptions of *Porphyra* populations, but these are sufficiently imprecise to be of any use in drawing up management proposals for *Porphyra*. Both authors allude to several forms of *Porphyra*, but conclude that all these fall within *P. capensis*. Despite the recent interest in South African *Porphyra* as a commercial crop (Anderson *et al.*, 1989), few hard data on *Porphyra* populations are available.

McQuaid (1985) has published descriptions of *Porphyra* population dynamics at Dalebrook, on the warmer, False Bay side of the Cape Peninsula. Overall, *Porphyra*

populations were correlated (with a 3 month lag) with heights of the lowest diurnal tide, and not with global radiation or sea surface temperature. *Porphyra* populations were present at two distinct heights in the upper eulittoral. The upper population showed a distinct seasonal pattern, with increased cover over the warm dry summer, and the lower was perennial.

Branch *et al.* (1990) observed changes in *Porphyra* populations in response to a freshwater flood event that killed most grazers. *Porphyra* populations expanded dramatically, along with *Ulva* and *Enteromorpha*. *Porphyra* rapidly colonized areas lower on the shore than where it had previously been found, and upper shore populations also increased. The upper boundary of *Porphyra* on the shore may have increased following herbivore death, though this is not altogether clear from the data presented. If so, then biotic interactions may have determined the upper as well as the lower boundaries of *Porphyra* in this ecosystem.

This chapter aims to identify patterns of *Porphyra* gametophyte population dynamics in the light of associated eulittoral community dynamics, to provide as baseline against which harvest treatments might be compared, and to predict possible effects of harvesting *Porphyra*. Dynamics of *Porphyra* populations across the eulittoral were monitored to assess: changes in *Porphyra* cover across the eulittoral; recruitment, fertility, and mortality patterns in *Porphyra*; composition of those *Porphyra* populations likely to be selected for harvesting; and correlation of *Porphyra* population dynamics with environmental changes. In addition, patterns of community organization were examined in an attempt to identify potential harvesting impacts. This chapter will only examine gametophytic *Porphyra*. At least one survey of sporophyte frequency is known (Martinez, 1990); however, the fugitive nature of *Porphyra* sporophytes makes quantitative assessment of sporophyte populations extremely difficult.

3.2 Methods

A site at Slangkoppunt on the Cape Peninsula (S 34°08′40″ E 18°19′10″) was regularly monitored to assess population dynamics of *Porphyra* species. A second site at Oudekraal (S 33°58′51″ E 18°21′47″) was initially monitored, but this was abandoned after oil pollution and clearing thereof by sandblasting destroyed the eulittoral community.

3.2.1 Parallel transects

Six roughly parallel permanent transects were placed down the shore. Each transect commenced and ended at roughly the same height relative to sea level: the top of each transect being approximately 50 cm above mean high tide at spring tide, and the bottom of the transect being approximately 20 cm above mean low tide at spring tide. Tidal range at Slangkoppunt ranges between approximately 1 m and 2 m, the latter at spring tide. Although there is no distinct seasonal pattern in tidal height, onshore winds and storm surge in the winter may cause tidal heights to increase, and offshore summer winds with associated high pressure cells may cause unusually low tides (Stegenga *et al.*, 1997). Transects were regularly examined, and *Porphyra* cover was recorded in contiguous 0.3×1.0 m quadrats (down and parallel to the shore respectively). The presence or absence of dominant intertidal species or taxa was recorded, as was epizooic or epiphytic growth of *Porphyra* on those species/taxa. Samples were collected during spring low tides during December 1993, February 1994, June 1994, August 1994 and December 1994.

Generally, data from six adjacent 0.3×1.0 m quadrats in permanent transects were pooled prior to analysis, as variability between quadrats was high. Frequency data were derived from pooled presence/absence data in smaller quadrats. To calculate summary statistics across the shoreline, transect length was standardised by discarding quadrats at regular intervals along each transect in all but the shortest transect, until all transects were the same length as the shortest one. When height on shore, sampling date or *Porphyra* cover were used as independent variables in statistical analysis, quadrats were assigned to height, date and cover classes. Quadrats within each class or combination thereof were treated as replicates. Each sample date formed a separate class. Transects were divided into five equally sized height classes, where height was expressed as distance from transect top. Cover classes were 0-10% cover, 10-20% cover, 20-30% cover, *et cetera.*

The effects of season and height on shore on *Porphyra* cover were tested using a repeated-measures analysis of variance following natural logarithmic transformation of data. Quoted p or α estimates from these tests were derived using Wilk's lambda from a type IV multivariate analysis of variance (Keppel, 1991).

In assessing changes in natural populations, various measures of diversity were used. Diversity is a widely used ecological concept, but is one that is difficult to precisely define, so that some have declared it a 'non-concept' (Hurlbert, 1971). The community that was monitored consisted of larger, dominant fauna and seaweeds, and changes in diversity were used to detect changes in the structure of the community. There is more than one type of diversity, and many indices have been proposed for each of these, several of which are in common use. Diversity indices may convey information on a given community's taxon (usually species) richness, or evenness or equitability, or both (Whittaker, 1972; Pielou, 1977; Magurran, 1988). This study uses several measures of alpha diversity, and one of beta diversity. Alpha diversity, analogous to MacArthur's (1965) within-habitat diversity, is the diversity within a homogeneous habitat (Whittaker, 1977). Beta diversity is a measure of the difference in diversity between communities (or along gradients) (Magurran, 1988). The measure of abundance used in the calculation of different diversity indices varies, and number of individuals, biomass, cover, number of modular units (e.g. shoots), and frequency of occurrence have all been commonly used (Magurran, 1988).

Of the indices of alpha diversity used in this study, two, the number of taxa and Margalef's richness index (Clifford & Stephenson, 1975), are functions of taxon richness (Margalef's index is an indication of taxon richness adjusted for sample size). The other two alpha diversity indices are based on the proportional abundance of the various taxa. The widely used Shannon index (Shannon & Weaver, 1949) derives from information theory, and, like the Simpson index, combines information on community richness and equitability. The Shannon index increases with increased richness and equitability, and is a measure of the probability of predicting the species of a randomly picked individual (Pielou, 1977). The Simpson index (Simpson, 1949) is strongly influenced by the abundance of the most common taxa, and hence is considered more useful as an indicator of dominance (effectively, the reverse of equitability).

The similarity indices used in the community analysis are an indication of beta diversity (Magurran, 1988). I have used the Bray-Curtis index only in this chapter. The Bray-Curtis index (Bray & Curtis, 1957), widely used in ecological studies, is not affected by joint absences of taxa in samples being compared. It gives greater weight to more abundant species without being strongly affected by abundant species (Field *et al.*, 1982). Transformation of data prior to index derivation will affect the weight given to abundant

and rare species. The Dice coefficient (Dice, 1945) is equivalent to the Bray-Curtis coefficient applied to binary (presence/absence) data (Sokal & Sneath, 1963).

Changes in eulittoral community structure were analysed by creating Bray-Curtis similarity matrices from taxa frequency data (Bray & Curtis, 1957). Binary data giving the presence of a taxon in a 0.3×1.0 m quadrat were pooled over six quadrats to give a measure of frequency of occurrence in the pooled quadrat. A Bray-Curtis similarity was calculated for each taxon using this pooled figure from the 1.8×1.0 m quadrat. Data on community structure were collected for the following eulittoral dominants: *Porphyra*, *Aeodes* (*A. orbitosa* (Suhr) Schmitz), *Gelidium* (*G. pristoides* (Turner) Kützing), *Mazzaella* (*M. capensis* (J. Agardh) Fredericq), *Gigartina/Sarcothalia* (mostly a mixture of *G. polycarpa* (Kützing) Setchell *et* Gardner and *Sarcothalia stiriata* (Turner) Hommersand *et al.* – when this survey commenced, *G. stiriata* had not yet been transferred to *Sarcothalia* by Hommersand *et al.*, 1993), *Nothogenia* (*N. erinacea* (Turner) Parkinson and *N. ovalis* (Suhr) Parkinson), ulvoid macrophytes (*Enteromorpha* spp. and *Ulva* spp.), kelps (*Ecklonia maxima*), mussels (predominantly *Mytilus galloprovincialis* Lamarck), barnacles (mostly *Tetraclita serrata* Darwin, with some *Octomeris angulosa* Sowerby and *Chthamalus dentatus* Krauss), eulittoral snails (mostly *Nodilittorina africana* Phillipi with some *Oxystele variegata* Anton), and limpets (mainly *Scutellastra granularis* Linnaeus, but *Cymbula granatina* Linnaeus, *Helcion pectunculus* Gmelin and other *Scutellastra* species were present).

A similar approach was used in an analysis of substrate choice by *Porphyra* in the six transects. Rather than recording the presence or absence of dominant taxa, the substrate of *Porphyra* growing epiphytically or epizooically was recorded. Taxa were grouped into the assemblies described above, with the addition of rock for *Porphyra* found growing epilithically. The analysis excluded ulvoid macrophytes and kelp, on which no eulittoral *Porphyra* was ever found growing epiphytically. However, *Porphyra* epiphytic on kelp (and epizooic on *Cymbula compressa* Linnaeus which is commonly found on kelp stipes) is common in the subtidal, but this is outside the scope of this analysis.

The data sets produced were too large to be tractable to a single analysis, so each transect was analysed separately, and a random subsample of the entire data set was used to test hypotheses relating to the entire shore. Similarity matrices for each transect were used as

input to an unweighted hierarchical average linkage agglomerative clustering procedure (UPGMA; Sokal & Sneath, 1963) and were ordinated in two and three dimensions using non-metric multidimensional scaling (NMDS; Kruskal & Wish, 1978), minimising global stress (after Field *et al.*, 1982; Minchin, 1987). NMDS was selected owing to its advantages over ordination methods such as principal co-ordinates, reciprocal averaging, and correspondence analysis for the reasons discussed in Field *et al.* (1982), most notably the flexibility conferred by input of a user-defined matrix of similarities/dissimilarities. Dendrograms produced by the clustering procedures were used to assist in analysis of the ordinations.

The co-occurrence of *Porphyra* and monitored taxa and substrates was determined using a cross-tabulation procedure. The data for this analysis were from 0.3×1.0 m quadrats.

The importance of the main effects of date of sampling, height on shore and *Porphyra* cover in determining community structure and *Porphyra* substrate selection was tested using a Mantel-type Monte Carlo analysis (ANOSIM) (Mantel, 1967; Clarke & Warwick, 1994). Within each height or date class, variance over sample date or height, respectively, was assessed using Warwick and Clarke's index of multivariate dispersion (Warwick & Clarke, 1993). Another permutation procedure was used to compare both similarity matrices produced for each transect (after Clarke & Ainsworth, 1993) to assess the extent of substrate specificity.

The percentage contribution of each taxon to similarity within and between height, date and cover classes was calculated to help identify marker taxa.

Traditional univariate diversity indices (number of taxa, Margalef's richness (d), Shannon diversity (H) and Simpson dominance (D), all calculated using the frequencies of taxa) were also computed (Shannon & Weaver, 1949; Simpson, 1949; Clifford & Stephenson, 1975). All logarithms used in deriving univariate diversity indices were natural logarithms. Kruskal-Wallis tests were used to assess differences in diversity indices between shore height classes, sampling date classes and *Porphyra* cover classes (Zar, 1984). The correlation of univariate diversity with gradients of height on shore and *Porphyra* cover was tested using Spearman's rank correlation (Zar, 1984). This combination of methods based on rank correlation and comparison between classes was employed so that the

presence of any correlation with cover and height could be assessed, and, if no simple correlation was present, the significance of any changes not correlated with height on shore or cover could be assessed.

The same combination of Kruskal-Wallis tests and Spearman's rank correlation was used to examine the relation between frequency of taxa and substrate and height on shore, sample date and *Porphyra* cover, to determine which taxa, if any, drove changes in taxon and substrate alpha diversity.

An analysis of covariance was used to test the importance of *Porphyra* cover alone in determining alpha diversity, without complications due to the correlation of height with cover (height, expressed as distance from transect top, was used as the covariate).

Bray-Curtis similarity matrices of shore and substrate beta diversity were compared using a Spearman's rank correlation to assess the correlation between taxon frequency and *Porphyra* substrate choice, and thereby to assess the whether substrate choice in *Porphyra* is specific or opportunistic. *Porphyra* (as a taxon) and rock (as a substrate) were excluded from this analysis, as were taxa on which *Porphyra* never grew. The latter include ulvoid macrophytes, too fragile to support epiphytic *Porphyra*, kelp, on which *Porphyra* is epiphytic but not present in significant quantities, and *Mazzaella*.

3.2.2 Random quadrats

In the second approach, 0.25×0.25 m quadrats were subjectively placed in patches of *Porphyra* taken to be representative of patches with high biomass, as these are most likely to attract harvesters. The wet biomass and reproductive status of plants in those quadrats was recorded. Samples were collected during spring low tides in September 1993, November 1993, February 1994, June 1994, November 1994, January 1995 and May 1995.

3.2.3 Recruitment, growth, and mortality

Finally, the size and reproductive status of ten haphazardly selected plants located in each of ten permanent 0.1×0.1 m quadrats was monitored to assess population dynamics in wild populations (five quadrats were initially monitored, but die-off of recruits necessitated an

increase in sample size). Plants selected for monitoring were ideally sporelings (approximately 1 mm long), but if no sporelings were present, larger thalli were selected. Once selected, thalli were monitored regularly until they died. Thalli were relocated using a clear plastic overlay that recorded their positions relative to the stainless steel screws at opposite corners of each quadrat. Data were collected at spring low tides from April 1995 until August 1996.

The surface area of thalli in permanent quadrats was estimated from maximum thallus length (l) and width (w) data using the empirically derived formula (1) presented below. Areas estimated using this formula correlated with measured areas of thalli that had shapes varying from linear to umbilicate ($p=0.032$), and areas were closely correlated with thallus mass ($p<0.001$). Relative growth rates (RGR) were calculated using (2), where n and $n\text{-}1$ are times of measurement, and Δt is the time elapsed between measurements (Brody, 1945; Schmalhausen, 1984).

(1) $$A = \pi(0.25(l + w))^2$$

(2) $$RGR = (\ln A_n - \ln A_{n\text{-}1})\Delta t$$

Thallus senescence frequently led to holdfasts alone remaining on the substrate. Holdfasts never regenerated into plants; as a result, growth rate calculations were not undertaken for holdfast remnants. Growth rates were calculated for all thalli originating as spores, and for all sporelings that survived to become fertile. Statistics on growth rates were calculated from when thalli were first recorded, and so cover the full lifespan of monitored thalli.

Data on thallus mortality were derived from the number of monitored thalli in each quadrat, the number of monitored thalli lost between samples, and the area of the quadrat. Instantaneous (or between sample) mortality is expressed as proportional mortality: the mean proportion of monitored thalli lost between samples.

Data on recruitment were derived from counts of the number of new sporelings in each quadrat. The maximum number of thalli monitored in each quadrat was ten (only 10% of samples had more than 10 recruits per quadrat). A measure of proportional recruitment was calculated as follows: proportional recruitment is the number of thalli recruited into each

quadrat over the maximum number of possible monitored recruits in that quadrat. The maximum number of possibly monitored recruits is the difference between the number of thalli surviving from the previous sample date and ten.

3.2.4 Weather effects

Data on maximum temperature, minimum temperature, humidity, wind speed, rainfall and cloud cover, collected at Cape Town (Cape Town International airport weather station, 33km from the sample site), were assessed to determine how great an influence meteorological conditions had on population dynamics, and to identify conditions that might lead to a collapse in *Porphyra* populations. Correlations between weather data and biotic data were examined using graphical analyses comparing weather data and biotic indices, and weather data was used as overlays on ordinations of biotic data.

3.3 Results

3.3.1 *Porphyra* cover in transects

Porphyra cover at Slangkoppunt varied significantly over time ($p<0.001$), and with height on the shore ($p<0.001$) (Figure 3-1). Differences between the replicate transects were also present ($p=0.049$), and two- and three-way interactions between transect, height on shore, and sampling date were also detected (height × date $p<0.001$; transect × date $p<0.001$; height × date × transect $p=0.001$). Though the three-way height on shore-date of sampling-transect number model accounts for much of the variation in *Porphyra* populations, the complexities of these interactions make interpretation of patterns of change in *Porphyra* cover difficult. Interactions involving transects will be ignored in this chapter as little is to be gained from considering them other than confirmation of the truisms that no strip of shore is the same as another, and that populations of *Porphyra* were likely to be influenced to some extent by populations present on the same site previously.

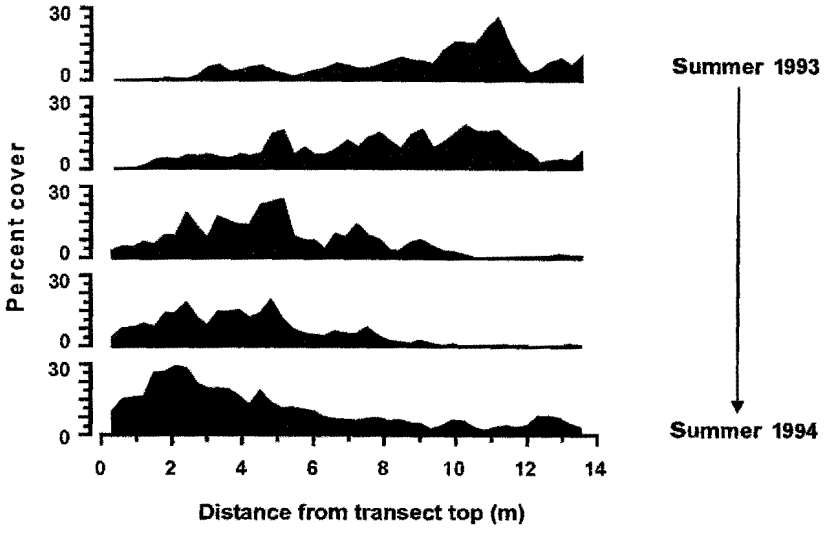

Figure 3-1 Mean cover of *Porphyra* in the eulittoral from summer 1993 until summer 1994.

In the austral 1993-1994 summer, *Porphyra* populations were concentrated in the low eulittoral (Figure 3-1). In autumn, the low eulittoral populations had changed little, but the upper eulittoral populations had increased. By winter, the low eulittoral population had dramatically decreased, but the upper eulittoral population continued to increase, and this population had changed little by spring. In the summer of 1994-1995, the low eulittoral population had again increased, but the upper eulittoral populations had maintained their high cover.

An examination of weather data found no obvious correlation between changes in *Porphyra* cover with changes in temperature, wind speed, humidity, rainfall, or cloud cover. This was unexpected, as there is a clear effect of date, and associated seasonal changes, on *Porphyra* cover, and all weather variables are correlated with season. It is possible that an examination of sea temperatures, which change seasonally, might give a better correlation. However, this study was too short to allow full assessment of the effect of weather variables with *Porphyra* cover. Only data from summer 1993 and summer 1994 allowed comparison across years within the same season. During November and December 1993, several periods with dry, hot, windy weather that lasted for several days were recorded, more so than in November and December 1994. This may account for the lower *Porphyra* cover in the upper eulittoral during summer 1993.

Porphyra populations in the low eulittoral were present during both summers but absent during the winter. The low eulittoral summer *Porphyra* populations seldom grew epilithically, but rather were epiphytic, most notably on *Aeodes orbitosa* but to a lesser extent on *Gelidium pristoides*, *Gigartina polycarpa* and *Sarcothalia stiriata*, or epizooic on *Scutellastra granularis* (Figure 3-3). Several of these substrates (*A. orbitosa*, *G. polycarpa* and *S. stiriata*) showed distinct seasonality, with populations that peaked in summer.

3.3.2 Community analysis

The data collected from transects was comprehensively analysed to detect patterns in community behaviour. The results of this analysis were extensive, and only the more important findings are presented in this thesis. Where they are presented, results from analyses of individual transects will be indicated; otherwise results are from the full data set, or from a random sample where analysis of all data was not possible.

Changes in the distribution of monitored eulittoral taxa with time are presented in Figure 3-2. The data presented in Figure 3-2 are frequencies derived from presence/absence data from six vertically adjacent 1×0.3m quadrats. These do not translate to biomass or cover data, as can be seen if cover and frequency data for *Porphyra* are compared (Figure 3-1 vs Figure 3-2). This is because frequency measurements do not reflect the presence of two or more individuals per 1×0.3m quadrat. For this reason, a minor decrease in frequency often reflects a much greater decrease in the number of individuals.

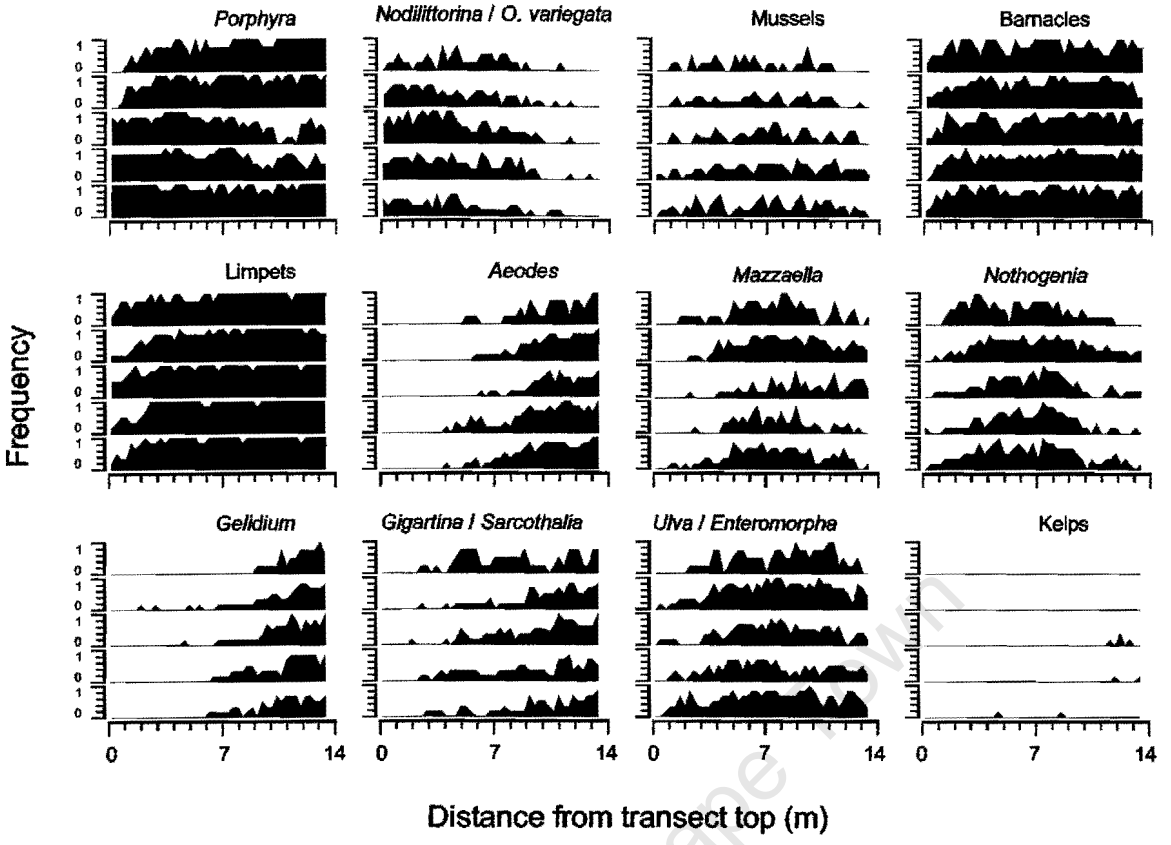

Figure 3-2 Frequency of taxa in 1×1.8m transect quadrats. Five sample times, from summer 1993 (top plot) to summer 1994 (bottom plot), are shown on each graph. Frequencies are plotted on a scale of 1 (occurring in 100% of quadrats) to 0 (in no quadrats). Only taxa attached in each quadrat are recorded.

Several of the taxa examined showed some indication of a seasonal pattern in their distribution, although all except kelp were present in at least one transect throughout the year (and extensive kelp beds were present year-round below the transects). For example, *Nothogenia* and snails were more frequent on the upper shore during winter, and *Mazzaella* was more frequent in the mid-shore during summer. In the case of mobile taxa, seasonal changes in distribution may indicate seasonal changes in migration patterns (Branch, 1975), or distributions may simply represent a tendency to remain sheltered and hidden, and hence unsampled, under rocks or in cracks during the day. However, of the taxa sampled, only limpets and snails are mobile. Inspection of hidden areas (deep crevices, under rocks etc) in areas where either of these taxa seemed absent revealed little, and it seems that samples of mobile taxa are representative. Changes in the distributions of sessile taxa clearly reflect the effects of population recruitment, growth and mortality.

Changes in *Porphyra*'s substrate choice with time and height on shore are presented in Figure 3-3. The majority of *Porphyra* thalli grew epilithically. After rock, limpets were the most common substrate; this occurred lower on the shore. In the study area, the only macrophyte commonly forming a substrate for *Porphyra* was *Aeodes orbitosa*. Smaller amounts were found on *Gelidium pristoides*. Low shore epiphytic and epizooic growth of *Porphyra* is clearly revealed as a summer phenomenon.

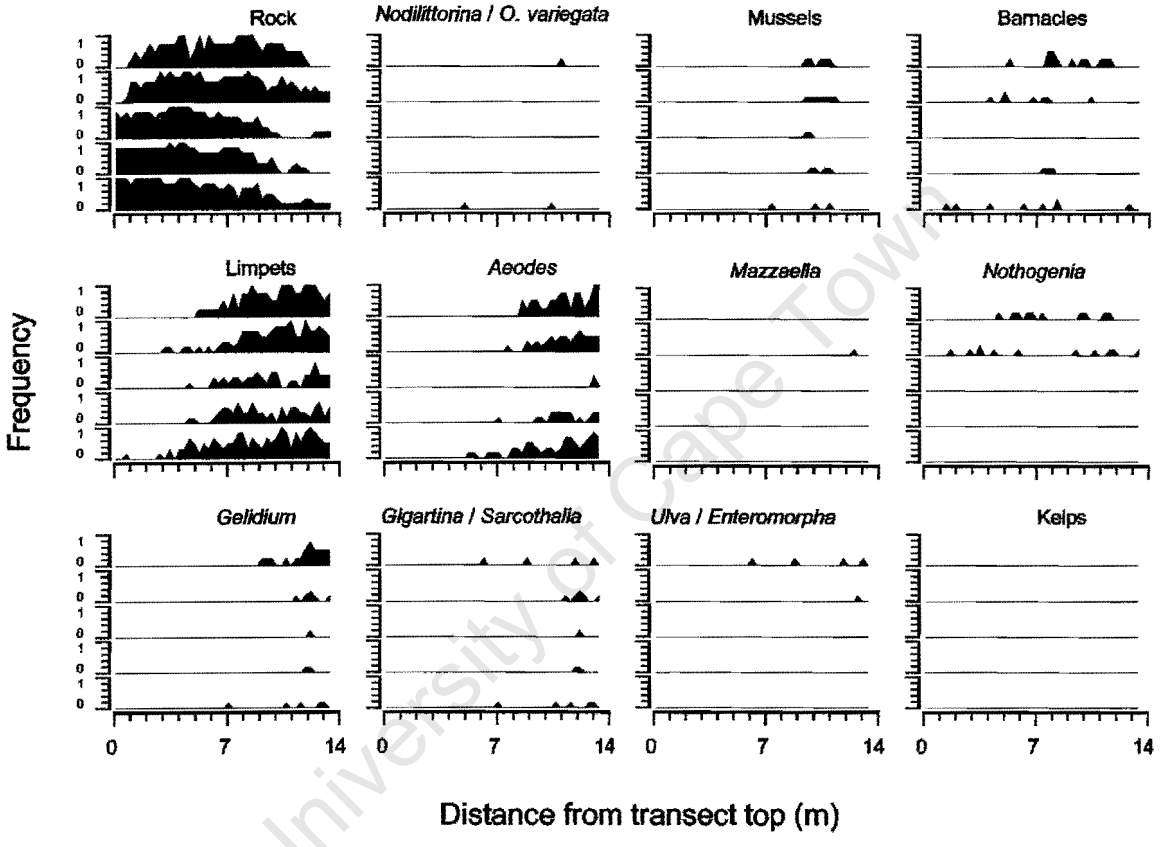

Figure 3-3 Frequency of *Porphyra* substrate choice in 1×1.8m transect quadrats. Five sample times, from summer 1993 (top plot) to summer 1994 (bottom plot), are shown on each graph. Frequencies are plotted on a scale of 1 (occurring in 100% of quadrats) to 0 (in no quadrats).

Overall co-occurrence of *Porphyra* with eulittoral taxa, as well as substrate choice of *Porphyra* is presented in Table 3-1. *Porphyra* is most frequently associated with taxa that are found throughout the eulittoral (limpets, barnacles and ulvoids). Taxa that are restricted to zones within the sampled eulittoral are less frequently associated. This is to be expected, as *Porphyra* is present, often at low levels, throughout the eulittoral. Data on frequency of

substrate choice show clearly that, beyond rock, only limpets and *Aeodes* commonly act as substrates for *Porphyra*.

Table 3-1 Co-occurrence of *Porphyra* and eulittoral taxa in quadrats. Data show the percentage of 0.3×1.0 m quadrats in which *Porphyra* and various eulittoral taxa co-occur, and the percentage of quadrats in which *Porphyra* grew epiphytically or epizooically on any taxon. All data are expressed as a proportion of the 0.3×1.0 m quadrats that contained *Porphyra* (78 % of all quadrats).

	Co-occurrence	Substrate
rock		75
Nodilittorina/*O. variegata*	34	0
mussels	25	2
barnacles	77	3
limpets	90	40
Aeodes	32	18
Mazzaella	35	0
Nothogenia	45	2
Gelidium	23	3
Gigartina/*Sarcothalia*	27	0
Ulva/*Enteromorpha*	52	0
kelp	0	0

An NMDS ordination of Bray-Curtis similarities of quadrats derived from untransformed frequency data of taxa frequency (Figure 3-4) suggests the same conclusions as does inspection of Figure 3-2. Changes in community composition and diversity are associated primarily with height on shore, though the effect of seasonal changes is apparent. No consistent evidence of taxon assemblages that may have been obscured by pooling data across transects in graphical analysis is evident.

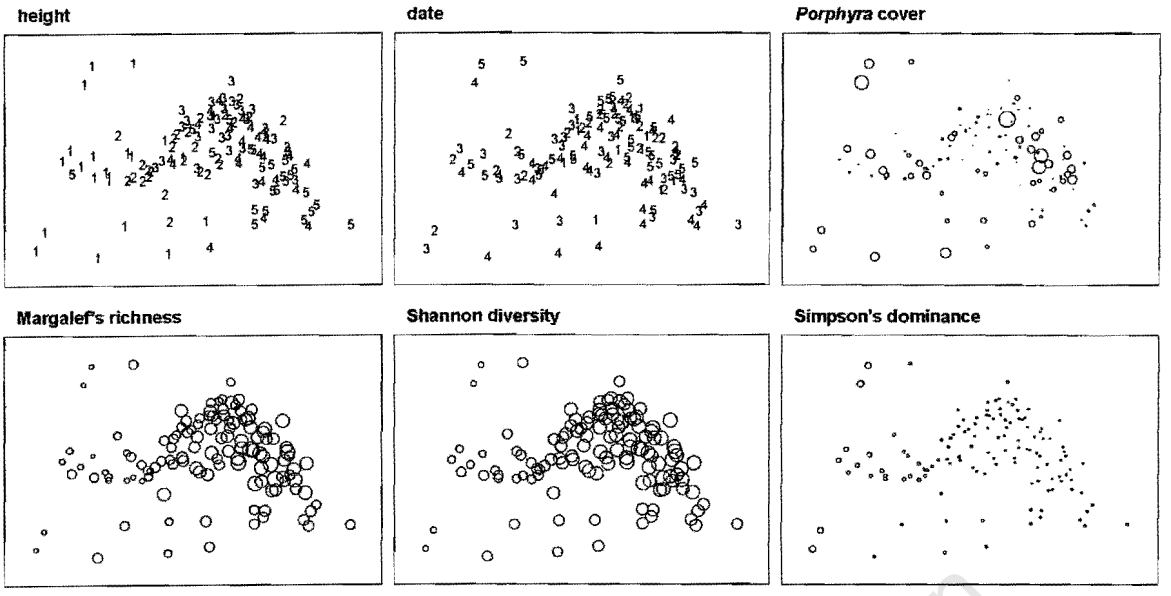

Figure 3-4 NMDS ordination of Bray-Curtis similarities of untransformed frequency data of taxa present in 1.0×1.8 m quadrats on the shore (one outlier excluded, stress is 0.14). Five height classes (1-upper 20% of transect; 2-20 to 40% down transect; 3-40 to 60% down transect; 4-60 to 80% down transect; 5-lower 20% of transect) and all dates (1-December 1993; 2-February 1994; 3-June 1994; 4-August 1994; 5-December 1994) are shown. Circles sizes are proportional to *Porphyra* cover, Margalef's richness, Shannon diversity and Simpson diversity.

When the ordination is examined, a clear trend is visible from high shore to low shore communities. Communities from the upper reaches of the shore generally have low diversity and are dominated by a few taxa. Examination of data from high shore communities reveals them to be generally dominated by one taxon, frequently either by *Porphyra* or by snails, though other taxa may be present. Ordination points from high shore communities are highly dispersed compared to those lower on the shore, suggesting a less homogenous community than lower on the shore (Figure 3-5). Such variation between replicates has been suggested as an indication of stress (Warwick & Clarke, 1993).

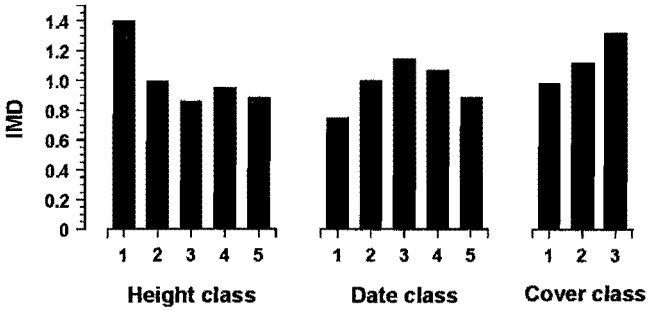

Figure 3-5 Index of multivariate dispersion (IMD) of shore taxon diversity in 1.0×1.8 m quadrats within height, date, and cover classes. Height classes: 1: upper 20% of transect; 2: 20-40% down transect; 3: 40-60% down transect; 4: 60-80% down transect; 5: lower 20% of transect. Dates: 1: December 1993; 2: February 1994; 3: June 1994; 4: August 1994; 5: December 1994. Cover classes: 1: 0-20% cover; 2: 20-40% cover; 3: 40-60% cover (60-100% cover-too few samples).

Changes in season on the ordination are apparent, with a shift from summer 1993 to winter 1993, and back (Figure 3-4). Communities in quadrats from summer 1994 did not all return to the states observed in summer 1993, and retained elements characteristic of winter communities (also see Figure 3-2). A number of points from summer 1994 lie with points from winter 1993 and spring 1993, although many have communities similar to those in summer 1993. Dispersion is greatest in winter, lowest in summer, and intermediate in autumn and spring.

No clear pattern that correlated with the amount of *Porphyra* cover was detected in the ordination, which indicates that no single characteristic community is associated with *Porphyra* in the eulittoral. Instead, dispersion of quadrats increased with increasing *Porphyra* cover.

When taxon frequencies are overlaid on the ordination, widely distributed taxa such as *Porphyra*, barnacles and limpets are least associated with the ordination pattern. Taxa that have clear seasonal or distribution patterns are best associated with the overall ordination.

Cluster analysis of Bray-Curtis similarities derived from taxon frequency show quadrat similarities to fall predominantly along a gradient, with few distinct clusters. The most distinct cluster contained nearly all quadrats from the upper 20% of the shore. Median *Porphyra* cover was highest in this cluster, and communities contained few taxa and were

strongly dominated. The cluster contained typically high-shore taxa, predominantly *Porphyra*, snails, barnacles, and limpets. The next distinct, large cluster also contained many samples with relatively high *Porphyra* cover (approximately 50% of samples), but samples were drawn predominantly from the lower 40% of the shore, where diversity and species richness were highest. The most common taxa were barnacles, limpets, *Aeodes*, *Gelidium*, *Gigartina*, *Porphyra* and ulvoid macrophytes. The remaining clusters were characterized by intermediate diversity and low *Porphyra* cover. Changes in season on the cluster plots are largely obscured by differences in height on shore; nevertheless, in some transects distinct summer and winter clusters were visible.

A Mantel-type Monte Carlo analysis of the combined effect of height on shore and season revealed height to be highly correlated with changes in community beta diversity ($p=0.001$). Significant differences between all height classes bar the two lowest classes were noted. Some correlation between season and patterns in community change was detected ($p=0.084$), though this is not significant at the 5% level. Pairwise tests between sample times showed winter 1993 to be unlike summer 1993 ($p=0.081$), autumn 1993 ($p=0.013$), and summer 1994 ($p=0.022$).

The lack of significance of results of tests on sample date may be constrained by sample size or the use of frequency as a measure of abundance. Frequency data are more easily collected than abundance data; however, they may be insensitive to changes in abundance. An examination of *Porphyra* frequency and cover data in Figure 3-1 and Figure 3-2 indicates the difference between the measures: plots of *Porphyra* frequency suggest that, with a few exceptions, *Porphyra* is common throughout all height groups and seasons; plots of *Porphyra* cover show considerable changes in *Porphyra* abundance with season and height on shore.

When this analysis was repeated using the Dice index rather than Bray-Curtis similarity, height ($p=0.001$) and sample date ($p=0.015$) were significantly correlated with similarity patterns. The conversion to Dice index means that quadrats are not differentiated on the basis of frequency values, only presence/absence of taxa, and so stresses the contribution of new populations and shifting boundaries of populations, were frequency is low. This similarity index might therefore be expected to be more sensitive to shifts in populations over environmental gradients, though less sensitive to changes within those populations.

Alpha diversity differed significantly between height classes (Margalef's richness $p=0.002$; Shannon diversity $p<0.001$; Simpson's dominance $p<0.001$). Margalef's richness and Shannon diversity peaked midway down the shore. Height is less correlated with changes in diversity indices in individual transects; the difference between the overall result and that from individual transects is apparently a function of the number of replicates in the test.

Changes in alpha diversity with season were not easily detected: overall, there were no significant effects of sample date on alpha diversity, and only in one transect, where all diversity indices changed significantly with time ($p<0.004$), were changes in any diversity index significantly correlated with changes in sample date.

Perhaps the most important, in light of potential harvesting of *Porphyra*, of the Mantel-type Monte-Carlo tests is the test for correlation between changes in *Porphyra* cover and patterns in beta diversity, as harvesting will, at least in the short term, act to decrease the cover of *Porphyra*. A good correlation was found between *Porphyra* cover classes and patterns of beta diversity ($p=0.006$). Pairwise comparisons of *Porphyra* cover class groups reveal little more, probably because the great majority of samples had low *Porphyra* cover.

As noted above, quadrats with high *Porphyra* cover, and therefore those most likely to attract harvesters, fell into two distinct classes: the high-shore quadrats with low diversity, and the low-shore quadrats with high diversity. High-cover communities high on the shore typically contained mostly *Porphyra*, snails, barnacles and limpets, and little else. High-cover communities low on the shore typically contained *Mazzaella*, *Aeodes*, *Gigartina/Sarcothalia*, *Gelidium*, limpets, mussels, and barnacles.

Changes in alpha diversity were closely associated with *Porphyra* cover classes (Margalef's richness $p=0.002$; Shannon diversity $p<0.001$; Simpson's dominance $p<0.001$). Although significant correlation existed between diversity indices and height on shore, the results were less significant, as indices peaked at intermediate cover values. Margalef's richness and Shannon diversity decreased with increased cover. Maximum richness and diversity were associated with 0-15% cover, and were at a minimum in quadrats with 45-100% cover.

A complicating factor in analyses using *Porphyra* cover as an independent variable is the correlation of *Porphyra* cover with height on the shore (overall p=0.016), and the correlation between diversity and height. Once the covariance of height had been accounted for, only Margalef's richness varied significantly between *Porphyra* cover classes (p<0.001). Richness decreased with increased cover.

The frequency of most macrophytes showed a negative correlation with *Porphyra* cover (*Aeodes* p<0.001; *Gelidium* p<0.001; *Gigartina/Sarcothalia* p<0.001; kelp p=0.011), although some positive correlations were detected (*Mazzaella* p=0.996; *Nothogenia* p<0.001; *Ulva/Enteromorpha* p=0.059). With the exception of *Ulva/Enteromorpha*, which grows ephemerally throughout most of the eulittoral, correlations seem to be a function of individual macrophyte seasonal and environmental preferences.

Porphyra cover was negatively correlated with most fauna (mussels p=0.015; barnacles p=0.879; limpets p=0.641), though cover was positively correlated with *Nodilittorina/O. variegata* frequency (p<0.001). Observations indicate that the fauna most commonly associated with *Porphyra* are amphipods (*Hyale* spp.), isopods (frequently (*Parisocladus* spp.), and snails (especially *Nodilittorina africana*). In particular, *Hyale* spp. were seldom found apart from *Porphyra*, and seem most likely to be adversely affected by widespread harvesting. Beyond the observations above, the use of broad assemblages of taxa used in this chapter make further predictions of the impact of harvesting on eulittoral organisms difficult.

The NMDS ordination of untransformed Bray-Curtis similarities of substrate choice is presented in Figure 3-6. The ordination has low stress (0.10), indicating that the data is well represented in two dimensions. A clear height gradient is less apparent, but a trend from high to low shore quadrats is still visible. The effect of date, although obscured by height, is nevertheless apparent, particularly in quadrats from the top 20% and bottom 20% of the shore.

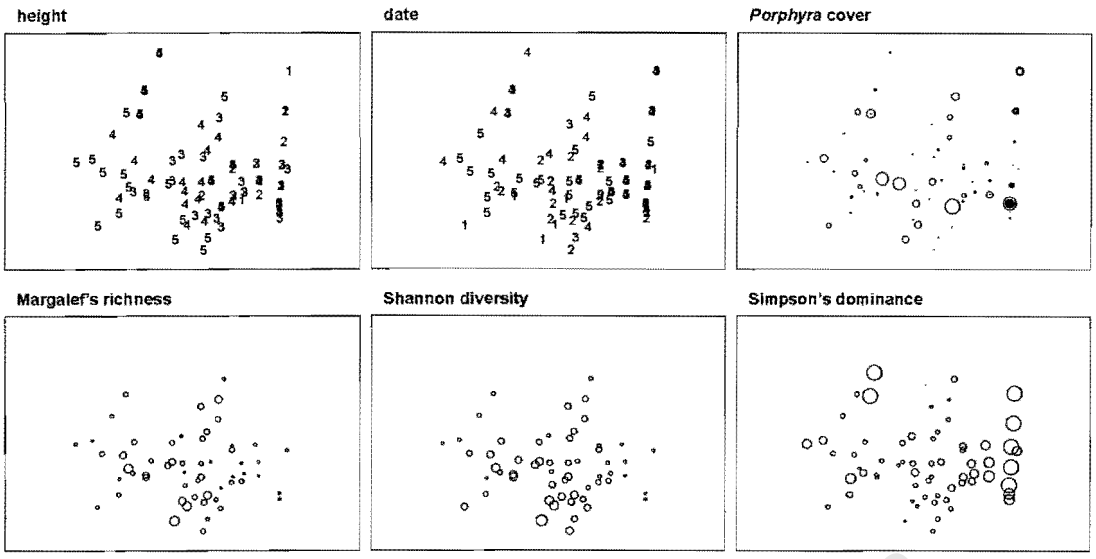

Figure 3-6 NMDS ordination of Bray-Curtis similarities of untransformed frequency data of *Porphyra* substrate choice in 1.0×1.8 m quadrats on the shore (one outlier excluded, stress is 0.10). Five height classes (1-upper 20% of transect; 2-20 to 40% down transect; 3-40 to 60% down transect; 4-60 to 80% down transect; 5-lower 20% of transect) and all dates (1-December 1993; 2-February 1994; 3-June 1994; 4-August 1994; 5-December 1994) are shown. Circles sizes are proportional to *Porphyra* cover, Margalef's richness, Shannon diversity and Simpson diversity.

Dispersion increases from high on the shore to low (Figure 3-7). The change in dispersion with height is likely a function of a more favourable environment and greater variety of substrates, combined with increased grazing pressure lower on the shore. Increased grazing pressure in combination with a more favourable environment apparently led to *Porphyra* growing more commonly on other taxa rather than on rock. There is no pattern of dispersion with time of sampling.

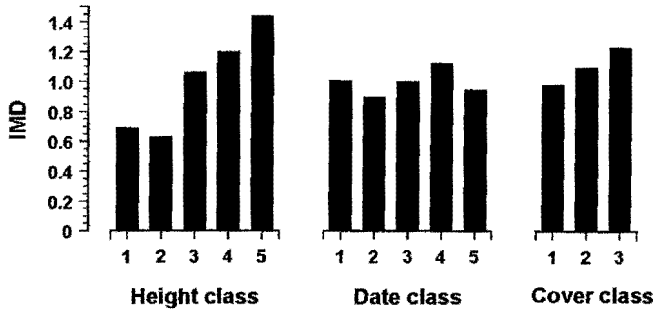

Figure 3-7 Index of multivariate dispersion (IMD) of substrate diversity in 1.0×1.8 m quadrats within height, date, and cover classes. Height classes: 1: upper 20% of transect; 2: 20-40% down transect; 3: 40-60% down transect; 4: 60-80% down transect; 5: lower 20% of transect. Dates: 1: December 1993; 2: February 1994; 3: June 1994; 4: August 1994; 5: December 1994. Cover classes: 1: 0-20% cover; 2: 20-40% cover; 3: 40-60% cover (60-100% cover-too few samples).

High-shore quadrats, where rock and barnacles are the only substrates, show high substrate dominance. Substrate richness and diversity is greatest below this, with the exception of a number of quadrats in the lowest 20% of the shore that were sampled between winter 1993 and summer 1994, where limpets and *Aeodes* dominated substrate choice. When substrate frequencies are overlaid on the ordination, a clear trend is seen from quadrats dominated by rock, through limpets, to those where *Aeodes* is the primary substrate.

The cluster plot produced a number of well-defined groups. One, with samples drawn almost entirely from the bottom 20% of the shore and from winter 1993 - summer 1994, contained samples where limpets, *Aeodes* and *Gelidium* were the sole substrates. Substrates from the upper 40% of the shore and autumn 1993 – spring 1993, with high, but patchy, *Porphyra* cover and rock as the only substrate were clearly distinguished from a cluster from the upper 40% of the shore, present year-round, more evenly distributed *Porphyra* cover, and a greater number of substrates, although rock was still the most important. Another clear cluster contained quadrats from the lower 60% of the shore, present year-round, with a range of substrates, of which limpets, rock and *Aeodes* were the most important.

A Mantel-type Monte Carlo analysis showed height on shore to be highly correlated with substrate choice in *Porphyra*. Pairwise tests did not distinguish between quadrats in the top 20% and 40% of the shore, or between the bottom 20% and 40% of the shore. As such, it

seems that substrate selection in *Porphyra* can be divided into three zones: high-shore, where rock, limpets and barnacles make up the substrate; mid-shore, where the substrates comprise rock, limpets, *Aeodes* and barnacles, and low shore, with a wide range of substrates. In the high-shore zone, rock makes up more than 95% of the substrate. The importance of rock decreases as a substrate thereafter: in the mid-shore, rock is 75% of the substrate, and limpets 20%, and in the low-shore limpets and, lower down, *Aeodes* are more common substrates.

The effect of date on substrate choice is apparent, but not statistically significant ($p=0.062$). When the Dice index, rather than the Bray-Curtis index was used, both height and sampling date were significantly correlated with substrate choice similarity data ($p<0.001$ in both cases). When individual transects were tested, height was significant in four transects, and sample date in five. Only in one transect was no significant effect of sample date detected ($p=0.098$). Pairwise comparisons of overall beta diversity revealed that all dates were significantly different ($p=0.012$ or less) except winter and spring 1994, which could not be statistically distinguished.

Porphyra cover and substrate diversity were closely correlated, but this was not significant when the Bray-Curtis index was used ($p=0.062$). When the Dice index was used, changes in substrate diversity between *Porphyra* cover classes were found to be highly significant ($p<0.001$).

Height on shore affected all measures of substrate choice alpha diversity: overall, Margalef's richness ($p=0.008$), Shannon diversity ($p=0.001$) and Simpson's dominance ($p<0.001$) all varied over five height classes. Margalef's richness and Shannon diversity increased from the upper 20% of the shore, peaked 60-80% down the shore, and decreased again in the lower 20% of the shore. In contrast to the significant shifts in substrate choice beta diversity with date of sampling, date had very little effect on alpha diversity indices. All the diversity indices tested showed no significant change with sample date, either overall or in individual transects. *Porphyra* cover had no overall significant correlation with any substrate choice alpha diversity index.

There was a close correlation between Bray-Curtis similarity matrices of taxon frequency and substrate choice frequency ($p=0.002$). This does not indicate necessarily that substrate

choice in *Porphyra* is opportunistic, but rather that height on shore and date alter *Porphyra* substrate selection to the same degree that they modify eulittoral communities.

3.3.3 High density populations

Porphyra patches with 100% cover were examined to determine what populations might attract harvesters. The mean wet biomass of dense patches was 1.96 $kg.m^{-2}$, with a density of 612 $thalli.m^{-2}$. The biomass and density of populations changed with time (Figure 3-8). Greater densities were consistently found over the summer period. There was no clear pattern of change of biomass with time.

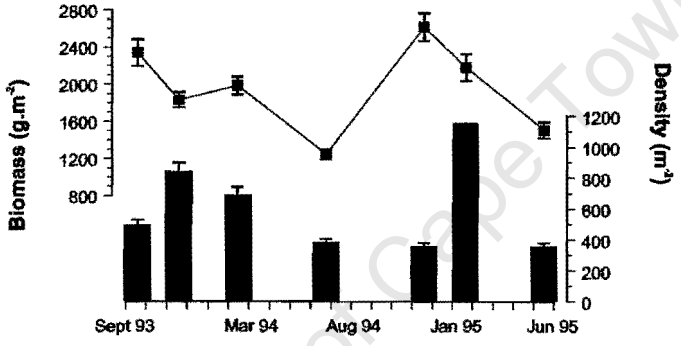

Figure 3-8 Biomass and density of high-density populations of *Porphyra* with time. Solid bars show the density (±standard error), and the line shows the biomass (±standard error).

Thalli in high-density quadrats were generally large, and usually were reproductively mature (Figure 3-9). The data from January 1995 differ sharply from those from other dates, as dense patches sampled then were younger, with smaller, more densely packed thalli, few of which were reproductively mature.

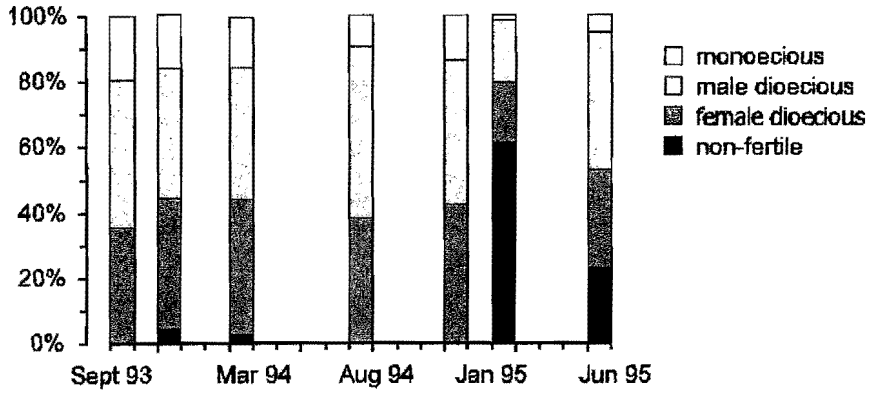

Figure 3-9 Proportions of monoecious, dioecious and non-fertile *Porphyra* thalli in high density populations over time.

Dense patches usually contained large populations of crustaceans, notably the amphipod *Hyale* and the isopod *Parisocladus*.

3.3.4 Recruitment, growth and mortality

Porphyra recruitment into permanent quadrats over the period April 1995 to August 1996 showed distinct peaks in September 1995 and March-April 1996 (Figure 3-10). Outside these temporal windows, recruitment was low to negligible. The main pattern in *Porphyra* mortality was due to increases in mortality that accompanied peaks in recruitment (Figure 3-10). These peaks in mortality were due to the very high mortality of new recruits (62% of sporelings did not survive to be sampled again). Mortality was relatively high in January 1996; this corresponded with hot, dry windy weather that may have increased stress in the eulittoral. Patterns of recruitment and mortality from April to July 1996 correspond well with those for April to July 1995, and suggest a seasonal pattern.

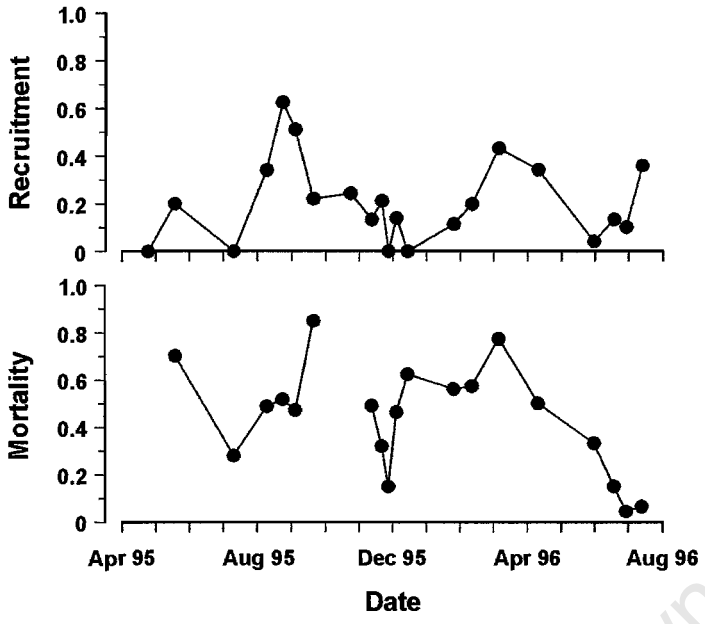

Figure 3-10 Proportional recruitment and mortality in *Porphyra* populations between April 1995 and August 1996. See text for definition of units.

The mean relative growth rate of *Porphyra* thalli following recruitment in permanent quadrats was initially very high, at 7.8±1.0 $\%.d^{-1}$ in the initial 22 days or so of growth (Figure 3-11). This equates to a doubling of thallus area every 8.9 days. The growth rate decreased with time after recruitment, until, approximately 70 days after recruitment, a negative growth rate was recorded. The growth rates presented here are the effective growth rates, and show the combined effect of growth and thallus wear, and do not show the potential growth rate in the absence of wear and grazing. An indicator of the potential growth rate of sporelings is provided by the maximum recorded growth rate, which was 23.1 $\%.d^{-1}$, giving a thallus area doubling time of 3.0 days.

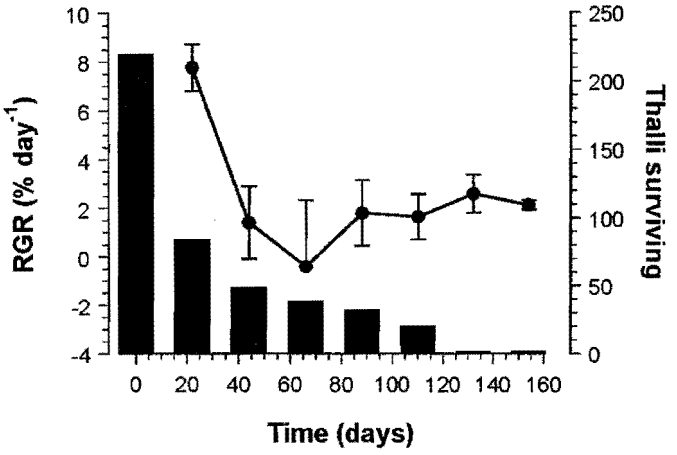

Figure 3-11 Growth rate and survival of *Porphyra* recruits. Solid bars show the number of recruits surviving with time after recruitment. The line shows the growth rate of recruits (±standard error) after recruitment.

The number of recruits surviving shows an exponential decrease with time after recruitment (Figure 3-11). The majority (62 %) of sporelings were lost before they could be resampled. Only 63% of 334 established recruits survived for a further 22 days. The survivorship pattern suggests that *Porphyra* plants have a size threshold above which survival is more likely. This was reached after approximately 40 to 60 days growth, when the mean RGR stabilises to a roughly constant value, and survival rates improve. Survival was better when a number of thalli established together as a cohort in one quadrat.

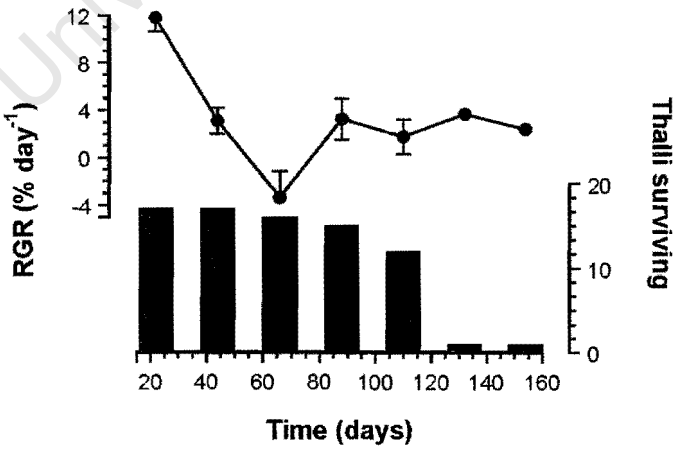

Figure 3-12 Growth rate and survival of *Porphyra* recruits that survived to become fertile. Solid bars show the number of recruits surviving with time after recruitment. The line shows the growth rate of recruits (±standard error) after recruitment.

The mean growth rate of all sporelings was compared to that of thalli that survived and grew to become fertile. Of these, time from establishment to fertility varied from 16 to 166 days, with a mean of time to fertility of 66±1.53 days. Fertile thalli showed a similar growth pattern to all thalli, with high initial growth decreasing to a negative value after approximately 66 days, then stabilising around 3%.d^{-1} (Figure 3-12). Growth rates in fertile thalli were initially approximately 50 % greater than the mean, but, after 66 days, fell to values lower than the population mean, before stabilising around the mean growth rate.

It is not clear whether initial growth patterns played a part in determining which plants grew large enough to become fertile. That the drop in growth rate approximately 66 days after establishment occurs in long-lived thalli indicates that this is not an artefact introduced by monitoring thalli that failed to establish. This period matches the window in which the majority of thalli became fertile, and the deceased growth rate after 66 days may be function of decreased growth consequent to the onset of fertility in the thallus.

3.4 Discussion

3.4.1 *Porphyra* populations

The relatively high *Porphyra* cover in the mid- to upper eulittoral during the winter of 1994 matches observations from later years that support the regular appearance of a dense *Porphyra* population in the mid- to upper eulittoral during winter. Observations suggest that the mid- to upper eulittoral winter population can be the densest *Porphyra* growth for any given year. The distribution of *Porphyra* at Slangkoppunt in summer differed in several respects from 1993 to 1994. In the summer of 1993, *Porphyra* cover was concentrated relatively low in the eulittoral, while in summer 1994 the high eulittoral *Porphyra* cover was greater than at any other time during this survey. Observations before and after this survey suggest that winter populations are capable of surviving into summer provided that environmental conditions do not become too harsh. The relatively dry, south-easterly winds common in summer in this region are often associated with a dramatic dieback in exposed macroalgae (Bolton & Joska, 1995; Stegenga *et al.*, 1997). A seasonal decrease in *P. columbina* biomass has also been attributed to 'the onset of unfavourable environmental conditions and the release of reproductive tissue' (Brown *et*

al., 1990). I attribute the survival of winter high eulittoral populations to a relatively mild 1994 summer, while summer 1993 had more, and longer, hot, dry, windy spells.

It is perhaps surprising that no greater effects of weather, beyond seasonal changes, on *Porphyra* populations were detected, as *Porphyra* life histories are closely keyed to environmental parameters. To illustrate, nori production may be predicted using weather data (Noda & Iwata, 1978). Weather data inform particularly on spore set and release, and thereby affect recruitment. In the harsh eulittoral of the study region, greater effects of short term, non-seasonal weather patterns were anticipated, as environmental effects are reported to impact heavily on gametophyte populations (Bolton & Joska, 1995; Stegenga *et al.*, 1997). The effect of seasonal weather patterns was apparent, as patterns in *Porphyra* cover in this study were very well explained by a combination of height on shore and date (which includes general seasonal change). McQuaid (1985) was not able to find a significant correlation between *Porphyra* biomass at Dalebrook and light (as global radiation) or sea surface temperature, although there was a correlation with height of lowest diurnal tide.

In the above discussion of weather effects on *Porphyra* populations, it is important to note that two effects of weather on *Porphyra* are considered. The first, affecting dieback of upper eulittoral populations in summer, can be considered a disturbance, and is a function of short-term weather conditions. The second effect of weather is as a trigger for life history stages. Most species of *Porphyra* that have been investigated show a seasonal recruitment pattern, in which annual gametophyte recruitment occurs in spring-summer or autumn-winter, leading to annual summer or winter gametophyte populations (Dickson & Waaland, 1985; Avila *et al.*, 1986; Waaland *et al.*, 1990). It is unusual for annual gametophyte populations to survive through a year, and most are present for a four to seven month window (Kurogi, 1961; Kapraun & Luster, 1980; Arasaki, 1981). Spore production in *Porphyra* responds to a number of environmental parameters with day length and temperature being perhaps the most important (e.g. see Tseng & Chang, 1956; Dickson & Waaland, 1985; Mitman & van der Meer, 1994). Seasonal recruitment in this study suggests that *Porphyra* is responding to environmental cues, although the nature of these cues has not been determined.

The seasonality detected in Chapter 2 and seasonal shifts in distribution suggest the presence of several *Porphyra* species in eulittoral populations. The biannual peaks in recruitment observed here are also consistent with the presence of several species of *Porphyra* in the monitored populations. Morphological forms that showed distinct, differing seasonalities were noted during the course of this survey, and it seems likely that these represent new species. The taxonomy of *Porphyra* in South Africa needs to be revised before comprehensive management plans can be devised for the genus, as nearly all the sampled thalli and populations (with the exception of a few low eulittoral populations) were part of the *P. capensis* species complex. Other *Porphyra* species encountered were *P. saldanhae*, *P. aeodis* and *P.* sp. indet. (*sensu* Stegenga *et al.*, 1997).

The continuous low levels of recruitment observed throughout the study probably derive either from vegetative archeospores produced by extant gametophytes, or from continually produced and released conchospores from sporophyte populations. Both these mechanisms have been noted frequently in *Porphyra* species (Kurogi, 1961; Cole & Conway, 1980; Nelson & Knight, 1996). Archeospore production has been reported in *P. capensis* (Graves, 1969), and I have observed conchocelis of unidentified *Porphyra* species in the field. Thalli counted as recruits had already grown to at least 1mm in length, and had survived for some time since spore settlement and germination. This definition of recruitment, where the period between spore settlement and recruitment depends on observer limitations, is widely used in studies in the intertidal and marine environments (Santelices, 1990*b*). As recruitment was assessed in the presence of herbivores it is to be expected that a number of sporelings had already been lost to grazing or to death by another means. The recruitment rates presented here therefore underestimate the number of actual recruits. As grazing of sporelings has emerged has a major factor regulating algal distribution (Branch & Griffiths, 1988; Cubit, 1984; Santelices, 1990*b*), it is likely that recruitment in the absence of grazers would be considerably higher.

If protected from grazers (specifically *Scutellastra cochlear* Born), *P. saldanhae* is able to develop in high densities in the low eulittoral (Joska in Stegenga *et al.*, 1997). Another indication of the importance of grazers in limiting growth of South African *Porphyra* was the establishment of dense beds of *Porphyra* following death of most grazers, including *S. granularis*, due to a freshwater flood (Branch *et al.*, 1990). Finally, grazer exclusion plots in False Bay result in increased growth of *P. capensis* or *Ulva/Enteromorpha*

(G. Maneveldt, pers. comm.). The impact of grazing on *Porphyra* establishment in the mid-lower eulittoral is suggested in this study by the predominantly epizooic and epiphytic growth habit of *Porphyra* in this habitat. An epizooic or epiphytic habit probably offers a refuge from the intense grazing pressure associated with bare rock substrata: this would explain the frequent growth of *Porphyra* on *S. granularis*, a major herbivore at the study site, when it cannot be found on bare rock in the same environment.

The absence of epiphytic or epizooic *Porphyra* in the low eulittoral during winter is unlikely to be a function of grazing pressure, as epiphytic/epizooic *Porphyra* is found in the low eulittoral during summer, and epiphytic/epizooic *Porphyra* is at least partially protected from grazers (especially immediately after recruitment) by its choice of substrate. It seems more likely that the relative absence of *Porphyra* from the winter low eulittoral is due to specific habitat requirements. This suggests that winter *Porphyra* populations have different environmental requirements to summer populations. Lower on the shore, *Porphyra* epiphytic on kelp may be found in this period. The absence during winter of *P. aeodis*, which grows epiphytically on *A. orbitosa*, may be due to a relative lack of substrate, as *A. orbitosa* is functionally a summer annual (Levitt *et al.*, 1995), or to its own seasonality: *P. nereocystis* Anderson, an epiphyte of *Nereocystis luetkeana* (Mertens) Postels *et* Ruprecht has a life history that is closely synchronized with that of *N. luetkeana* (Dickson & Waaland, 1985). Though isolated *P. aeodis* plants may survive through winter where their substrate does too, recruitment was only observed during spring.

Competition and other direct interactions between macrophytes may explain some of the negative correlations between *Porphyra* cover and macrophyte occurrence. However, in most cases negative correlations are more likely an effect of the various factors that restrict any macrophyte to a zone in the eulittoral, and not directly due to interactions between the taxa in question. *Porphyra* is common in the upper eulittoral, where, regardless of the presence of *Porphyra*, few other macrophytes grow. This appears to be due to the capacity of *Porphyra* to endure the stress of life in the upper eulittoral where other macrophytes cannot [see Davison & Pearson (1996) and references therein for a discussion of the effects of stress in modulating interactions between eulittoral seaweeds]. On the south-western coast, *Porphyra* is capable of growth in the lower eulittoral and of apparently outcompeting other macrophytes (at least in the short term), provided that grazer influence is excluded (Branch *et al.*, 1990; Joska in Stegenga *et al.*, 1997; G. Maneveldt, pers.

comm.). The absence of *Porphyra* from the lower eulittoral seems therefore to be attributable to grazing pressure [see Paine (1990) for a review of mechanisms mediating competition in macrophytes]. The lower limit of *Porphyra* on this shore therefore seems determined by biotic factors. The upper limit of *Porphyra* on the shore may be set by stress, as has been observed in other eulittoral macroalgae (e.g. Lubchenco, 1980, and references therein). However, grazing pressure, especially in the presence of stress, may also act to lower the upper boundary of *Porphyra* (e.g. Cubit, 1984). Data presented in Branch *et al.* (1990) suggest that grazers may modify the upper limit of *Porphyra* in the region.

Of the macrophytes examined in this study, *Nothogenia* and *Ulva/Enteromorpha*, frequently found together with *Porphyra*, are most likely to interact with *Porphyra*. Such interactions may be competitive; however, it is also possible that the damper conditions in stands of *Porphyra* might facilitate sporeling establishment, particularly higher on the shore. Otherwise, competition between *Porphyra* and other macrophytes seems to be largely ruled out, apparently due to the impact of herbivores on *Porphyra* populations. The observations of Branch *et al.* (1990) suggest that competition between *Porphyra* and other macrophytes, in particular *Ulva/Enteromorpha*, is likely in the absence of grazing.

Porphyra species are often classed as ephemerals or stress tolerators, according to the schemes of Grime (1979) and Connell and Slatyer (1977), or as opportunists, according to Littler and Littler's (1980) scheme. Santelices (1990*a*) suggested two strategies in macroalgae: species that are capable of pre-empting and occupying space, and those that are adapted for a patchy occupation of the environment. These do not completely correspond to Littler and Littler's (1980) opportunists and late successional forms. Santelices (1990*a*) uses *Porphyra* as an example of a taxon that combines elements of both of opportunists and late successional forms. *Porphyra* is a taxon that is adapted for patchy occupation of environments according to Santelices's (1990*a*) proposal.

Generally, *Porphyra* is not regarded as a competitive dominant. It rather avoids competition by growing in environments where stress levels reduce the number of potential competitors, or occurs as an early successional form that is soon replaced. A number of studies have demonstrated the considerable influence of herbivores in mediating population behaviour in *Porphyra* gametophytes. The results of this study, taken together

with others (Branch *et al.*, 1990; Joska in Stegenga *et al.*, 1997; G. Maneveldt, pers. comm.), suggest that South African *Porphyra* is capable of competing with other macroalgae, when the influence of herbivores is removed. The long-term outcome of such competition is unknown. Positive correlations with other macroalgae are likely to be primarily driven by environmental factors (including grazing pressure), as both *Nothogenia* and *Ulva/Enteromorpha*, commonly found in the same quadrats as *Porphyra*, were often found where *Porphyra* is absent. If, as I speculate here, the densities of other macrophytes are little affected by the presence of *Porphyra*, and vice versa, then harvesting *Porphyra* should have few or no negative effects on other macrophytes.

3.4.2 Eulittoral community analyses

The frequency measure of taxon abundance employed in this chapter is relatively crude, but has the advantage that it is rapidly assessed. This metric is skewed in that the presence of a single individual of a taxon in a 0.3 m^2 quadrat scores the same as many individuals of that taxon. It follows that maximum frequency, after pooling of adjacent quadrats, can be achieved by as little as six individuals in a 1.8 m^2 quadrat. As such, frequency will stress the contribution of taxa that have a relatively low abundance but are dispersed across a 1.8 m^2 quadrat (such as *A. orbitosa*), while underestimating those taxa that occur in dense, but spatially restricted populations (e.g. high-shore populations of *Porphyra* or *Nodilittorina*). Some effects of this bias will occur in analyses presented here: however, my observations on the shore suggest that few occurrences of any taxa were spatially clustered sufficiently often so as to significantly decrease their contribution to quadrat similarity. The primary effects of using this estimate of frequency were therefore to underestimate absolute abundance of taxa, in particular patchily distributed taxa such as high shore *Porphyra* and *Nodilittorina*. In downscaling the importance of high abundances, it can be argued that the use of frequency as a measure of abundance affects analyses in a similar, if less precise, fashion to the transformation of abundance data.

When Mantel-type Monte-Carlo analyses were undertaken, the Dice index proved more sensitive to changes in height on shore and date than did the Bray-Curtis index. From this, one may infer that changes with height on shore and, in particular date, are better detected by examining the absolute co-occurrence of taxa, and changes therein, rather than by incorporating information on abundance (as frequency).

Warwick and Clarke's IMD was used here as a measure of variation between replicates, with a view to using it as an indicator of disturbance due to harvesting. IMD, on the whole, proved sensitive to changes across gradients, and may be useful as an indicator of disturbance owing to harvesting.

I do not propose to discuss the community dynamics of the eulittoral community in depth. The analyses in this chapter were undertaken to assess the likely impact on the eulittoral community of harvesting *Porphyra*, and will be discussed in this light. Data collected are insufficient to undertake a full analysis of community dynamics. Broad generalisations from the data are presented below. Implications for harvesting are discussed later.

Changes in communities are associated most with a gradient of height on shore, and, to a lesser extent, with seasonal changes. That height on shore is such a major gradient is not surprising, as height is a sharp gradient that extends from areas that are exposed for almost all of the tidal cycle and experience extremes of heat and dehydration to those that are rarely exposed. Changes in alpha diversity clearly reflect this gradient.

The effect of date may be due to seasonal changes in recruitment and mortality (or preferred location in mobile taxa). Changes with date also reflect seasonal weather patterns. Winters are generally cooler and damper, and the dehydration gradient represented by height on shore may be moderated in winter by cooler, wetter weather. As an example of the possible effect of this, a breakdown in the steep summer dehydration gradient may allow taxa whose upper distribution limits are environmental to survive higher on the shore.

3.4.3 Implications for harvesting

Porphyra is present throughout the year, and no seasonal pattern in harvestable biomass availability was detected. Growth is rapid, and recruitment depends primarily on seasonal recruitment. This window of recruitment is, from the viewpoint of *Porphyra* biology, perhaps the major factor limiting potential yields. As ongoing non-seasonal recruitment is low, and holdfasts do not regrow, harvested populations will not recover or be replaced until the next spring or autumn. The growth rate of *Porphyra* has therefore no implication

for postharvest recovery of populations. Harvesting shortly after recruitment, before recruits are fertile, may limit the number of carpospores released, and therefore the size of the sporophyte population. The longevity of sporophytes in the wild is not known; however, in laboratory cultures sporophytes of *P. capensis* have survived for two years. Nevertheless, until data on wild sporophyte populations is available, it would be wise to manage harvesting in order that a proportion of gametophytes survive to fertility. This will assure sufficient recruitment into sporophyte populations that in turn should enable continued gametophyte recruitment.

The analysis of substrate choice was undertaken to explore factors influencing *Porphyra*'s location on the shore, and to determine whether *Porphyra* grew epiphytically or epizooically to such an extent that harvesting would be likely to impact on the substrate organism. *Porphyra* grew predominantly on rock, then limpets, and then *A. orbitosa*. Limpets were seldom removed when *Porphyra* was hand-picked, and limpet removal during harvesting would seem to be a negligible risk. When *Porphyra* on *A. orbitosa* is hand-picked, a part of the *A. orbitosa* thallus is frequently detached. The extent of damage to *A. orbitosa* following *Porphyra* harvesting is likely to be a function of *Porphyra* availability higher on the shore (epiphytic populations are lower and can only be collected at low tide), and the density and size of *P. aeodis* thalli (sparse populations are unlikely to attract harvesters).

As species richness decreased with increasing *Porphyra* cover, it appears that *Porphyra* excludes most other taxa from dense patches. Areas with high *Porphyra* cover had few associated taxa, and only snails (*N. africana* in particular) were commonly associated with *Porphyra*. However, both *N. africana* and *Oxystele variegata* were found in areas with little or no *Porphyra*. Branch *et al.* (1990) found that the development of dense growths of *Porphyra* and *Ulva* displaced *N. africana*, which was subsequently found higher on the shore than usual. They suggest that this may be due to a decrease in microalgal food availability consequent on macroalgal overgrowth, or to physical removal by foliar sweeping during high tides. Ephemeral amphipods and isopods were observed to be common in dense *Porphyra* in this study, although no attempt was made to quantify their abundance. Branch *et al.* (1990) found large numbers of both in *Porphyra* and *Ulva* beds that formed after grazer death, and it seems that dense beds of these macroalgae in particular support large populations of isopods and amphipods in the mid-eulittoral.

It seems unlikely, based on my results here and on the literature, that harvesting eulittoral *Porphyra* will have any impact on other eulittoral macroalgae. *Porphyra* appears to function as an ephemeral and as a stress-tolerator in the environment studied, and there was no clear indication that *Porphyra* might be a competitive dominant or that it might modify the environment in such a way as to facilitate the growth of other macroalgae. The only potential impact of harvesting is mechanical damage to those macroalgae acting as a substrate for epiphytic *Porphyra*.

During harvesting, harvesters may cause damage by trampling eulittoral organisms. However, as regrowth of *Porphyra* is seasonal, harvesters are unlikely to revisit a section of shore within any six month period. The impact is likely to be greatest on those organisms found associated with *Porphyra*, and in particular *N. africana*.

One possible impact of harvesting *Porphyra* may be the removal of sheltered sites favorable to other species. *Porphyra* patches may offer a refuge from extreme environmental conditions, and may possibly play a role in the life histories of other taxa present (e.g. spore or larval settlement). Branch *et al.* (1990) note the establishment of the mussel *Aulacomya ater* Molina in the mid-shore following the development of extensive beds of *P. capensis*, *Ulva* and *Enteromorpha*. They also note apparent increased recruitment of the false limpet *Siphonaria aspera* Krauss after algal bed establishment.

The importance of the contribution of *Porphyra* sporelings, and even spores, to the diet of intertidal herbivores is not known, though grazing of sporelings is well documented (Cubit, 1984; Branch & Griffiths, 1988; Santelices, 1990*b*). Harvesting of gametophytes may impact on sporeling recruitment rates, if ongoing, non-seasonal recruitment of *Porphyra* is due to vegetative archeospores produced from gametophyte populations, or if gametophyte harvesting reduced sporophyte populations and thereby reduces conchospore production. The temporal and spatial nature of *Porphyra* spore clouds is not known; though large spore clouds have been recorded for a number of intertidal algae (Santelices, 1990*b*). Martìnez (1990) notes that *Porphyra* sporophyte population densities do not necessarily correspond with those of gametophytes. The impact of harvesting *Porphyra* gametophytes on *Porphyra* propagule recruitment and thereby on grazers is not known. Monitoring of grazer

communities on harvested shores should reveal any impact of large-scale removal of *Porphyra* on food resources.

4 Impact of harvesting *Porphyra*

4.1 Introduction

Porphyra has been used as a food in many of the regions where it occurs, and small-scale harvesting has been recorded from a number of places that *Porphyra* is found (references in Chapter 1). Small-scale harvesting is seldom well documented, and quantities harvested are often unknown, although records do exist for countries with larger-scale, more commercial harvesting. Many records are in the grey literature (reports etc.) and so are not easily available.

The effect of harvesting on *Porphyra* and associated eulittoral communities is generally not understood, as few published studies on the impacts of harvesting wild *Porphyra* are available. Woessner (1981) assessed the potential of *P. nereocystis* in central California for harvest. He pointed out that 'we are still not ready to exploit this resource' as the effects of harvesting on *P. nereocystis* (or on its host *Nereocystis luetkeana*) were not known. The same can be said for most wild populations of *Porphyra*.

The relative lack of formal studies is perhaps not surprising, as the prime consumer of *Porphyra*, the nori industry, relies on predominantly farmed material from few species, and has for some time (Miura, 1975). Beyond *Porphyra* production in Asia, harvests are generally small and localised. When localised harvesting is examined, it is often as an assessment of the suitability of local species for the nori market (e.g. González & Santelices, 2003).

Porphyra columbina is harvested in Chile for human consumption, and at times large quantities have been removed (1119 tons in 1994; Alveal, 1998). Although technologies have been developed for farming of this species in Chile (Santelices, 1996), farming has not yet been implemented, possibly as *Porphyra* is of relatively minor importance as a seaweed crop in that country. Santelices (1996) considered that *P. columbina* had little ecological importance as a habitat, and that the impact on other eulittoral organisms of harvesting *Porphyra* would be low. *Porphyra columbina* requires a specific set of environmental conditions to recruit, and therefore is more likely to be more sensitive to

disturbance due to harvesting than *Ulva* spp. (Santelices, 1996); nevertheless, *P. columbina* can act as an opportunist and rapidly colonise open space (Santelices & Martínez, 1988).

Roland and Coon (1984) assessed the effect of harvesting on *Porphyra* populations in British Columbia, consisting predominantly of *Porphyra perforata* J. Agardh (probably *Porphyra abbottae* Krishnamurthy (S. Lindstrom pers. comm.)). Recovery of populations (in terms of *Porphyra* biomass) was complete one year after harvesting. The authors suggest that only severe reduction of gametophyte populations would impact on sporophyte populations, and conclude that sustainable yields will be maintained provided that thalli are handpicked.

Nelson and Conroy (1989) assessed the impact of harvesting wild *Porphyra* in New Zealand. They found harvest methods to affect regeneration: when 5mm thallus stubs were left after harvesting, greater post-harvest regeneration than in completely cleared quadrats was obtained. They do not attribute the greater recovery entirely to thallus stub regrowth, but note that stubs did regenerate tissue. They suggest, for greater yield per unit effort, harvesting once only, late in the *Porphyra* growing season. Several species were included in harvests, including two undescribed taxa. The authors attribute some of the differences in post-harvest recovery between sites to differences in species composition. Their study was too short to fully assess the recovery of harvested populations.

Both of the above papers report on the impact of harvesting populations containing several species of *Porphyra*. The species reported on differ from those found in South Africa. In both, harvesting impact on *Porphyra* populations only is considered. In this chapter, I assess the impact of harvesting *Porphyra* at differing harvest frequencies over the period of one year. Eulittoral grazers of *Porphyra* are identified, and the impacts of harvesting on *Porphyra* populations and on associated eulittoral taxa are examined.

4.2 Methods

The work described in this chapter aims to assess the effect of three harvesting regimes (three-, six- and twelve-month harvest intervals) on populations of *Porphyra* at Slangkoppunt on the Cape Peninsula. The rationale behind the analyses used in this chapter

is discussed at length in Chapter 3, as a similar approach is taken to many of the analyses of harvesting effects. Where methodologies differ, it is indicated below.

Six replicate, non-abutting 2×1 m permanent quadrats were haphazardly placed in apparently homogeneous populations of *Porphyra* in an approximately 50 m broad stretch of the mid-eulittoral. Each 2×1 m quadrat was subdivided into eight 0.5×0.5 m quadrats. Within each 2×1 m quadrat, each of three randomly selected 0.5×0.5 m quadrats was assigned to one harvest treatment. The remaining 0.5×0.5 m quadrats were randomly assigned as controls.

Every three months following the start of the experiment (24 April 1995), one control quadrat per replicate was harvested, along with appropriate treatment quadrats. The sampling regime is presented in Table 4-1.

Table 4-1 Sampling regime for 0.5×0.5 m quadrats in each 2×1 m replicate quadrat. Harvest times for treatment quadrat after experiment start are shown.

	Time after experiment start (months)				
	0	3	6	9	12
Treatment					
harvest: 3 month interval	×	×	×	×	×
harvest: 6 month interval	×		×		×
harvest: 12 month interval	×				×
Control					
start	×				
3 month		×			
6 month			×		
9 month				×	
12 month					×

Harvesting of treatment and control quadrats was destructive. In an attempt to simulate the actions of commercial harvesters, all plants greater than *ca.* 3 cm in length were hand-picked; if thalli tore, the holdfast and torn remnants were left if they were shorter than *ca.* 3 cm. It was common that thalli tore, and holdfasts were frequently left behind; however, thallus remnants longer than *ca.* 1 cm in length were relatively rare. Picking smaller plants meant much effort was expended for little *Porphyra*, and I considered it highly unlikely that commercial harvesters would continue to harvest an area when thalli were less than *ca.*

3 cm long. Roland and Coon (1984) reported harvesters collecting only thalli longer than 5 cm.

While the entire quadrat was harvested, only material with holdfasts in the central 0.25×0.25 m was retained for analysis, in order to minimise edge effects. The wet mass of retained plants was determined after soaking plants for 15 min in seawater, and then removing superficial water first by spinning plants in a salad spinner until no more water was collected, then by blotting them with paper towels. During the harvest, the presence, if any, of fauna in the harvested quadrats was noted. Those fauna removed with harvested thalli were collected by thoroughly rinsing thalli in seawater to dislodge fauna, and identified and counted. In an attempt to simulate the action of commercial harvesters, no fauna were collected beyond those trapped among *Porphyra* thalli and unable to escape.

4.2.1 *Porphyra* biomass and density

The effect of treatments on average thallus mass, thallus density and biomass per unit area was evaluated using an analysis of variance on transformed data (seventh root transformation of average plant mass, fourth root transformation of thallus density, and natural logarithmic transformation of total quadrat biomass). Null hypotheses of no difference between treatment and control populations, no change in control populations over time, and no difference between treatments after 12 months were tested using contrast analysis (Keppel, 1991). Thallus density data from control populations were further analysed to determine whether *Porphyra* in the mid-upper eulittoral shows patchy growth by plotting frequency distributions on various scales. The effect of patchiness on thallus growth was assessed by testing for correlation between thallus density and mean thallus size and stand biomass using Spearman's rank correlation (Zar, 1984).

4.2.2 Harvesting and eulittoral communities

In order to assess potential impacts of *Porphyra* harvesting on rocky shore fauna, the abundances of fauna in experimental quadrats on the shore, and the abundance fauna collected with harvested thalli were examined for changes with time and treatment using an unweighted hierarchical average linkage agglomerative clustering procedure (UPGMA; Sokal & Sneath, 1963) and non-metric multidimensional scaling (NMDS), minimising

global stress, of similarity matrices (after Field *et al.*, 1982; Minchin, 1987). Similarity matrices were calculated using the Dice coefficient for shore fauna (Dice, 1945), and the Bray-Curtis coefficient (Bray & Curtis, 1957) of fourth root-transformed abundances for collected fauna (use of the Dice coefficient effectively transformed abundances to frequencies). Species from the shore were grouped to higher taxonomic levels (mussels, limpets, snails, chitons, anemones, starfish, barnacles, amphipods, isopods, and polychaete worms), as accurate identification of all fauna in field conditions was not always possible. Shore fauna abundances were transformed to presence/absence data to include information on isopods and amphipods that were abundant in *Porphyra* patches and difficult to count and identify in the field. As collected fauna were more easily counted and identified, data on this component were aggregated only to genus level, and abundance data were used in the analyses.

The number of taxa, Margalef's richness (d), Shannon diversity (H) and Simpson's dominance (D) were calculated for all quadrats (Shannon & Weaver, 1949; Simpson, 1949; Clifford & Stephenson, 1975). The correlations of these indices with *Porphyra* biomass, mean thallus mass, and density were tested using Spearman's rank correlation test (Zar, 1984). A Mantel-type Monte Carlo analysis was used to test hypotheses of treatment and time effect on community structure (Clarke, 1993).

4.2.3 Grazers and *Porphyra*

Numerically dominant fauna associated with *Porphyra* (the amphipod *Hyale grandicornis* Krøyer, the isopod *Parisocladus stimpsonii* Heller, the snails *Nodilittorina africana* and *Oxystele variegata*, and the limpets *Scutellastra granularis* and *Helcion pectunculus*) were collected from haphazardly selected patches of *Porphyra*, identified, and dissected. Semipermanent slides of gut contents were made, and examined microscopically for *Porphyra*. The presence of positively identified *Porphyra* fragments (well preserved material in transverse 'section' or reproductive material present), tentatively identified *Porphyra* fragments (surface view of thallus or degraded thallus fragments present) and fragments of other algae were noted. Ten individuals of each species were examined in this way, except for *S. granularis* (nine individuals) and *H. pectunculus* (five individuals).

4.3 Results

4.3.1 Harvesting and *Porphyra*

All thalli harvested during this experiment were *P. capensis* (*sensu* Stegenga *et al.*, 1997), and no representatives of *P. saldanhae*, *P. aeodis* or the linear ('*augustinae*') form of *P. capensis* were collected in experimental or control quadrats.

Significant changes in the biomass of control populations of *Porphyra* ($p=0.005$) over time were largely a function of changes in population density ($p<0.001$), as there was no significant change over time in mean thallus mass ($p=0.783$) (Figure 4-1). This suggests that the mechanism of biomass loss was thinning of control populations, where biomass was lost as discrete thalli, rather than by gradual decrease in the size of thalli. The decrease in density in control quadrats was not due to even thinning of *Porphyra* populations, but was rather a function of the loss of patches of *Porphyra*. Distribution of *Porphyra* was patchy: frequency analysis of *Porphyra* densities in control plots reveals a bimodal frequency distribution with peaks at zero thalli.m^{-2}, and approximately 300 thalli.m^{-2} (Figure 4-2). There was no gradual shift in the frequency distribution with time. This pattern is what would be expected from a combination of high-density within-patch samples, samples where *Porphyra* is absent, and relatively few samples from thinning patches or patch margins. The zero thalli.m^{-2} mode was derived mostly from samples from the later half of the experiment, when most initial populations had disappeared, and the 304 thalli.m^{-2} within-patch mode was mostly from samples taken from extant populations six months or less after the start of the experiment. When data from within patches and patch margins only are examined (data from quadrats with density of zero are excluded), no significant change in *Porphyra* density ($p=0.316$) or stand biomass ($p=0.603$) over time was detected. The density recorded within patches is lower than that recorded in the 100% cover quadrats discussed in Chapter 3.

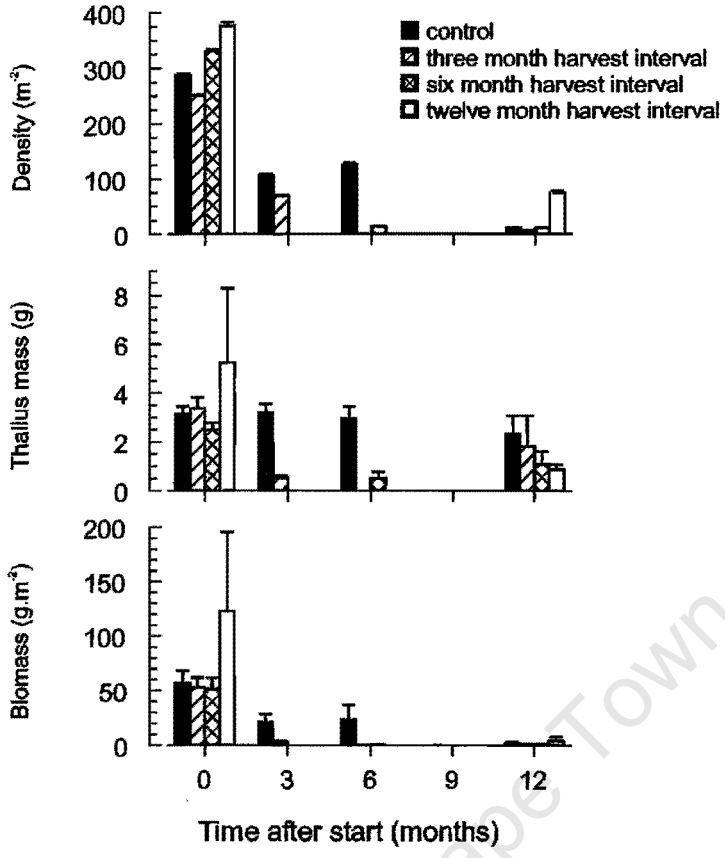

Figure 4-1 Density (thalli.m^{-2}), mean thallus mass (g) and biomass (g.m^{-2}) of *Porphyra* thalli in three harvest treatments and control quadrats. All data are mean ± standard error.

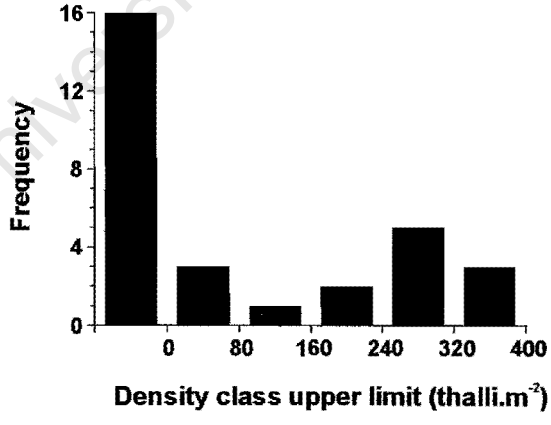

Figure 4-2 Frequency distribution of *Porphyra* thallus density in control quadrats pooled over time.

Recruitment into experimental quadrats was intially low, but increased towards the end of the experiment (Figure 4-1). Control populations may have experienced some recruitment

into the *ca* 3 cm size harvestable class throughout the study period, as evidenced by the recruitment of thalli into treatment quadrats.

When compared to the controls, harvesting significantly decreased the biomass of *Porphyra* in treatment quadrats ($p=0.005$). Overall, mean thallus mass in harvested quadrats was lower than in contemporary control plots ($p<0.001$). As harvesting removes all thalli longer than *ca* 3 cm, those thalli in harvested quadrats either grew as new recruits from spores, or from a sporeling understorey that survived the harvest treatment. I consider it unlikely that regrowth from torn thallus fragments and holdfasts remaining after harvesting contributed significantly to the regrowth of *Porphyra* in treatment quadrats for the reasons discussed in Chapter 3; however, improved recruitment due to archeospores produced by holdfasts and torn plant remnants, and improved recruitment of sporelings in the sheltered environment provided by the holdfasts may have contributed to regrowth.

Differences in thallus density between harvest and control plots were less significant ($p=0.088$). Harvested populations showed a pronounced decrease in density after the first harvest; however, a parallel, delayed decrease in density was evident in control populations (Figure 4-1). The dramatic decrease in control population density between six and nine months after the experiment started can be attributed largely to the early summer mid-eulittoral die-back of *Porphyra* that occurs most years. At this time, eulittoral algal populations established during the winter are exposed to hot, dry spring-summer south-easterly winds (Bolton & Joska, 1995; Stegenga *et al.*, 1997).

No significant differences between any harvest treatments were found.

There was no recruitment during the monitored period sufficient to bring control or treatment quadrat densities to match the density encountered at the start of the experiment. This is partially a function of the experimental design: experimental quadrats were not randomly placed in the eulittoral, but were placed into areas that had large, relatively uniform populations of *Porphyra*. After twelve months, although regrowth of winter populations of *Porphyra* had begun, relatively little regrowth had occurred in the permanent quadrats. That regrowth had begun in some quadrats indicates that harvesting does not prevent recruitment; and the low average recruitment after twelve months was due rather to the stochastic spatial distribution of *Porphyra* patches. Decreased post-harvest

recruitment as a result of stochastic recruitment patterns was noted by both Roland & Coon (1984) and Nelson & Conroy (1989).

4.3.2 Herbivores and *Porphyra*

Positive and tentative identifications of *Porphyra* from the guts of various herbivores are presented in Table 4-2. These data are undoubtedly underestimates, as they rely on the presence of recognisable material in the gut. In addition, those herbivores that had not consumed *Porphyra* prior to sampling may at other times.

Table 4-2 Proportions of sampled herbivorous fauna with *Porphyra* fragments or fragments of other macroalgae in their gut. Positive and tentative identifications of *Porphyra* are presented separately.

	Porphyra			Other macroalgae
Species	Positive	Tentative	Total	
Hyale grandicornis	0.9	0.1	1.0	0.3
Parisocladus stimpsonii	1.0	0.0	1.0	0.4
Nodilittorina africana	0.0	0.0	0.0	1.0
Oxystele variegata	0.0	0.3	0.3	1.0
Scutellastra granularis	0.8	0.2	1.0	1.0
Helcion pectunculus	0.2	0.8	1.0	1.0

Both crustaceans collected from *Porphyra* patches, *Hyale grandicornis* and *Parisocladus stimpsonii*, grazed heavily on *Porphyra*, and occasionally had fragments of other macroalgae in their guts. The snail *Nodilittorina africana* was the only herbivore that was not found to graze at all on *Porphyra*, although identification of *Porphyra* in the gut of another snail *Oxystele variegata* was only tentative. Both limpets (*Scutellastra granularis* and *Helcion pectunculus*) are generalist grazers, and may be important *Porphyra* herbivores. That *Hyale*, numerically dominant to *Parisocladus*, is likely an important herbivore of *Porphyra* is supported by the match, in size, shape and markings, between *Porphyra* fragments from the gut of *Hyale* and those found in faecal pellets collected from *Porphyra* patches on the shore. Gut contents of both crustaceans contained apparently fertile spermatia and carposporangia, and *Porphyra* is therefore not protected from these herbivores by passing a size threshold beyond which grazing does not occur.

Apart from *Porphyra*, macroalgal fragments in herbivores' guts were generally *Ulva* or *Enteromorpha* species, or unidentified crustose macroalgae.

4.3.3 Associated fauna

The faunal taxa apparently most affected by a reduction in *Porphyra* biomass were amphipods (*Hyale* spp) and isopods (mostly *Parisocladus* spp) (Figure 4-3). When the initial dense *Porphyra* stands were reduced by natural population changes and by harvesting, both amphipods and isopods, previously present in very high numbers, were nearly always absent. Snails (predominantly *N. africana*) were less frequent in treatment quadrats, and this may indicate a response to harvesting. Snail populations in treatment quadrats had recovered 12 months after the start of the experiment. Limpets appeared unaffected by harvesting. Limpet densities were generally high in dense *Porphyra* stands, but high limpet densities also occured without seaweed cover. Other taxa occurred too infrequently and their frequencies were too variable to suggest a harvesting impact.

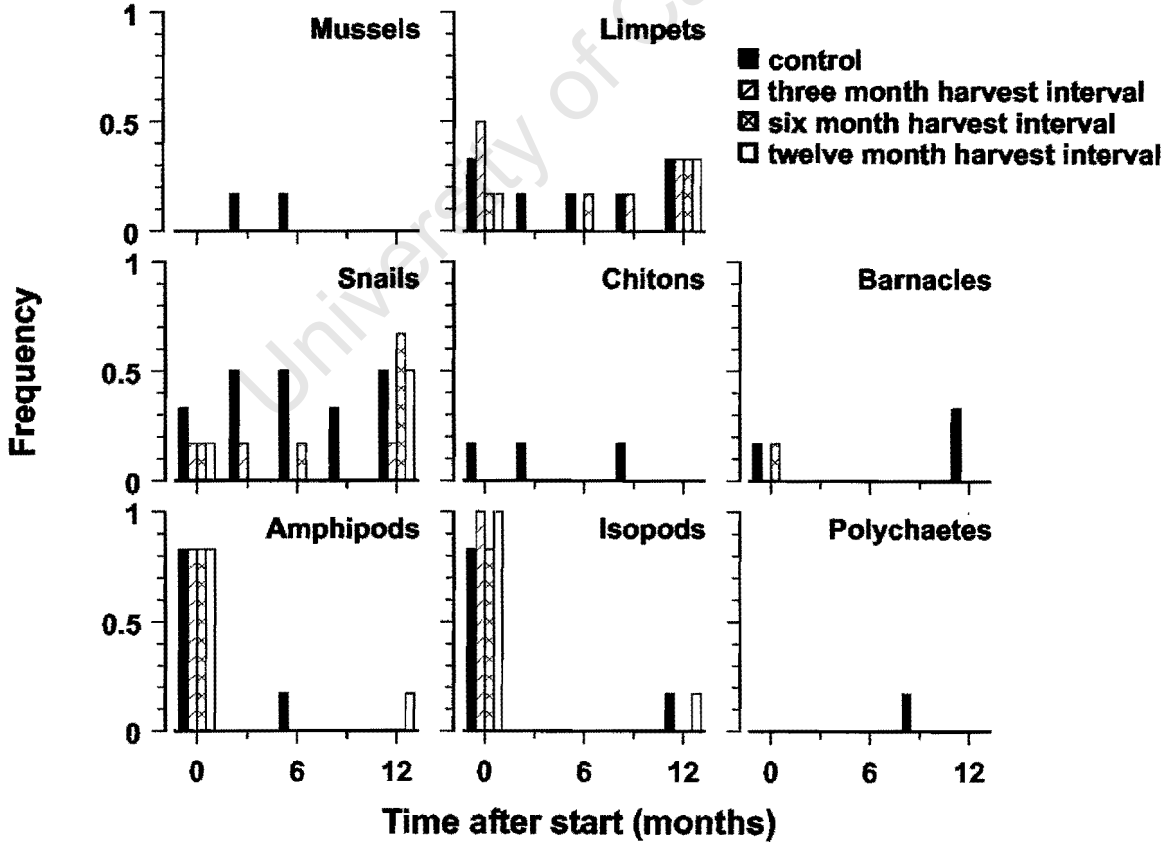

Figure 4-3 Frequency of eulittoral faunal taxa in experimental quadrats under three harvest and one control treatments, over time. Frequency is expressed as the proportion of quadrats containing a

given taxon. As all quadrats were examined, the absence of data from any period that should be present according to the experimental design indicates a zero and not missing data.

The NMDS ordination of quadrats based on eulittoral fauna presence is presented in Figure 4-4. The relatively high global stress (0.22) indicates that relations between the quadrats are essentially multivariate and cannot be completely resolved in two dimensions.

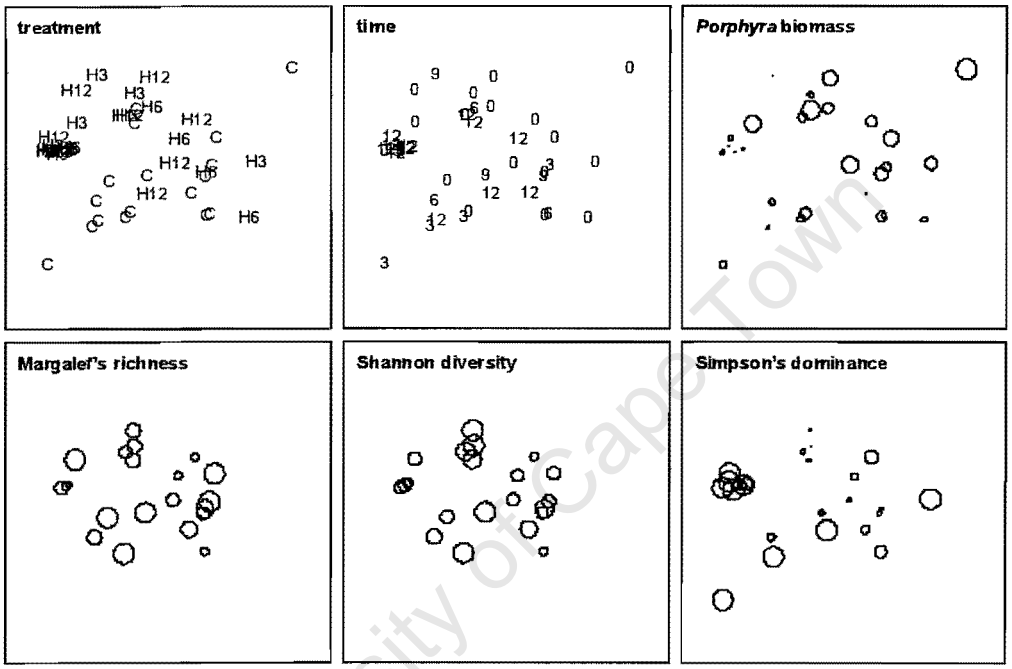

Figure 4-4 NMDS ordination, minimising global stress, of fauna present in harvest and control quadrats. Overlays show treatment, time after start, *Porphyra* biomass, Margalef's richness, Shannon diversity and Simpson's dominance indices. Numeric variables are proportional to circle diameter. Global stress is 0.22.

A gradient of time is visible in the ordinations, as control quadrats from later in the study lie to the bottom of the ordination. The latter quadrats have low *Porphyra* biomass, low Margalef's richness and Shannon diversity, and are highly dominated. These characteristics are in common with the harvested quadrats, which are densely clustered in the ordination. Nevertheless, the two are clearly distinguished by their associated fauna. This indicates that changes due to *Porphyra* harvesting differ from those due to natural population dieback. Snails, in particular, were more common in control quadrats. One possible reason for this difference is that during natural *Porphyra* biomass decreases,

Porphyra density decreased, and remaining thalli were generally large. Only holdfasts and thallus stubs remained after harvesting, leaving a stand consisting of small, short thalli only. Differences between controls and treatments might also partially derive from edge effects around harvested quadrats, where fauna from adjacent undisturbed populations were able to migrate into treatment quadrats, whereas control quadrats where natural populations had decreased would be less likely to be surrounded by *Porphyra* and any associated fauna. Although steps were taken to decrease edge effects, they could not be entirely avoided without clearing unacceptably large areas of *Porphyra* from the shore.

A Mantel-type Monte Carlo analysis using a one-way design did not detect any significant changes in the Dice similarity matrix underlying the Figure 4-4 plots with time and treatment. Aggregation to the level of the taxa used here represents a considerable simplification in that species- and genus-level variation will not be detected. When the analysis was repeated using a Bray-Curtis similarity matrix based on the original unaggregated species abundance data (excluding amphipods and isopods), changes as a result of time and treatment were highly significant ($p=0.006$).

Somerfield and Clarke (1995), commenting on the efficacy of aggregating data in marine community studies, noted that the effects of aggregating data depended on the community in question. In some circumstances aggregation to phylum level did not diminish the ability of the Mantel test to discriminate between stations, and in others aggregation above genus level reduced the test's power. It seems that the eulittoral fauna associated with *Porphyra* are insufficiently diverse to maintain all information after aggregation. However, the effects of time and treatment on aggregated data were still detectable, as evidenced by the Figure 4-4 ordinations.

When the abundances of fauna collected with harvested *Porphyra* were examined, the taxa most commonly removed were amphipods (all *Hyale* spp.), snails (predominantly *Nodilittorina africana*) and isopods (largely *Parisocladus* spp.) (Figure 4-5). In particular, large numbers of *Hyale* were removed with harvested *Porphyra*.

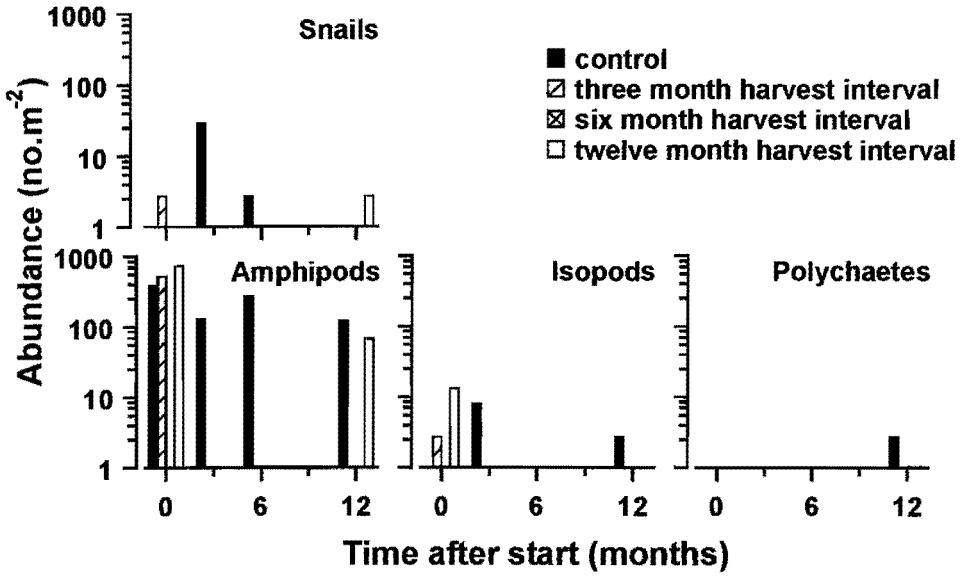

Figure 4-5 Abundance of fauna collected with harvested *Porphyra* from harvest and control quadrats over time. Abundance is expressed as the number of individuals per unit area harvested. Data are aggregated to taxon level.

Bray-Curtis similarities of fauna collected with harvested *Porphyra* were ordinated, and the results are presented in Figure 4-6. Only one point in this ordination indicates a post-harvest treatment quadrat, as few fauna were collected from treatment quadrats following the first harvest. As such, the ordination shows only natural variability, and not the effects of the harvest treatments. As a result, control and harvest treatments could not be statistically differentiated.

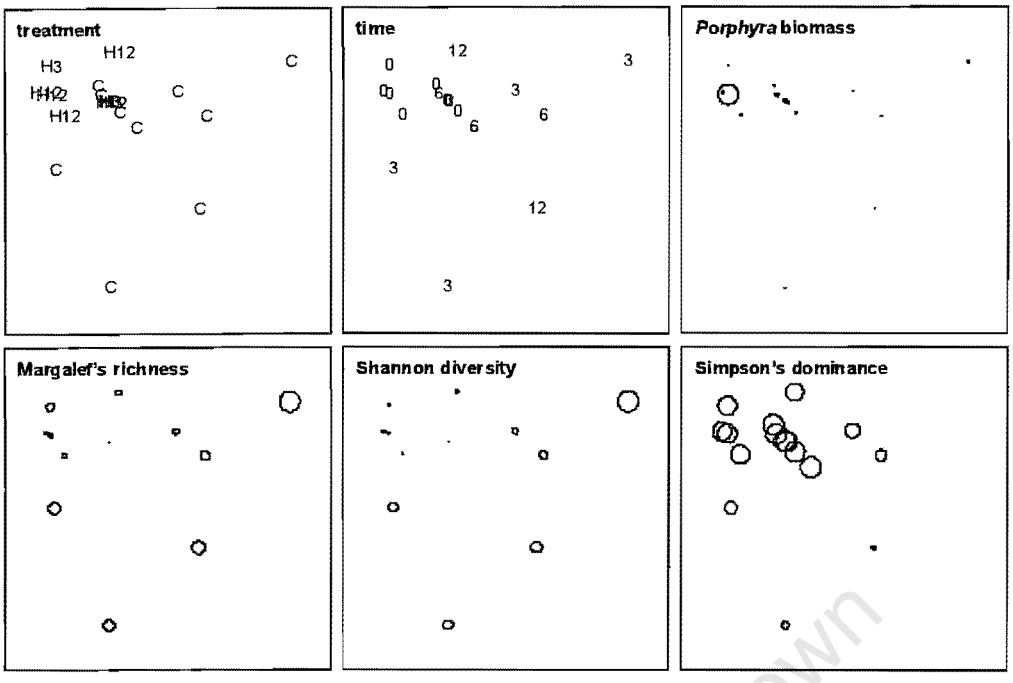

Figure 4-6 NMDS ordination, minimising global stress, of fauna collected with *Porphyra* from harvest and control quadrats. Overlays show treatment, time after start, *Porphyra* biomass, Margalef's richness, Shannon diversity and Simpson's dominance indices. Numeric variables are proportional to circle diameter. Global stress is 0.09.

In dense *Porphyra* populations from the start of the experiment Margalef's richness and Shannon diversity were low, and quadrats were not dispersed on the ordination (Figure 4-6). These quadrats were highly dominated by *Hyale*. In the sparser *Porphyra* patches after the start of the experiment, richness and alpha diversity were greater, and quadrats were considerably more dispersed. However, variability between quadrats in each time/treatment group was high, and no statistically significant change in these parameters with time or treatment was detected. The total biomass of *Porphyra* was highly correlated with the number of individuals and the number of genera of collected fauna ($p<0.001$).

Warwick and Clarke's Index of Multivariate Dispersion (IMD), a measure of variability between replicates, was highest for treatments at the start of the experiment, but beyond this does not distinguish between controls and treatments or the effect of time (Figure 4-7). In many cases the IMD could not be calculated for treatment quadrats, as insufficient shore fauna were present.

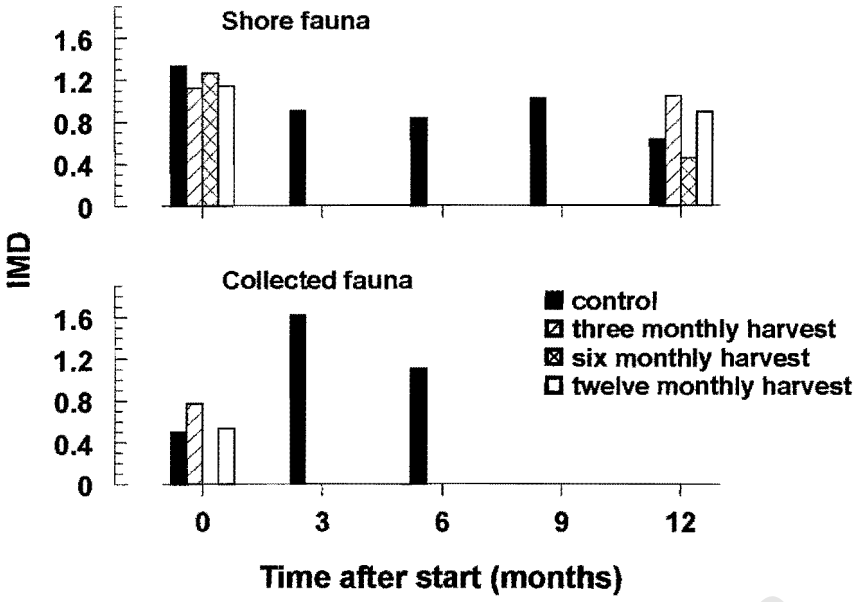

Figure 4-7 Index of multivariate dispersion (IMD) derived from faunal taxa present on the shore and collected with harvested *Porphyra*.

4.4 Discussion

Harvesting of *Porphyra* had a detectable impact on *Porphyra* populations and on the fauna in harvested quadrats. Harvesting decreased the biomass of *Porphyra* present, and also reduced the abundance of fauna on the shore. Harvesting anticipated the natural die-back of *Porphyra* populations by three to six months. The effects of harvesting were largely indistinguishable from those due to natural population collapse, with the exception of the impact of harvesting on snails: fewer snails were found in harvested quadrats than in controls, even after *Porphyra* in control populations had died back to levels similar to those in harvested quadrats.

The reasons for this impact of harvesting on snails, the vast majority of which were *Nodilittorina africana*, are unknown. Sampled *N. africana* had no fragments of *Porphyra* in their gut, and it seems that they do not graze on *Porphyra*. *Nodilittorina africana* may be found in areas of the shore where no *Porphyra* stands are present (see Chapter 3). Dense stands of *Porphyra* have even been reported to exclude *N. africana* (Branch *et al.*, 1990). One may speculate that removal of *Porphyra* stands removes damp patches that might favour the growth of other food resources, or that *Porphyra* holdfasts left after harvesting

interfere in some way with *N. africana*, or that physical removal of *N. africana* with harvested *Porphyra* decreased local *N. africana* populations.

Of the herbivores whose gut contents were examined, only the crustaceans *Hyale grandicornis* and *Parisocladus stimpsonii* seemed to rely largely on *Porphyra* as a food resource. Both of the crustaceans are potentially more mobile than the molluscan grazers, and appear capable of grazing at least on other thin macroalgae (both had fragments of *Ulva* in their gut), and perhaps on thicker species too (e.g. see Buschmann & Vergara, 1993). Branch (1971) reports a wide range of food resources for *Scutellastra granularis*, and Branch & Griffiths (1988) report sporelings of macroalgae (rather than established thalli) as being an important limpet food resource. It is clear that harvesting of *Porphyra* will remove a food resource from the intertidal; however, all herbivores examined appear capable of utilizing other food resources.

That no difference was detected between harvest treatments appears to be due to the lack of regrowth from holdfasts and to patterns of seasonal recruitment together with relatively low non-seasonal recruitment. During the study period, a seasonal peak in recruitment give rise to winter populations. However, these were not within the areas under study. This was because *Porphyra* recruits at different heights on the shore in summer and in winter (Chapter 3). Non-seasonal recruitment was detected, particularly in the first few months of the study period, but was insufficient to allow significant recovery of harvest populations. As a result, localised recovery of harvested populations only occurred after 12 months.

One aim of the work described in this chapter was to assess the impact of harvesting on *Porphyra* gametophyte populations. Due to the limited spatial scope of the experiment, detectable impacts on neighbouring gametophyte or sporophyte populations, and therefore on future spore availability, were not anticipated. Impacts of harvesting on gametophyte populations were therefore not anticipated to last longer than one year-hence the duration of the harvest trial.

That *Porphyra* is restricted to spatially patchy populations has been remarked on by other workers (Arasaki, 1981; McQuaid, 1985; Branch *et al.*, 1990; Santelices, 1990*a*). *Porphyra* species, often common or dominant in the mid- to upper eulittoral throughout their range (e.g. Lubchenco & Cubit, 1980; Roland & Coon, 1984), are tolerant of the

unusual and extreme conditions there (e.g. see Lipkin *et al.*, 1993). Although environmental stress undoubtedly acts to some extent in determining the upper limit of growth of many macroalgae in the mid- to upper eulittoral (e.g. see Stekoll & Deysher, 1996), I believe that the spatially patchy growth of *Porphyra* at Slangkoppunt is at least partially a function of disturbance patterns when thalli are sporelings, and of grazing patterns in particular. Grazers may act to clear young sporelings from areas shortly after their recruitment. This hypothesis is supported by observations that, following dense sporeling recruitment, sporelings would commonly survive until a disturbance removed all of them. Seldom were few recruits lost; either they survived or died together. Apart from grazers, no other disturbances that might have resulted in spatially localised removal of established sporelings are known at the study site. Also supporting this hypothesis is the observation by Branch *et al.* (1990) that, after freshwater floods had killed most grazers present at Steilhoogte on the South African west coast, initially patchily distributed *Porphyra* was replaced by a *Porphyra* bloom with a cover of 100%. The high-shore grazers at Steilhoogte were dominated by *Scutellastra granularis*, *Oxystele variegata* and *Siphonaria* spp., all of which were common at Slangkoppunt. Examination of the gut contents of these grazers at Slangkoppunt shows that *S. granularis* definitely grazes on *Porphyra*, and *O. variegata* may graze on *Porphyra* (the gut contents of *Siphonaria* were not examined). *Scutellastra granularis*, where it is present, may be an important herbivore of *Porphyra* and may decrease macroalgal recruitment by grazing sporelings (Branch, 1971; Bustamante & Branch, 1996). Patchiness in intertidal macroalgal distribution has often been attributed to herbivore grazing patterns (Santelices, 1990*b*).

Survival in patches in the upper eulittoral would seem to benefit plants in patches in that they would reduce water loss by presenting a relatively small surface area for evaporation (Levitt & Bolton, 1991), and so would potentially allow prolonged photosynthesis following emersion (Hay, 1981; Herbert & Waaland, 1988; Hanelt *et al.*, 1993; Scrosati & DeWreede, 1998). The extensive contact between thalli in patches should also facilitate efficient gamete transfer. That growth in patches seems to improve the growth of *Porphyra*, despite potential competition for space and light, is supported by the positive correlation between thallus density and mean thallus size and stand biomass within the range of control densities recorded in this study. Hruby and Norton (1979) found that high-density recruitment improved survival high on the shore in *Blidingia minima* (Nägeli) Kylin.

The *Porphyra* densities observed in this study were well below those predicted by the ultimate biomass-density line (Weller, 1987; Scrosati & DeWreede, 1997). However, since smaller thalli were not harvested, conclusions regarding the relationship of biomass and density in *Porphyra* patches cannot be drawn from these data, beyond noting that the growth of thalli longer than *ca.* 3 cm seems to be favoured in patches.

Nelson & Conroy (1989) found that regrowth of *P. columbina* was greatly improved if 5 mm thallus stubs were left after harvesting. While they did not specifically attribute all regrowth to these thallus stubs, they did note that the stubs regenerated new tissue. Santelices (1996) reports growth from thallus stubs of *P. columbina*, and notes that leaving holdfasts to facilitate regrowth is a harvesting strategy in Chile. No regrowth from holdfasts was noted during this study. However, there was evidence of slow continual recruitment regardless of season, with peaks in spring and autumn (recruitment defined here as the appearance of sporelings, not visibly derived from holdfasts, which were visible to the naked eye). Such ongoing recruitment may account for the increase in biomass in harvest (and potentially in control) quadrats.

It has been suggested that amphipod grazing may facilitate recruitment of grazed *Mazzaella laminaroides* (Bory) Fredericq (as *Iridaea laminaroides* Bory) by distributing spores that remain viable after passing through its gut (Buschmann & Vergara, 1993). *Porphyra* that has been grazed by molluscs may produce protoplasts capable of germination from faecal pellets (Santelices & Ugarte, 1987). The effects of amphipod grazing on South African *Porphyra* and the potential contribution of grazing patterns to recruitment are unknown. However, given the grazing of *Porphyra* by *Hyale* and *Parisocladus*, and the presence of apparently viable zygotospores in faecal pellets collected from the shore, it seems that some recruitment of *Porphyra* after grazing is likely.

5 *Porphyra aeodis*: a new species of *Porphyra* epiphytic on *Aeodes orbitosa*

5.1 Introduction

Porphyra capensis has occasionally been recorded growing epiphytically on *Aeodes orbitosa* in the mid- to lower eulittoral (Graves, 1969; Stegenga *et al.*, 1997). On closer inspection, I found *Porphyra* epiphytic on *A. orbitosa* to be morphologically distinct from *P. capensis*, and more similar to *P. saldanhae*. Morphological and ecological differences between epilithic *P. saldanhae* and the epiphyte of *A. orbitosa* suggested that the epiphyte was probably distinct from both *P. capensis* and *P. saldanhae*.

Traditional morphological characters, some of which vary with environmental conditions (Suto, 1972) have proved inadequate to delimit the more than 130 *Porphyra* species (Lindstrom & Cole, 1992*b*; Stiller & Waaland, 1993; Brodie *et al.*, 1998), and so I used isozyme electrophoresis to test the hypothesis that the epiphyte and *P. saldanhae* are the same species. Isozyme electrophoresis has proved valuable in resolving the taxonomy of *Porphyra* species even in areas where comprehensive morphological studies have been undertaken (Lindstrom & Cole, 1990*a*, 1990*b*, 1992*a*, 1992*b*, 1992*c*; Hwang *et al.*, 1998).

5.2 Materials and methods

Fresh material was collected from Kommetjie (S34°09′06″ E18°19′22″) and Rocklands (S33°54′28″ E18°23′25″) on the Cape Peninsula, South Africa, and pressed specimens and microscopic slides were prepared from this material. Sections were prepared by hand or with a freezing microtome from fresh tissue, and semipermanent slides were made by mounting rinsed sections in 43 Standard Glucose Syrup [African Products (Pty) Ltd]. Photomicrographs were taken using a Zeiss Large Universal Research Microscope equipped with bright-field and phase-contrast illumination. Line drawings were prepared using a camera lucida.

Fertile spermatangial tissue from fresh specimens was fixed in 1:2 acetic acid:ethanol, squashed, and stained for chromosomes using the acetic acid-iron-haematoxylin-chloral hydrate method (Wittmann, 1965). The same fixing and staining methods were employed

when dried material was examined (after Coll & Oliveira Filho, 1977). Overall, 117 cells from three *P. saldanhae* thalli and 225 cells from six *P. aeodis* thalli were examined.

Haphazardly selected *A. orbitosa* thalli with epiphytic *Porphyra* were collected in September 1994 ($n = 5$) and February 1995 ($n = 6$), and the number, thallus area and reproductive status of epiphytic *P. aeodis* were determined. On large thalli, the surface area was determined by direct measurement using a leaf-area meter; on smaller cordiform thalli surface area (A) was calculated from the length (l) and breadth (w) using the empirically derived formula presented below (1).

(1) $A = 0.75\ l \cdot w$

Terminology relating to spores and sporangia follows Guiry (1990) and Magne (1991).

5.2.1 Isozyme electrophoresis

The methods used were modified from Conkle *et al.* (1982), Cheney (1985), Lindstrom & South (1989) and Lindstrom & Cole (1990*a*). Fresh thalli of *P. saldanhae* ($n = 5$) and *P. aeodis* ($n = 7$) were collected from Kommetjie and brought to the laboratory (one dried, pressed, two-week old *P. saldanhae* specimen, also from Kommetjie, was successfully processed using the same methods, after being hydrated for 15 minutes prior to extraction). Five discs (15 mm diameter) were cut with a cork-borer from each thallus, then ground by hand in liquid nitrogen in a porcelain mortar. Fifteen to twenty drops of extraction buffer were added (0.1 M Tris-HCl, 5 % w/v PVP40, 4 mM Na_2EDTA, 20 mM Na metabisulphite, 200 mM Na ascorbate, 4 mM mercaptoethanol, adjusted to pH 7.7; modified from Lindstrom & South, 1989) and the samples were ground further. Extraction products were adsorbed onto 15×3 mm chromatographic paper wicks (six wicks were prepared per extraction), and immediately cooled to -18°C.

Starch gels were prepared from 12 % starch (Starchart) with 3 % sucrose. Full-strength Tris-EDTA-borate (TEB) buffer (0.2 M Tris-HCl, 4 mM Na_2EDTA, 80 mM boric acid, adjusted to pH 8.8) was used for the electrode buffers, and quarter-strength TEB buffer was used for the preparation of the gel (modified from Lindstrom & South, 1989). Each gel was 220 mm wide × 150 mm long × 9 mm thick. After pouring, cooled gels were sliced

vertically across their width 45 mm from the anodal end, and the two pieces were separated. Wicks were loaded evenly into the gap. Each specimen was replicated twice on each gel, and several equally spaced wicks with marker dye (bromophenol blue) for front location were also loaded. The two gel slices were then carefully placed back into contact without creating air pockets, and held together by squeezing a plastic drinking straw between the gel mould and the anodal end of the gel. Gels were run horizontally under 150 V-250 V, keeping power below 8 W to reduce heating, and were cooled using ice packs and by running gels in a temperature-controlled room at 4°C. After gels had been run for 20 min, the wicks were removed, and the run continued. Gel runs were stopped when the front was approximately 20 mm from the cathodal end (after approximately 5 hours), when gels were immediately sliced and stained.

The isozyme systems examined were GOT/AAT (glutamate oxaloacetate transaminase, also known as aspartate aminotransferase), G6PD (glucose-6-phosphate dehydrogenase), GDH (glutamate dehydrogenase), PGI (phosphoglucoisomerase), MNR (menadione reductase), MDH (malate dehydrogenase), IDH (isocitrate dehydrogenase) and LDH (lactate dehydrogenase). The following stain recipes were used; if published recipes were modified, they are listed in full: GOT/AAT (Lindstrom & South, 1989); G6PD (Lindstrom & South, 1989); GDH (Lindstrom & South, 1989); PGI (Lindstrom & South, 1989); MNR (Conkle *et al.*, 1982); MDH (20 ml 1 M Tris-HCl pH 8.0, 20 ml 1.5 M DL malic acid pH 7.0, 30 mg NAD, 20 mg MTT, 4 mg PMS, 60 ml H_2O; incubation in dark); and IDH (Lindstrom & South, 1989).

The genetic identity (I*) and distance (D*) between *P. aeodis* and *P. saldanhae* were calculated according to the formulas of Nei (1972) as modified by Hillis (1984).

5.3 Results

5.3.1 Description

Porphyra aeodis Griffin, Bolton *et* Anderson.

Figure 5-1-Figure 5-4.

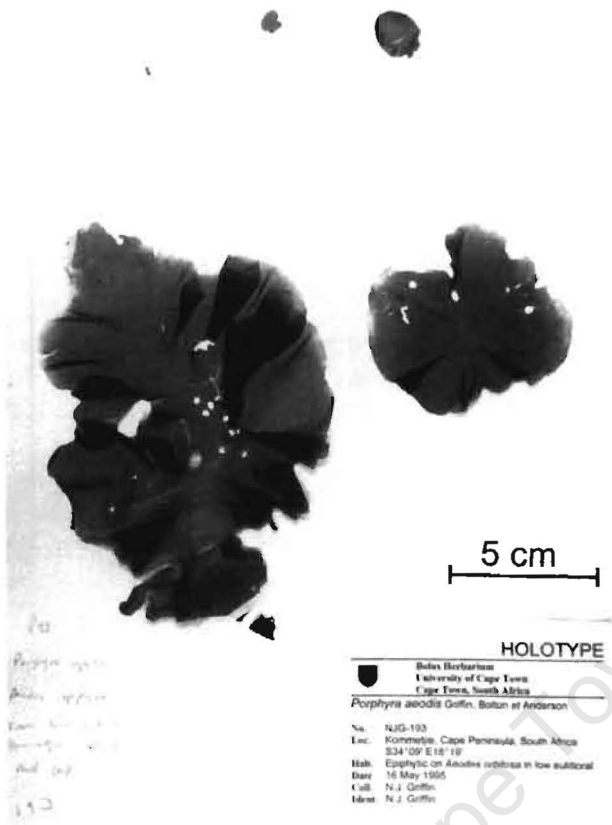

Figure 5-1 Holotype of *Porphyra aeodis* sp. nov., collected at Kommetjie, Cape Peninsula, South Africa, on 16 May 1995.

Thallus ovatus usque cordiformis, ad usque 35 cm longus et 25 cm latus, ubi juvenis purpureus, postea cordiformis usque umbilicatus, basaliter olivaceus, distaliter lateritius. Thallus monostromaticus, 60-140 μm diametro, cellulis vegetativis in sectione transversali oblongis, 20-35 μm × 8-10 μm, in superficie ovatis prismaticisve; prope hapteron, ubi thallus crassior, in sectione ovatis, omnis cum filo rhizoideo ad hapteron extenso. Cellulae vegetativae chloroplastos duos quosque pyrenoide una continent. Plantae monoeciae, maturae fertiles sexualiter circum margines, praeter prope hapteron. Margo fertilis ex maculis irregulariter formatis consistit; maculis stramineis spermatangios continentibus, maculis roseis zygotosporangios continentibus. Carpogonia fusiformia prototrichogynibus duabus brevibus acutis. Zygotosporangia in sectione transversali oblonga ellipticave, 65-100 μm × 25-40 μm, ubi matura ordinibus octo usque sedecim. Spermatangia in sectione transversali fusiformia, 40-70 μm × 5-15 μm, ordinibus octo usque sedecim. Archeospora e margine basili liberata. Numerus haploideus chromosomatum quatuor. Gametophyta annua solstitialia, in *Aeodes orbitosa* (Suhr) Schmitz epiphytice crescentia.

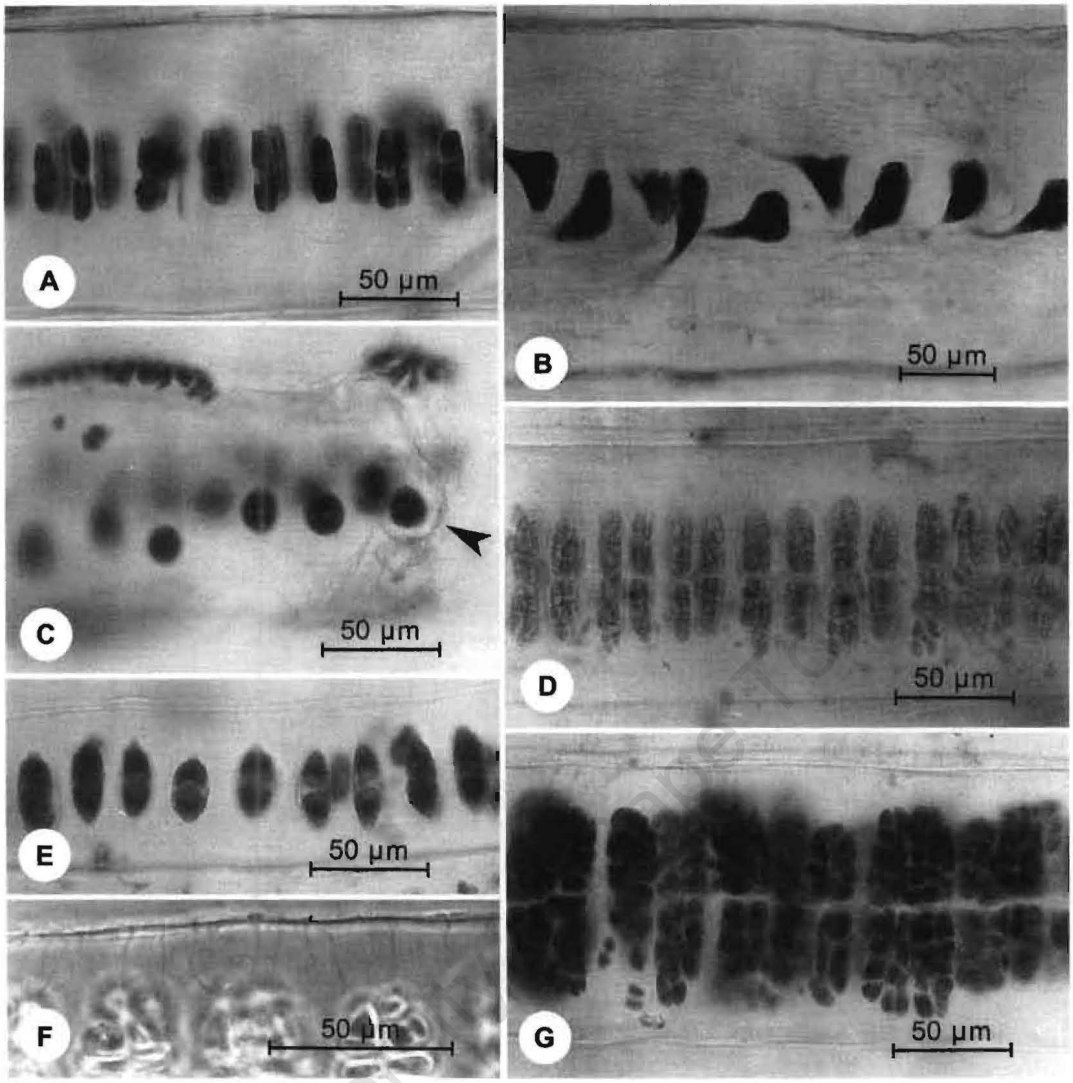

Figure 5-2 Transverse sections of *Porphyra aeodis* sp. nov. gametophytes. A). Vegetative cells. B) Basal rhizoidal cells in the holdfast region (holdfast is to the left). C) Archeospore production at the basal margin (arrowhead shows marginal archeospores release). D) Mature spermatangia. E) Carpogonia. F) Fertilization channels leading from the thallus surface to young zygotosporangia (phase-contrast). G) Mature zygotosporangia.

Thallus ovate to cordiform, up to 35 cm long and 25 cm wide, red-brown when young, later cordiform to umbilicate, olive green basally, brownish-red distally. Thallus monostromatic, 60-140 µm thick, with vegetative cells oblong in cross-section, 20-35 µm × 8-10 µm, in surface view ovate to prismatic; near the holdfast, where the thallus is thicker, ovate with rhizoidal filaments growing towards the holdfast. Vegetative cells containing two chloroplasts, each with one pyrenoid. Plants monoecious, mature thalli sexually fertile

around the margins except immediately adjacent to the holdfast. Fertile margin made up of irregular patches; yellow patches containing spermatangia and red patches containing zygotosporangia. Carpogonia fusiform, each with two short, acute prototrichogynes. Zygotosporangia in cross-section oblong or elliptic, 65-100 μm × 25-40 μm, with 8-16 tiers at maturity. Spermatangia in cross-section fusiform, 40-70 μm × 5-15 μm, with 8-16 tiers. Archeospores released from basal margins. Haploid chromosome number four. Gametophytes summer annuals, epiphytic on *Aeodes orbitosa* (Suhr) Schmitz.

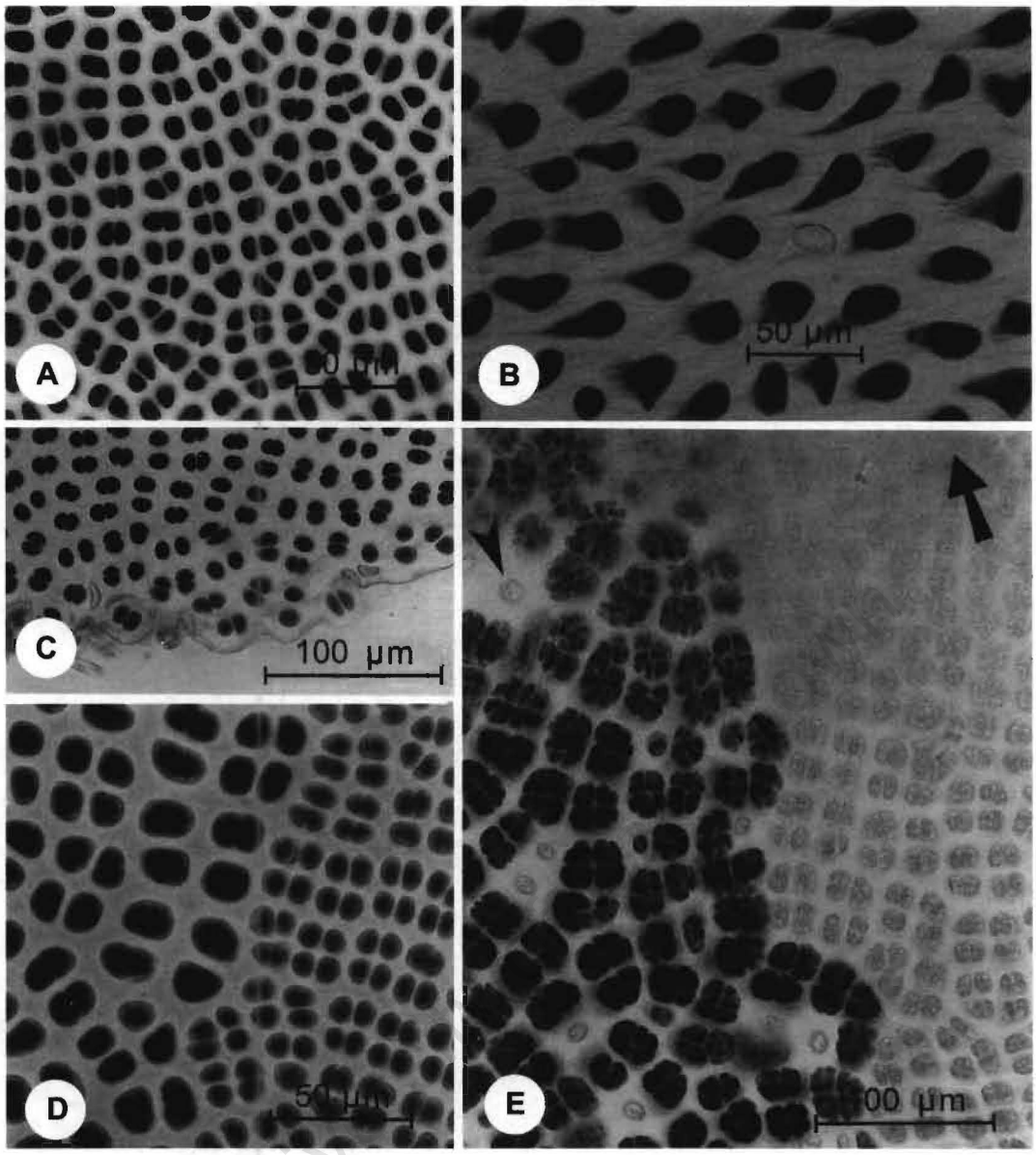

Figure 5-3 Surface view of *Porphyra aeodis* sp. nov. gametophytes. A) Vegetative cells. B) Rhizoidal cells near the holdfast (holdfast is to left). C) Archeospore production at the basal margin. D) Immature procarpogonia (larger, darker cells to left). E) Mature zygotosporangia (dark clusters to left) and spermatangia (pale clusters to right). Zygotosporangia and spermatangia mature from the bottom of the figure and release their contents at the top (arrow indicates margin). Single pale cells among the zygotosporangia are unfertilized carpogonia (arrowhead).

Holotype: NJG-193 (Figure 5-1), collected from *A. orbitosa* by N. Griffin at Kommetjie, Cape Peninsula, South Africa on 16 May 1995 (BOL).

Isotypes: NJG-190, NJG-191 (BOL).

Other material examined: NJG-10 (Kommetjie, 2 Nov. 1993), NJG-16 (Kommetjie, 16 Nov. 1993), NJG-24 (Kommetjie, 1 Dec. 1993), NJG-25 (Kommetjie, 1 Dec. 1993), NJG-156 (Kommetjie, 23 Jan. 1994), NJG-190 (Kommetjie, 16 May 1995), NJG-191 (Kommetjie, 16 May 1995), NJG-305 (Rocklands, 22 Oct. 1995), NJG-368 (Kommetjie, 3 June 1996), NJG-369 (Kommetjie, 3 June 1996) (BOL).

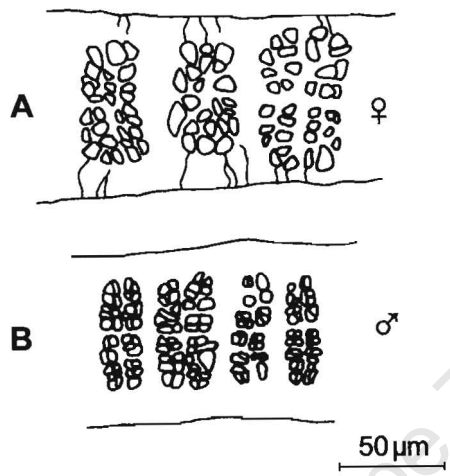

Figure 5-4 Drawings of mature zygotosporangia and spermatangia of *Porphyra aeodis* sp. nov. in transverse section to show detail. A) Mature zygotosporangia with fertilization channels. B) Mature spermatangia.

5.3.2 Habitat

Porphyra aeodis is epiphytic on *A. orbitosa*, which grows in the mid- to lower eulittoral zone and the shallow subtidal. *Porphyra aeodis* is found on the south-western and western coasts of South Africa. Extensive populations of *Porphyra* have been recorded growing epiphytically on *A. orbitosa* as far north as Möwe Bay, Namibia (S 19°3' E 12°42') (H. Engledow, pers. comm.), and it is likely that the range of *P. aeodis* extends to northern Namibia.

5.3.3 Seasonality

Aeodes orbitosa, a southern African endemic, is an annual species (Bolton & Levitt, 1992; Levitt *et al.*, 1995). Thalli are recruited during winter, and grow to a large size by early summer, when they become reproductive. The vast majority of thalli die before the winter,

although a minority survive into winter. In late winter to spring, numerous small *P. aeodis* thalli appear, growing epiphytically on *A. orbitosa* laminae. The *P. aeodis* plants at this stage are cordiform, relatively flat and red-brown. As they age, thalli become larger, the basal area becomes thicker and more olive-green, and marginal areas become more folded and begin to show the distinct patchwork pattern characteristic of fertile and near-fertile tissues (Table 5-1). Recruitment of gametophytes continues throughout the summer, providing circumstantial support for the continued production of archeospores by extant gametophytes.

Table 5-1 Density, size (± standard error) and reproductive status (proportion of plants with visible patches of spermatangia and/or zygotosporangia) of *Porphyra aeodis* sp. nov. thalli growing on *Aeodes orbitosa* at Kommetjie in spring (September) 1994 and late summer (February) 1995.

	spring	late summer
density (per *A. orbitosa* thallus)	9.8	5.3
mean thallus area (cm^2)	3.7±0.8	57.1±7.4
proportion fertile	0%	81%

5.3.4 Etymology

Porphyra aeodis is named after its host or substrate organism, the rhodophyte *Aeodes orbitosa*.

5.3.5 Electrophoresis

The relative front (R_f) distances run by the various isozyme systems are presented in Table 5-2. Only one band was detected at each locus in each individual. Of the loci surveyed, only GOT/AAT did not differ between the species. *Porphyra* species generally exhibit only one band on zymograms, and are often relatively invariant within and between conspecific populations (Lindstrom & Cole, 1992*a*; Lindstrom, 1993). Exceptions found to this generalisation were G6PD and GDH in *P. aeodis* and MNR in *P. saldanhae*, all of which varied within populations. G6PD and GDH also showed intraspecific variation in some species from the North Atlantic and the North Pacific (Lindstrom & Cole, 1992*a*). GOT/AAT, which did not vary here even between species, is frequently variable and often has two loci in *Porphyra* species (Lindstrom, 1993). In their electrophoretic survey of 21

species and two subspecies of *Porphyra*, Lindstrom & Cole (1992*b*) observed a single GOT/AAT locus only in three obligately and one facultatively epiphytic *Porphyra* species.

Table 5-2 Relative front (R_f) distances of alleles in *P. aeodis* sp. nov. and *P. saldanhae*. Numbers in parentheses are the proportions of resolved thalli with each allele.

Isozyme system	*P. aeodis*	*P. saldanhae*
GOT/AAT	0.53 (1.00)	0.53 (1.00)
G6PD	0.49 (0.29)	0.53 (1.00)
	0.51 (0.71)	
GDH	0.37 (0.29)	0.23 (1.00)
	0.39 (0.71)	
PGI	0.48 (1.00)	0.45 (1.00)
MNR	0.68 (1.00)	0.53 (0.33)
		0.58 (0.67)
MDH	0.39 (1.00)	0.31 (1.00)
IDH	0.38 (1.00)	0.42 (1.00)

Genetic similarity between the species was low, as the genetic identity (I*) was 0.143, and distance (D*) was 1.946. A wider sampling of loci or individuals might improve the accuracy of I* and D* (Nei, 1978). In comparison, Gottlieb (1977), using Nei's (1972) measure of genetic identity (I), reports a mean identity between conspecific plants of 0.95 and congeneric plants of 0.67. The genetic identity between *P. aeodis* and *P. saldanhae* was as low as the least measured between five North Pacific and five North Atlantic species of *Porphyra* (Lindstrom & Cole, 1992*a*). As *P. aeodis* and *P. saldanhae* were growing sympatrically, geographic separation cannot account for the genetic separation between the populations, and the considerable genetic divergence suggests that barriers to genetic exchange are well established. As zymograms may underestimate genetic variation [band similarity does not guarantee genetic identity (Gottlieb, 1977)], genetic separation between the two species may be greater than indicated here.

5.4 Discussion

That I was able to obtain active isozyme material from dried, pressed specimens of *Porphyra* indicates the utility of isozyme electrophoresis in studies on the systematics and population biology of *Porphyra* species. Many *Porphyra* species are tolerant of drying

(Smith *et al.*, 1986; Lipkin *et al.*, 1993), and if dried rapidly without using fixatives may survive being pressed on herbarium sheets, as occurred here. The tolerance of many *Porphyra* species to drying has been commercially applied: 'nursery-nets' with young gametophytes growing on them are commonly dried until the thalli are 20-40% of their wet weight and then stored indefinitely at -6°C to -30°C during the commercial production of *Porphyra* species (Miura, 1975). Though survival will decrease with storage time, especially if stored at room temperature (Lipkin *et al.*, 1993), I found that viable isozymes may be obtained from relatively fresh, unfixed herbarium specimens. However, since this work commenced, the increasing utility and availability of molecular methods, the growing availability of molecular data for comparison, and the possibility of obtaining valid data from older herbarium material (Goff & Moon, 1993; Brodie *et al.*, 1998; Hughey *et al.*, 2002) suggest molecular methods as probably more appropriate for systematic studies.

Porphyra gametophytes are generally thought to be genetic chimeras, as meiosis is delayed until conchospore germination, which results in a sporeling comprising four genotypes derived from the meiotic tetrad (Ma & Miura, 1984; Ohme *et al.*, 1986; Burzycki & Waaland, 1987; Ohme & Miura, 1988; Tseng & Sun, 1989; Mitman & van der Meer, 1994). Although reports of other sites of meiosis in *Porphyra* species exist (Ishikawa, 1921; Dangeard, 1927; Tseng & Chang, 1955; Migita, 1967; Giraud & Magne, 1968; Kito, 1974), more evidence for meiosis at conchospore germination has accumulated in recent years. When sampling thalli for electrophoresis, five discs of tissue were taken from different parts of the thallus with the aim of detecting any genetic chimeras. Despite three loci that were polymorphic within species, no evidence of chimeras was obtained. Not all species of *Porphyra* have obligately sexual life histories (e.g. see Kornmann & Sahling, 1991), in which conchocelis formation is necessarily preceded by gametogenesis, and conchospore formation or germination involves a meiotic division (Kapraun & Freshwater, 1987) that may lead to a chimera. The chromosome number in the conchocelis of *P. aeodis* and *P. saldanhae* has not been determined, and the role of sexual reproduction in the life history of these species is unknown, although the presence of sexual reproduction is suggested by the presence of apparent fertilization channels leading from spermatia on the thallus surface to carpogonia and zygotosporangia (Figure 5-2F, Figure 5-4A). Another possible explanation for the low genetic diversity within thalli may derive from breeding system theory: thalli are monoecious, with spermatangial and zygotosporangial sectors that mature simultaneously, and, if thalli are self-compatible, then inbreeding may act to lower

diversity (Gottlieb, 1977). After the tide subsides, thalli lie damp and folded on themselves, which would greatly facilitate self-fertilisation if it occurs. Self-fertilisation rates in *P. yezoensis* ranged from 45-57 % of conchocelis produced when thallus fragments were co-cultured in test tubes (Shin & Miura, 1990). As the latter data were derived under arguably less favourable conditions for self-fertilisation than are found in *P. saldanhae* and *P. aeodis* growing in the eulittoral, high rates of self-fertilisation in these species seem plausible.

Porphyra aeodis and *P. saldanhae* overlap morphologically to the extent that identifying specimens on the basis of gametophyte morphology alone may be difficult. However, there are distinct ecological differences between their gametophytes that help to distinguish the species. *Porphyra saldanhae* is a winter annual, while *P. aeodis* is a summer annual. *Porphyra saldanhae* grows on rock or other hard substrates, like mussel or limpet shells, while *P. aeodis* is an algal epiphyte, growing on *A. orbitosa*, though it may be capable of establishing on several other macroalgae [similar forms have relatively rarely been recorded on *Nothogenia erinacea*, *Gelidium pristoides*, *Sarcothalia stiriata* and *Gigartina polycarpa*. None of these was chosen for electrophoresis, and their specific affinity is not confirmed]. These differences between *P. aeodis* and *P. saldanhae* considerably facilitate species identification, particularly in the field.

The two species may be confused as they are of similar size and colour; both produce spermatangia and zygotosporangia in patches around most of the margin; they have similar cell shape and size; both produce archeospores from basal margins; both have short fusiform prototrichogynes extending above and below carpogonial cells; neither has bumps on the thallus surface above zygotosporangia (cf. *P. capensis*); they exhibit relatively similar division patterns of spermatangia and zygotosporangia; and they have the same haploid chromosome number. However, *P. aeodis* always has two clearly separated chloroplasts in vegetative cells (except cells in the region of the holdfast, where chloroplasts cannot be clearly distinguished), whereas *P. saldanhae* more commonly has one central chloroplast only, although cells with two chloroplasts do occur. Stegenga *et al.* (1997) report two stellate chloroplasts per cell in their description of *P. saldanhae*, although some cells with one chloroplast are present in the isotype. It is not clear whether the number of chloroplasts per cell varies in *P. saldanhae*; however, in specimens I examined the majority of cells had only one central stellate chloroplast, and cells with two

chloroplasts may be due to chloroplast division prior to cell division (for another example see Lindstrom & Cole, 1992*c*).

Spermatangial and zygotosporangial division patterns have been widely used as taxonomic characters in *Porphyra*, though it has long been recognised that they are not always reliable (Hus, 1902; Krishnamurthy, 1972). Mature spermatangia in *P. saldanhae* generally have only eight tiers (Stegenga *et al.*, 1997), while the spermatangia of *P. aeodis* have eight to sixteen tiers of spermatia (Figure 5-2D, Figure 5-4B). Also, zygotosporangia in *P. saldanhae* have only two distinct tiers because all but the first divisions of the zygotosporangia are oblique, which produces a characteristic ovate zygotosporangium. Although zygotosporangium formation in *P. aeodis* often involves oblique divisions, these do not occur to the same extent as in *P. saldanhae*, giving rise to a more oblong or elliptic zygotosporangium in which the number of tiers may be better determined (Figure 5-2G, Figure 5-4A).

The thalli of *P. saldanhae* are lanceolate or linear-lanceolate, often with highly ruffled margins, while *P. aeodis* thalli tend to be more ovate or cordiform, and, at maturity, may have an umbilicate appearance. Although margins of *P. aeodis* are commonly ruffled, particularly in larger plants, they are seldom folded to the same extent as in *P. saldanhae*.

I compared the morphology of *P. aeodis* with that of other obligately and facultatively epiphytic *Porphyra* species around the world (descriptions from Krishnamurthy, 1972; Coll & Cox, 1977; Tseng, 1984; Bird & McLachlan, 1992; Lindstrom & Cole, 1992*b*; Nelson, 1993; Hwang & Lee, 1994; Womersley, 1994; Stegenga *et al.*, 1997; Nelson *et al.*, 1998) and found none that resembled *P. aeodis*. Generally, most other epiphytic *Porphyra* species, in particular obligate epiphytes, were considerably smaller and/or more delicate than *P. aeodis*, and most had different morphologies and/or arrangements of spermatangia and carposporangia. Even without microscopic examination, they would not easily be mistaken for *P. aeodis*.

Many species of *Porphyra* grow epiphytically, and several of these are apparently obligate epiphytes with high host specificity (Krishnamurthy, 1972; Dickson & Waaland, 1985; Nelson, 1993). *Porphyra aeodis* apparently exhibits some degree of host specificity and, like *P. nereocystis* (Dickson & Waaland, 1985), seems to have a life history that is

synchronised with that of its host. In addition to *P. aeodis*, at least four other epiphytic forms of *Porphyra* have been recorded from the region (epiphytic on a range of algae including kelps, *Cladophora capensis* (C. Agardh) De Toni, and several intertidal Florideophyceae), and indications are that the number of *Porphyra* species from this region has been under-reported. The lumping of *Porphyra* species in southern Africa into *P. capensis* is in common with historical *Porphyra* taxonomy in other localities [for example, New Zealand (Nelson & Adams, 1990) and South America (Oliveira Filho & Coll, 1975; Coll & Oliveira Filho, 1976)] where the application of early species concepts gave rise to widely distributed form species that are only now being resolved (Bird & van der Meer, 1993).

6 Biodiversity in South African *Porphyra*

6.1 Introduction

Authors prior to 1997 have generally agreed that only one species of *Porphyra* is present in South Africa. That species, *P. capensis*, was described as having considerable morphological and ecological plasticity. The extent of variation attributed to *P. capensis* is higher than that in most *Porphyra* species.

When this study commenced, *P capensis* was the only species of *Porphyra* reliably reported from South Africa. Stegenga *et al.* (1997) later described *P. saldanhae*, recorded *P. gardneri* and *P suborbiculata* (as *P. carolinensis*), and described but did not formally name, *P.* sp., a kelp epiphyte. The work in Chapter 5 led to the description of *P. aeodis*. *Porphyra capensis* still contains forms that are umbilicate to linear, pale yellow to near-black, and may be found all year round, either as epiphytes or epilithic throughout the eulittoral and low supralittoral from Namibia to the eastern coast of South Africa. The vast majority of *Porphyra* on the south-western and western coasts are currently attributed to *P. capensis*.

In a recent review, Yoshida *et al.* (1997) listed 133 species of *Porphyra*, and the number of species has grown since then (e.g. see Coll & Oliveira, 2001; Nelson *et al.*, 2001; Neefus *et al.*, 2002; Lindstrom & Fredericq, 2003). The genus is currently understood to consist of species with a largely regional distribution (Yoshida *et al.*, 1997; Nelson *et al.*, 2001, Broom *et al.* 2002, Guiry & Nic Dhonncha, 2002), although this was not always thought to be the case. Examples of apparently widely distributed species include *P. purpurea* [records from Australia, Pakistan, Sri Lanka (Silva *et al.*, 1996), France, Belgium (Coppejans, 1995), Arctic Canada and the Maritimes, north Norway to Portugal, Iceland, and possibly the Baltic Sea (Bird & McLachlan, 1992)], and *P. leucosticta* Thuret [records from south Newfoundland to North Carolina, eastern north Atlantic from Iceland and northern Norway to the Canary Islands, Mediterranean Sea, Black Sea (Bird & McLachlan, 1992), Helgoland (Kornmann & Sahling, 1991), the Arctic, the Azores, Brazil to the Falkland Islands (Schneider & Searles, 1991), and Uruguay (Coll & Oliveira Filho, 1976)]. There is doubt about the validity of such widespread taxa: for example, *P. leucosticta*

seems likely to be restricted to the Northern Atlantic and Mediterranean Sea (Brodie & Irvine in Broom *et al.*, 2002).

It seems likely that wide distributions are in some cases due to species misidentifications. Simple morphology and a long evolutionary history (Campbell, 1980; Freshwater *et al.*, 1994; Ragan *et al.*, 1994) have resulted in convergent morphological characters in *Porphyra* that often complicate species identification (Stiller & Waaland, 1993). Morphological distinctions between species may be difficult to establish unless all stages in the life history are known and examined (Yoshida *et al.*, 1997).

The discovery of a large number of regional species was facilitated by the use of characters from isozyme electrophoresis in differentiating species. Before the availability of this technique, *Porphyra* species were generally described using gametophyte morphology, and occasionally including details from chromosomes and life history studies. A full understanding of the life history requires time, and most species were described using gametophyte morphology only. Isozyme electrophoresis made a new suite of easily assessed characters available to *Porphyra* taxonomists. Lindstrom and Cole (1990*a*, 1990*b*, 1992*a*, 1992*b*, 1992*c*, 1993) used isozyme electrophoresis in a revision of *Porphyra* species largely occurring in British Columbia. In many cases, new species described had previously been reported as members of apparently widely distributed taxa, such as *P. miniata* (C. Agardh) C. Agardh, *P. purpurea* and *P. variegata* (Kjellman) Kjellman. However, there are limitations to the use of isozyme electrophoresis as a tool for species resolution (Avise, 1974; Felsenstein, 1985*b*; Lindstrom & Cole, 1992*b*; Sosa & Lindstrom, 1999). For example, redundancy of the genetic code means that not all genetic changes are reflected in changes to isozymes, and identical electrophoretic mobility does not guarantee that isozymes are identical. As a result, genetic differentiation of taxa is underestimated by electrophoretic methods. Another disadvantage of isozyme electrophoresis is its requirement for fresh material, ruling out electrophoretic comparison with pressed specimens.

Genomic data, particularly DNA sequences, have more recently been used in *Porphyra* taxonomy (Stiller & Waaland, 1993, 1996; Brodie *et al.*, 1996; 1998; Woolcott & King, 1998; Kunimoto *et al.*, 1999*a*, 1999*b*; Neefus *et al.*, 2002). Combined use of morphological, life history and DNA data has been useful in work on *Porphyra*

systematics in the New Zealand region (Broom *et al.*, 1999, 2002; Nelson *et al.*, 2001, 2003). DNA data has been used as support for new species, and has also proved useful in distinguishing misidentified species and identifying superfluous species (Brodie *et al.*, 1998; Broom *et al.*, 2002; Nelson *et al.*, 2003).

One of the sequences widely used in Bangialean taxonomy is nuclear-encoded 18S small subunit ribosomal DNA, or nSSU. This is despite ribosomal RNA being among the oldest macromolecules in living systems (Sogin & Gunderson, 1987) and therefore a very conservative marker of speciation events (Hillis & Dixon, 1991; Stiller & Waaland, 1993). Initial phylogenetic investigation of the Bangiales using this sequence revealed, firstly, an unusually high degree of primary structural divergence within the Bangiales, and secondly, that *Porphyra* and *Bangia* are not distinct sister genera, as previously supposed (Oliveira *et al.*, 1995). To illustrate the extent of sequence divergence in the nSSU rDNA exon in the Bangiales, Oliveira *et al.* (1995) noted that, over the most conserved regions of Bangialean nSSU rDNA, pairwise identities range from 95.5% to 99.3%. In comparison, pairwise identities between the slime mold *Acanthamoeba castellanii* (Douglas) Volkonsky and the soy bean *Glycine max* (Linnaeus) Merrill are 92.25% over virtually the same region. Müller *et al.* (1998) compared data on sequence divergence within the Rhodophyta, and found that nSSU rDNA and *rbc*L sequence divergence between members of the Bangiales was considerably greater than interspecific divergence in other Rhodophyte taxa, and occasionally greater than interfamilial or interordinal divergence.

The order Bangiales is monophyletic, but the relationship between genera is not at all clear, as *Porphyra* appears to be polyphyletic within *Bangia* (Müller *et al.*, 1998, 2001; Broom *et al.*, 1999; Oliveira & Bhattacharya, 2000). In one of the most comprehensive analysis of the Bangiales to date, Broom *et al.* (1999) found three distinct groups in the Bangiales. One contained North American *Bangia* samples, the second, and largest, group contained *Porphyra* and *Bangia* species from disparate geographical regions, and the third contained *Bangia* (including all freshwater samples), *P. purpurea*, *P. umbilicalis* and several undescribed New Zealand *Porphyra* isolates. A merger of *Porphyra* and *Bangia* would create a monophyletic but molecularly divergent genus, in which the name *Bangia* would have priority. This would create havoc in the nori industry (Oliveira *et al.*, 1995). Until now, this merger has not been formally proposed [though it has been suggested (Woolcott & King, 1998)], and neither have any other genera within the Bangiales been

proposed. The consensus seems to be to maintain a *Porphyra/Bangia* complex until more light has been cast on systematic problems in the order (Oliveira *et al.*, 1995; Müller *et al.*, 2001).

This chapter aims to assess the diversity of *Porphyra* in South Africa using sequences of the nSSU rDNA exon. This gene was selected as it has been widely used in studies on the phylogenetics of *Porphyra* and *Bangia* (Oliveira *et al.*, 1995; Yamazaki *et al.*, 1996; Müller *et al.*, 1998; Broom *et al.*, 1999, 2002; Kunimoto *et al.*, 1999*a*, 1999*b*; Nelson *et al.*, 2001, 2003), and more complete Bangialean nSSU sequences are available for comparison than any other gene. This allows the comparison of local sequences with the widest possible number of *Porphyra* and *Bangia* sequences. *Bangia* is recorded from South Africa only as marine *B. atropurpurea* (Roth) C. Agardh (Stegenga *et al.*, 1997), and South African *Bangia* was not sampled for this survey.

This biodiversity assessment has three parts. Firstly, South African samples were compared with a wide range of Bangiales to determine where local *Porphyra* is located within the phylogeny of the Bangiales, and to determine whether any of the local samples might match any current species for which sequences were available. Secondly, nSSU variation in local species was compared with that of other *Porphyra* species to determine whether variation in local species was within the bounds of expected nSSU variation in species of *Porphyra*. Finally, the phylogeny of *P. capensis* was examined in greater detail.

6.2 Methods

Samples were collected by the author, W. Jones, D. Jones, C. Sapsford, and S. Fredericq. DNA extraction, amplification, sequencing and preliminary assessment were undertaken by Wynne Jones and Judy Broom in laboratories at the University of Otago, New Zealand.

Sixteen sites between St Helena Bay on the west coast (S32°44′50″ E18°01′16″) and Sheffield Beach on the east coast (S29°28′02″ E31°16′21″) were examined in late January-early February 2001. *Porphyra* was present and was collected at ten sites (Figure 6-1). Fifty-eight samples of *Porphyra* were collected, and voucher specimens were prepared [available at the herbarium of the Museum of New Zealand Te Papa Tongarewa (WELT)]. A portion of this material was desiccated in silica gel for molecular analysis. Forty-five

samples were selected for DNA analysis, after samples with very similar morphology from the same site were excluded.

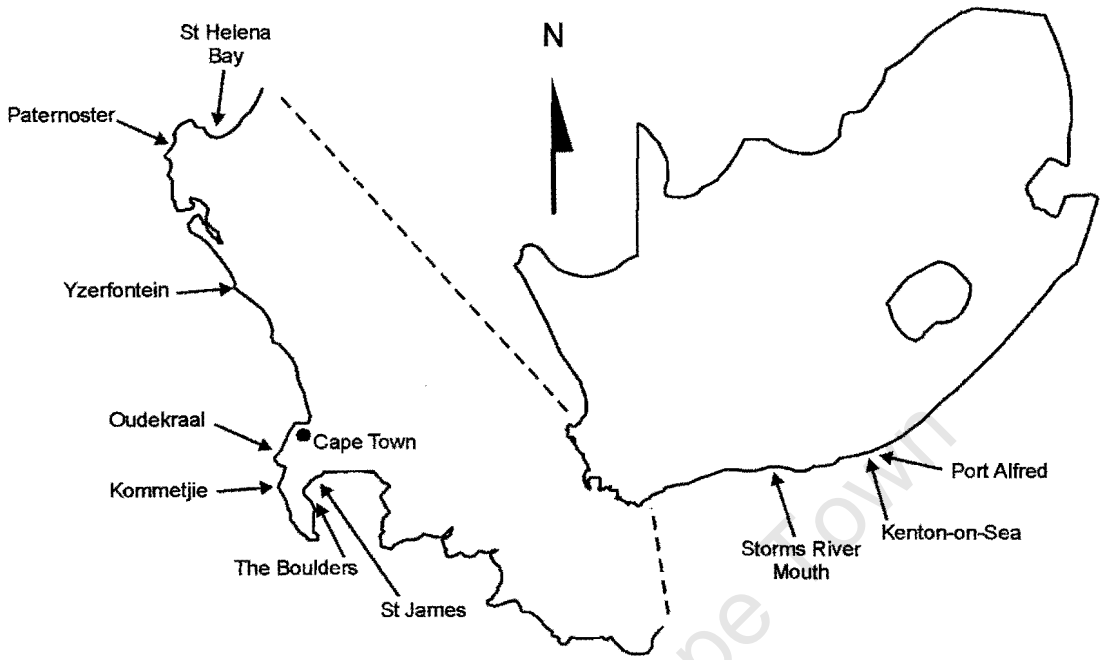

Figure 6-1 Sites on the South African coast where *Porphyra* was collected for biodiversity assessment. Site coordinates: St Helena Bay (S32°44′50″ E18°01′16″); Paternoster (S32°44′08″ E17°54′37″); Yzerfontein (S33°20′47″ E18°09′04″); Oudekraal (S33°58′51″ E18°21′47″), Kommetjie (S34°09′00″ E18°19′17″); The Boulders (S34°12′46″ E18°27′34″); St James (S34°07′14″ E18°27′28″); Storms River Mouth (S34°01′05″ E23°54′21″); Kenton-On-Sea (S33°41′07″ E26°40′31″); Port Alfred (S33°35′20″ E26°53′27″).

DNA was extracted from desiccated samples using Goff and Moon's (1993) Chelex extraction method. Two overlapping fragments in the nuclear SSU rDNA region were amplified: R [*ca.* 1170bp, primers 18E (Hillis & Dixon, 1991) and NS4 (White *et al.*, 1990)], and Qs [*ca.* 850bp, primers G04 (Saunders & Kraft, 1994) and J04 (Broom *et al.*, 1999)] (Figure 6-2). All amplifications were performed in a Stratagene Robocyler (Stratagene Corporation, La Jolla, CA) according to Broom *et al.* (1999). Sizes and yields of PCR products were assessed by electrophoresis through a 1% agarose gel. Reaction products were purified by PEG precipitation (Hillis, 1996) and sequenced using an ABI 377 automatic sequencer (Perkin Elmer Applied Biosystems Foster City, CA) using recommended standard methods. Where possible, approximately 485bp of the 3′ region of Qs was initially sequenced, using primer G06 (Saunders & Kraft, 1994). This region, here

designated Xs, contains the variable V9 region of the gene (Neefs *et al.*, 1993), and has been found to be a useful proxy for variation in the whole gene (Broom *et al.* 1999). Region R was also used for initial matches where amplification of Qs was problematic.

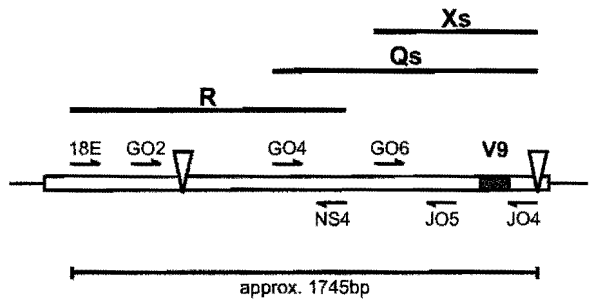

Figure 6-2 Relative positions of regions R, Qs, and Xs in nSSU as used in this study (not to scale). The variable region V9, used for initial screening of samples, is shaded. Triangles mark the insertion points of two Group I introns commonly present in *Porphyra* species. Positions of all primers are shown. Figure modified after Jones *et al.* (in press).

Sequences obtained from the variable Xs region were compared with *Porphyra* sequences in a local database using the GCG software package (Genetics Computer Group, 1994). Complete nSSU sequences were compared with existing sequences in GenBank using BLAST (Altschul *et al.*, 1997). Near-complete nSSU sequences were obtained for one or more samples that exhibited novel Xs sequences. Internal primers G02 (Saunders & Kraft, 1994) and J05 (Broom *et al.*, 1999) were used to complete the R and Qs sequences respectively.

All unique sequences except one contained a Group I intron inserted at the SSU position equivalent to base 516 in *Escherichia coli* (ZEK881 had no introns). The upstream intron from each entity was sequenced in all samples except ZAE953, which proved problematic. The existence of the second Group I intron downstream was not investigated. Intron sequences were not used in any analyses as little data was available for comparison with South African sequences. In addition, the absence of an intron in ZEK881 limited comparisons within the group.

Sequences were aligned using ClustalX (Thompson *et al.*, 1997). Forty-five *Porphyra* sequences, 18 *Bangia* sequences, and 10 outgroup sequences (Table 6-1) were included in

the alignment. Putative introns were removed from all sequences prior to alignment. Following automated alignment, adjustments were made by eye. After alignment, four pairs of taxa were found to be identical: *B. atropurpurea* (AT) and *B. atropurpurea* (NL); *P. tenera* (S) and *P. tenera* (K); *B. fuscopurpurea* (A) and *B.* sp. (Alaska); and *P. yezoensis* (O) and *P. yezoensis* (H). *Bangia atropurpurea* (NL), *P. tenera* (S), *B.* sp. (Alaska), and *P. yezoensis* (O) were excluded from further analysis. Areas that could not be unambiguously aligned were noted. Two data matrices were prepared from this alignment: one with sequence ends trimmed to the length of the shortest sequence and regions where the alignment was possibly ambiguous removed from the base matrix, leaving a matrix of 1436 bp and 81 taxa-this is referred to as the abbreviated matrix; and one with sequence ends trimmed, but all intermediate base pairs, excluding introns, present, leaving a matrix of 1721 bp and 82 taxa-this is referred to as the full matrix. The sample sequence ZDR980 matched that of ZDR966 in the abbreviated data matrix, and was excluded from that matrix.

The abbreviated matrix was used in phylogenetic reconstructions of the full data set, where positional homology of bases could not be guaranteed (after Swofford & Olsen, 1990). The full matrix was used to examine more closely the *P. capensis*, *P. suborbiculata*, *P. leucosticta* and *P. miniata* subclades, once it had been confirmed that there were no areas of ambiguous alignment within each species.

Table 6-1 Sequences used in comparisons with South African *Porphyra* samples. Taxon name, Genbank accession number, and notes, where available, on deposited sequences are presented.

Name	GenBank no.	Notes
Porphyra		
Porphyra abbottae	AF175545	
Porphyra acanthophora	L26197	Strain: Acanthophora, collection site: Ubatuba, Sao Paulo, Brazil
Porphyra amplissima	L36048	Individual isolate from Sandy Cove, Halifax County, Nova Scotia
Porphyra cinnamomea	AF136419	Strain: BRU107, gametophyte, collection site: Bruce's Rock, Otago, New Zealand, S 45°59', E 170°17'
Porphyra coleana	AF136423	Isolate: PAP052, gametophyte, collection site: Leigh, Northland, New Zealand, S 36°17', E 174°48'

Name	GenBank no.	Notes
Porphyra dentata	AB013183	Gametophyte, collection site: Koga Fukuoka, Japan
Porphyra fallax subsp. *fallax*	AF175541	
Porphyra haitanensis	AB013181	Gametophyte, collection site: Yuge Ehime, Japan
Porphyra kanakaensis	AF175556	
Porphyra katadae	AB013184	Gametophyte, collection site: Kawatana Yamaguchi, Japan
Porphyra kuniedai	AF123051	Strain: PK-50
Porphyra leucosticta (P)	L26199	
Porphyra leucosticta (S)	AF342746	Strain: SAG B 55.88, collection site: Helgoland
Porphyra linearis	AF175539	Strain: CCAP 1379/1, collection site: Nova Scotia, Canada
Porphyra miniata (L)	L26200	
Porphyra miniata (C)	AF175547	Strain: CCAP 1379/2, collection site: Nova Scotia, Canada
Porphyra miniata (N)	AF175540	Isolate: NF, collection site: Newfoundland, Canada
Porphyra nereocystis	AF175542	
Porphyra pseudolanceolata	AF175543	
Porphyra pseudolinearis (PK)	AF116913	Strain: PK-48
Porphyra pseudolinearis (TT)	AB013185	Gametophyte, collection site: Tohaku Tottori, Japan
Porphyra purpurea (A)	L26201	Strain: Avonport, collection site: Avonport, Nova Scotia
Porphyra purpurea (N)	AF175550	Isolate: NLBr
Porphyra rakiura	AF136425	Isolate: RAK049, gametophyte, collection site: Kaikoura, New Zealand, S 42°31′, E 173°30′
Porphyra sp. (cf. *plocamiestris*)	AF175555	Strain: CCMP 673, collection site: Palmer Station, Antarctica
Porphyra sp. (GRB108)	AF136420	Isolate: GRB108, gametophyte, collection site: Otago New Zealand, S 45°08′, E 171°58′
Porphyra sp. (LGD030)	AF136422	Isolate: LGD030, gametophyte, collection site: Wellington, New Zealand, S 41°21′, E 174°48′
Porphyra sp. (Marseilles)	AF175546	Collection site: Marseilles, France
Porphyra sp. (PK-49)	AF117239	Strain: PK-49
Porphyra sp. (Shimonoseki)	AB013182	Gametophyte, collection site: Shimonoseki Yamaguchi, Japan
Porphyra sp. (ROS054)	AF136426	Isolate: ROS054, gametophyte, collection site: Kaikoura, New Zealand, S 42°31′, E 173°30′
Porphyra sp. (SSR053)	AF136427	Isolate: SSR053, gametophyte, collection site: Kaikoura, New Zealand, S 42°31′, E 173°30′

Name	GenBank no.	Notes
Porphyra sp. (SSR091)	AF136428	Isolate: SSR091, gametophyte, collection site: Otago, New Zealand, S 45°57', E 170°20'
Porphyra sp. (Wales SW1)	AF175554	Isolate: Wales SW1, collection site: Wales, United Kingdom
Porphyra suborbiculata (PK)	AF117306	Strain: PK-723
Porphyra suborbiculata (K)	AB013180	Gametophyte, collection site: Kawatana Yamaguchi, Japan
Porphyra suborbiculata (L)	AF136424	Gametophyte, collection site: Wellington, New Zealand, S 41°21', E 174°47'. Deposited as *P. lilliputiana*
Porphyra spiralis var. *amplifolia*	L26177	Collection site: Ilhado Cardoso, Sao Paulo, Brazil
Porphyra tenera (K)	AB013176	Gametophyte, collection site: Kawaura Kumamoto, Japan
Porphyra tenera (S)	AB013175	Gametophyte, collection site: Shinwa Kumamoto, Japan
Porphyra torta	AF175552	Isolate: BC, collection site: British Columbia, Canada
Porphyra umbilicalis	AB013179	Gametophyte, collection site: Nahant Massachussets
Porphyra virididentata	AF136421	Isolate: LGD018, gametophyte, collection site: Wellington, New Zealand, S 41°21', E 174°48'
Porphyra yezoensis (O)	AB013178	Gametophyte, collection site: Ogatsu Miyagi, Japan
Porphyra yezoensis (H)	AB013177	Gametophyte, collection site: Hakodate Hokkaido, Japan
Bangia		
Bangia atropurpurea (AT)	AF169339	Isolate: AT17, collection site: Austria, freshwater collection
Bangia atropurpurea (BN)	L36066	
Bangia atropurpurea (NL)	AF169341	Isolate: NL, collection site: Ysselmeer, Netherlands, freshwater collection
Bangia fuscopurpurea (A)	AF175530	Collection site: Antarctica
Bangia fuscopurpurea (F)	AF175535	Collection site: Nice, France
Bangia gloiopeltidicola	AB053490	Isolate: B7, collection site: Shinori, Hakodate, Hokkaido, Japan, thallus dioecious, epiphytic on *Gloiopeltis furcata*
Bangia sp. (Alaska)	AF043355	Isolated from Alaska, Greenland and Northwest Territories
Bangia sp. (north BC)	AF043360	Isolated from northern British Columbia
Bangia sp. (California)	AF043356	Isolated from California
Bangia sp. (freshwater)	AF043365	Freshwater samples from Lake Ontario, Lake Erie, Lake Huron, Lake Michigan, Lake Simcoe, St. Lawrence River, Italy, Ireland and England (River Thames)
Bangia sp. (Massachusetts)	AF043362	Strain UTEX LB741, collection site: Woods Hole, Massachusetts
Bangia sp. (New Hampshire)	AF043353	Isolated from New Hampshire

Name	GenBank no.	Notes
Bangia sp. (Newfoundland)	AF043357	Isolated from Newfoundland
Bangia sp. (North Carolina)	AF043363	Isolated from North Carolina
Bangia sp. (Oregon)	AF043358	Isolated from Oregon
Bangia sp. (Rhode Island)	AF043354	Isolated from Rhode Island
Bangia sp. (Texas)	AF043361	Isolated from Texas
Bangia sp. (Victoria BC)	AF043359	Isolated from Victoria, British Columbia
Bangia sp. (Virgin Islands)	AF043364	Isolated from the Virgin Islands
Outgroup		
Boldia erythrosiphon	AF055299	
Compsopogon coeruleus	AF342748	Strain: SAG B 36.94
Compsopogonopsis leptoclados	AF087125	Isolate: Hawaii 14, collection site: Hawaii
Dixoniella grisea	L26187	
Erythrocladia sp.	L26188	
Erythrotrichia carnea	L26189	
Rhodella maculata	U21217	Strain: CCMP 736, collection site: Southend-On-Sea, Essex
Rhodella violacea	AF168624	Strain: UTEX LB 2427
Stylonema alsidii	L26204	
Smithora naiadum	AF087126	Collection site: California

Several measures of pairwise distance were computed using the full data matrix. The number of nucleotide substitutions, the number of point insertions/deletions, and the proportional distance excluding and including gaps were calculated for each sequence pair. Unless otherwise indicated, distance values given in the test are proportional distance, excluding gaps.

MRBAYES 3.0b4 (Huelsenbeck & Ronquist, 2001) was used to construct trees by Bayesian inference using a GTR+I+Γ evolutionary model, as suggested by Modeltest 3.06 (Posada & Crandall, 1998). Default program priors were used. Eight incrementally heated chains (temperature parameter: 0.2) were run in a Metropolis-coupled Markov chain Monte Carlo analysis to explore the likelihood surface more thoroughly. The model was run for 10^6 generations, sampling every 100 generations. Trees created prior to model stabilization ('burn-in') were discarded.

Parsimony analysis was undertaken using PAUP 4.0b10 (Swofford, 1998). Parsimony trees were inferred using a heuristic search with random sequence addition (100 replicates) and tree bisection-reconnection branch swapping. Zero-length branches were collapsed, all minimal-length trees were retained during branch swapping, gaps were treated as missing data, and all sites were weighted equally. Consensus trees (50% majority rule) were generated for all most parsimonious trees. Nonparametric bootstrap analysis of the data set (Felsenstein, 1985*a*) was undertaken (100 replicates, random sequence addition) to assess support for parsimony trees. Character states for those characters that varied within the *P. capensis*, *P. miniata* [excluding *P. miniata* (C)], *P. leucosticta* and *P. suborbiculata* clades were reconstructed for the full matrix consensus tree.

A third matrix consisting only of species from the *P. capensis* clade was used to examine relations in this clade more closely. This matrix was aligned and trimmed to the length of the shortest *P capensis* sequence, which made an extra 148 bp available for analysis compared to the full matrix. The *P. capensis* matrix was examined by parsimony analysis using a branch and bound search, with gaps treated as a new character state, and support was assessed by bootstrap analysis (1000 replicates). Bayesian analysis of this data set was also undertaken, using a GTR+I evolutionary model. Model and priors were chosen using Modeltest 3.06.

6.3 Results

6.3.1 Unique sequences: gross morphology and habitat

Eleven unique South African sequences were detected from 18 nearly complete nSSU sequences during preliminary analysis by W.A. Jones and J.E. Broom. Collection data for all unique nSSU sequences is presented in Table 6-2. Where any one sequence was found in several samples, the samples follow the sequence in the table.

Table 6-2 Sequence and sample codes, WELT herbarium numbers, and collection data for all unique South African nSSU sequences. Sample codes of matching partial sequences are also presented. Data presented in this table were supplied by W.A. Jones, J.E. Broom and W.A. Nelson.

Sequence and sample code	WELT number	Collection notes
ZDR980		
KM8	A23100	Kommetjie, mid-low eulittoral, epilithic
Xs matches: KM5, OK1		
ZIR970		
KM2	A23105	Kommetjie, mid-upper eulittoral, epilithic
KM10	A23109, A23110	Kommetjie, low eulittoral, epilithic
Xs matches: KM4, KM7, KMD2, PN2, PN3, PN6, SH4, SH7, YF2		
ZPP956		
PA2	A23093	Port Alfred breakwater, epilithic
KS3	A23091	Kenton-On-Sea, mid eulittoral, epilithic
Xs matches: PA1, PA4, SR2		
ZIR901		
SH2	A23078	St Helena Bay, mid-low eulittoral, epizooic on mussels
YF1	A23074	Yzerfontein, high eulittoral, epilithic
Xs matches: BB1, PN7		
ZEK881		
PN8	A23104	Paternoster, mid-low eulittoral, epizooic on mussels
KMD3	A23103	Kommetjie, epiphytic on *Ecklonia maxima*
Xs matches: YF7		
ZLI1045		
PN9	A23073	Paternoster, epizooic on *Cymbula compressa* on *Ecklonia maxima*
ZCE965		
BB2	A23090	The Boulders, high eulittoral, epilithic
ZAE953		
PN1	A23101	Paternoster, in tidal pool, epiphytic on *Aeodes orbitosa*
PN4	A23102	Paternoster, in tidal pool, epiphytic on *Aeodes orbitosa*
YF8	A23072	Yzerfontein, low eulittoral, epilithic on dolerite
Xs matches: YF10, YF5		

Sequence and sample code	WELT number	Collection notes
ZBS900		
SH1	A23077	St Helena Bay, mid-high eulittoral, epilithic
SH3	A23081	St Helena Bay, mid-low eulittoral, epizooic on mussels
Xs matches: SH5, PN5		
ZGR903		
SH6	A23079	St Helena Bay, mid-high eulittoral, epilithic
R matches: KM1		
ZDR966		
BB4	A23095	The Boulders, mid-high eulittoral, epilithic
Xs matches: SJ1, SJ2, SJ3		

Herbarium sheets with one specimen from each unique sequence group are presented in Figure 6-3 to show gross morphology.

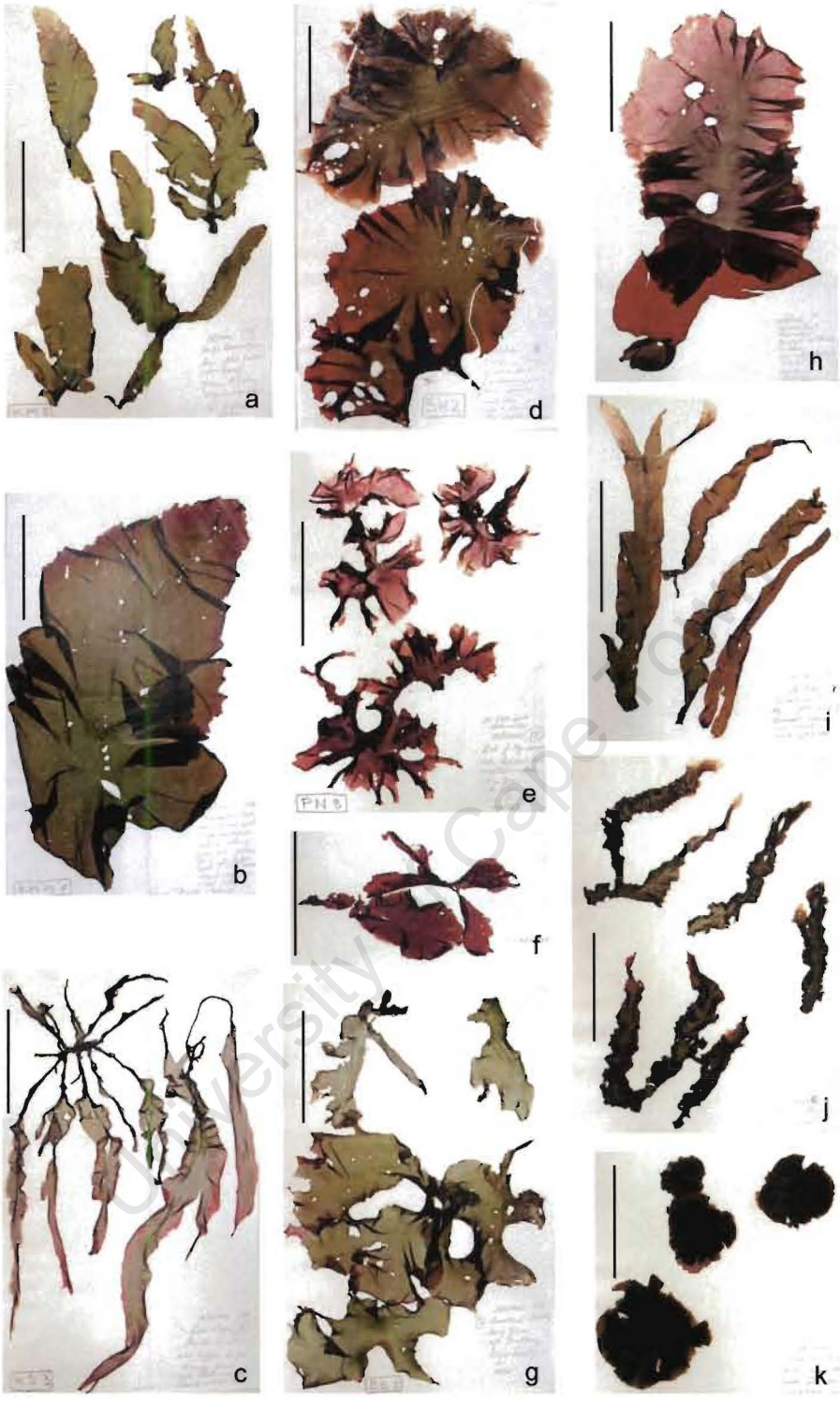

Figure 6-3 Herbarium sheets with specimens from each unique sequence group. Sample numbers, in parentheses, follow the sequence code. A) ZDR980 (KM8); B) ZIR970 (KM10F); C) ZPP956 (KS3); D) ZIR901 (SH2); E) ZEK881 (PN8); F) ZLI1045 (PN9); G) ZCE965 (BB2); H) ZAE953 (PN1); I) ZBS900 (SH1); J) ZGR903 (SH6); K) ZDR966 (BB4). Scale bars are 10 cm. Images supplied by T.J. Farr from voucher specimens housed in WELT, Museum of New Zealand Te Papa Tongarewa.

Brief descriptions of the gross morphology and habitat of samples in each unique sequence group are given below. Indication is given where additional data from partial sequence matches were used. Collection data includes partial sequences matches. All data used in compiling the brief descriptions below were contributed by W.A. Jones and W.A. Nelson. W.A. Nelson also supplied comments on morphologies.

A) ZDR980

Thalli ovate to lanceolate, may tear to produce several blades. Blades up to 20 cm long. Color yellowish green to brownish green. Apparently dioecious. Grew epilithically in the mid-low eulittoral. Collected from the west coast of the Cape Peninsula (Table 6-2).

B) ZIR970

Ovate or lanceolate to laciniate linear, up to 50 cm in length. Color ranges from dark greenish brown to reddish purple, often iridescent when submerged. Apparently dioecious or monoecious (several Xs partial sequence matches). Grew epilithically and epizooically on mussels (Xs match). Samples were collected from the low to the mid-upper eulittoral. Collected from the west coast (Table 6-2).

C) ZPP956

Linear lanceolate, tending to a rosette form in worn plants, with numerous blades produced from each holdfast. Margins have tendency to roll in along the long axis, particularly near the holdfast. Thalli range from clusters of blades up to 50 cm in length, to rosettes approximately 10 cm in diameter. Thalli pale green to pale yellow-green. Monoecious. Grew epilithically in mid eulittoral. Collected only from the south-eastern coast of South Africa (Table 6-2).

D) ZIR901

Ovate to narrowly elliptic, occasionally with pleated margins. Less than 30 cm in length. Olive-green basally to greenish-brown distally, usually iridescent when submerged. Usually monoecious, with spermatangia and zygotosporangia in broad sectors. Grew

epilithically or epizooically on mussels. Collected from the west coast and False Bay (Table 6-2).

E) ZEK881

Thalli lanceolate or cordiform, occasionally umbilicate. Margins highly pleated. Thalli up to 15 cm long. Thalli olive green-red basally and along the midrib, pink to red distally. Monoecious, with small interspersed patches of spermatangia and zygotosporangia. Grew epiphytically on *E. maxima*, and epizooically on mussels in the low eulittoral. Collected from the west coast (Table 6-2).

F) ZLI1045

Thalli ovate to lanceolate, up to 10 cm in length. Deep purple-red in color. Monoecious, with small spermatangial patches in a zygotosporangial matrix. Grew epizooically on *Cymbula compressa* (itself epiphytic on *E. maxima*). Collected from Paternoster, near St Helena Bay on the west coast (Table 6-2).

G) ZCE965

Thalli ovate to lanceolate, apparently tearing to produce several blades from one holdfast. Blade may be twisted along long axis. Blade less than 40 cm in length. Thalli pale olive-green to grass-green. Monoecious. Grew epilithically in the upper eulittoral. Collected only on warmer, False Bay side of Cape Peninsula (Table 6-2).

H) ZAE953

Thallus cordiform to umbilicate, occasionally lanceolate, with pleated margins. Thallus up to 30 cm in length. Olive green basally and around the midrib, brown-red to pale rose-red distally. Monoecious, with small interspersed patches of spermatangia and zygotosporangia. Grew epiphytically on *Aeodes orbitosa*, epilithically and epizooically on mussels in tidal pools (Xs partial sequence matches). Collected from the west coast (Table 6-2).

I) ZBS900

Linear to linear-lanceolate, thalli occasionally tearing roughly parallel to the long axis of the thallus towards the holdfast. Approximately 30 cm long. Color from brownish green to yellowish brown. Apparently monoecious. Grew epilithically and epizooically on mussel shells, in the mid-eulittoral. Collected from the west coast (Table 6-2).

J) ZGR903

Linear to linear-lanceolate, with pleated margins. Up to 20 cm long. Somewhat translucent green to yellow green in color. Apparently dioecious. Grew epilithically in the mid-upper eulittoral. Collected from the west coast (Table 6-2).

K) ZDR966

Thalli umbilicate to roseate, and 5-10 cm in diameter (20 cm in Xs partial sequence match). Color olive green to brown-green. Monoecious. Grew epilithically in the mid-upper eulittoral. Collected from warmer, False Bay coast of Cape Peninsula (Table 6-2).

6.3.2 Phylogenetic analysis of sequences

Most South African samples in this analysis fell into the current definition of *P. capensis*. Several representatives of umbilicate to ovate thalli ('*capensis*' form) and linear to lanceolate thalli ('*augustinae*' form) were present. Other species apparently present were *P. aeodis* and/or *P. saldanhae* (ZAE953). There were no examples of *P. gardneri* or *P. suborbiculata*.

Several of the analysed sequences were very similar (Table 6-3). For example, ZDR966 differs from ZDR980 by only one point insertion/deletion (though introns were markedly different); ZIR901 from ZIR970 by one point substitution and one insertion/deletion, ZBS900 from ZIR901 by 4 point substitutions, and ZBS900 from ZIR970 by three point substitutions and one insertion/deletion. Sequences from ZEK881, ZAE953 and ZLI1045 are distinctly different to the other eight South African sequences.

Table 6-3 Condensed nSSU sequences of South African *Porphyra*. Reference sequence is ZIR970. Dashes represent a gap, and dots represent an identical match to the reference sequence. 211 variable positions are shown and invariant positions are omitted. Data from full sequence matrix.

```
ZIR970    CTT--GTACC TTA-AC-AAA T-CACTTCG- -----TGCTT --CCGCTTTG GTGAGCACAC TA-CGCGTGT TACGC
ZIR901    ..........  .........  .........  .........  .........  .........  .........  .....
ZBS900    ..........  .........  .........  .........  .........  .........  .........  .....
ZGR903    ..........  .........  .........  .........  .........  .........  .......A..  ...A.
ZCE965    ..........  ..........  -C.......  .......T..  .........  .........  .TT...AA..  ...AT
ZPP956    ..........  ..........  -C.......  .........  .........  .........  .....G....  ...A.
ZDR966    .......G..  ..........  -C...C....  .........  .......AC.  .........  .........  .....
ZDR980    .......G..  ..........  -C...C....  .........  .......AC.  .........  .........  .....
ZEK881    AC.AG--..T  --..-.A.G-  .CTG..CTTT  CTAGAC..AC  AG-T..C..T  CCTG..GTT.  CG.TC....C  CG...
ZAE953    ACAAG--..T  --TT-.AGG-  .CTG.CC.GC  AAGGACA.AC  AG-TATA..T  CCTG..GTT.  CG.TC....C  CGGA.
ZLI1045   AC.AG--CAT  --..-AA.G-  .CTGT.C.TT  CGGGACA.AC  TG-...C..T  CCTGATG.T-  ..ATC...AC  CG...

ZIR970    GTTCG GCTCTT-T-- TT-CCTGAAG GT-CTTA--C TATC-CTGAG CTCACA---- GAGTGGCGTT ACAGGGTCAC
ZIR901    ..... .......... .......... .......... .......... .......... .......... ..........
ZBS900    ..... .......... .......... .......... .......... .......... .......... ..........
ZGR903    ..... .......... .......... .......... .......... .......... .......... ..........
ZCE965    ..... ...G.-.... C..G...G.. .......... .......... ..T....... .......... ...T..C...
ZPP956    ..... .......... .......... .......... .......... ....G..... .......... ...T..C..T
ZDR966    ..C.. -.C....C.. AC........ .......... ..C........ ..T..G.... .......... ..GT..C..T
ZDR980    ..C.. ..C....C.. AC........ .......... ..C........ ..T..G.... .......... ..GT..C..T
ZEK881    CCC.C CGCT..C.TT C-G..A.GGT T.GTCCT.GT CG.T.GG.CT TGTG.GTTAC .C.GAATCAA TTG.ATCTGT
ZAE953    C.C.T CGCTC.C.AT .-G.T..GG. A.G.CCTTG. CG.T.GGACT .GTG.GTTAC .C.GAA.CA. TTG.ATC.GT
ZLI1045   TC.TC CGCTC.C.TT .-G..GCGG. TGGTCCTTGT CG.TG-G.CT .GT..GTTAC ACAGAATCAA TTG.ATCTGT

ZIR970    CAATGGGTTC -AGTGATATC CTGTC-TATA CATAAGCCTA GCCATATTCT T-CTGGTTTC C
ZIR901    .......... .......... ...-...... .......... .....T.... .......... .
ZBS900    .......... .......... ...-...... .......AC. ..T....... .......... .
ZGR903    .......A.. ..........  .C.-...... .......AC. .......... .......... .
ZCE965    .......A.. ..........  .C.-...... .......AC. .....T.... A......... .
ZPP956    .......A-. T.........  ...-...... .......AC. .....T.... A......... .
ZDR966    ......A... ..........  TC.-...... .......TC. ..T..T.... .........A .
ZDR980    ......A... ..........  TC.-...... .......TC. ..T..T.... .........A .
ZEK881    TTTG....A. .TA.T...CT  .C.-AG.-CG TGCGTCTA.T CT.GATA.A. GT-.T..A.A .
ZAE953    T.TGAA...T .TT...C.CT  ...-AG-.CG .GCGTCTAAT CT.GATA.A. GT-.T.CACA .
ZLI1045   TTTG....A. .GAAAT.TC.  ACA-AG.-CG TGCGTC.AAT CT.GATACAC AT-CTA...A T
```

Distances between sequences from South African entities range from 0.1% to 9.6% (including gaps) and 0% to 8.0% (excluding gaps) (Table 6-4). The greatest distance between *Porphyra* sequences used in this analysis was between *P. kuneidai* Kurogi and *P.* sp. (PK-49), and was 16.8% (excluding gaps), or 19.6% (including gaps).

Table 6-4 Distances between unique nSSU sequences from South African *Porphyra* (samples identified as *P. capensis* in block at top left). Data in the upper right triangle are proportional distances, excluding gaps (topmost figure) and including gaps (bottom figure), and data in the lower left triangle are nucleotide substitutions (topmost figure) and insertions/deletions (bottom figure). All data were derived from the full sequence matrix.

	P. capensis										
	ZIR-970	ZIR-901	ZBS-900	ZGR-903	ZCE-965	ZPP-956	ZDR-966	ZDR-980	ZEK-881	ZAE-953	ZLI-1045
ZIR970		0.1	0.2	0.4	1.2	0.7	1.5	1.5	7.0	7.1	7.7
		0.1	0.2	0.4	1.4	0.9	1.6	1.6	8.5	8.7	9.4
ZIR901	1		0.3	0.4	1.1	0.6	1.4	1.4	7.0	7.1	7.7
	1		0.2	0.4	1.3	0.8	1.5	1.5	8.4	8.6	9.3
ZBS900	3	4		0.3	1.1	0.6	1.4	1.4	7.1	7.1	7.7
	1	0		0.3	1.3	0.8	1.5	1.4	8.5	8.7	9.4
ZGR903	6	7	5		0.8	0.6	1.6	1.6	7.1	7.2	7.8
	1	0	0		1.0	0.8	1.6	1.6	8.5	8.7	9.4
ZCE965	19	18	18	13		0.9	1.8	1.8	7.2	7.3	8.0
	5	4	4	4		1.0	1.9	1.8	8.7	8.9	9.6
ZPP956	11	10	10	9	14		1.5	1.5	7.1	7.1	7.7
	5	4	4	4	4		1.6	1.5	8.6	8.7	9.4
ZDR966	24	23	22	25	29	24		0.0	7.0	7.2	7.7
	4	3	3	3	3	3		0.1	8.5	8.7	9.4
ZDR980	24	23	22	25	29	24	0		7.1	7.2	7.8
	3	2	2	2	2	2	1		8.5	8.7	9.4
ZEK881	111	110	112	113	113	112	111	112		2.8	2.9
	35	34	34	34	36	36	35	34		2.8	3.0
ZAE953	113	112	113	114	116	112	113	114	45		4.5
	37	36	36	36	38	38	37	36	4		4.6
ZLI1045	122	121	122	123	126	121	122	123	46	72	
	40	39	39	39	39	41	40	39	5	7	

Patterns in similarity reveal a cluster of eight sequences within which similarity was high. This contains samples identified as *P. capensis*. The remaining sequences were from other, generally epiphytic, species. Within the large cluster, four entities (ZIR970, ZIR901, ZBS900 and ZGR903) were particularly closely grouped. Two others (ZDR980 and ZDR966) resembled each other closely, but were distinct from the four-sequence cluster. The three sequences from species not identified as *P. capensis* showed a greater affinity for

each other than for the *P. capensis* cluster, though similarities between the three were relatively low compared to those within the *P. capensis* cluster.

Phylogenetic trees inferred from parsimony and Bayesian analyses of the abbreviated sequence matrix revealed essentially the same topology (Figure 6-4, Figure 6-5). These analyses were used to locate the South African entities within the Bangiales. The Bangiales resolve as monophyletic, and contain two major subclades, and one minor, basal subclade. *Bangia* sp. (Virgin Islands) and *P.* sp. (PK-49) resolved basally in the Bangiales, and were not placed in either of the major clades. The latter two taxa resolved consistently within the Bangiales, but are markedly dissimilar to other sequences in this analysis.

The smaller of the major Bangialean subclades contains all the South African *P. capensis* samples, as well as *P. umbilicalis*, *P. purpurea*, *P. coleana* Nelson, *P.* sp. (Wales SW1), *P.* sp. (GRB108), *P.* sp. (LGD030), and a number of *Bangia* species (including most of the *Bangia* collected from freshwater habitats). The second subclade contains the great majority of *Porphyra* sequences, as well as a number of *Bangia* sequences. The three South African samples not identified as *P. capensis* fall into this subclade. Resolution in the latter subclade is considerably lower than in the former, as many intermediate level branches are not supported by bootstrap or posterior probability analysis. Basal division of the Bangiales into two major clades not corresponding to *Porphyra* and *Bangia* is widely recognized, as is difficulty in resolving intermediate level branches using data from nSSU rDNA (Oliveira *et al.*, 1995; Müller *et al.*, 1998, 2001; Broom *et al.*, 1999). Overall tree topology accords well with results from other large phylogenetic analyses of the Bangiales (Müller *et al.*, 1998, 2001; Broom *et al.*, 1999; Lindstrom & Fredericq, 2003).

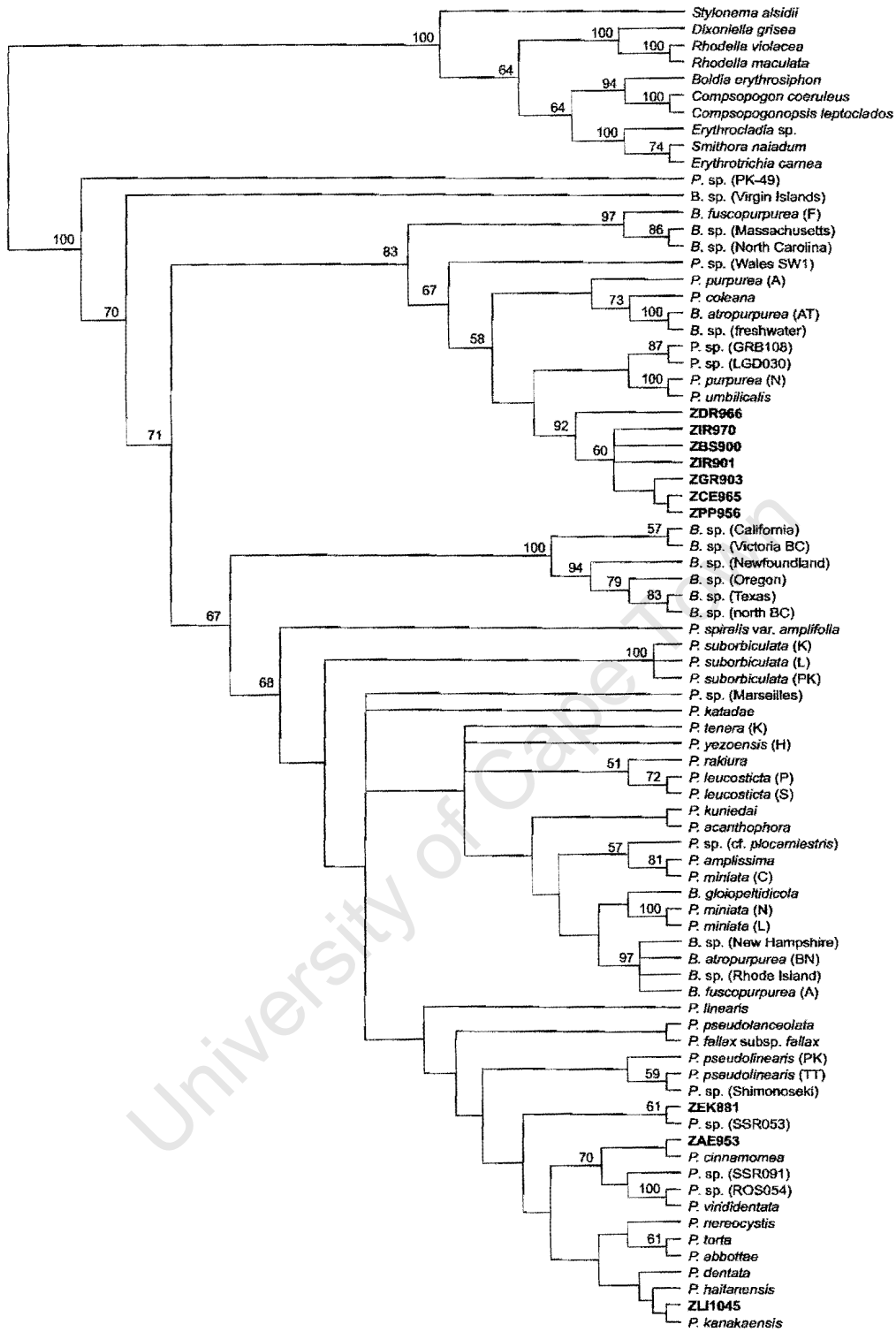

Figure 6-4 Fifty percent majority rule consensus tree from 10000 most parsimonious trees, based on 342 phylogenetically informative characters from a 1436 bp matrix. Numbers above internal branches show bootstrap support (100 replicates). Only bootstrap values greater than 50% are presented. Tree length 1412, consistency index 0.537, retention index 0.778. South African samples are shown in bold.

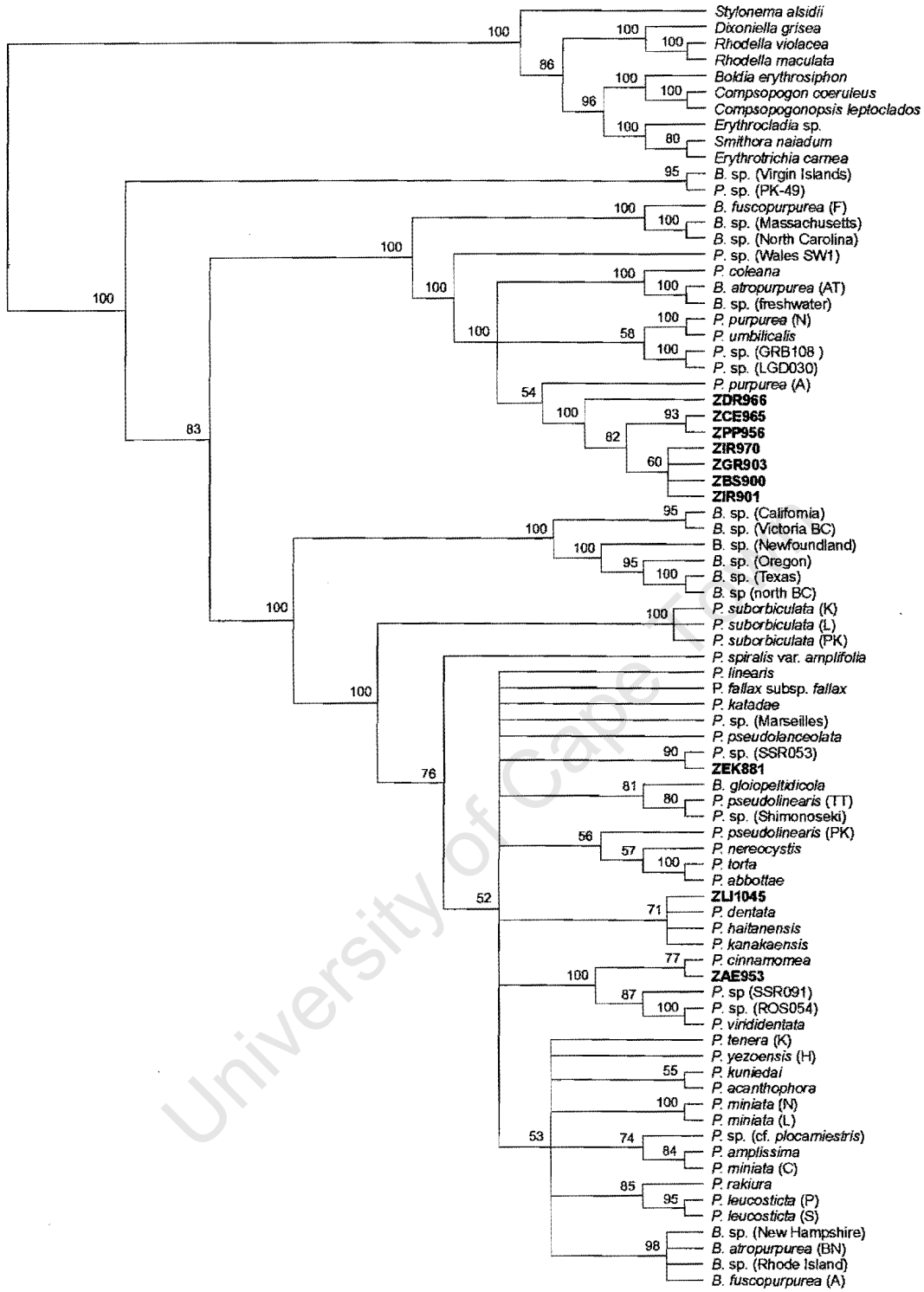

Figure 6-5 Fifty percent majority rule consensus tree from 9001 trees inferred from Bayesian analysis of a 1436 bp matrix. Numbers above internal branches show posterior probabilities. Only probabilities greater than 50% are presented. South African samples are shown in bold.

The three South African entities not associated with *P. capensis* fall into different parts of the larger Bangialean subclade. ZAE953 is closely associated with *P. cinnamomea* Nelson,

inside a clade that also contains *P.* sp. (SSR091), *P. virididentata* Nelson, and *P.* sp. (ROS054). ZEK881 is associated with *P.* sp. (SSR053). ZLI1045 is closely associated with *P. kanakaensis* Mumford, in a clade with *P. dentata* Kjellman and *P. haitanensis* Chang *et* Zheng. The latter clade is not supported by bootstrap analysis, though it appears in all most parsimonious trees and has a posterior probability of 99%.

The *P. capensis* group constitutes a discrete assemblage within the smaller Bangialean subclade that appears endemic to South Africa. The placement of the *P. capensis* clade within the smaller Bangialean subclade is not clearly resolved in either analysis. Within the *P. capensis* clade, ZDR966 diverges basally, and ZCE965 and ZPP956 are associated (well supported by posterior probabilities, but not by bootstrap values), but their position within the clade is not clear. The positions of other South African entities within the *P. capensis* clade cannot reliably be determined from these analyses.

Parsimony analysis of the full sequence matrix presented the same overall topology as that in Figure 6-4 and Figure 6-5. The results from this analysis were used to calculate a distance matrix between sequences, and to reconstruct apomorphies in the *P. capensis*, *P. suborbiculata*, *P. leucosticta* and *P. miniata* clades, for use as an aid in comparisons of within-species variation. *Porphyra suborbiculata* had a maximum of two autapomorphies, and *P. leucosticta* and *P. miniata* each had a maximum of four autapomorphies. Apomorphies in the *P. capensis* clade are presented below (Figure 6-6).

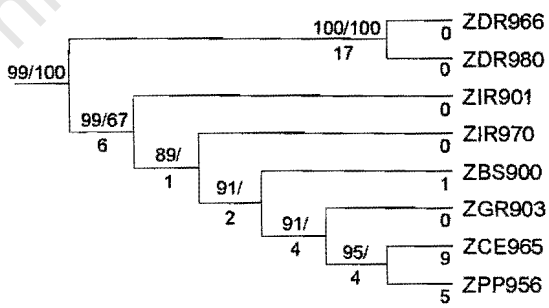

Figure 6-6 *Porphyra capensis* clade as inferred from 50% majority rule consensus trees of 97 most parsimonious trees from parsimony analysis (28 parsimony-informative characters; tree length 49, consistency index 0.7959, and retention index 0.7561), and 9000 trees from Bayesian analysis. Both analyses used a 1721 bp matrix. Numbers above internal branches show support (posterior probability/bootstrap); only support values greater than 50% are presented. Numbers below branches show the number of inferred non-gap apomorphies.

In order to use all available data to maximize resolution within the *P. capensis* clade, it was re-analysed in isolation using longer sequences than were possible when a wide range of sequences were used. Gaps were treated as fifth character states in parsimony analysis of this matrix. Clade topology, as inferred from parsimony and Bayesian analyses, was identical, and is presented below (Figure 6-7).

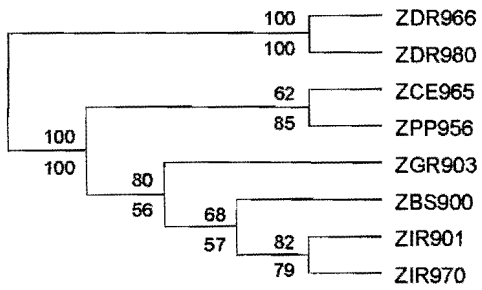

Figure 6-7 Single most parsimonious tree from the *Porphyra capensis* clade, based on 32 parsimony-informative characters from a 1716 bp matrix; also most probable tree (p=0.383). Numbers above internal branches show bootstrap support, numbers below branches show posterior probability (only support values greater than 50% are presented). Tree is unrooted, with length 59, consistency index 0.8644, and retention index 0.8367.

In the two analyses of the *P. capensis* clade presented above, the association of ZDR966 and ZDR980 is consistent and well supported. Relationships between the remaining six entities are less clear. ZCE965 and ZPP956 are consistently paired, as they were in the abbreviated matrix analyses, but are placed either terminally or basally in the clade with the remaining six entities. This uncertainty is due to differences between the trees in the placement of the ZDR966/ZDR980 clade relative to the other six entities. Of the *P. capensis* trees presented, that in Figure 6-7 is most consistently supported by bootstrap values and posterior probabilities.

6.4 Discussion

There is some debate about the degree of support provided by posterior probabilities and nonparametric bootstrap values, as the former are often greater. Based on simulation studies, Suzuki *et al.* (2002) claimed that posterior probability values considerably overestimated phylogenetic signal, and that nonparametric bootstrap values were slightly

conservative and preferable. Wilcox *et al.* (2002) stated that, under the conditions they investigated, using simulated data, nonparametric bootstrap values were excessively conservative and posterior probabilities were better indicators of phylogenetic accuracy.

The two measures are not directly comparable. The nonparametric bootstrap is a measure of uncertainty based on resampling the data matrix (Felsenstein, 1985*a*). It has been found not to measure confidence in the traditional, hypothesis-testing context (Efron *et al.*, 1996; Sanderson & Wojciechowski, 2000). Corrected measures (e.g. parametric bootstrap) exist, but are computationally expensive, and may still exhibit some bias (Newton, 1996). Posterior probabilities estimate uncertainty based on a specified evolutionary model in combination with the given data, and thus are affected by the model of evolution chosen (Huelsenbeck *et al.*, 2002). Huelsenbeck *et al.* (2001) note an example where parametric bootstrap and posterior probabilities agreed, and both were considerably greater than nonparametric bootstrap values. Determination of differences between the two measures in terms of their relative stability and accuracy, particularly on long trees, awaits further study (Huelsenbeck *et al.*, 2002; Suzuki *et al.*, 2002).

Several authors have published analyses where more than one sample per species of *Porphyra* is considered. These may be used to provide an estimate of nSSU variation within species of *Porphyra*. Only studies where data from other sources were used to corroborate the results of nSSU analysis will be discussed.

Porphyra yezoensis Ueda and *P. tenera* Kjellman are perhaps the best understood of current species of *Porphyra*, as a result of their commercial importance. They are acknowledged to be very similar species, and are capable of cross-fertilization and hybrid formation (Suto, 1972). Kunimoto *et al.* (1999*b*) examined nSSU variation between nine *Porphyra* species, and included two samples each of *P. tenera* and *P. yezoensis* in their analysis. They observed that the nSSU exon differed between species but was identical within the species they examined, though introns varied within species. Kunimoto *et al.* (1999*a*) published a survey of molecular divergence within *P. yezoensis* in which fifteen putative *P. yezoensis* sequences were included. Twelve of the sequences were identical, two had three point substitutions, and one had five point substitutions. The authors concluded that the latter three sequences were not from *P. yezoensis*. Differences in ITS1 sequences from the same specimens supported this conclusion.

Broom *et al.* (2002) used nSSU in a review of *P. suborbiculata*. Of eight haplotypes derived from analysis of nSSU, ITS1, ITS2 and two introns, seven were invariant in the nSSU exon. The eighth differed from the rest by 3 point substitutions. Based on analysis of all the sequences examined, the authors concluded that all haplotypes were conspecific. As samples in this analysis were collected from Japan, China, Australia, New Zealand, and the east and west coasts of North America, this level of nSSU variation may be taken as a reasonable estimate of variation within a species containing geographically and therefore reproductively isolated populations. Although the widespread occurrence of one haplotype suggests very recent dispersal, probably by shipping, the species appears to have a longstanding distribution in the Pacific.

Before discussing the results of my analyses, it should be noted that sequences labeled as a particular species may not have been correctly identified or labeled. An example of incorrect identification can be found in the work by Hendriks *et al.* (1991), where nSSU was used in evaluating the evolutionary position of *P. umbilicalis*, but where the sample used was later found to be *Palmaria palmata* (Linnaeus) Kuntze (Y. van de Peer, pers. comm.). This is an extreme example, but misidentifications within the Bangiales are easily made. Some authors have avoided these problems by using DNA from type specimens in their analyses (Brodie *et al.*, 1998; Hughey *et al.*, 2002), but this approach is not always possible or practical.

Given the above, variation within apparent conspecifics in the data set used in this chapter was assessed. Apparent conspecifics were identified as follows: sequences were derived from specimens labeled as conspecific; and sequences resolved together, with support from bootstrap and posterior probabilities, in all phylogenetic reconstructions. Apparent conspecifics were used as a standard against which South African sequences were compared. This approach was chosen to derive estimates of intraspecific variation that were based on the same data matrix and alignment as that used for analysis of South African *Porphyra*. Ideally, such assessments of variation would be based on sister clades (Nadler, 2002). However, insufficient data is available for this approach, and, as a result, South African *Porphyra* were compared with apparent conspecifics that had been identified as *Porphyra*. To minimize alignment effects, gaps were treated as missing data in this comparison.

Two sequences of each of *P. tenera* and *P. yezoensis* were found to be identical after excision of introns and alignment. These are not present in Figure 6-4 and Figure 6-5 as duplicate sequences were not used in phylogenetic reconstructions. There was little difference between *P. yezoensis* and *P. tenera* (two point substitutions, or a distance of 0.1%). This is in agreement with Kunimoto *et al.* (1999*a*; 1999*b*).

Porphyra purpurea is represented by two sequences in the data matrix. Distance between the two is high (91 point substitutions, or 5.8%), and the two do not resolve together in phylogenetic reconstructions. There are also two sequences labeled as *P. pseudolinearis* Ueda in the data matrix. Dissimilarity between the two is 3.6%, or 58 point substitutions, and the relationship between them is unresolved. Such low similarity between apparent conspecifics and their disjunction in phylogenetic reconstructions suggests strongly that neither pair is conspecific. Brodie *et al.* (1996) and Brodie & Irvine (1997) note that the name *P. purpurea* has frequently been misapplied.

Other apparent conspecifics include two species each of *P. leucosticta* and *P. miniata* (excluding *P miniata* (C)). *Porphyra miniata* (C) is not used to estimate intraspecific variation, as it seems likely that it is a misidentification of *P. amplissima* (Kjellman) Setchell *et* Hus. Of the three sequences labeled as *P. miniata* in these analyses, *P. miniata* (C) clustered with *P. amplissima* in all analyses and not with the remaining *P. miniata* samples. Brodie *et al.* (1998) note that *P. miniata* records from Great Britain appear to be misidentifications of *P. amplissima*.

Porphyra leucosticta and *P. miniata* each contain a pair of sequences that are 0.3% dissimilar (five point substitutions), with a maximum of four autapomorphies in any one sequence. Intraspecific similarity is lower than that published for *P. suborbiculata* or *P. yezoensis* (Kunimoto *et al.*, 1999*a*; 1999*b*; Broom *et al.*, 2002), and greater than that between *P. tenera* and *P. yezoensis* (Kunimoto *et al.*, 1999*a*; 1999*b*; this analysis).

Porphyra suborbiculata is represented by three sequences in the data matrix. Dissimilarities within *P. suborbiculata* were 0.1% (2 point substitutions) or less. This level of variation is within the bounds of variation of *P. suborbiculata* as reported by Broom *et*

al. (2002). Character state reconstruction shows two autapomorphies in *P. suborbiculata* (PK), and none in *P. suborbiculata* (K) or *P. suborbiculata* (L).

These apparent conspecifics can be used to set an estimate of acceptable intraspecific variation for comparison with *P. capensis*, based on the same data and alignment. The maximum dissimilarity between conspecific sequences is 0.3%, or five base pair substitutions, and the greatest number of intraspecific autapomorphies is four.

South African sequences from outside the *P. capensis* species complex, with the exception of ZAE953 (*P. saldanhae*/*P. aeodis*), seem to be representative of either new species of *Porphyra* or of species not recorded from South Africa. The placement of ZEK881, ZLI1045 and ZAE953 in phylogenetic analyses is stable and fairly well resolved. ZAE953 differs considerably from *P. cinnamomea* (34 bp), with which it is most closely associated. In the same way, ZLI1045 is clearly different from *P. dentata* (44 bp). ZEK881, on the other hand, differs little from *P.* sp. (SSR053) from New Zealand (1 bp). It is not possible to separate the latter sequence pair into different species based solely on evidence from nSSU sequences.

Following the standard set by the literature and by analysis of apparent conspecifics in the full data matrix, the *P. capensis* clade was further examined. The association between ZDR980 and ZDR966 is strongly supported in all analyses (barring abbreviated matrix analyses, where ZDR980 was excluded). ZDR966 and ZDR980 are at least 1.4% dissimilar to all other entities in the *P. capensis* complex. Character state reconstruction reveals 23 apomorphies separating the clade containing ZDR980 and ZDR966 from other *P. capensis* sequences, with 17 on the branch leading to the ZDR980/ZDR966 clade. By any of the standards presented above, ZDR980 and ZDR966 are not likely to be conspecific with the remaining *P. capensis* sequences. Sequences from this pair differ only by one insertion/deletion. However, gross morphology differs between the two. Further examination will be necessary to elucidate the relationship between them. It would be useful in this regard to examine a more variable part of the genome in combination with nSSU.

Relationships between the remaining six entities within the *P. capensis* clade are not as clearly defined in phylogenetic reconstructions. Differences between trees produced are

largely due to variation in where the ZDR966/ZDR980 clade joins the clade with the other six *P. capensis* entities. This results in two general topologies for the remaining six entities: either ZCE965 and ZPP956 form a sister clade to the remaining four entities, or they are placed terminally in a clade together with the remaining four entities.

ZCE965 and ZPP956 are associated in all trees, with moderate to strong support from posterior probabilities, though bootstrap support is only found when *P. capensis* is examined in isolation. Minimum dissimilarity between members of this clade and all other *P. capensis* sequences is 0.6%. Dissimilarity between ZCE965 and ZPP956 is relatively high at 0.9%. Character state reconstruction suggests nine terminal autapomorphies in ZCE965, and five in ZPP956, and four apomorphies on the branch leading to this clade. Compared to the standards derived from *P. miniata*, *P. suborbiculata*, and *P. leucosticta*, both ZCE965 and ZPP956 are likely to represent new species. Differences in gross morphology support this conclusion.

Distances between pairs of the four remaining *P. capensis* entities range from 0.1% to 0.4%, or 1-7 base pair substitutions. Although ZGR903 is the most distinct entity in this group, no entity or group thereof is sufficiently different from its neighbors to suggest that it may represent a different species according to the standards derived from apparent conspecifics. However, all entities are different, and so all may represent unique species, according to the standard of Kunimoto *et al.* (1999*a*, 1999*b*). If these results are compared to those of Broom *et al.* (2002), only ZGR903 might represent a different species. However, all bar ZGR903 are represented by more than one unique sequence (excluding partial sequence matches), suggesting that these haplotypes are fixed. All were collected from the western coast of South Africa, and three were present at St Helena Bay (four, if partial sequence matches are included). As such, these entities have the same or overlapping distributions. This suggests either nSSU polymorphism in one or more species, or the presence of reproductive barriers between these entities. There is considerable gross morphological variation between these entities. ZBS900 and ZGR903 are both linear to linear-lanceolate, and representative of the '*augustinae*' form of *P. capensis*. ZIR970 and ZIR901 are both ovate to lanceolate and, of the South African samples, are most representative of the '*capensis*' form of *P. capensis*.

The extent of nSSU variation within the *P. capensis* complex suggests strongly that several species are present. It should be noted again at this point that nSSU is a conservative gene, and, as a result of its presence in multi-copy arrays undergoing concerted evolution, shows a strong tendency to homogenization of sequences within breeding populations (Page & Holmes, 1998; Graur & Li, 2000). Within-species polymorphism of the nSSU exon is therefore low, and usually transitory (Hillis & Dixon, 1991). This would suggest that *P. capensis* entities ZBS900, ZGR903, ZIR970 and ZIR901 are likely to be different species. This complex needs to be assessed in the light of more evidence, preferably molecular evidence from a more variable part of the genome, to determine the number of species present.

It seems therefore that of the 18 full sequences examined in this chapter, at least seven, by a conservative standard, are likely to drawn from unique species, and there many be as many as eleven unique species in the sample set. Of these, three are not part of the *P. capensis* complex. The *P. capensis* complex, as represented by these samples, may therefore contain four to eight species, in a clade apparently unique to South Africa.

The samples used in this survey were identified as *P. capensis*, *P. saldanhae* and *P. aeodis*. No representatives of *P. gardneri* or *P. suborbiculata* were present. On macromorphological grounds, ZLI1045 represents *Porphyra* sp. indet. (*sensu* Stegenga *et al.*, 1997). As *P. gardneri* and *P. suborbiculata* were not sampled, there may be nine to thirteen species of *Porphyra* present in South Africa.

It was not possible in this survey to reliably differentiate between *P. saldanhae* and *P. aeodis*. There are few morphological differences, and, in the absence of electrophoretic data, the species are distinguished using a combination of anatomical and ecological characters. ZAE953 was defined here using three full nSSU sequences. As samples were collected growing on rock and on *A. orbitosa*, with gross morphologies that correspond to both *P. aeodis* and *P. saldanhae*, and as time of sample collection is not a reliable indicator of seasonality, it seems possible that *P. aeodis* and *P. saldanhae* share the same nSSU sequence. *Porphyra* species sharing nSSU sequences have been observed before (Kunimoto *et al.* 1999*a*), and recently diverged species with identical nSSU sequences have been observed in *Gelidium* (Bailey & Freshwater, 1997). As *P. saldanhae* and *P. aeodis* appear to have identical nSSU sequences, the estimate of the number of species

present in South Africa should therefore be elevated by one, as ZAE953 seems to represent two species. The final estimate of the minimum number of *Porphyra* species in South Africa is therefore ten to fourteen.

Condensing differences between sequences into a scalar measure of pairwise dissimilarity (or similarity) has the disadvantage of irretrievable loss of information (Penny, 1982). Beyond the reduction of complex information to a single number, simple dissimilarity does not account for repeat changes in any one position (Swofford & Olsen, 1990). However, dissimilarity data do provide a useful measure that allows comparison with published data where full sequences and their alignments are not available. Character state reconstruction and derived apomorphy counts, as used here, do account for character reversion, and location of change within the reconstructed tree. However, results are affected by the methods used to reconstruct the tree. Use of either measure to predict variation within a clade, based on results in another clade, makes assumptions about rate equivalence in sequence divergence and speciation patterns. As such, it is inadvisable to use either measure alone to estimate whether two sequences are from conspecifics or not, and comparisons should be accompanied by full phylogenetic reconstructions (ideally based on the same sequences, when such data are available). Use of such a 'genetic yardstick' may be avoided altogether by collecting data from another source. Morphological data may be used in this regard, although, given the extent of morphological convergence in the Bangiales, it may be wiser to use sequences from a more variable region of the genome in combination with morphological data.

This analysis of biodiversity has been based on evidence from sequences from nuclear nSSU. Although nSSU is a conservative gene, and tends to being fixed within breeding populations, it is important to bear in mind that all phylogenetic reconstructions presented in this study are based on a single gene. As the *P. capensis* clade is monophyletic, it is possible, though, given the extent of nSSU variation, extremely unlikely, that all variation can be attributed to the presence of nSSU haplotypes within a single species. This would be more probable if the various haplotypes had been drawn from a number of geographically isolated populations. This was not the case in *P. capensis*, as different entities were commonly collected from the same site. In comparison, a comprehensive study on *P. suborbiculata* collected from sites around the world found only two nSSU haplotypes (Broom *et al.*, 2002). Probabilistically, therefore, it is extremely unlikely that

the nSSU variation within *P. capensis* is due to fixed intraspecific nSSU haplotypes. Even if speciation has occurred, gene sorting may result in a gene tree that is incongruent with the species tree (Nei, 1987). Without examining data from other sources, it is not possible to conclusively state that phylogenetic reconstructions presented here represent a species rather than a gene tree. However, differences in gross morphology support the conclusion that *P. capensis* is in fact a species complex.

The majority of *Porphyra* species are seasonal (Kurogi, 1972; Noda & Iwata, 1978; Miura, 1988), and, as all samples used in this analysis were collected in summer, these results will underestimate the number of *Porphyra* species present in South Africa. This strongly indicates the need for a comprehensive review of *Porphyra* taxonomy in South Africa, as the number of species suggested by these results clearly exceeds previous estimates. In many ways, *Porphyra* taxonomy in South Africa seems to parallel the situation in New Zealand, where, until 1998, one epilithic species was recognized (Nelson *et al.*, 1998). Since then, a number of new species have been described, and evidence strongly suggests that at least ten epilithic species and five epiphytic species are present (Broom *et al.*, 1999). Not all of these have been described.

All of the local entities, except perhaps for ZEK881, seem to be endemic to southern Africa. Although *P capensis* has been reported from outside southern Africa, records are considered to be to misidentifications (see Chapter 1), and examination of *P. capensis* collected from elsewhere is necessary to confirm the endemic status of *P. capensis*.

It is of interest to note that two local entities have strong affinities with species from New Zealand. ZEK881 is only 0.1% dissimilar to *P.* sp. (SSR053) from New Zealand. ZAE953 is associated with four other sequences, all collected in New Zealand (distance 2.1-4.8%). Of these sequences, two are unidentified, one is from *P. cinnamomea* and one is from *P. virididentata*. Both species are only known from the New Zealand region (Nelson *et al.*, 2001). ZLI1045 is associated with three species, with pairwise dissimilarities of 2.4-2.7%. Two of these were collected in Japan, and the collection site for the third sequence is unknown. *Porphyra haitanensis* is known from China, and *P. dentata* from the coasts of China, Korea and Japan (Tseng, 1984). *Porphyra kanakaensis* occurs on the Pacific coast of America and Canada (Garbary *et al.*, 1980). The biogeographic affinities of the entities

outside *P capensis* support the proposal that a proportion of South African west coast red algae have origins in Australasia (Hommersand 1986; Hommersand & Fredericq, 2003).

Porphyra capensis itself is associated with a range of sequences in this analysis. These sequences derive from widely reported species (mostly Atlantic samples), some from New Zealand, and some *Bangia* species. No clear biogeographic affiliations are suggested. The *P. capensis* species complex was clearly monophyletic in all reconstructions, and seems to represent a radiation of *Porphyra* that is confined to southern African shores. This complex has aged sufficiently that distances of 32 bp, or 1.9%, are recorded here between entities in the complex.

These results present a picture of Bangialean phylogeny that is consistent with other published studies (Oliveira *et al.*, 1995; Yamazaki *et al.*, 1996; Müller *et al.*, 1998, 2001; Broom *et al.*, 1999; Klein *et al.*, 2003; Lindstrom & Fredericq, 2003). Neither *Porphyra* nor *Bangia,* as currently understood, are holophyletic, and species of *Porphyra* may be genetically more similar to *Bangia* than to other *Porphyra* species. Nevertheless, species of *Porphyra* and *Bangia* are normally found in clusters of congenerics across the terminal branches of phylogenetic trees. This suggests that *Porphyra* and *Bangia* consist in fact of several morphologically similar but genetically disparate entities. There may be an evolutionary constraint on this order leading to the repeated development of morphologies characteristic of either *Porphyra* or *Bangia*. It is clear that much work is needed before the nature of *Porphyra* and *Bangia* are resolved.

7 General discussion

7.1 Harvest ecology

Porphyra was found at all sites examined during the course of this survey, with much spatial and temporal variation in *Porphyra* biomass distribution. Estimated *Porphyra* biomass was generally greatest in the summer at west and south-west coast sites, and in the winter at sites on the Cape Peninsula. Generally, the biomass of *Porphyra* on the west coast and Cape Peninsula, as extrapolated from the sampling programme, was greater than elsewhere. A considerable proportion of the estimated biomass is protected from harvesting owing to its location within reserves or restricted areas. This proportion is likely to increase with time due to current national policy that aims to increase the proportion of the shore protected within marine protected areas. Most rocky shores between St Helena Bay and Cape Agulhas have potential for the collection of *Porphyra*, and harvest site selection will likely be dictated more by operational factors, for example site access and transport distance versus potential return, than by the biomass of *Porphyra* present.

Porphyra gametophytes showed biannual recruitment peaks with low, continuous recruitment throughout the year. Recruitment peaked in spring and autumn. Few recruits survived to fertility. Those that did grew fast initially, after which their growth rates decreased with time until a stable, mature growth rate of approximately 2 $\%.d^{-1}$ was reached. The survival of plants in clumps that established together was greater than that of isolated thalli. No pattern in the mortality of larger plants was detected, although peaks of sporeling mortality followed recruitment peaks. Regrowth of blades from holdfasts was not observed during the course of this investigation.

Porphyra populations varied with season and height on shore. Generally, the greatest biomass of *Porphyra* was found in the mid-eulittoral. Low-shore populations appeared annually in the summer, growing mostly epiphytically and epizooically. High shore populations were very variable, their success apparently mainly dependant on grazer impact during recruitment, and environmental conditions. There was much variability in the size of *Porphyra* populations along the shore.

Porphyra was associated with a number of eulittoral flora and fauna. The number of organisms growing with *Porphyra* decreased from the bottom to the top of the eulittoral. *Porphyra* grew epilithically most frequently, but also was also recorded growing epizooically on limpets, mussels, and barnacles, and epiphytically on *Aeodes orbitosa*, *Gigartina/Sarcothalia* spp, and *Gelidium pristoides* (other substrates were recorded, but these were relatively infrequent). Epilithic growth was more prevalent higher in the eulittoral.

Generally, areas with a dense cover of *Porphyra* had a low diversity of other organisms present, but a high number of organisms acting as substrates for epiphytic/epizooic *Porphyra* (in some cases I recorded a correlation between *Porphyra* biomass and the number of faunal taxa present). Harvesting would probably have the greatest direct impact on substrate organisms, which may be damaged or removed by harvesters, and those organisms (in particular *Nodilittorina*, *Hyale* and *Parisocladus*) that are common in dense *Porphyra*.

Harvesting significantly reduced *Porphyra* populations, as well as those of organisms associated with *Porphyra*. However, annual population changes also reduced *Porphyra* populations, and the main effect of harvesting was to reduce *Porphyra* populations in advance of natural population collapses. Harvesting reduced *Hyale* and *Parisocladus* populations; however, these changes could not be distinguished from those following *Porphyra* population collapse. *Nodilittorina* populations were more affected by harvesting than by *Porphyra* die-back, but recovered after 12 months. Harvesting *Porphyra* just prior to natural population decreases (late summer and winter) should therefore have little added impact on *Porphyra* or associated organisms. Due to the relatively low rates of non-seasonal recruitment, and consequent lack of recovery of harvested populations, it seems unlikely that any stretch of shore could be harvested more frequently than twice per year.

Porphyra at Slangkoppunt grew higher on the shore than other macrophytes, which seem largely to be absent from this environment. As such, in the mid- to upper eulittoral at least, *Porphyra* generally does not appear to compete with other macroalgae. The large-scale removal of *Porphyra* from the mid- to upper eulittoral should impact little on other eulittoral macroalgae. Small-scale removal of *Porphyra* in this study had no effect on eulittoral macroalgae. Lower on the shore, *Porphyra* is apparently excluded by grazers,

unless it grows epizooically or epiphytically and so is to an extent protected from grazers, particularly in the vulnerable, sporeling stage. No impacts on eulittoral macroalgae in the lower eulittoral due to harvesting *Porphyra* are anticipated (beyond possible tearing of substrate taxa by harvesters).

Extensive patches of *Porphyra* may displace *Nodilittorina africana*, a process Branch *et al.* (1990) believe to be mediated by either physical removal of the snails by algal sweeping due to wave action, or suppression of edible microalgal growth in *Porphyra* patches. Littorinid snails have been found to eat and prefer *Porphyra* species in other studies (Norton *et al.*, 1990, and references therein), although I found no *Porphyra* fragments in the gut of *N. africana*. Physical removal as a result of wave action is a risk for this small snail (McQuaid, 1980), and the presence of a stand of macroalgae would seem to exacerbate the risk of removal due to the sweeping effect of algal fronds (e.g. Santelices, 1990*a*). Nevertheless, I frequently found *N. africana* in *Porphyra* patches (though samples were collected at low tide, when no disturbance due to wave action occurs, and patches may act as a shelter against desiccation). Harvesting decreased localised populations of *N. africana* in this study; however, these populations recovered after 12 months. The reason for this decrease is not known, though it may be a result of physical removal of large numbers of this snail when *Porphyra* was harvested. If so, impacts could be reduced by rinsing or shaking harvested thalli to remove attached snails. Despite the observed impact on local *N. africana* populations, no major impact on *N. africana* due to wide-scale harvesting of *Porphyra* is anticipated, as *N. africana* does not graze on *Porphyra* and may be found where *Porphyra* is absent.

All molluscan grazers collected from dense *Porphyra* patches had fragments of other macroalgae in their guts, whether or not identifiable fragments of *Porphyra* were present. As such, it appears that none of the dominant molluscan grazers in patches of *Porphyra* at Slangkoppunt subsisted entirely on *Porphyra*. *Scutellastra granularis*, the dominant midshore grazer in experimental plots, is a generalist grazer that will graze wherever rocks are moist (Branch, 1971). The primary effect of *S. granularis* grazing on macroalgae is the removal of sporelings (Branch, 1971). The removal of *Porphyra* should not greatly affect molluscan grazers unless *Porphyra* patches provide shelter, or shelter for microalgae that may be grazed by these organisms, or some other resource. For example, Branch *et al.* (1990) observed increased recruitment of *Aulacomya ater* and *Siphonaria aspera* in dense

Porphyra and *Ulva* beds. However, observations during this study did not reveal any association between *Porphyra* and any mollusc (beyond *N. africana*), or any obvious recruitment of molluscs into *Porphyra* beds.

Harvesting *Porphyra* will nevertheless remove a food resource from these organisms. Extensive harvesting may reduce ongoing *Porphyra* sporeling recruitment by reducing archeospore or conchospore production, and thereby decrease the number of sporelings or mature thalli available for grazing. As it is not known to what extent year-round recruitment depends on archeospores from gametophytes, or conchospores from sporophytes, this impact cannot be predicted.

Crustacean grazers were present around and in patches of high and mid-shore *Porphyra*, but these did not entirely suppress *Porphyra*'s recruitment and growth. My observations suggest that pressure from these grazers does accelerate the demise of *Porphyra* patches, as, shortly before patches break down and are lost, thalli can be seen to be perforated and seem to have been heavily grazed. These fauna, found associated with *Porphyra* patches, were uncommon elsewhere in the eulittoral in this study. Branch *et al.* (1990) record that, in dense algal beds formed after the death of largely molluscan grazers, several cryptic fauna (isopods, amphipods, and polychaetes) reached high densities. These organisms may be adversely affected by large-scale *Porphyra* harvesting. *Hyale* and *Parisocladus*, at least, grazed heavily on *Porphyra*, but both seem to be capable of utilizing other food resources. Insufficient data on the ecology of these taxa is available to predict the impact of harvesting *Porphyra* on them, and their densities in harvested areas should be monitored. Large numbers of *Hyale* were accidentally collected along with harvested *Porphyra*, and commercial harvesting may remove many of these amphipods from the shore.

In certain regards, gametophytic *Porphyra* seems to fit Grime's (1979) definition of a stress-tolerator. Eulittoral *Porphyra* species are commonly tolerant of desiccation (Smith *et al.*, 1986; Lipkin *et al.*, 1993), and often grow higher in the eulittoral than other macrophytes (McQuaid, 1985). However, as a taxon that seems to respond rapidly and profoundly to disturbance, and to be capable of rapid establishment and growth in disturbed sites throughout the eulittoral, *Porphyra* has many characteristics of a ruderal, or ephemeral life history strategy (Dayton, 1975; Lubchenco, 1978). In the absence of grazers, *Porphyra* seems capable of competitive dominance, at least in the short term

(Lubchenco, 1983; G. Maneveldt pers. comm.). With the possible, and notable, exception of *Porphyra* in the upper eulittoral, *Porphyra* at Slangkoppunt cannot be considered a keystone or secondary species (after Dayton, 1975), and its removal should have little impact on the eulittoral ecosystem.

It is important that commercial-scale harvesting operations be accompanied by monitoring programs to assess the impact of widespread or intense harvesting on the populations of *Porphyra* or associated organisms. This study assesses the localised impact of harvesting *Porphyra* only, and it is not possible from these results to fully predict the potential impact of complete and repeated removal of *Porphyra* from the shore. To illustrate: harvesting in this study was found to have an impact on *Nodilittorina africana*, but populations recovered one year after harvesting. As harvested quadrats were small, recovery may have been due to migration from neighbouring populations. If the entire shore is harvested, all populations of *N. africana* may be equally affected by harvesting, and recovery due to migration from neighbouring populations is unlikely. As *N. africana* does not graze on *Porphyra*, occurs where there is no *Porphyra*, and may in fact be excluded by *Porphyra* (Branch et al., 1990), recovery of *N. africana* populations after widespread *Porphyra* harvesting is anticipated. However, this has not been tested in this study, and monitoring of harvested areas is necessary to confirm this prediction. In this light, a comment on the suitability for monitoring of the methods I employed is appropriate.

Generally, I found that much information was lost through the conversion of abundance data on species to binary taxon presence/absence data. However, the collection of presence/absence data is considerably easier than collecting abundance data, particularly for cryptic and highly mobile fauna, and the aggregation of species data to some higher level considerably facilitates field work. Calculating beta diversity as similarity matrices, and testing the correlation of patterns of beta diversity with the experimental design or environmental factors proved a valuable approach in tracking changes in community structure. This was particularly so where changes were not evident after inspection of the data and initial graphical analysis. However, particularly when few taxa are present and presence/absence data are used, a large number of replicates are needed if changes are to be statistically detected. The Warwick and Clarke index of multivariate dispersion (IMD) did not discriminate between controls and treatments, and seems inappropriate for monitoring programs. This is largely due to the low number of taxa found together with

epilithic *Porphyra*: IMD is less sensitive when very few taxa are present in control populations.

No attempt was made to survey populations of *Porphyra*'s sporophyte in this survey. It would be extremely difficult to accurately assess sporophyte populations, owing to the fugitive growth of the sporophyte in shells. The extent of harvesting in Chapter 4 is too low to affect sporophyte populations. It is possible that intense or widespread harvesting may lead to decreased sporophyte populations and thence to reduced gametophyte recruitment. However, Martìnez (1990) reports that populations of *Porphyra* gametophytes and sporophytes may be spatially disjunct, and it is therefore possible that sporophyte populations do not rely on continual recruitment from zygotospores produced by gametophytes. Sexual reproduction is important in most *Porphyra* species, and the seasonal peaks of recruitment observed in this study suggest the seasonal conchospore release typical of most species of *Porphyra* (Noda & Iwata, 1978). Long-term monitoring of gametophytes on relatively broad, intensely harvested shores should indicate whether sporophyte populations are reduced by harvesting of gametophytes, as the biannual peaks in gametophyte recruitment recorded here are likely to be reduced should sporophyte populations shrink sufficiently.

If *Porphyra* were to be harvested for the consumption of tank-reared abalone, it would be desirable for harvesting sites and farms to be close together, to facilitate the delivery of fresh *Porphyra* to the abalone farms. Projections of raw *Porphyra* biomass should therefore be considered in the light of the locations of abalone farms. Most abalone farms are south-east of Cape Town, focussed around Hermanus and Danger Point. On the west coast there are farms around Cape Columbine. Harvesting pressure is likely to be intense around the farms, and harvesting *Porphyra* from sites far removed from the farms may not prove economically viable. In this case, the biomass of *Porphyra* found, for example, near Cape Town, may remain untouched, unless farms are established in this area.

Where farms use *Ecklonia maxima* for abalone fodder, dried thalli have been used where and when fresh *E. maxima* was not available. Eulittoral species of *Porphyra* are well adapted to drying, as, where present, they are commonly one of the seaweeds found highest in the intertidal, and are capable of remaining viable for some time after they have been dried (Smith *et al.*, 1986, Lipkin *et al.*, 1993). Any *Porphyra* collected on a

commercial scale in South Africa would of necessity comprise intertidal species, as these are more easily collected and much larger and more common than subtidal forms. Thalli could therefore be collected, air- or sun-dried, and, once dry, transported to the farms, where they could be rehydrated and fed to abalone. This may spread the impact of harvesting *Porphyra* over a wider area, and may make larger harvests per farm possible.

Should harvesting of *Porphyra* for abalone fodder be permitted, the demand is likely to be high. Kelp harvests have rapidly increased following the onset of abalone farming in South Africa (Anderson *et al.*, 2003; Rotmann *et al.*, 2003) and may be approaching the limits of sustainability in areas where farms are concentrated. Demand by abalone farmers to harvest *Porphyra* has also been high. No other seaweed beyond kelp has a regional biomass large enough to support harvesting as a primary fodder for abalone farms. Should *Porphyra* harvesting be permitted, the relatively small quantities of *Porphyra* available will probably dictate that *Porphyra* is used as a specialist fodder, probably to increase the growth of smaller abalone.

Impacts of harvesting *Porphyra* may be mitigated by polyculture of *Porphyra* and abalone. Current research on the use of a mixed diet of *Porphyra*, *Ulva* and/or *Gracilaria* together with kelp clearly indicates the value to abalone farmers of mixed seaweed diets (D. Robertson-Andersson pers. comm.; K. Naidoo, pers. comm.). Nutrient uptake rates of *Porphyra* are high (Chopin *et al.*, 1999), and, if grown in polyculture, *Porphyra* may act to decrease nutrient pollution due to abalone farming. Research into such polyculture systems is still at an early stage, however, and the potential benefits, difficulties and costs of maintaining a polyculture system have not been fully explored.

One point that arises repeatedly in this thesis is the difficulty of predicting *Porphyra* biomass and ecology, associated community diversity, the potential for sustainable yield of *Porphyra*, without unacceptable impact on *Porphyra* and other organisms, using data from few sites in a long and heterogeneous coastline, given the difficulty in extrapolating coastline community data (Jara & Moreno, 1984; Foster, 1990). For this reason, monitoring programs are essential if the impact of harvesting *Porphyra* on eulittoral communities is to be minimised, while the yield of *Porphyra* is to be maximised and sustainable. Clearly, sustainable harvests of wild material cannot exceed the capacity of

ecosystems, and if greater yields are required, an alternative to harvesting wild material will need to be found.

Porphyra in South Africa has been found to consist not of a single species, as has long been believed, but of a number of largely undescribed species. It was not possible to undertake a full taxonomic revision of South African *Porphyra* prior to assessing the potential for harvest of *Porphyra*. If *Porphyra* in South Africa is to be managed on a sustainable basis, deriving full benefit from the range of species available, taxonomic revision is critical.

7.1.1 Specific management recommendations

The following tentative management recommendations are proposed for areas being harvested. The recommendations are conservative, and are suggested until specific effects of harvesting under extensive harvesting regimes at a number of sites are known. In formulating these, I have aimed to limit impacts on *Porphyra* populations and associating eulittoral organisms, even in relatively short stretches of shore.

1. No more than 80 % of the harvestable biomass of *Porphyra* present in any 50m stretch of shore should be removed by harvesters.
   This should ensure that sufficient *Porphyra* gametophytes remain, at regular intervals, to offset impacts both on *Porphyra* sporophyte populations and on any organisms that rely on mature *Porphyra* gametophytes as a resource. This will also maintain a level of archeospore production and consequent sporeling recruitment.
2. Between 50-75 % of the *Porphyra* left unharvested at harvest sites should be in dense patches. Unharvested *Porphyra* should be representative of all components of the original *Porphyra* population.
   Porphyra growth and survival in patches is better than outside patches. Together with (1), this will act to offset impacts of harvesting on *Porphyra* populations and on organisms (e.g. amphipods) that use *Porphyra* patches as a resource.
3. Once harvested, a site should remain undisturbed thereafter for a minimum of six months.
   As recruitment levels outside of biannual recruitment peaks are low, re-harvesting a

site before a recruitment peak will yield little. This six-month limit on re-harvests will increase the probability that *Porphyra* recruited during the rest period reach fertility.

4. Harvesting should take place as late in the growing season of *Porphyra* gametophytes as possible, viz. late summer or winter.

   Late season harvests will anticipate the natural collapse of gametophyte populations. Yields should still be high, and impact on organisms associated with *Porphyra* will be minimised. Harvesting early in the growing season will yield small infertile gametophytes only, and will largely clear *Porphyra* from the harvested area until the next biannual recruitment peak. This recommendation may be prove impractical should fresh harvests of *Porphyra* be required year-through, and may be dropped in these circumstances, provided that other recommendations limiting harvesting are followed.

5. Harvesters may collect *Porphyra* by hand plucking or using shears or knives or similar instruments.

   As no regrowth from holdfasts was found, a harvest method that leaves some holdfast (e.g. shears, knives) after harvesting need not be specified.

6. Harvesters should minimise removal or damage of substrate fauna or flora when harvesting epiphytic/epizooic *Porphyra*.

   Certain organisms commonly act as substrates for *Porphyra*, and impact on these substrate organisms should be minimised. Substrate organisms such as *Aeodes orbitosa*, which tears easily when epiphytic *Porphyra* is plucked, may be severely damaged by harvesting (esp. hand plucking) in areas where many thalli have *Porphyra* epiphytes. Harvesting of *Porphyra* may occasionally dislodge or remove limpets, another common *Porphyra* substrate. Hand plucking (or other harvest methods) need not affect substrate organisms provided that care is taken. For example, limpet removal during hand plucking of *Gelidium pristoides* is minimised by tapping on limpets prior to plucking, causing limpets to adhere more strongly to the rock. This results in a cleaner seaweed crop, and less damage to limpets.

7. Harvesters should take steps (e.g. shaking or rinsing thalli) to avoid the removal of those fauna associated with *Porphyra* during harvesting.

   Similar to (6). This will minimise impacts on those fauna found in association with *Porphyra* and easily removed with harvested thalli (in particular *Nodilittorina africana* and a number of amphipod/isopod crustaceans).

8. Selected sites under regular or frequent harvesting regimes must be continuously monitored to assess the long-term effect of harvesting on *Porphyra* populations and other eulittoral fauna/flora.

   As this study is a preliminary one, in which the specific affinities of *Porphyra* populations were not determined, and where the effect of no large or long-term harvest treatment was assessed, it can only serve to highlight likely impacts of harvesting. Monitoring is essential to ensure that impacts of harvesting on *Porphyra* and other eulittoral organisms remain acceptable. Monitoring programmes must include taxa identified in this study as being potentially vulnerable to harvesting impact as well as *Porphyra* populations. Though monitoring programmes will need to be designed around local conditions and taxa, potentially vulnerable eulittoral taxa include *Nodilittorina africana*, *Hyale grandicornis*, *Parisocladus stimpsonii* (and other crustaceans commonly associated with *Porphyra*), and *Aeodes orbitosa*.

7.2 Taxonomy and biodiversity

When this thesis commenced, only one species of *Porphyra*, *P. capensis*, was recorded from South Africa. Soon thereafter, Stegenga *et al.* (1997) recorded *P. garderi* and *P. suborbiculata* (as *P. carolinensis*) along with *P. capensis*, and described *P. saldanhae*. The work presented here has added *P. aeodis* to the list of *Porphyra* species recorded from South Africa, and has revealed an unexpectedly high biodiversity of *Porphyra* in South Africa. Beyond simply locating likely new species, the biodiversity survey revealed an unusually high level of genetic variation within *P. capensis* that indicates the urgent need for a review of this species.

Porphyra capensis seems to consist of a complex of closely related species that are endemic to the region. The *P. capensis* species complex contains by far the most common types of *Porphyra* encountered in South Africa. *Porphyra capensis* contains forms that live over a range of heights in the eulittoral, and generally grow epilithically or epizooically. Thalli range from linear or falcate through ovate or lanceolate to umbilicate or reniform. The degree of marginal folding varies widely, with some thalli having extensively folded margins. Colours range from deep, almost black purple-green through to translucent yellowish-green. Thalli are thick for *Porphyra*, with thalli ranging from 60 µm up to 180 µm in distal cells. All are monostromatic. Most vegetative cells are diplastidic, though

monoplastidic thalli do occur. Cell length to width ratio in sections ranges from 7.5:1 to 2:1. Both monoecious and dioecious thalli are encountered. Dioecious thalli have sexes segregated on separate plants; no evidence of temporal segregation of sexes on single thalli was found. Monoecious plants always have zygotosporangia and spermatangia in broad marginal sectors, and never in smaller, interspersed patches as seen in *P. saldanhae*, *P. aeodis*, and *P.* sp. (*sensu* Stegenga *et al.*, 1997). Female gametes vary considerably in the extent to which prototrichogynes are present. Often prototrichogynes cannot be detected; however, a range from brief fusiform prototrichogynes to extended prototrichogynes reaching almost to the thallus surface inside pronounced superficial swellings also occur. Zygotosporangia range from fusiform to terete, with four to sixteen tiers of zygotospores. Terete zygotosporangia usually occur with long fertilisation channels that run from the thallus surface. Fusiform zygotosporangia generally have abbreviated fertilisation channels, and develop from gametes with prototrichogynes. Spermatangia are composed of eight to 32 tiers, and are always terete.

Three unique sequences outside the *P. capensis* species complex were detected. One of these appears to be common to *P. saldanhae* and *P. aeodis*. The remaining two sequences represented new, apparently undescribed species, and do not correlate morphologically with species described or reported from South Africa.

All South African *Porphyra* from outside the *P. capensis* complex are morphologically easily distinguished from *P. capensis*. All are monoecious, with male and female patches intermingled on the thallus. Thalli are thinner, and redder in colour. Prototrichogynes and associated superficial bumps are not present, except in *P. gardneri*. Zygotosporangia are ovate to terete, with distinct fertilisation channels. Most species grew in environments where they would remain continuously hydrated or submerged, or nearly so, and most were epiphytes.

After the publication of the work of Stegenga *et al.* (1997), *P. capensis*, *P. saldanhae*, *P. gardneri* and *P. suborbiculata* (as *P. carolinensis*) were reported from South Africa. I have since described *P. aeodis*. The biodiversity survey revealed eleven unique nSSU sequences, from samples that included only *P. capensis*, *P. aeodis*, and *P. saldanhae*. Comparison with variation in other *Porphyra* species suggests that a conservative minimum of seven of these represent unique species. However, it appears that one

sequence represents two species, *P. aeodis*, and *P. saldanhae*. Thus, the minimum number of species suggested by the biodiversity survey is eight. Adding species that were not sampled during the survey gives ten as a conservative minimum estimate of the number of *Porphyra* species in South Africa. Five of these await description.

The range of morphologies and habitats attributed to *P. capensis* is very large. The drawings of *P. capensis* and *P. augustinae* in Kützing (1869) show different morphologies, with *P. capensis* being ovate, and *P. augustinae* being linear to falcate. Graves (1969) noted a wide range of morphologies in *P. capensis*, and other authors have made similar observations (Isaac, 1957; Molloy, 1990; Stegenga *et al.*, 1997). On morphological grounds alone, it has proved impossible to tease apart this range of morphologies into discrete groups that might be used to describe new species. It is clear from the results of this thesis that several different species do occur. It seems too that those within the *P. capensis* species complex have evolved locally from a common ancestor.

Fossils of *Porphyra* conchocelis have been described that date back 425 million years, and fossils that resemble bangiophytes have found in strata dating from the Proterozoic (up to 1.2 billion years old) (Campbell, 1980; Butterfield *et al.*, 1990). The degree of divergence in molecular data within the Bangiophyceae and within *Porphyra* is very high (Ragan *et al.*, 1994; Oliveira *et al.*, 1995), and this may be to some extent a function of the age of the order. However, molecular divergence is not paralleled by morphological diversity. The lack of morphological diversity within *Porphyra* has severely hampered taxonomy of the genus since it was described. As members of the *P. capensis* species complex derive from a recent common ancestor, some degree of shared morphology, beyond that found between two randomly selected *Porphyra* species, is likely. This may explain the tendency of previous authors to maintain the *P. capensis* complex.

As it seems that *P. aeodis* and *P. saldanhae* share the same nSSU exon, but can nevertheless be clearly distinguished on the basis of isozyme electrophoresis, this species pair might also be recently diverged. Insofar as both are recently described and so unlikely to have been widely cited, they are also known only from southern Africa, though they are closely related to entities from New Zealand. That isozyme electrophoresis proved more sensitive that nSSU in this example is not surprising: despite the proven utility of nSSU at species level in *Porphyra*, it remains a highly conserved part of the genome, and *Porphyra*

species with the same nSSU sequence have been recorded (Kunimoto *et al.* 1999*a*). Isozyme electrophoresis, on the other hand, is commonly used to detect differences between populations (Sosa & Lindstrom, 1999), and is a significantly more sensitive measure.

When *P. capensis* and *P. augustinae* were described, no illustrations were given and no types were designated. Kützing (1869) presented drawings of both in a later publication. I located isotypes of both taxa at the Rijksherbarium in Leiden. In both cases, samples were from Kützing's herbarium, had been identified as *P. capensis* or *P. augustinae* by Kützing, and had been collected at the same site and by the same pair of collectors as given in Kützing (1843). As such, these conform to the definition of isotypes given in Greuter *et al.* (1984). None matched the illustrations in Kützing (1869). Future taxonomic work on *P. capensis* will require lectotypification of the species. As the *P. capensis* isotypes match Kützing's concept of *P. capensis*, they would be useful for designating an appropriate lectotype. It seems appropriate to suggest that a *nomen novum* be designated for *P. augustinae*, as it appears Agardh (1890) may well have been incorrect in declaring *P. augustinae* a synonym of *P. capensis*. Again, this will have to be lectotypified.

This thesis demonstrates two approaches using genetic data that can be used to identify and define species of *Porphyra*. Of the two, gene sequencing is the most advantageous in that samples can easily be matched against the growing number of *Porphyra* sequences that are available in public databanks. The decreasing cost and increasing availability of facilities for sequencing make this approach more attractive. Despite the remarkable divergence of nSSU in the Bangiales and its frequent use in addressing taxonomic questions in the order, data from nSSU proved insufficient to draw firm conclusions on the phylogeny of the *P. capensis* species complex. A more comprehensive approach, using nSSU together with at least one more variable sequence as was utilized by Broom *et al.* (2002) in their reassessment of *P. suborbiculata*, would enable better resolution of members of the *P. capensis* species complex.

7.3 Revision of objectives

This study was largely able to address the objectives that were initially set. Information is provided on the distribution of biomass of *Porphyra* on the south western shores of the

country, data are presented on seasonal variation in a *Porphyra* gametophyte population, and an understanding of the position of *Porphyra* within the eulittoral community is gained. This, combined with an examination of the various impacts of harvesting *Porphyra*, has led to the development of recommendations for management of the taxon.

Further research into the population biology of *Porphyra* was halted as it became apparent that a number of species, that were not easily distinguished, were present. Initially, the feasibility of an assessment of biodiversity using isozyme electrophoresis was examined. Techniques developed in this phase were used to confirm the validity of an apparently new species, *P. aeodes*. Isozyme electrophoresis was finally rejected in favour of gene sequencing as a method for a broad biodiversity survey, largely as sequence data were more easily collected (fresh material was not required), and sequences from South African *Porphyra* could easily be compared with already available sequences from elsewhere in the world. A survey of diversity revealed a breadth of variation within *Porphyra* that was unsuspected at the start of this study, and that has profound implications for management of the taxon.

Although the work presented in this thesis had led to the production of a list of recommendations for the sustainable management of *Porphyra*, these recommendations must be viewed as provisional. The number of species of *Porphyra* in South Africa is not known, and most would appear to be undescribed. Consequently, their population biology, their role in eulittoral ecosystems and their response to harvesting can only be inferred from this study. Further taxonomic study will be required in order to circumscribe species so that better management plans can be drawn up.

7.4 Conclusions

There is a large biomass of *Porphyra* year-round on rocky shores in South Africa. However, the demand for fresh seaweed by abalone farms is high, and there may not be enough *Porphyra* to meet this demand. If so, *Porphyra* could only be used in limited quantities, probably as a specialist feed for smaller abalone, if it is to be used at all. If *Porphyra* is harvested according to the guidelines presented above, the impacts of harvesting should be low, as the impact of harvesting, on a local scale, seems to be minor, and to mimic the effects of periodic, usually seasonal, crashes in *Porphyra* gametophyte

populations. Long term monitoring will be necessary to determine the full impact of, especially, widespread or intense harvesting.

For effective management planning, however, taxonomic revision of South African *Porphyra* is critical. South Africa has a large diversity of cryptic *Porphyra* species, and the ecology of individual species is not known. Observation suggests that members of the *P. capensis* species complex share a functional niche, and this premise is used in drawing up the management guidelines presented here. Nothing is known of the population biology of any of South African *Porphyra* species, however, and the impact of harvesting on individual species cannot be predicted until those species can be identified and further studied.

8 References

Acleto, C.O. 1998. The seaweed resources of Peru. In: *Seaweed resources of the world*, eds Critchley, A.T. & Ohno, M., Japan International Cooperation Agency, Yokusuka: 343-346.

Adams, N.M. 1994. Seaweeds of New Zealand. Canterbury University Press.

Agardh, C. 1823. *Species Algarum* 1(2). Lund.

Agardh, C. 1824. *Systema Algarum*. Lund.

Agardh, J.G. 1890. Til algernes systematic. Nya bidrag. *Lunds Universitets Årsskrift,* Afd 2, 26: 63-64.

Akatsuka, I. 1992. Seaweed farming in Japan. In: *Proceedings of the first international workshop on sustainable seaweed resource development in sub-Saharan Africa*, eds Mshigeni, K. E., Bolton, J. J., Critchley, A. T. & Kiangi, G., University of Namibia, Windhoek: 1-18.

Aken, M.E., Griffin, N.J. & Robertson, B.L. 1993. Cultivation of the agarophyte *Gelidium pristoides* in Algoa Bay, South Africa. *Hydrobiologia* 268: 169-178.

Altschul, S.F., Madden, T.L., Schaffer, A.A., Zhang, J., Zhang, Z., Miller, W. & Lipman, D.J. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research* 25: 3389-3402.

Alveal, K. 1998. The seaweed resources of Chile. In: *Seaweed resources of the world*, eds Critchley, A.T. & Ohno, M., Japan International Cooperation Agency, Yokusuka: 347-365.

Anderson, R.J., Bolton, J.J., Molloy, F.J. & Rotmann, K.W.G. 2003. Commercial seaweeds in South Africa. In: *Proceedings of the 17th International Seaweed Symposium*, eds Chapman, A.R.O., Anderson, R.J., Vreeland, V.J. & Davison, I.R., Oxford University Press, Oxford: 1-12.

Anderson, R.J., Simons, R.H. & Jarman, N.G. 1989. Commercial seaweeds in southern Africa: a review of utilization and research. *South African Journal of Marine Science* 8: 277-299.

Arasaki, S. 1981. A comparison of the phenology of intertidal *Porphyra* on the coasts of Japan and North America. In: *Proceedings of the 8th International Seaweed Symposium*, eds Fogg, G.E. & Eifion Jones, W., The Marine Science Laboratories, Menai Bridge: 273-277.

Ardissone, F. 1888. Le alghe della Terra del Fuoco raccolte dal prof. Spegazzini. *Realle Istituto Lombardo Science e Lettere Rendiconti*, ser. 2, 21: 579-586.

Areschoug, J.F. 1851. *Phyceae capensis*. Upsala.

Avila, M., Santelices, B. & McLachlan, J. 1986. Photoperiod and temperature regulation of the life history of *Porphyra columbina* (Rhodophyta, Bangiales) from central Chile. *Canadian Journal of Botany* 64: 1867-1872.

Avise, J.C. 1974. Systematic value of electrophoretic data. *Systematic Zoology* 23: 465-481.

Bae, S.H. 1991. The origin and development process of the laver culture industry in Korea. Laver culture history until the end of Chosun dynasty. *Bulletin of the Korean Fisheries Society* 24: 153-166.

Bailey, J.C. & Freshwater, D.W. 1997. Molecular systematics of the Gelidiales: inferences from separate and combined analyses of plastid rbcL and nuclear SSU gene sequences. *European Journal of Phycology* 32: 343-352.

Barton, E.S. 1893. A provisional list of the marine algae of the Cape of Good Hope. *Journal of Botany, London* 31: 53-56, 81-84, 110-114, 138-144, 171-177, 202-210.

Bergdahl, J.C. 1990. Nori (*Porphyra* C. Ag.: Rhodophyta) mariculture research and technology transfer along the northeast Pacific coast. In: *Introduction to Applied Phycology*, ed. Akatsuka, I., SPB Academic Publishing, The Hague: 519-551.

Bird, C.J. & McLachlan, J.L. 1992. *Seaweed Flora of the Maritimes. 1. Rhodophyta - the Red Algae*. Biopress Ltd, Bristol.

Bird, C.J. & van der Meer, J.P. 1993. Systematics of economically important marine algae: a Canadian perspective. *Canadian Journal of Botany* 71: 361-369.

Bolton, J.J. & Joska, M.A.P. 1995. Population studies on a South African carrageenophyte: *Iridaea capensis* (Gigartinaceae, Rhodophyta). *Hydrobiologia* 260/261: 191-195.

Bolton, J.J. & Levitt, G.J. 1992. South African west coast carrageenophytes. In: *Proceedings of the first international workshop on sustainable seaweed resource development in sub-Saharan Africa*, eds Mshigeni, K.E., Bolton, J.J., Critchley, A. & Kiangi, G., K.E. Mshigeni, Windhoek: 37-49.

Branch, G.M. 1971. The ecology of *Patella* Linnaeus from the Cape Peninsula, Africa I. Zonation, movements and feeding. *Zoologica Africana* 6: 1-38.

Branch, G.M. 1975. Mechanisms reducing intraspecific competition in *Patella* spp.: migration, differentiation and territorial behaviour. *Journal of Animal Ecology* 44: 575-600.

Branch, G.M. & Branch, M. 1981. *The living shores of southern Africa.* C. Struik Publishers, Cape Town.

Branch, G.M., Eekhout, S. & Bosman, A.L. 1990. Short-term effects of the 1988 Orange River floods on the intertidal rocky-shore communities of the open coast. *Transactions of the Royal Society of South Africa* 47: 331-354.

Branch, G.M. & Griffiths, C.L. 1988. The Benguela ecosystem, part 5. The coastal zone. *Oceanography and Marine Biology: an annual review* 26: 395-486.

Bray, J.R. & Curtis, J.T. 1957. An ordination of the upland forest communities of southern Wisconsin. *Ecological Monographs* 27: 325-349.

Brodie, J., Hayes, P.K., Barker, G.L. & Irvine, L.M. 1996. Molecular and morphological characters distinguishing two *Porphyra* species (Rhodophyta: Bangiophycidae). *European Journal of Phycology* 31: 303-308.

Brodie, J., Hayes, P.K., Barker, G.L., Irvine, L.M. & Bartsch, I. 1998. A reappraisal of *Porphyra* and *Bangia* in the northeast Atlantic based on the rbcL-rbcS intergenic spacer. *Journal of Phycology* 34: 1069-1074.

Brodie, J. & Irvine, L.M. 1997. A comparison of *Porphyra dioica* sp. nov. and *P. purpurea* (Roth) C. Ag. (Rhodophyta: Bangiophycidae) in Europe. *Cryptogamie, Algologie* 18: 283-297.

Brody, S. 1945. *Bioenergetics and growth.* Van Nostrand Reinhold, New York.

Broom, J.E., Jones, W.A., Hill, D.F., Knight, G.A. & Nelson, W.A. 1999. Species recognition in New Zealand *Porphyra* using 18s rDNA sequencing. *Journal of Applied Phycology* 11: 421-428.

Broom, J.E., Nelson, W.A., Yarish, C., Jones, W.A., Aguilar Rosas, R. & Aguilar Rosas, L.E. 2002. A reassessment of the taxonomic status of *Porphyra suborbiculata*, *Porphyra carolinensis* and *Porphyra lilliputiana* (Bangiales, Rhodophyta) based on molecular and morphological data. *European Journal of Phycology* 37: 227-235.

Brown, M.T., Fraser, A.W.J., Brasch, D.J. & Melton, D.L. 1990. Growth and reproduction of *Porphyra columbina* Mont. (Bangiales, Rhodophyceae) from southern New Zealand. *Journal of Applied Phycology* 2: 35-44.

Burzycki, G.M. & Waaland, J.R. 1987. On the position of meiosis in the life history of *Porphyra torta* (Rhodophyta). *Botanica Marina* 30: 5-10.

Buschmann, A.H. & Vergara, P.A. 1993. Effect of rocky intertidal amphipods on algal recruitment: a field study. *Journal of Phycology* 29: 154-159.

Bustamante, R.H. & Branch, G.M. 1996. The dependence of intertidal consumers on kelp-derived organic matter on the west coast of Africa. *Journal of Experimental Marine Biology and Ecology* 196: 1-28.

Butterfield, N.J., Knoll, A.H. & Swett, K. 1990. A bangiophyte red alga from the Proterozoic of Arctic Canada. *Science* (Washington DC) 250: 104-107.

Campbell, S.E. 1980. *Paleoconchocelis starmachii*, a carbonate boring microfossil from the Upper Silurian of Poland (425 million years old): implications for the evolution of the Bangiaceae (Rhodophyta). *Phycologia* 19: 25-36.

Cannon, M.I. 1984. New findings on the systematics, cytology and reproductive strategies of *Porphyra* (Bangiaceae, Rhodophyta) in Hawaii and California. *Pacific Science* 38: 358.

Chamberlain, Y.M. 1965. Marine algae of Gough Island. *Bulletin of the British Museum of Natural History* 3: 175-232.

Chapman, A.R.O. 1986. Population and community ecology of seaweeds. *Advances in Marine Biology* 23: 1-161.

Chapman, A.R.O. & Johnson, C.R. 1990. Distribution and organisation of macroalgal assemblages in the Northwest Atlantic. *Hydrobiologia* 192: 77-121.

Chapman, V.J. 1969. *The marine algae of New Zealand. Part 3 Rhodophyceae. Issue 1 Bangiophycidae and Florideaphycidea (Nemalionales, Bonnemaisoniales, Gelidiales).* Verlag von J. Cramer.

Chapman, V. J. 1970. *Seaweeds and their uses* (2nd ed.). Methuen, London.

Cheney, D.P. 1985. Electrophoresis. In: *Handbook of phycological methods: Ecological field methods: Macroalgae*, eds Littler, M.M. & Littler, D.S., Cambridge University Press, Cambridge: 87-119.

Chopin, T. 1998. The seaweed resources of eastern Canada. In: *Seaweed resources of the world*, eds Critchley, A.T. & Ohno, M., Japan International Cooperation Agency, Yokusuka: 273-302.

Chopin, T., Yarish, C., Wilkes, R., Belyea, E., Lu, S. & Mathieson, A. 1999. Developing *Porphyra*/salmon integrated aquaculture for bioremediation and diversification of the aquaculture industry. *Journal of Applied Phycology* 11: 463-472.

Clarke, K.R. 1993. Non-parametric multivariate analyses of change in community structure. *Australian Journal of Ecology* 18: 117-143.

Clarke, K.R. & Ainsworth, M. 1993. A method of linking multivariate community variables structure to environmental variables. *Marine Ecology Progress Series* 92: 205-219.

Clarke, K.R. & Warwick. R.M. 1994. Similarity-based testing for community pattern: the two-way layout with no replication. *Marine Biology* 118: 167-176.

Clifford, H.T. & Stephenson, W. 1975. *An introduction to numerical classification.* Academic Press, London.

Cole, K. & Conway, E. 1980. Studies in the Bangiaceae: reproductive modes. *Botanica Marina* 23: 545-553.

Coll, J. & Cox, J. 1977. The genus *Porphyra* C. Ag. (Rhodophyta, Bangiales) in the American North Atlantic. I. New species from North Carolina. *Botanica Marina* 20: 155-159.

Coll, J. & Oliveira Filho, E.C. de. 1976. The genus *Porphyra* C. Ag. (Rhodophyta-Bangiales) in the American South Atlantic. II. Uruguayan species. *Botanica Marina* 19: 191-196.

Coll, J. & Oliveira Filho, E.C. de. 1977. Chromosome counting on 79-year-old dried seaweed, *Porphyra leucosticta* (Rhodophyta). *Experientia* 33: 102.

Coll, J. & Oliveira, E.C. 2001. *Porphyra drewiana*, a new species of red algae (Bangiales, Rhodophyta). *Phycological Research* 49: 67-72.

Conkle, M.T., Hodgskiss P.D., Nunnally, L.B. & Hunter, S.C. 1982. *Starch Gel Electrophoresis of Conifer Seeds: a Laboratory Manual.* General Technical Report PSW-64, U. S. D. A. Pacific Southwest Forest and Range Experimental Station, Berkeley.

Connell, J.H. 1972. Community interactions on marine rocky intertidal shores. *Annual Review of Ecology and Systematics* 3: 169-192.

Connell, J.H. & Slatyer, O.R. 1977. Mechanisms of succession in natural communities and their role in community stability and organization. *American Naturalist* 111: 1119-1144.

Conway, E. 1965. Juvenile stages in the genus *Porphyra*. In: *Proceedings of the 5th International Seaweed Symposium*, eds Young, E.G. & McLachlan, J.L., Pergamon Press, Oxford: 102-105.

Conway, E., Mumford, T.F. & Scagel, R.F. 1975. The genus *Porphyra* in British Columbia and Washington. *Syesis* 8: 185-244.

Coppejans, E. 1995. *Flore algologique des côtes du Nord de la France et de la Belgique*. Scripta Botanica 9, National Botanic Garden of Belgium, Meise.

Cubit, J.D. 1984. Herbivory and the seasonal abundance of algae on a high intertidal rocky shore. *Ecology* 65: 1904-1917.

Cuomo, V., Merrill, J., Palomba, I. & Perretti, A. 1993. Systematic collection of *Ulva* and mariculture of *Porphyra*: biotechnology against eutrophication in the Venice Lagoon. *International Journal of Environmental Studies* 43: 141-149.

Dangeard, P. 1927. Recherches sur les *Bangia* et les *Porphyra*. *Botaniste* 18: 183-244.

Davison, I.R. & Pearson, G.A. 1996. Stress tolerance in intertidal seaweeds. *Journal of Phycology* 32: 197-211.

Day, J.H. 1969. *A guide to marine life on South African shores*. A.A. Balkema, Cape Town.

Dayton, P.K. 1975. Experimental evaluation of ecological dominance in a rocky intertidal algal community. *Ecological Monographs* 45: 137-159.

De Toni, J.B. 1897. *Sylloge floridearum* 1. Padua.

de Zaixso, A.B., Ciancia, M. & Cerezo, A.S. 1998. The seaweed resources of Argentina. In: *Seaweed resources of the world*, eds Critchley, A.T. & Ohno, M., Japan International Cooperation Agency, Yokusuka: 372-384.

Delf, E.M. & Michell, M.R. 1921. The Tyson collection of marine algae. *Annals of the Bolus Herbarium* 3: 89-119.

Dice, L.R. 1945. Measures of the amount of ecological association between species. *Ecology* 26: 297-302.

Dickson, L.G. & Waaland, J.R. 1985. *Porphyra nereocystis*: a dual-daylength seaweed. *Planta* 165: 548-553.

Drège, J.F. 1843. Zwei pflanzengeographische Documente. *Besondere Beigrabe zur Flora*: 26.

Drew, K.M. 1949. Conchocelis-phase in the life history of *Porphyra umbilicalis* (L.) Kütz. *Nature* 164: 748-749.

Efron, B., Halloran, E. & Holmes, S. 1996. Bootstrap confidence levels for phylogenetic trees. *Proceedings of the National Academy of Sciences of the United States of America* 63: 13429-13434.

Felsenstein, J. 1985*a*. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39: 783-791.

Felsentein, J. 1985*b*. Phylogenies from gene frequencies: a statistical problem. *Systematic Zoology* 34: 300-311.

Field, J.G., Clarke, K.R. & Warwick, R.M. 1982. A practical strategy for analysing multispecies distribution patterns. *Marine Ecology Progress Series* 8: 37-52.

Foster, M.S. 1990. Organization of macroalgal assemblages in the Northeast Pacific: the assumption of homogeneity and the illusion of generality. *Hydrobiologia* 192: 21-33.

Freshwater, D.W., Fredericq, S., Butler, B.S., Hommersand, M.H. & Chase, M.W. 1994. A gene phylogeny of the red algae (Rhodophyta) based on plastid *rbcL*. *Proceedings of the National Academy of Sciences of the United States of America* 91: 7281-7285.

Garbary, D.J., Hansen, G.I. & Scagel, R.F. 1980. The marine algae of British Columbia and northern Washington: division Rhodophyta (Red Algae), class Bangiophyceae. *Syesis* 13: 137-195.

Genetics Computer Group. 1994. *Program manual for the GCG package*. Madison, Wisconsin.

Giraud, A. & Magne, F. 1968. La place de la méiose dans le cycle de développement de *Porphyra umbilicalis*. *Compte Rendu de l'Academie des Sciences, Paris, Séries D.* 267: 586-588.

Goff, L.J. & Moon, D.A. 1993. PCR amplification of nuclear and plastid genes from algal herbarium specimens and algal spores. *Journal of Phycology* 29: 381-384.

González, A. & Santelices, B. 2003. A re-examination of the potential use of central Chilean *Porphyra* (Bangiales, Rhodophyta) for human consumption. In: *Proceedings of the 17th International Seaweed Symposium*, eds Chapman, A.R.O., Anderson, R.J., Vreeland, V.J. & Davison, I.R., Oxford University Press, Oxford: 249-255.

Gottlieb, L.D. 1977. Electrophoretic evidence and plant systematics. *Annals of the Missouri Botanical Gardens* 64: 161-180.

Graur, D. & Li, W.-H. 2000. *Fundamentals of molecular evolution* (2nd ed.). Sinauer Associates, Sunderland, Massachusetts.

Graves, J.M. 1969. The genus *Porphyra* on South African coasts: I. Observations on the autecology of *Porphyra capensis* sensu Isaac (1957), including a description of dwarf plants. *Journal of South African Botany* 35: 343-362.

Greuter, W., Barrie, F.R., Burdet, H.M., Chaloner, W.G., Demoulin, V., Hawksworth, D.L., Jørgensen, P.M., Nicholson, D.H., Silva, P.C., Trehane, P. & McNeill, J. 1994. International code of botanical nomenclature (Toyko Code). *Regnum Vegetabile* 131: 1-83.

Grime, J.P. 1979. *Plant strategies and vegetation processes*. John Wiley and Sons Inc., New York.

Guiry, M.D. 1990. Sporangia and spores. In: *Biology of the red algae*, eds Cole, K.M. & Sheath, R.G., Cambridge University Press, Cambridge: 347-376.

Guiry, M.D. & Hession, C.C. 1998. The seaweed resources of Ireland. In: *Seaweed resources of the world*, eds Critchley, A.T. & Ohno, M., Japan International Cooperation Agency, Yokusuka: 210-216.

Guiry, M.D. & Nic Dhonncha, E. 2002. *Algaebase*. World wide web electronic publication (www.algaebase.org). 25 Nov 2002.

Hanelt, D., Huppertz, K. & Nultsch, W. 1993. Daily course of photosynthesis and photoinhibition in marine macroalgae investigated in the laboratory and field. *Marine Ecology Progress Series* 97: 31-37.

Harley, C.D.G. 2002 Light availability indirectly limits herbivore growth and abundance in a high rocky intertidal community during the winter. *Limnology and Oceanography*: 47: 1217-1222.

Harvey, W.H. & Hooker, J.D. 1844. Algae. In: *The botany of the Antarctic voyage of the H.M. Discovery Ships* Erebus *and* Terror *in the years 1839-1843, under the command of Captain Sir James Clark Ross, Kt., R.N., F.R.S., etc. Vol. 1 Flora Antarctica, Part 1 Botany of Lord Auckland's Group and Campbell's Island.* Reeve Brothers, London.

Harvey, W.H. & Hooker, J.D. 1847. Algae. In: *The botany of the Antarctic voyage of the H.M. Discovery Ships* Erebus *and* Terror *in the years 1839-1843, under the command of Captain Sir James Clark Ross, Kt., R.N., F.R.S., etc. Vol. 1 Flora Antarctica, Part 2 Botany of Fuegia, the Falklands, Kerguelen's Land, etc.* Reeve Brothers, London.

Hawkins, S.J. & Hartnoll, R.G. 1985. Factors determining the upper limits of intertidal canopy-forming algae. *Marine Ecology Progress Series* 20: 265-271.

Hay, C.H., Adams, N.M. & Parsons, M.J. 1985. The marine algae of the subantarctic islands of New Zealand. *National Museum of New Zealand Miscellaneous Series* 11: 1-70.

Hay, M.E. 1981. The functional morphology of turf-forming seaweeds: persistence in stressful marine habitats. *Ecology* 62: 739-750.

Hendriks, L., de Baere, R., van de Peer, Y., Neefs, J., Goris, A. & de Wachter, R. 1991. The evolutionary position of the rhodophyte *Porphyra umbilicalis* and the basidiomycete *Leucosporidium scottii* among other eukaryotes as deduced from

complete sequences of small ribosomal subunit RNA. *Journal of Molecular Evolution* 32: 167-177.

Herbert, S.K. & Waaland, J.R. 1988. Photoinhibition of photosynthesis in a sun and shade species of the red algal genus *Porphyra*. *Marine Biology* 97: 1-7.

Hillis, D.M. 1984. Misuse and modification of Nei's genetic distance. *Systematic Zoology* 33: 238-240.

Hillis, D.M. 1996. Inferring complex phylogenies. *Nature* 383: 130-131.

Hillis, D.M. & Dixon, M.T. 1991. Ribosomal DNA: molecular evolution and phylogenetic evidence. *Quarterly Review of Biology* 66: 411-453.

Hommersand, M.H. 1986. The biogeography of the South African marine red algae: a model. *Botanica Marina* 29: 257-270.

Hommersand, M.H. & Fredericq, S. 2003. Biogeography of the marine red algae of the South African West Coast: a molecular approach. In: *Proceedings of the 17th International Seaweed Symposium*, eds Chapman, A.R.O., Anderson, R.J., Vreeland, V.J. & Davison, I.R., Oxford University Press, Oxford: 325-336.

Hommersand, M.H., Guiry, M.D., Fredericq, S. & Leister, G.L. 1993. New perspectives in the taxonomy of the Gigartinaceae (Gigartinales, Rhodophyta). *Hydrobiologia* 260/261: 105-120.

Hruby, T. & Norton, T.A. 1979. Algal colonization on rocky shores in the Firth of Clyde. *Journal of Ecology* 67: 65-77.

Huelsenbeck, J.P., Larget, B., Miller, R.E. & Ronquist, F. 2002. Potential applications and pitfalls of Bayesian inference of phylogeny. *Systematic Biology* 51: 673-688.

Huelsenbeck, J.P. & Ronquist, F.R. 2001. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17: 754-755.

Huelsenbeck, J.P., Ronquist, F., Nielsen, R. & Bollback, J.P. 2001. Bayesian inference of phylogeny and its impact on evolutionary biology. *Science* 294: 2310-2314.

Hughey, J.R., Silva, P.C. & Hommersand, M.H. 2002. ITS1 sequences of type specimens of *Gigartina* and *Sarcothalia* and their significance for the classification of South African Gigartinaceae (Gigartinales, Rhodophyta). *European Journal of Phycology* 37: 209-216.

Hurlbert, S.H. 1971. The non-concept of species diversity: a critique and alternative parameters. *Ecology* 52: 577-586.

Hus, H.T.A. 1902. An account of the species of *Porphyra* found on the Pacific coast of North America. *Proceedings of the California Academy of Science* 2: 173-241.

Hwang, M.S., Han, M. & Lee, I.K. 1998. Allozyme variation and species relationships in the genus *Porphyra* (Bangiales, Rhodophyta) from Korea. *Algae* 13: 447-459.

Hwang, M.S. & Lee, I.K. 1994. Two species of *Porphyra* (Bangiales, Rhodophyta), *P. koreana* sp. nov. and *P. lacerata* Miura from Korea. *Korean Journal of Phycology* 9: 169-177.

Isaac, W.E. 1942. Seaweeds of possible economic importance in the Union of South Africa. *Journal of South African Botany* 8: 225-236.

Isaac, W.E. 1957. The distribution, ecology and taxonomy of *Porphyra* on South African coasts. *Proceedings of the Linnean Society (London)* 168: 61-65.

Isaac, W.E. & Molteno, C.J. 1953. Seaweed resources of South Africa. *Journal of South African Botany* 19: 85-92.

Ishikawa, M. 1921. Cytological studies on *Porphyra tenera* Kjellm. *Botanical Magazine, Tokyo* 35: 206-218.

Istini, S., Zatnika, A. & Sujatmiko, W. 1998. The seaweed resources of Indonesia. In: *Seaweed resources of the world*, eds Critchley, A.T. & Ohno, M., Japan International Cooperation Agency, Yokusuka: 92-97.

Jackson, L.F. & Lipschitz, S. 1984. *Coastal sensitivity atlas of southern Africa.* Department of Transport, Pretoria.

Jara, H.F. & Moreno, C.A. 1984. Herbivory and structure in a midlittoral rocky community: a case in Southern Chile. *Ecology* 65: 28-38.

Jensen, A. 1993. Present and future needs for algae and algal products. *Hydrobiologia* 260/261: 15-23.

Jones, J.M.K. & Holt, T. 1998. The seaweed resources of Britain. In: *Seaweed resources of the world*, eds Critchley, A.T. & Ohno, M., Japan International Cooperation Agency, Yokusuka: 217-225.

Jones, W.A., Griffin, N.J., Jones, D.T., Nelson, W.A., Farr, T.J. & Broom, J.E. Phylogenetic diversity in South African *Porphyra* (Bangiales, Rhodophyta) determined by nuclear SSU sequence analyses. *European Journal of Phycology* 39: in press.

Kang, J.W. 1971. Species of cultivated *Porphyra* in Korea. In: *Proceedings of the 5th International Seaweed Symposium*, ed. Nisizawa, K., University of Tokyo Press, Tokyo: 108-110.

Kang, J.W. & Koh, N.P. 1977. *Algal Mariculture*. Taehwa Publishing Company, Pusan.

Kapraun, D.F. & Freshwater, D.W. 1987. Karyological studies of five species of *Porphyra* (Bangiales, Rhodophyta) from the North Atlantic and Mediterranean. *Phycologia* 26: 82-87.

Kapraun, D.F. & Luster, D.G. 1980. Field and culture studies of *Porphyra rosengurtii* Coll et Cox (Rhodophyta, Bangiales) from North Carolina. *Botanica Marina* 23: 449-457.

Keppel, G. 1991. *Design and analysis: a researcher's handbook* (3rd ed.). Prentice-Hall, Englewood Cliffs, New Jersey.

Kito, H. 1974. Cytological observations on the conchocelis-phase in three species of *Porphyra*. *Bulletin of the Tohoku Regional Fisheries Research Laboratory* 33: 101-117.

Klein, A.S., Mathieson, A.C., Neefus, C.D., Cain, D.F., Taylor, H.A., Teasdale, B.W., West, A.L., Hehre, E.J., Brodie, J., Yarish, C. & Wallace, A.L. 2003. Identification of north-western Atlantic *Porphyra* (Bangiaceae, Bangiales) based on sequence variation in nuclear SSU and plastid *rbc*L genes. *Phycologia* 42: 109-122.

Kornmann, P. & Sahling, P.-H. 1991. The *Porphyra* species of Helgoland (Bangiales, Rhodophyta). *Helgoländer Meeresuntersuchungen* 45: 1-38.

Kraemer, G.P. & Yarish, C. 1999. A preliminary comparison of the mariculture potential of *Porphyra purpurea* and *Porphyra umbilicalis*. *Journal of Applied Phycology* 11: 473-477.

Krishnamurthy, V. 1972. A revision of the algal genus *Porphyra* occurring on the Pacific Coast of North America. *Pacific Science* 26: 24-49.

Kruskal, J.B. & Wish, M. 1978. *Multidimensional scaling.* Sage Publications, Beverley Hills, California.

Kunimoto, M., Kito, H., Kaminishi, Y., Mizukami, Y. & Murase, N. 1999*a*. Molecular divergence of the ssu rRNA gene and internal transcribed spacer 1 in *Porphyra yezoensis* (Rhodophyta). *Journal of Applied Phycology* 11: 211-216.

Kunimoto, M., Kito, H., Yamamoto, Y., Cheney, D.P., Kaminishi, Y. & Mizukami, Y. 1999*b*. Discrimination of *Porphyra* species based on small subunit ribosomal RNA gene sequence. *Journal of Applied Phycology* 11: 203-209.

Kurogi, M. 1961. Species of cultivated *Porphyra*s and their life histories. *Bulletin of the Tohoku Regional Fisheries Research Laboratory* 18: 91-115.

Kurogi, M. 1972. Systematics of *Porphyra* in Japan. In: *Contributions to the systematics of benthic marine algae of the North Pacific*, eds Abbott, I.A. & Kurogi, M., Japanese Society of Phycology, Kobe: 167-196.

Kützing, F.T. 1843. *Phycologia generalis*. F.A. Brockhaus, Leipzig.

Kützing, F.T. 1849. *Species Algarum*. F.A. Brockhaus, Leipzig.

Kützing, F.T. 1869. *Tabulae phycologicae* 19. Nordhausen.

Landsborough, D. 1857. A popular history of British seaweeds (3rd ed.). London Reeve, London.

Leister, G.L. 1977. *Taxonomy and reproductive morphology of* Iridaea cordata *(Turner) Bory and* Iridaea crispata *Bory (Gigartinaceae, Rhodophyta) from southern South America*. Ph.D thesis, Duke University.

Levitt, G.J. & Bolton, J.J. 1991. Seasonal patterns of photosynthesis and physiological parameters and the effects of emersion in littoral seaweeds. *Botanica Marina* 34: 403-410.

Levitt, G.J., Bolton, J.J. & Anderson, R.J. 1995. Potential harvestable biomass of four carrageenan-producing seaweeds of the south-western Cape, South Africa. *South African Journal of Marine Science* 15: 49-60.

Lewmanomont, K. 1998. The seaweed resources of Thailand. In: *Seaweed resources of the world*, eds Critchley, A.T. & Ohno, M., Japan International Cooperation Agency, Yokusuka: 70-78.

Lindstrom, S.C. 1993. Inter- and intrapopulation genetic variation in species of *Porphyra* (Rhodophyta: Bangiales) from British Columbia and adjacent waters. *Journal of Applied Phycology* 5: 53-62.

Lindstrom, S.C. 1998. The seaweed resources of British Columbia, Canada. In: *Seaweed resources of the world*, eds Critchley, A.T. & Ohno, M., Japan International Cooperation Agency, Yokusuka: 266-272.

Lindstrom, S.C. & Cole, K.M. 1990*a*. An evaluation of species relationships in the *Porphyra perforata* species complex (Bangiales, Rhodophyta) using starch gel electrophoresis. *Hydrobiologia* 204/205: 179-183.

Lindstrom, S.C. & Cole, K.M. 1990*b*. *Porphyra fallax*, a new species of Rhodophyta from British Columbia and northern Washington. *Japanese Journal of Phycology* 38: 371-376.

Lindstrom, S.C. & Cole, K.M. 1992*a*. Relationships between some North Atlantic and North Pacific species of *Porphyra* (Bangiales, Rhodophyta): evidence from isozymes, morphology, and chromosomes. *Canadian Journal of Botany* 70: 1355-1363.

Lindstrom, S.C. & Cole, K.M. 1992*b*. A revision of the species of *Porphyra* (Rhodophyta: Bangiales) occurring in British Columbia and adjacent waters. *Canadian Journal of Botany* 70: 2066-2075.

Lindstrom, S.C. & Cole, K.M. 1992*c*. The *Porphyra lanceolata-P. pseudolanceolata* (Bangiales, Rhodophyta) complex unmasked; recognition of new species based on isozymes, morphology, chromosomes and distributions. *Phycologia* 31: 431-448.

Lindstrom, S.C. & Cole, K.M. 1993. The systematics of *Porphyra*: character evolution in closely related species. *Hydrobiologia* 260/261: 151-157.

Lindstrom, S.C. & Fredericq, S. 2003. *rbc*L gene sequences reveal relationships among north-east Pacific species of *Porphyra* (Bangiales, Rhodophyta) and a new species, *P. aestivalis*. Phycological Research 51: 211-224.

Lindstrom, S.C. & South, G.R. 1989. Evidence of species relationships in the Palmariaceae (Palmariales, Rhodophyta) based on starch gel electrophoresis. *Cryptogamic Botany* 1: 32-41.

Lipkin, Y., Beer, S. & Eschel, A. 1993. The ability of *Porphyra linearis* (Rhodophyta) to tolerate prolonged periods of desiccation. *Botanica Marina* 36: 517-523.

Lipkin, Y. & Friedlander, M. 1998. The seaweed resources of Israel and other Eastern Mediterranean countries. In: *Seaweed resources of the world*, eds Critchley, A.T. & Ohno, M., Japan International Cooperation Agency, Yokusuka: 156-163.

Littler, M.M. & Littler, D.S. 1980. The evolution of thallus form and survival strategies in benthic marine macroalgae: field and laboratory studies of a functional form model. *The American Naturalist* 116: 25-44.

Lubchenco, J. 1978. Plant species diversity in a marine intertidal community: importance of herbivore food preference and algal competitive abilities. *American Naturalist* 112: 23-39.

Lubchenco, J. 1980. Algal zonation in the New England rocky intertidal community: an experimental analysis. *Ecology* 61: 333-344.

Lubchenco, J. 1983. *Littorina* and *Fucus*: effects of herbivores, substratum heterogeneity, and plant escapes during succession. *Ecology* 64: 1116-1123.

Lubchenco, J. & Cubit, J. 1980. Heteromorphic life histories of certain marine algae as adaptations to variations in herbivory. *Ecology* 61: 676-687.

Lubchenco, J. & Menge, B. 1978. Community development and persistence in a low rocky intertidal zone. E*cological Monographs* 59: 67-94.

Ma, J.H. & Miura, A. 1984. Observations of the nuclear division in the conchospores and their germlings in *Porphyra yezoensis* Ueda. *Japanese Journal of Phycology* 32: 373-378.

MacArthur, R.H. 1965. Patterns of species diversity. *Biological Reviews* 40: 510-533.

Magne, F. 1991. Classification and phylogeny in the lower Rhodophyta: a new proposal. *Journal of Phycology* 27 (Suppl.): 46.

Magurran, A.E. 1988. *Ecological diversity and its measurement.* Croom Helm, London.

Mantel, N. 1967. The detection of disease clustering and a generalized regression approach. *Cancer Research* 27: 209-220.

Martinez, E. 1990. The conchocelis-phase of *Porphyra* (Rhodophyta) in the intertidal of San Juan Island, Washington, U.S.A. *Phycologia* 29: 391-395.

McQuaid, C.D. 1980. *Spatial and temporal variations in rocky intertidal communities.* Ph.D. thesis, University of Cape Town.

McQuaid, C.D. 1985. Seasonal variation in biomass and zonation of nine intertidal algae in relation to changes in radiation, sea temperature and tidal regime. *Botanica Marina* 38: 539-544.

Merrill, J.E. 1993. Development of nori markets in the western world. *Journal of Applied Phycology* 5: 159-154.

Merrill, J.E. & Waaland, R.J. 1998. The seaweed resources of the United States of America. In: *Seaweed resources of the world,* eds Critchley, A.T. & Ohno, M., Japan International Cooperation Agency, Yokusuka: 303-323.

Migita, S. 1967. Cytological studies on *Porphyra yezoensis* Ueda. *Bulletin of the Faculty of Fisheries, Nagasaki University* 24: 55-64.

Minchin, P.R. 1987. An evaluation of the relative robustness of techniques for ecological ordination. *Vegetatio* 69: 89-107.

Mitman, G.G. & van der Meer, J.P. 1994. Meiosis, blade development, and sex determination in *Porphyra purpurea* (Rhodophyta). *Journal of Phycology* 30: 147-159.

Miura, A. 1975. *Porphyra* cultivation in Japan. In: *Advance of phycology in Japan,* eds Tokida, J. & Hirose, H., Dr. W. Junk Publishers, The Hague: 273-304.

Miura, A. 1988. Taxonomic studies of *Porphyra* species cultivated in Japan, referring to their transition to the cultivated variety. *Journal of the Tokyo University of Fisheries* 75: 311-325.

Molloy, F.J. 1990. Utilized and potentially utilizable seaweeds from the Namibian coast: biogeography and accessibility. *Hydrobiologia* 204/205: 293-299.

Müller, K.M., Oliveira, M.C., Sheath, R.G. & Bhattacharya, D. 2001. Ribosomal DNA phylogeny of the Bangiophycidae (Rhodophyta) and the origin of secondary plastids. *American Journal of Botany* 88: 1390-1400.

Müller, K.M., Sheath, R.G., Vis, M.L., Crease, T.J. & Cole, K.M. 1998. Biogeography and systematics of *Bangia* (Bangiales, Rhodophyta) based on the Rubisco spacer, *rbc*L gene and 18S rRNA gene sequences and morphometric analyses. 1. North America. *Phycologia* 37: 195-207.

Mumford, T.F. 1990. Nori cultivation in North America: growth of the industry. *Hydrobiologia* 204/205: 89-98.

Nadler, S.A. 2002. Species delimitation and nematode biodiversity: phylogenies rule. *Nematology* 4: 615-625.

Nang, H.Q. & Dinh, N.H. 1998. The seaweed resources of Vietnam. In: *Seaweed resources of the world,* eds Critchley, A.T. & Ohno, M., Japan International Cooperation Agency, Yokusuka: 62-69.

Neefs, J.-M., van de Peer, Y., De Rijk, P., Chapelle, S. & De Wachter, R. 1993. Compilation of small ribosomal subunit RNA structures. *Nucleic Acids Research* 21: 3025-3049.

Neefus, C.D., Mathieson, A.C., Klein, A.S., Teasdale, B., Gray, T., & Yarish, C. 2002. *Porphyra birdiae* sp. nov. (Bangiales, Rhodophyta): a new species from the northwest Atlantic. *Algae* 17: 203-216.

Nei, M. 1972. Genetic distance between populations. *American Naturalist* 106: 283-292.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* 89: 583-590.

Nei, M. 1987. *Molecular evolutionary genetics.* Columbia University Press, New York.

Nelson, W.A. 1993. Epiphytic species of *Porphyra* (Bangiales, Rhodophyta) from New Zealand. *Botanica Marina* 36: 525-534.

Nelson, W.A. & Adams, N.M. 1990. A new species of *Porphyra* (Bangiales, Rhodophyta) from the Three Kings Islands, Northern New Zealand. *Botanica Marina* 33: 3-7.

Nelson, W.A., Brodie, J. & Guiry, M.D. 1999. Terminology used to describe reproduction and life history stages in the genus *Porphyra* (Bangiales, Rhodophyta). *Journal of Applied Phycology* 11: 407-410.

Nelson, W.A., Broom, J.E. & Farr, T.J. 2001. Four new species of *Porphyra* (Bangiales, Rhodophyta) from the New Zealand region described using traditional characters and 18S rDNA sequence data. *Cryptogamie, Algologie* 22: 263-284.

Nelson, W.A., Broom, J.E. & Farr, T.J. 2003. *Pyrophyllon* and *Chlidopyllon* (Erythropeltidales, Rhodophyta): two new genera for obligate epiphytic species

previously placed in *Porphyra*, and a discussion of the orders Erythropeltidales and Bangiales. *Phycologia* 42: 308-315.

Nelson, W.A. & Conroy, A.M. 1989. Effect of harvest method and timing on yield and regeneration of Karengo (*Porphyra* spp.) (Bangiales, Rhodophyta) in New Zealand. *Journal of Applied Phycology* 1: 277-283.

Nelson W. A. & Knight, G. A. 1996. Life history in culture of the obligate epiphyte *Porphyra subtumens* (Bangiales, Rhodophyta) endemic to New Zealand. *Phycological Research* 44:19-25.

Nelson, W.A., Knight, G.A. & Hawkes, M.W. 1998. *Porphyra lilliputiana* sp. nov. (Bangiales, Rhodophyta): a diminutive New Zealand endemic with novel reproductive biology. *Phycological Research* 46: 57-61.

Newton, M.A. 1996. Bootstrapping phylogenies. *Biometrika* 83: 315-328.

Noda, H. & Iwata, S. 1978. A guide to the improvement of nori products. National Federation of Nori and Shellfish Fisheries Cooperative Association.

Norton, T.A., Hawkins, S.J., Manley, N.L., Williams, G.A. & Watson, D.C. 1990. Scraping a living: a review of littorinid grazing. *Hydrobiologia* 193: 117-138.

Ohme, M., Kunifuji, Y. & Miura, A. 1986. Cross experiments of the color mutants in *Porphyra yezoensis* Ueda. *Japanese Journal of Phycology* 34: 101-106.

Ohme, M. & Miura, A. 1988. Tetrad analysis in conchospore germlings of *Porphyra yezoensis* (Rhodophyta, Bangiales). *Plant Science* 57: 135-140.

Ohno, M. & Largo, D.B. 1998. The seaweed resources of Japan. In: *Seaweed resources of the world*, eds Critchley, A.T. & Ohno, M., Japan International Cooperation Agency, Yokusuka: 1-14.

Oliveira Filho, E.C. de & Coll, J. 1975. The genus *Porphyra* C. Ag. (Rhodophyta-Bangiales) in the American South Atlantic. I. Brazilian species. *Botanica Marina* 18: 191-197.

Oliveira, M.C. & Bhattacharya, D. 2000. Phylogeny of the Bangiophycidae (Rhodophyta) and the secondary endosymbiotic origin of algal plastids. *American Journal of Botany* 87: 482-492.

Oliveira, M.C., Kurniawan, J., Bird, C.J., Rice, E.L., Murphy, C.A., Singh, R.K., Gutell, R.R. & Ragan, M.A. 1995. A preliminary investigation of the order Bangiales (Bangiophycidae Rhodophyta) based on sequences of nuclear small-subunit ribosomal RNA genes. *Phycological Research* 43: 71-79.

Oohusa, T. 1993. Recent trends in nori products and markets in Asia. *Journal of Applied Phycology* 5: 155-159.

Page, R.D.M. & Holmes, E.C. 1998. *Molecular evolution-a phylogenetic approach.* Blackwell Scientific Publications, Oxford.

Paine, R.T. 1990. Benthic macroalgal competition: complications and consequences. *Journal of Phycology* 26: 12-17.

Papenfuss, G.F. 1964. Catalogue and bibliography of Antarctic and sub-Antarctic benthic marine algae. *Antarctic Research Series* 1: 1-76.

Penny, D. 1982. Towards a basis for classification: the incompleteness distance measures, incompatibility analysis and phenetic classification. *Journal of Theoretical Biology* 96: 129-142.

Pielou, E.C. 1977. *Mathematical ecology.* John Wiley and Sons, New York.

Posada, D. & Crandall. K.A. 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics* 14: 817-818.

Ragan, M.A., Bird, C.J., Rice, E.L., Gutell, R.R., Murphy, C.A. & Singh, R.K. 1994. A molecular phylogeny of the marine red algae (Rhodophyta) based on the nuclear small-subunit rRNA gene. *Proceedings of the National Academy of Sciences of the United States of America* 91: 7276-7280.

Ramírez, M.E. & Santelices, B. 1991. *Catálogo de las algas marinas bentónicas de la costa temperada del Pacífico de Sudamérica.* Monografías Biológicas 5, Facultad de Ciencias Biológica, Pontificia Universidad Católica de Chile.

Roland, W.G. & Coon, L.M. 1984. Postharvest recovery of beds of the edible red alga *Porphyra perforata*. *Canadian Journal of Botany* 62: 1968-1970.

Roth, A.W. 1797. *Catalecta botanica*, Fasc. I. Leipzig.

Rotmann, K.W.G., Ruscoe, W.M. & Rotmann, H.G. 2003. The economic importance of *Ecklonia maxima* and other kelps in the Southern Cape region of South Africa. In: *Proceedings of the 17th International Seaweed Symposium*, eds Chapman, A.R.O., Anderson, R.J., Vreeland, V.J. & Davison, I.R., Oxford University Press, Oxford: 131-136.

Sanderson, M.J. & Wojciechowski, M.F. 2000. Improved bootstrap confidence limits in large-scale phylogenies, with an example from Neo-*Astragalus* (Leguminosae). *Systematic Biology* 49: 671-685.

Santelices, B. 1990*a*. Patterns of organisation of intertidal and shallow subtidal vegetation in wave exposed habitats of central Chile. *Hydrobiologia* 192: 3558.

Santelices, B. 1990*b*. Reproduction, dispersal and recruitment in seaweeds. *Oceanography and Marine Biology: an Annual Review* 28: 177-276.

Santelices, B. 1996. Seaweed research and utilization in Chile: moving into a new phase. *Hydrobiologia* 326/327: 1-14.

Santelices, B. & Martínez, E. 1988. Effects of filter-feeders and grazers on algal settlement and growth in mussel beds. *Journal of Experimental Marine Biology and Ecology* 118: 281-306.

Santelices. B., Montalva, S. & Oliger, P. 1981. Competitive algal community organization in exposed intertidal habitats from Central Chile. *Marine Ecology Progress Series* 6: 267-276.

Santelices, B. & Ugarte, R. 1987. Algal life-history strategies and resistance to digestion. *Marine Ecology Progress Series* 35: 267-275.

Saunders, G.W. & Kraft, G.T. 1994. Small-subunit rRNA gene sequences from representatives of selected families of the Gigartinales and Rhodymeniales (Rhodophyta). 1. Evidence for the Plocamiales *ord. nov. Canadian Journal of Botany* 72: 1250-1263.

Schiel, D.R. & Nelson, W.A. 1990. The harvesting of macroalgae in New Zealand. *Hydrobiologia* 204/205: 25-33.

Schmalhausen, I.I. 1984. *Growth and differentiation.* Vol. 1 and 2, Naukova Dumka, Kiev.

Schneider, C.W. & Searles, R.B. 1991. *Seaweeds of the southeastern United States: Cape Hatteras to Cape Canaveral.* Duke University Press, Durham.

Schonbeck, M.W. & Norton, T.A. 1978. Factors controlling the upper limits of fucoid algae on the shore. *Journal of Experimental Marine Biology and Ecology* 31: 303-313.

Scrosati, R. & DeWreede, R.E. 1997. The dynamics of the biomass-density relationship and frond biomass inequality for *Mazzaella cornucopiae* (Gigartinaceae, Rhodophyta): implications for the understanding of frond interactions. *Phycologia* 36: 506-516.

Scrosati, R. & DeWreede, R.E. 1998. The impact of frond crowding on frond bleaching in the clonal intertidal alga *Mazzaella cornucopiae* (Rhodophyta, Gigartinaceae) from British Columbia, Canada. *Journal of Phycology* 34: 228-232.

Sea Fisheries. 1996. *Marine conservation: do's and don'ts* [pamphlet]. Sea Fisheries, Roggebaai.

Seagrief, S.C. 1984. *A Catalogue of South African Green, Brown and Red Marine Algae.* Memoirs of the Botanical Survey of South Africa No. 47, Botanical Research Institute, Pretoria.

Shannon, C.E. & Weaver, W. 1949. *The mathematical theory of communication.* University of Illinois Press, Urbana, Illinois.

Shin, J.-A. & Miura, A. 1990. Estimation of the degree of self-fertilization in *Porphyra yezoensis* (Bangiales, Rhodophyta). *Hydrobiologia* 204/205: 397-400.

Silva, P.C., Basson, P.W. & Moe, R.L. 1996. *Catalogue of the Benthic Marine Algae of the Indian Ocean.* University of California Publications in Botany 79, University of California, Berkeley.

Simpson, B.J.A. 1994. *An investigation of diet management strategies for the culture of the South African abalone,* Haliotis midae. M. Sc. thesis, University of Cape Town.

Simpson, E.H. 1949. Measurement of diversity. *Nature* 163: 688.

Smith, C.M., Satoh, K. & Fork, D.C. 1986. The effects of osmotic tissue dehydration and air drying on morphology and energy transfer in two species of *Porphyra. Plant Physiology* 80: 843-847.

Sogin, M.L. & Gunderson, J.H. 1987. Structural diversity of eukaryotic small subunit ribosomal RNAs: evolutionary implications. In: *Endocytobiology* 3, eds Lee, J.J. & Frederick, J.F., New York Academy of Sciences, New York: 125-139.

Sohn, C.H. 1998. The seaweed resources of Korea. In: *Seaweed resources of the world,* eds Critchley, A.T. & Ohno, M., Japan International Cooperation Agency, Yokusuka: 34-46.

Sokal, R.R. & Sneath, P.H.A. 1963. *Principles of numerical taxonomy.* W.H. Freeman and Company, San Francisco.

Somerfield, P.J. & Clarke, K.R. 1995. Taxonomic studies, in marine community studies, revisited. *Marine Ecology Progress Series* 127: 113-119.

Sosa, P.A & Lindstrom, S.C. 1999. Isozymes in macroalgae (seaweeds): genetic differentiation, genetic variability and applications in systematics. *European Journal of Phycology* 34: 427-442.

Sousa, W.P. 1984. Intertidal mosaics: patch size, propagule availability, and spatially variable patterns of succession. *Ecology* 65: 1918-1935.

Sousa-Pinto, I. 1998. The seaweed resources of Portugal. In: *Seaweed resources of the world,* eds Critchley, A.T. & Ohno, M., Japan International Cooperation Agency, Yokusuka: 176-184.

Stegenga, H., Bolton, J.J. & Anderson, R.J. 1997. *Seaweeds of the South African West Coast.* Contributions from the Bolus Herbarium No. 18, Bolus Herbarium, University of Cape Town, Cape Town.

Stekoll, M.S. 1998. The seaweed resources of Alaska. In: *Seaweed resources of the world*, eds Critchley, A.T. & Ohno, M., Japan International Cooperation Agency, Yokusuka: 258-265.

Stekoll, M.S. & Deysher, L. 1996. Recolonisation and restoration of the upper intertidal *Fucus gardneri* (Fucales, Phaeophyta) following the Exxon Valdez oil spill. *Hydrobiologia* 326/327: 311-316.

Stepto, N.K. & Cook, P.A. 1996. Feeding preferences of the juvenile South African abalone *Haliotis midae* (Linnaeus, 1758). *Journal of Shellfish Research* 15: 653-657.

Stiller, J.W. & Waaland, J.R. 1993. Molecular analysis reveals cryptic diversity in *Porphyra* (Rhodophyta). *Journal of Phycology* 29: 506-517.

Stiller, J.W. & Waaland, J.R. 1996. *Porphyra rediviva* sp. nov. (Rhodophyta): a new species from northeast Pacific salt marshes. *Journal of Phycology* 32: 323-332.

Suto, S. 1972. Variation in species characters of *Porphyra* under culture conditions. In: *Contributions to the systematics of benthic marine algae of the North Pacific*, eds Abbott, I.A. & Kurogi, M., Japanese Society of Phycology, Kobe: 193-201.

Suzuki, Y., Glazko, G.V. & Nei, M. 2002. Overcredibility of molecular phylogenies obtained by Bayesian phylogenetics. *Proceedings of the National Academy of Sciences of the United States of America* 99: 16138-16143.

Swofford, D.L. 1998. *PAUP*. Phylogenetic Analysis Using Parsimony (*and other methods)*. Version 4. Sinauer Associates, Sunderland, Massachusetts.

Swofford, D.L. & Olsen, G.J. 1990. Phylogenetic reconstruction. In: *Molecular systematics*, eds Hillis, D.M. & Moritz, C., Sinauer Associates, Sunderland, Massachusetts: 411-501.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F. & Higgins, D.G. 1997. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* 24: 4876-4882.

Trono, G. 1998. The seaweed resources of the Philippines. In: *Seaweed resources of the world*, eds Critchley, A.T. & Ohno, M., Japan International Cooperation Agency, Yokusuka: 47-51.

Tseng, C.K. 1981. Commercial cultivation. In: *Biology of seaweeds*, eds Lobban, C.; Wynne, M.J., Blackwell Scientific Publications, Oxford: 680-725.

Tseng, C.K. 1984. *Common seaweeds of China*. Science Press, Berkeley.

Tseng, C.K. & Chang, T.J. 1955. Studies on *Porphyra*. III. Sexual reproduction in *Porphyra*. *Acta Botanica Sinica* 4: 153-166.

Tseng, C.K. & Chang, T.J. 1956. Conditions of *Porphyra* conchospores formation and discharge and the discharge rhythm. *Acta Botanica Sinica* 5: 33-48.

Tseng, C.K. & Sun, A. 1989. Studies on the alternation of the nuclear phases and chromosome numbers in the life history of some species of *Porphyra* from China. *Botanica Marina* 32: 1-8.

Turner, N.C. 1973. The ethnobotany of the Bella Coola Indians of British Columbia. *Syesis* 6: 193-220.

Turner, N.C. & Bell, M.A.M. 1971. The ethnobotany of the Coast Salish Indians of Vancouver Island. *Economic Botany* 25: 63-104.

Turner, N.C. & Bell, M.A.M. 1973. The ethnobotany of the southern Indians of British Columbia. *Economic Botany* 27: 257-310.

Turner, N.J. 2003. The ethnobotany of edible seaweed (*Porphyra abbottae* and related species; Rhodophyta: Bangiales) and its use by First Nations on the Pacific coast of Canada. *Canadian Journal of Botany* 81: 283-293.

Ueda, S., Iwamoto, K. & Miura, A. 1963. *Suisan syokubutu-gaka*. Kouseisya Kouseikaku, Tokyo.

Underwood, A.J. & Jernakoff, P. 1984. The effects of tidal height, wave-exposure, seasonality and rock-pools on grazing and the distribution of intertidal macroalgae in New South Wales. *Journal of Experimental Marine Biology and Ecology* 75: 71-96.

Waaland, J.R., Dickson, L.G. & Duffield, E.C.S. 1990. Conchospore production and seasonal occurrence of some *Porphyra* species (Bangiales, Rhodophyta) in Washington State. *Hydrobiologia* 204/205: 453-459.

Warwick, R.M. & Clarke, K.R. 1993. Increased variability as a symptom of stress in marine communities. *Journal of Experimental Marine Biology and Ecology* 172: 215-226.

Weller, D.E. 1987. A reevaluation of the 3/2 power rule of plant self-thinning. *Ecological Monographs* 57: 23-43.

White, T.J., Bruns, T., Lee, S. & Taylor, W.J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: *PCR protocols: A guide to methods and applications*, eds Innis, M.A., Gelfand, D.H., Sninsky, J.J. & White, T.J., Academic Press, San Diego: 315-322.

Whittaker, R.H. 1972. Evolution and measurement of species diversity. *Taxon* 21: 213-251.

Whittaker, R.H. 1977. Evolution of species diversity in land communities. In: *Evolutionary Biology*, vol 10, eds Hecht, M.K., Steere, W.C. & Wallace, B., Plenum, New York: 167.

Wilcox, T.P., Zwickl, D.J., Heath, T.A. & Hillis, D.M. 2002. Phylogenetic relationships of the dwarf boas and a comparison of Bayesian and bootstrap measures of phylogenetic support. *Molecular Phylogenetics and Evolution* 25: 361-371.

Wittmann, W. 1965. Aceto-iron-haematoxylin-chloral hydrate for chromosome staining. *Stain Technology* 40: 161-164.

Woessner, J. 1981. The measurement and harvest of the marine crop plant, *Porphyra nereocystis*. In: *Proceedings of the 8th International Seaweed Symposium*, eds Fogg, G.E. & Eifion Jones, W., The Marine Science Laboratories, Menai Bridge: 764-769.

Womersley, H.B.S. 1994. *The Marine Benthic Flora of Southern Australia. Rhodophyta. Part 3. Bangiophyeae and Florideophyceae (Acrochaetiales, Nemaliales, Gelidiales, Hildenbrandiales and Gigartinales* sensu lato*)*. Australian Biological Resources Study, Canberra.

Woolcott, G.W. & King, R.J. 1998. *Porphyra* and *Bangia* (Bangiaceae, Rhodophyta) in warm temperate water of eastern Australia: morphological and molecular analyses. *Phycological Research* 45: 111-123.

Wu, C.Y. 1998. The seaweed resources of China. In: *Seaweed resources of the world*, eds Critchley, A.T. & Ohno, M., Japan International Cooperation Agency, Yokusuka: 34-46.

Yamazaki, S., Kitade, Y., Maruyama, T. & Saga, N. 1996. Phylogenetic position of *Porphyra yezoensis* (Bangiales, Rhodophyta) based on the 18S rDNA sequence. *Journal of Marine Biotechnology* 4: 230-232.

Yoshida, T., Notoya, M., Kikuchi, N. & Miyata, M. 1997. Catalogue of species of *Porphyra* in the world, with special reference to the type locality and bibliography. *Natural History Research* 3 (special edition): 5-18.

Zar, J.H. 1984. *Biostatistical analysis* (2nd ed.). Prentice-Hall, Englewood Cliffs.

Appendix A Biomass of *Porphyra* in Western Cape

This Appendix contains tabulated data of seasonal *Porphyra* biomass, and projected seasonal *Porphyra* biomass from the forty sample sites in Chapter 2.

Table A-1 Wet biomass of *Porphyra* per metre of shore at each of forty sample sites during winter and summer.

	Biomass ($kg.m^{-1}$)	
	Winter	Summer
West coast		
Hannasbaai	0.95	0.35
Middelbaai	2.92	0.65
Britannia Point	1.71	4.28
Groot Paternosterpunt	1.62	3.08
Abdolsbaai	0.60	1.50
Tietiesbaai	0.26	2.69
Rooisteen	0.20	0.83
Jacobsbaai	0.84	3.81
Yzerfontein	0.37	0.22
Wintersteen	0.19	0.29
Melkbosstrand	0.59	3.69
Bloubergstrand	0.01	0.27
Cape Peninsula		
Mouille Point	2.83	1.67
Three-anchor Bay	0.24	0.24
Rocklands	1.02	0.49
Graaf's Pool	0.58	0.87
Sunset Beach	0.21	0.87
Camp's Bay	1.18	0.21
Oudekraal	0.83	0.16
Kommetjie north	8.79	1.48
Kommetjie Kom	0.93	2.92
Slangkoppunt	21.44	1.01
Soetwater pool	15.08	3.16
Soetwater south	10.10	8.74

	Biomass ($kg.m^{-1}$)	
	Winter	Summer
Misty Cliffs	0.48	0.83
Scarborough	8.10	7.81
Miller's Point	0.00	0.00
Glencairn	0.43	0.60
South-west coast		
Rooi Els	0.32	2.10
Pringle Bay	0.53	1.21
Hangklip	0.19	0.04
Silver Sands	0.53	1.12
Kleinmond	0.00	0.14
Harry's Bay	0.02	0.01
Sandbaai	0.28	0.88
Hermanus	0.14	0.06
Stanford's Cove	0.04	0.40
Danger Point	0.20	0.37
Franskraal	0.34	1.19
Pearly Beach	2.05	3.79

Table A-2 Extrapolated wet biomass of *Porphyra* in the eulittoral around each of forty sample sites during the winter and summer. Data from each sample site has been extrapolated to adjacent rocky shores.

	Extrapolated biomass (kg)					
	Reserves and restricted areas excluded		Reserves excluded		Total rocky shore	
	Winter	Summer	Winter	Summer	Winter	Summer
West coast						
Hannasbaai	5585	2062	5585	2062	5585	2062
Middelbaai	5257	1169	6717	1494	6717	1494
Britannia Point	1709	4283	3503	8781	3503	8781
Groot Paternosterpunt	1053	2004	9317	17724	9317	17724
Abdolsbaai	1223	3077	4175	10508	4175	10508
Tietiesbaai	627	6462	2430	25040	2430	25040
Rooisteen	264	1114	1494	6312	1494	6312
Jacobsbaai	27246	122771	40214	181206	48535	218703

	Extrapolated biomass (kg)					
	Reserves and restricted areas excluded		Reserves excluded		Total rocky shore	
	Winter	Summer	Winter	Summer	Winter	Summer
Yzerfontein	4925	2961	4925	2961	4925	2961
Wintersteen	4694	7342	4694	7342	4694	7342
Melkbosstrand	878	5536	878	5536	878	5536
Bloubergstrand	134	3313	134	3313	134	3313
Cape Peninsula						
Mouille Point	6932	4100	6932	4100	6932	4100
Three-anchor Bay	339	335	339	335	339	335
Rocklands	767	366	767	366	767	366
Graaf's Pool	727	1085	727	1085	727	1085
Sunset Beach	676	2832	676	2832	676	2832
Camp's Bay	6861	1214	6861	1214	6861	1214
Oudekraal	23556	4546	23556	4546	23556	4546
Kommetjie north	20666	3473	20666	3473	20666	3473
Kommetjie Kom	1111	3506	1111	3506	1111	3506
Slangkoppunt	26802	1266	26802	1266	26802	1266
Soetwater pool	10558	2214	10558	2214	10558	2214
Soetwater south	32309	27974	32309	27974	32309	27974
Misty Cliffs	1128	1943	1128	1943	1128	1943
Scarborough	8100	7810	8100	7810	207352	199928
Miller's Point	33	37	33	37	77	87
Glencairn	1264	1756	1264	1756	3578	4971
South-west coast						
Rooi Els	7577	50479	7577	50479	8903	59312
Pringle Bay	6489	14816	6489	14816	6489	14816
Hangklip	1955	440	1955	440	1955	440
Silver Sands	3262	959	3262	6959	5946	12683
Kleinmond	20	768	28	1090	28	1090
Harry's Bay	0	0	93	88	93	88
Sandbaai	1709	5446	2122	6764	2480	7906
Hermanus	619	254	619	254	1260	518
Stanford's Cove	728	7146	728	7146	728	7146
Danger Point	1862	3450	1862	3450	1862	3450
Franskraal	1889	6629	1889	6629	1889	6629
Pearly Beach	15053	27892	32564	60338	32564	60338

	Extrapolated biomass (kg)					
	Reserves and restricted areas excluded		Reserves excluded		Total rocky shore	
	Winter	Summer	Winter	Summer	Winter	Summer
Total						
west coast	53594	162095	84066	272281	92388	308778
Peninsula	141828	64456	141626	64456	343439	259839
south-west coast	41163	124280	59169	158453	64197	174416
overall	236586	350831	285083	495190	500024	744033