A TAXONOMIC, ECOLOGICAL AND NUTRITIONAL STUDY OF *PORPHYRA CAPENSIS* KÜTZING POPULATIONS FROM THE NAMIBIAN COAST

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ABSTRACT

The Namibian *Porphyra capensis* Kützing was studied as very little was known of its taxonomy, ecology, phylogeny or nutritional status. It was hypothesized that it was phlogenetically related to the South African *P. capensis* as they share common ancestry. DNA was extracted from thalli and partial sequences obtained from their 18SrDNA and ITS regions. GenBank sequences of interest were incorporated, alignments made and phylogenetic trees were generated using MEGA 5.1. The results showed the existence of a Namibian *P. capensis* clade, implying preliminarily that it is a subspecies. It was also hypothesized that the abundance (standing crop biomass and cover) of *P.capensis* along Lüderitz and Swakopmund shores would be similar because of similar environmental conditions created by the cold Benguela Current System. This was tested in tandem with another hypothesis that the frequeny of harvesting thalli from the field would not significantly affect annual yield as nutrients and propagules would constantly be replenished from the system. A best fit model describing the relationship between standing crop and percentage foliar cover of Namibian *Porphyra* was developed. Cover and standing crop biomass were not significantly different between Lüderitz and Swakopmund but standing crop biomass varied significantly with seasons (p=0.001). Harvesting frequency increased the mean annual yield (p=0.008). The regression power equation $Y = 2.5429x^{0.9932}$ with R^2 = 0.649 was proposed as the best model for estimating biomass (Y, g) from cover (X, %) in the field while being cognizant of the relatively low coefficient of determination which resulted from its patchy distribution and multilayering of thalli. The hypothesis that the nutritional content of *P. capensis* was comparable to that of lettuce was tested and laboratory cultures on the Namibian *P. capensis* life cycle were also initiated.

Nutritional analyses were carried out according to standard procedures and it was established that fibre and calcium were significantly higher in *P. capensis* (p=0.016 and p=0. 036 respectively) while fat was significantly higher in lettuce (p=0. 0026). Magnesium, phosphorus, crude protein, iron, carbon and β–carotene were not significantly different. The life cycle of *P. capensis* in laboratory cultures was completed. The study proposed a subspecies status to the Namibian *P. capensis*; assessed its nutritional content while opening up possibilities for its mariculture to conserve wild populations. The study contributes to the promotion of *P. capensis* as a 'health food'. Further taxonomic exploration of local *Porphyra* populations was recommended and a prudent use of the abundance model encouraged.

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LIST OF ABBREVIATIONS

AOAC	Association of Analytical Chemists
ITS	Internal transcribed spacer
ITS1	Internal transcribed spacer region 1 located between SSU and 5.8S rDNA
MFMR	Ministry of Fisheries and Marine Resources
PCR	Polymerase Chain Reaction
PSU	Practical Salinity Units
rDNA	Ribosomal DNA
SSUrDNA	Small Subunit of the ribosomal nuclear DNA (18S)

DEFINITIONS

Terms used in the dissertation defined in the study's context:

Archeospores: Spores formed by differentiation of a vegetative mature thallus cell (n) or conchocelis filament (2n).

Coriolis effect/ Force: The apparent deflection of a body of water in motion with respect to the earth, caused by the rotation of the earth and appearing as a deflection to the right in the Northern Hemisphere and a deflection to the left in the Southern Hemisphere.

Dioecious: Male and female gametangia on separate thalli

Epilithic: Growing attached to rock, stones or boulders.

Evolutionary distance: The number of base substitutions per nucleotide site that occurred since the separation of the species.

Gametophyte: In algae: the thallus that bears the gametangia (male and female gametes)

Genome: All the genes of an organism as contained in the nucleus or organelles such as mitochondria or chloroplasts.

Genetic variability: A measure of the tendency of individual genotypes in a population to vary from one another

Monoecious: Male and female gametangia on same thallus

Monophyletic: Groups that descended from a common ancestor including the founder species.

Neutral spore:	Asexual spores (n) formed by cell differentiation from a vegetative thallus.
Paraphyletic:	Groups that are derived from a common ancestor but, do not contain all the descendants of that ancestor
Photoperiod	The daily ratios of hours of light to hours of dark in a 24 hour period
Phylogeny:	The evolutionary history of related species / taxa.
Polyphyletic:	Groups that descend from several different ancestors / have different lines of descent
PSU	The practical salinity units scale that defines salinity in terms of the conductivity ratio of a sample, to that of a solution of 32.4356 g of KCl at 15°C in a 1 kg solution (standard for seawater is 35psu) same as parts per thousand (ppt/‰).
Subspecies	A taxonomic rank subordinate to species, where variation is due to geographical isolation.
Yield	The part of productivity utilized by humans or other animals.
Zygotospores (2n):	Spores produced by cell division of a zygote and on germination develop into the sporophyte phase (the conchocelis) in *Porphyra* species.

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DEDICATION

To my husband Frank; our children Juliet, Alan, Faith and Daniel for their support, encouragement, patience and LOVE. To my siblings for their encouragement.

To my God: “You are worthy my Lord and God, to receive glory and honour and power, for you made the whole universe; by your will, when it did not exist, it was created” (Rev 4: 11; The New Jerusalem Bible).

DECLARATIONS

I, Devota Kavishe, hereby declare that this study is a true reflection of my own research, and that this work, or part thereof has not been submitted for a degree in any other institution of higher education.

…… ……

Devota F. Kavishe Date

CHAPTER 1

1.0 INTRODUCTION

1.1 General introduction to seaweeds

Seaweeds are multicellular benthic marine macroalgae widely distributed throughout the world. They photosynthesize like terrestrial plants but differ morphologically and structurally. They have leaf like structures (thalli) within which photosynthesis takes place. They lack internal transport system made up of xylem and phloem vessels present in higher plants. Their thalli are immersed in seawater and as such materials move in and out of their tissues by diffusion. Seaweeds are not embryophytes and reproduction is facilitated by differentiated fertile cells within their thalli.

The distribution and abundance of seaweeds is influenced by factors such as availability of substrata for attachment, temperature, tidal amplitude, currents and wave action, nutrients, salinity, propagule availability and grazing pressure (Bustamante, Branch, & Eekhout, 1997; Cervin, Undergarth, Vlejo, & Aberg, 2004; Stegenga, Bolton, & Anderson, 1997). There are about ten thousand known seaweed species world-wide (Braune & Guiry, 2011). In a collection of benthic marine algae carried out in 1983 from three sites south of Swakopmund on the Namibian coast, 63 seaweed species were identified (Wayne, 1986). Lawson, Simmons & Isaac (1990), recorded 134 seaweed species from the Namibian coast, while Engledow, Bolton & Stegenga (1992), added 45 more species making a record of 179. More extensive excursions however need to be carried out to confirm the current status.

1.1.1 Classification of Algae

Algae in general form a polyphyletic group and the taxonomy of its various taxa is continuously being revised as more genetic and structural information is obtained in addition to established phenotypic characterization (Barsanti, & Gualtieri, 2006; Braune, & Guiry, 2011; Brodie, Mortensen, Ramirez, Russell, & Rinkel, 2008). The grouping of the algae in two kingdoms and eleven divisions by Barsanti & Gualtieri (2006) has been adopted in this study. This classification is based on phenotypic descriptions (morphological and anatomical) and life cycles. The types of pigments present, nature of photosynthetic storage products, organization of thylakoids, structure of cell walls, presence and structure of flagella are the main characteristics used. Following these criteria, the algae fall into two kingdoms; the kingdom Prokaryota eubacteria and the kingdom Eukaryota. Prokaryota eubacteria consists of 2 divisions namely Cyanophyta (blue green algae) and Prochlorophyta.

The Kingdom Eukaryota is made up of the divisions: Glaucophyta; Rhodophyta (red algae); Heterokontophyta; Haptophyta (coccolithophorids); Cryptophyta (cryptomonads); Clorarachniophyta; Dinophyta (dinoflagellates); Euglenophyta (euglenoids) and Chlorophyta (green algae). The division Heterokontophyta is made up of the classes; Chrysophyceae, Xanthophyceae, Eustigmatophyceae, Bacillariophyceae, Raphidophyceae, Dictyochophyceae and Phaeophyceae (brown algae). Taxonomic classification of various taxa currently incorporates molecular studies. Brodie & Lewis (2007); Brodie, Hayes, Barker & Irvine (1996); Broom, Jones, Hill, Knight & Nelson (1999); Broom, Nelson, Yarish, Jones & Aguilar (2002); Jones et al. (2004); Kunimoto et al. (1999a); Milstein & Oliveira (2005); Stiller & Waaland (1993); Sutherland et al.

(2011), established the taxonomic status of different *Porphyra* species by assessing their molecular phylogenetic relationships. A recent revision of the order Bangiales split the genus *Porphyra* into eight taxa namely; *Porphyr*a, *Boreophyllum* gen nov., *Clymene* gen. nov., *Fuscifolium* gen., *Lysithea* gen nov., *Miuraea* gen. nov., *Pyropia*, and *Wildemania* (Sutherland et al., 2011).

1.1.2 Economic importance of macroalgae (seaweeds)

The uses of macroalgae have been well reviewed (Barsanti, & Gualtieri, 2006; Dos Santos, & Mshigeni, 1992; Guiry, & Blunden, 1992; Mshigeni, 1992, 2001; Oliveira, 1992; Rabesandratana, & Rabesandratana, 1992; Turner, 2003; Vencatasamy, 1992; Wamukoya, 1992). Seaweeds are used for human consumption, as animal feed, in medicine, in processing industries, agriculture and forestry as well as for other commercial purposes. The genera utilized for human consumption have been listed by Barsanti & Gualtieri (2006) and include *Porphyra*, *Pyropia, Palmaria*, *Chondrus, Gracilaria*, *Gigartina*, *Callophyllis*, *Asparagopsis*, *Alaria*, *Laminaria*, *Undaria*, *Hizikia*, *Cladosiphon*, *Sargassum, Codium*, *Monostroma*, *Enteromorpha*, *Ulva* and *Caulerpa*.

Seaweeds have been used as flavorings to noodles, soups (Appendix 1) garnishes, incorporated in mixed vegetable dishes, served as snacks, as salads, or side dishes (Teas, Pino, Critchley, & Braverman, 2004). The same authors reported that in Japan 21 species of seaweeds are routinely included in the diet, whereas in Korea, 40 kinds of seaweeds are used as food while in Hawaii and Polynesian islands, 29 kinds of seaweeds are consumed. In the United States a study of marketing trends in 2003, reported that seaweeds had become common foods and food supplements. It was also established that 15% of Americans enjoy Japanese cooking which incorporated a variety of seaweeds

(Sloan, 2003). Seaweeds with medicinal applications from various parts of the world have been listed in Kiangi, (1992) and include members from the following genera; *Porphyra*, *Laminaria*, *Ulva*, *Caulerpa*, *Durvillea*, *Palmaria*, *Dictyopteris*, *Hypnea*, *Chondria*, *Acetabularia*, *Sargassum*, *Lithothamnium*, *Digenia*, and *Caloglossa*. Seaweeds containing agar, alginates and carageenan are harvested and these colloids industrially extracted for processing into useful products.

Agar, extracted from some species of red algae (Rhodopyhta) such as *Pterocladia*, *Gelidium* and *Gracilaria*, is used as a gelling agent (Barsanti, & Gualtieri, 2006; Critchley, & Rotmann, 1992). Alginates are extracted from the cell walls of some brown algae (Pheophyceae) and these have various industrial uses mainly in the textile industry, paper industry, in production of 'inks', paints, cosmetics, insecticides, pharmaceuticals, and as stabilizing agents in ice-creams and milkshakes (Barsanti, & Gualtieri, 2006). Alginates are obtained mainly from species of *Laminaria*, *Ecklonia*, *Macrocystis*, *Lessonia* and *Ascophyllum* (Barsanti, & Gualtieri, 2006). Carageenan is a polysaccharide extracted from *Chondrus*, *Euchema*, *Ahnfeltia*, *Gigartina*, *Hypnea*, *Kappaphycus* and *Iridaea* species for the production of special gels.

Jensen (1992), reported on the use of seaweeds in the production of livestock feed from *Ascophyllum*, *Laminaria*, *Fucus*, *Macrocystis* and *Nereocystis*. Fresh *Macrocystis*, *Gracilaria*, *Porphyra*, *Palmaria* and *Ulva* have been used as feed for various species of cultivated abalone (Barsanti, & Gualtieri, 2006; Dlaza, Manevelt, & Viljoen, 2008). The potential of seaweeds in agroforestry was discussed by Nshubemuki & Mshigeni (1992). It has been reported that coastal people in the United Kingdom, France and Argentina

collect; *Ascophyllum*, *Laminaria*, *Ecklonia*, *Fucus*, and *Sargassum* species cast ashore during high tide to be processed as fertilizers and soil conditioners (Barsanti, & Gualtieri, 2006). Mshigeni (1992, 2001) reported that *Eucheuma spp.* from Tanzania is exported for the extraction of carrageenan. Msuya, (2011) reiterated how seaweed value added products increased the profit margin to farmers where, I kilogram of seaweed powder was sold at US$ 6.7 compared US$ 0.2 – 0.3 for a kilogram of dry seaweed. The same author reported that the powder was also used locally to make soaps, jam, juices, lotions and a variety of products adding more value to the dried seaweed (see Plate 1.1; a-d).

The Western Indian Ocean (WIO) region (Tanzania, Kenya, Madagascar, Mozambique and Mauritius) has focused on producing value added products from seaweeds for utilization and trade (Msuya et al., 2014)

Plate 1.1 (a-d) *Eucheuma spp.* products; (a) seaweed powder and soap by Tusifemoyo women cooperative from Kidoti village; (b) jams and cakes from the Fujoni group; (c & d) flavoured mini soaps containing seaweed powder, from the Zanzibar seaweed centre at Paje, for sale at local hotels.

South African seaweeds have been exploited commercially (Anderson, Simons, & Jarman, 1989) and they include *Gracilaria*, *Gelidium*, *Ecklonia*, and *Laminaria.* The utilization potential of Namibian seaweeds has been studied (Molloy, 1990). Apart from some beach cast collection of *Gracilaria* sp. for agar extraction carried out by Taurus (Pty) Ltd in Rotman (1992) there's no report of any Namibian seaweed being utilized on a commercial basis. Currently, Taurus (Pty) is no longer in operation in Namibia but some *Gracillaria* sp beach cast is collected, dried and sold to undisclosed buyers (J.Fleidl, personal communication, August 2013).

Several species of *Porphyra* C. Agardh (Rhodophyta) are harvested from the natural populations or grown through mariculture for human food (see Plates 1.2 (a & b) in different countries and are generally known by a variety of names depending on the culture. They are known as Nori in Japan, Kim in Korea and Zicai in China, Limu pahe′e in Hawaii, Slake in Ireland and commonly as Laver or Purple Laver in Wales as reported in Anderson et al. (1989); Barsanti & Gualtieri (2006); Indergaard (1983); Mumford & Miura (1988) among others. The production of various species of *Porphyra / Pyropia*

such as *Pyropia yezoensis*, *Pyropia haitanensis*, *Pyropia leucosticta*, *Porphyra linearis*, *Porphyra umbilicalis* for human consumption by aquaculture, supports multimillion-dollar industries in Japan, China and Korea (Barsanti, & Gualtieri, 2006; Guiry, & Blunden, 1992; Mshigeni, 1992). Edible *Porphyra* species have been discussed in detail (Chapman, & Chapman, 1980; Darcy–Vrillon, 1993; McDermid, & Stuercke, 2003; Turner, 2003).

A complex polysaccharide 'Porphyran' has been isolated from *Porphyra* species, and it is claimed to have anti-allergy, anti-tumour, antioxidant and immuno-modulating as investigated by Bhatia et al. (2008); Jiang et al. (2012); Noda (1993) & Yamamotu, Maruyama & Takahashi (1987). The potential health benefit of this algal polysaccharide continues to be evaluated.

Porphyra capensis Kützing is one of the seaweed species occurring along the Namibian coast. It was first described by Kützing (1843) and belongs to the order Bangiales, family Bangiaceae and class Bangiophyceae. It was listed as having economic potential (Molloy, 1990) but it is not utilized locally yet. This study was thus undertaken to address some knowledge gaps about the species.

Plate 1.2 (a) Hoshi–nori; dried rectangular sheets of *Porphyra* sp. used to make sushi.

Plate 1.2 (b) Dried, flavored, crisp seaweed snacks from *Porphyra* sp.

1.2 Statement of the Problem

Edible *Porphyra* and *Pyropia* (formerly *Porphyra)* species are distributed in several countries of the world where they are either harvested from the wild or cultivated to be consumed locally or for commerce. There is potential for production and value-addition of local *P. capensis* for consumption and commercialization in Namibia. Such a venture would improve the livelihoods of local communities, especially coastal communities, if this potential comes to full realisation. Until now, very little is known about the general biology and nutritional value of this species and how it compares and relates to the neighbouring South African *P. capensis*. Nutritional studies carried out on different *Porphyra/ Pyropia* species have claimed high fibre and protein content as well as substantial amounts of vitamins and minerals (Barsanti, & Gualteri, 2006; McDermid, & Stuercke, 2003). Turner (2003) referred to *Pyropia abbottiae* and *Pyropia torta* food products as healthy. Namibian *P. capensis* is not eaten locally and its nutritional content had not yet been analyzed prior to the current study. Previous researchers on Namibian seaweeds referred to the Namibian *Porphyra* species as *P. capensis* (Engledow et al., 1992; Engledow, 2003; Molloy, 1990; Mshigeni, 1992 & Wayne, 1986), without taxonomic verification as it morphologically resembled the South African *P. capensis.*

The South African *P. capensis* had been taxonomically described (Graves, 1969; Stegenga et al., 1997), but the Namibian species had not. The morphological variations observed in Namibian populations of *P. capensis* thalli (personal observations 21st April 2009, Swakopmund; were similar to morphological diversities reported in the South African *P. capensis.* Interestingly, it has been reported that most *Porphyra* species have similar morphological variations making identification difficult (Blouin et al., 2007).

Promoting the use of biological resources has to go hand in hand with recommendations on management strategies so as to maintain a viable gene pool in the wild and avoid over exploitation. The ecological status of South African *Porphyra capensis* had been studied and management proposals on sustainable harvesting documented (Griffin, 1999). Such critical assessment of the Namibian *Porphyra* populations was lacking.

1.3 Objectives of the Study

The main aim of this study was to address some knowledge gaps about the Namibian populations of *Porphyra capensis* so as to disseminate the information to interested groups.

The specific objectives were:

1. To compare the genetic variability between *P. capensis* growing along the Namibian Coast and the South African *P. capensis* from Western Cape in S. Africa, by phylogenetic analyses of the nuclear rDNA region.
2. To determine the nutritional composition of Namibian *P. capensis* and evaluate its nutritional value for human consumption.
3. To compare the abundance of *P. capensis* between the southern coast (Lüderitz) and central coast (Swakopmund) of Namibia.
4. To investigate the effect of different harvesting regimes of *P. capensis* thalli on biomass production in the field.
5. To establish a predictive model of *P. capensis* abundance in the field from percentage cover and standing crop biomass data.
6. To initiate laboratory based growth cultures of the Namibian *P. capensis* for mariculture trials.

1.4 Research Hypotheses

The phylogeny of the Namibian *P. capensis* has not been established and information on its ecological and nutritional status is lacking. This study aims to test the following hypotheses.

1. Despite apparent morphological variations reported in *P. capensis*, the populations from the Namibian coast as well as those in Western Cape area of South Africa are phylogenetically related as they share common ancestry.
2. The nutritional content of the Namibian *P. capensis* is comparable to that of lettuce because they are both "leafy" vegetables.
3. The abundance of *P. capensis* in Lüderitz is equitable to that of Swakopmund owing to similar distribution of nutrient rich upwelled waters as they both belong to the same Benguela region.
4. Frequency of harvesting of *P. capensis* in the field, does not affect annual biomass production (yield) as nutrients and propagules are constantly replenished from the marine environment.
5. The biomass of *P. capensis* in the field can be estimated from its foliar cover since plant productivity is a function of its photosynthetic area.
6. The initiation of laboratory cultures of *P. capensis* for mariculrure trials can be achieved locally.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 The coastal ecosystem

The coastal area is ecologically unique as land meets ocean and the two systems merge harmoniously to form an interesting ecosystem where oceanographic, physical and biological factors interrelate. Oceanographic factors involve movement of water masses in the form of currents, waves and tides as driven by winds and other forces caused by latitudinal global positioning and subsequent movement of air masses. Water masses carry with them mineral elements and nutrients. Physical factors like topography, geology, temperature and light create microhabitats for fauna and flora. The micro climatic conditions are part of climatic patterns accross wider latitudinal ranges. Interactions between all these factors, the adjacent terrestrial environment and their biological components make the coastal ecosystem a suitable habitat for *Porphyra* species and other seaweeds.

The Namibian Coast in particular (see Figure 1.1) has a coastline of about 1570 kilometers (Robertson, Jarvis, Mendelsohn, & Swart, 2012) extending from the Orange River in the south to the Kunene River at the border with Angola, in a slight south-northwest orientation. It is a fairly straight coastline apart from a few bays. The coastline consists of 54% sandy beaches, 28% mixed sand and rocky shores, 16% rocky shores and 2% lagoons (Robertson et al., 2012). The sand is constantly shifting and thus provides a very unstable substratum for benthic organisms like seaweeds. The rocky substrates are however stable and appropriate as attachment surfaces. Rocky outcrops

that lie within the sub-tidal and intertidal zones provide substrata for seaweeds even though they are exposed only during low tides.

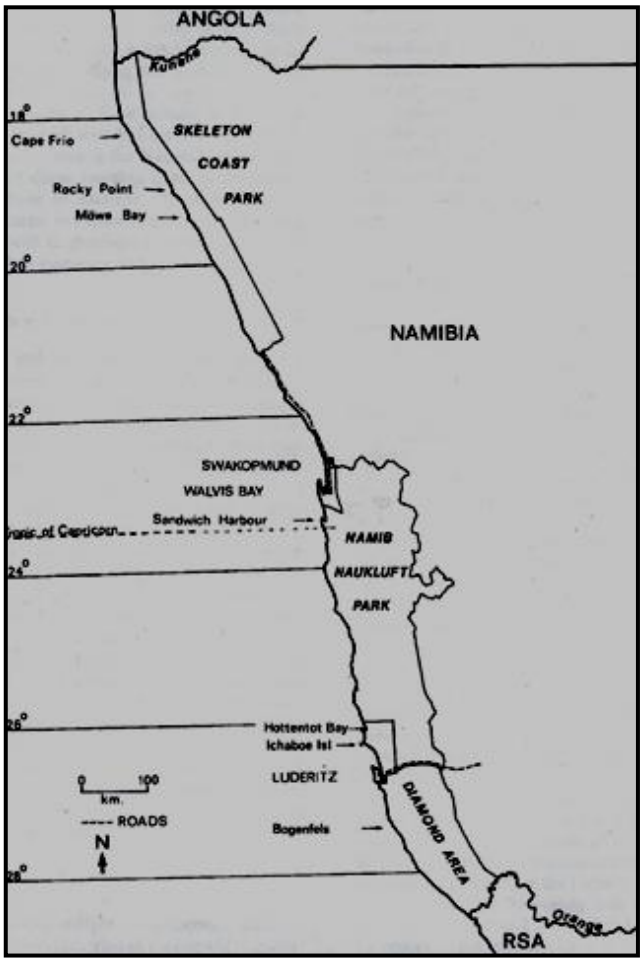

Figure 1.1 A sketch map of the Namibian Coast (extracted from Burke, 2004).

The Namibian coastal topography is believed to have been formed as a result of fragmentation of Gondwanaland that started some 135 million years ago separating Namibia from Brazil; and later modified by tectonic uplifts (Stegenga et al., 1997). The bedrock along the coast varies from hard gneisses and quartzites of the Namaqua metamorphic complex in some places to basic meta–sediment formations of the Gariep

group in others (Robertson et al., 2012). Apart from sandy shores there are a few salt pans, lagoons and estuaries along the coast. The continental shelf along the Namibian coast is about 50m–600m deep, while the width is between 100-140 km except at Cape Frio near the Angolan border where it is 90km wide (Bianchi et al., 1999). It is about 180 km wide off the Orange River delta (Robertson et al., 2012). The area of the continental shelf influences the nutrient load within coastal waters. Detritus, dead organisms and organic matter from benthic organisms within the euphotic zone are the source of the sediments on the continental shelf floor, from which nutrients are released.

2.1.1 Climatic conditions

Climate is an average of weather conditions in a specific geographical area over a period of time whereas weather is a summary of its attributes such as temperature, rainfall (precipitation), moisture and wind movement, assessed daily. Atmospheric air is always in motion due to unequal latitudinal heating of the atmosphere and the earth's surface by solar radiation. World climate falls under three main climatic groups depending on the predominant air masses (Smith, 1996). These include the tropical and equatorial air masses that lie within the tropics of Cancer and Capricorn. Their air masses are driven by winds within the Inter-tropical convergence zone (ITCZ). The polar air masses of the North and South Pole regions lie within the temperate zones. The air masses over the middle latitudes between the tropics and the Polar Regions are within the Subtropical High pressure zones. These three regions receive solar radiation at different angles containing varying amounts of heat, affecting local climate differently.

Mendelson, Jarvis, Roberts & Robertson (2009) stated that "Namibia is exposed to air movements driven by the three climatic systems; the Inter-Tropical Convergence Zone

the Subtropical High Pressure Zone and the Temperate Zone" (page 70). The Namibian coastal climate is however different from the interior of the country. This is caused by the effect of the two major high pressure systems: the South Atlantic Anticyclone system from the Atlantic Ocean and the Botswana Anticyclone system originating from the interior of South Africa (Robertson et al., 2012). The South Atlantic anticyclone is a high pressure cell that causes winds to blow up from the south and southwest upwards along the coast pushing the cold Benguela currents northwards along the coast, as well as blowing sand from the beaches to the interior forming the sand dunes. These winds carry cool air from the South Atlantic making the coastal area generally cool and windy.

The Botswana Anticyclone high pressure system on the other hand, controls air movements in the interior of mainland Namibia and is the source of the hot east winds (Berg winds). The Bergs travel at speeds of between 50–60 km/hr, from inland to the coast during winter carrying sand that causes destruction of biodiversity at the coast, where seaweeds are bleached due to sand friction and alleviated temperatures (Robertson et al., 2012).

The Namibian coastal climate is characterized by low rainfall (Mendelson et al., 2009). The cool moist air from the sea arising from the South Atlantic winds and the cool Benguela Current System does not rise higher above the warm air layer from the desert to develop into rain clouds but this moisture over the coastal shoreline forms low clouds or fog. Their moisture soon evaporates as the day gets warmer. Moisture carrying winds from the north and north eastern parts of the country that originate from warm moist air moving south and west of the ITCZ region carrying rain clouds, lose most of their water as the wind blows over the hot interior escarpments. Its water vapour evaporates limiting

the chances of forming rain clouds when the wind reaches the coast. Ambient temperatures along the Namibian coast are fairly mild throughout the year and the highest ambient temperatures along the coast are experienced during winter when the hot and sand bearing Berg winds blow fiercely to the coast from the escarpment (Robertson et al., 2012).

2.1.2 Wind action

Wind is moving air and it blows because of the differences in atmospheric pressure between two locations as it blows from areas of high pressure to areas of low pressure (Mendelson et al., 2009). The larger the difference in pressure between two points the stronger the wind will be. Warm air is at low pressure and as it rises it cools and its water vapour condenses to form precipitation. Wind action influences local climatic conditions of temperature and rainfall affecting local ecosystems by moving air masses (Smith, 1996).

The Namibian coast experiences strong winds originating from the high pressure South Atlantic Anticyclone moving easterly to the south west of the country then in a northern direction to blow over the coastal shoreline bringing cool air and winter rains in the south (Stegenga et al., 2007). These winds facilitate movement of the Benguela currents. The Bergs on the other hand move from the interior as easterly winds heading for the coast raising the temperature and causing aridity (Mendelson et al., 2009). Lüderitz and the mouth of the Kunene River experience the strongest winds along the Namibian coast from the Atlantic Ocean, and create the strongest upwelling cells.

2.1.3 Currents and upwelling events

An ocean current is a continuous movement of water generated by forces acting on the water such as wind, tides, temperature, Coriolis forces and differences in salinity. On the surface, currents are driven by wind while deep water movements are caused by water density and gravity as well as upwelling. The Benguela Current System is a shallow stream of cold water that forms part of a huge rotating current called a gyre, which flows anticlockwise around the Atlantic Ocean (Robertson et al., 2012). It is one of four major eastern boundary upwelling systems of the world that flow upwards along the western margins of continents (Hutchings et al., 2008). The Benguela Curent System is the major current along the Namibian coast that affects its ecosystem.

The Benguela Current System and its effects have been reviewed (Andrews, & Hutchings, 1980; Bartholomae, & van der Plas, 2007; Bianchi et al., 1999; Boyd, 1987; Nelson, & Hutchings, 1983; Shannon, 1985). The Benguela Current System can be subdivided into two; the first one starts from south of Angola between 14^{o}S and 16^{o}S to Lüderitz (26^{o}S) in Namibia and the second one starts from Lüderitz to the south-western coast of Africa, extending over the Agulhas bank in South Africa (Bianchi et al., 1999; Stegenga et al., 1997). The mean sea surface temperatures over the southern Benguela Current System, between Lüderitz and Sea point in Cape Town range between 11.5 and 14°C compared to the Central - Northern Namibian coast around Walvis bay which ranges between 12 and 18.4°C (Engledow & Bolton, 2003)

The Namibian coastal waters also receive some warm water intrusions from the Angolan counter current increasing the sea surface temperature within the Northern region. These micro-temperature gradient effects affect seaweed species diversity along the Namibian

coast. Benguela Niños occur when warm waters from the Angolan current intrude into the cold Benguela Current System, causing blooms of phytoplankton. This leads to lowering of oxygen levels in the water causing the death of marine organisms as reported in Robertson et al. (2012). El Niño per se is the unusual warming of cool near surface waters off the west coast of South America, causing a disturbance in coastal ecosystems (Diaz, & Markgraf, 1992)

Whereas the Benguela Current System is initiated by the South Atlantic gyre, the flow of water currents northwards is as a result of the South Atlantic Anticyclone high pressure system assisted by the low pressure generated along the coast as air moves over the hot desert (Stegenga et al., 1997). Upwelling along the Namibian coast occurs when strong northwards blowing winds across the ocean surface near the coast experience deflection to the left caused by Coriolis forces (Mendelson et al., 2009). The deflection of surface water away from the shore drags cool dense water from the deep, laden with nutrients to replace the deflected water mass (Figure 1.2).

The major nutrients that are upwelled are nitrates and phosphates; products of decomposed organic matter that had sunk to the bottom of the continental shelf. Rates of upwelling depend on the width and depth of the continental shelf, the local wind direction and strength as well as the coastal topography (Robertson et al., 2012, Stegenga et al., 1997). Currents affect coastal ecosystems as they modify local temperatures, salinity, as well as distribute nutrients and propagules.

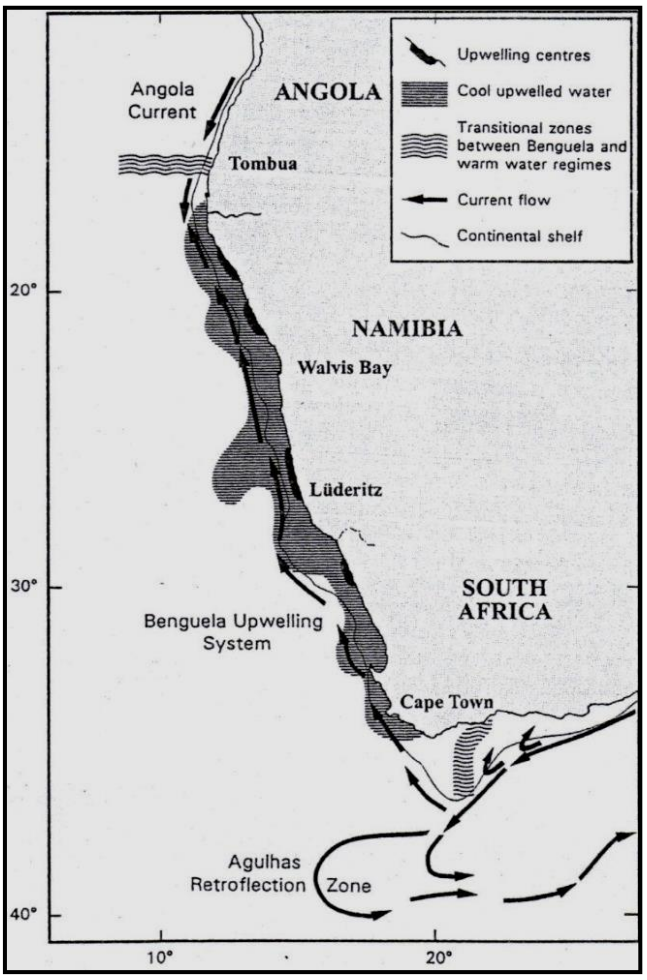

Figure 1.2 Map showing the upwelling currents and cells along the Namibian coast [Payne & Crawford (cited & adapted in Bianchi et al., 1999, p 2)].

2.1.4. Ocean nutrients and salinity

Nutrients in water bodies are derived from inflow or from the decomposition of organic matter within the sediments. Most sediments are deposited on the continental shelf and the wider and shallower the continental shelf the more sediments deposited and consequently the higher the nutrient levels (Stegenga et al., 1997). During upwelling nutrients are transported from the bottom waters into the surface waters. Algae within the

intertidal zone receive nutrients from such waters. Upwelling causes rapid growth of primary producers, resulting in high biological productivity (biomass).

The salinity of seawater is a measure of the concentration of dissolved salts in it. The salts are derived from eroded rocks in catchment areas and from the ocean's floor. Salinity is measured in practical salinity units (PSU) or parts per thousand (ppt) or $^{o}/_{oo}$ meaning the amount of salts in grams in a kilogram of seawater (Plaschke, & Morgan, 1999). Ecologically, salinity is important since the distribution of fauna and flora is dependent on their ability to physiologically adapt to local salinity fluctuations. Marine organisms are exposed to an osmotic pressure of 2MPa, but algae have higher internal, osmotic pressures of between. 2.6 - 3 MPa (Lüning, 1990), which allows them to uptake water and solutes from the sea to maintain their turgor pressure.

Extreme salinity values can interfere with physiological processes in organisms that do not have special osmoregulatory mechanisms. Sublittoral seaweeds tolerate salinities of between 0.5 – 1.5 times that of normal seawater while eulittoral and supralittoral seaweeds have a tolerance range of between 0.1 – 3.5 times of normal seawater (Lüning, 1990). This has enabled eulittoral seaweeds including *Porphyra* sp to survive local fluctuations in salinity resulting from varied periods of emersion during low tide. Salinity along Swakopmund ranges between 34.00 and 35.5 PSU (data from MFMR, 1999 – 2008).

2.1.5 Tides

Tides are the rise and fall of sea surface water levels about a reference point referred to as the mean sea level (MSL) and they are a natural phenomenon caused by the

gravitational attraction between the moon, the earth and the sun (Branch, & Branch, 1981). The same authors explain that spring tides occur during full moon and new moon as the earth is aligned with the moon and the sun and experiences a greater gravitational pull. Tides are referred to as diurnal when there is one high tide and one low tide within 24 hours but semi-diurnal when there are two high tides and two low tides (Stegenga et al,. 1997). The tidal zone descriptions in this document follow the classification by Stegenga et al. (1997). The high water mark reached during a spring tide is called the mean high water spring (MHWS) and tidal ranges are high. When the spring tides retreat the seawater level reaches the mean low water spring (MLWS) mark also called Chart Datum or mean low water level at spring tides. During the rest of the lunar cycle when the sun is not aligned with the moon and the earth, there is less gravitational pull towards the earth and as such the tidal ranges are smaller. These are the neap tides. The highest water mark during the neap tides is referred to as the mean high water neaps (MHWN) while the lowest levels are called the mean low water neaps (MLWN).

Tidal levels and cycles dictate the duration of exposure or submergence of the different zones on the intertidal shore thus affecting attached organisms (Branch & Branch, 1981). When storm surges cause water to rise above the normal high tide level, areas that should have otherwise been exposed are submerged and when atmospheric pressure is higher than normal, depressing the Chart Datum level, water is pushed away from the shore resulting in over-exposure of sublittoral seaweeds (Stegenga et al., 1997). The former interferes with spore release of eulittoral seaweeds as well as photosynthesis of sublittoral species due to elevation of the photic zone while the latter could cause bleaching and eventual destruction of seaweeds.

2.1.6 Vertical zonation

The upper layer of the sea where light penetrates is referred to as the euphotic zone and the one below it as the aphotic zone. The euphotic zone is further subdivided with respect to mean tidal levels such as the supralittoral (splash / spray) zone that extends from the MHWS tide mark upwards; the littoral zone, which is the main intertidal zone and the sublittoral zone (the lowest part of the shore, exposed only during extremely low tides. Lüning, (1990) and Stegenga et al. (1997) describe the eulittoral zone as the area just above the littoral zone below the splash zone. Different authors have used different terminologies to describe vertical divisions of the coastal shores.

Branch & Branch (1981) described zonation along the South African shores with respect to dominant fauna. The first zone is the Littorina zone which is occupied by *Nodilittorina Africana* Knysnaensis Phillipi snails and covers the upper eulittoral zone up to the splash zone. Barnacles and limpets occupy the Balanoid zone, found below the Littorina zone. The Cochlear zone, inhabited by the limpet *Scutellastra cochlear* Born on the other hand, lies below the Balanoid zone. The sea urchin (*Parechinus* sp) inhabits the sub littoral zone. Exposed rocky shores are inhabited by bivalves such as the mussel (*Perna* sp). This type of zonation as described by the authors above does not hold true for all rocky shores though some aspects of this description are true to zonation along the Namibian shore line as observed in the current study. Littorina snails, limpets and mussels are more abundant within certain intertidal areas as one ascends gentle slopes but in other areas they are absent.

Stengenga et al. (1997) while considering schemes of zonation along the South African coast by previous investigators (Stephenson et al., 1949 & Luning, 1990) summarized

zonation along a typical gentle slope rocky shore exposed to moderate wave action, into the supralittoral zone, the eulittoral zone and the sublittoral zone. In this description the supralittoral zone incorporates the area covered by sea spray to the upper most limit reached by waves and tides while the eulittoral zone represents the main intertidal belt, with an upper eulittoral zone characterized by patches of *Porphyra* sp and a lower eulittoral zone characterized by coralline red algae. The sublittoral zone is however, characterized by the kelp communities.The zonation along the Namibian coast has not been thoroughly described but the presence of the granular limpet (*Patella granularis* Linnaeus) from the middle intertidal to the high spring tide zones, along the entire coast was reported in Bianchi et al. (1999). The description of rocky shore zonation in Stegenga et al. (1997) is followed in the current study.

2. 1.7 Marine flora adaptations

Marine flora forms part of coastal ecosystems and its diversity as well as composition relies on its adaptation to dynamic environmental factors of temperature, nutrients, salinity resulting from emergence and submergence caused by tidal movements. The general distribution of algae at a global scale has been described very well by Braune & Guiry (2011) where taxa distribution follows a latitudinal pattern. Stephenson & Stephenson (1949) stated that upper zonal distribution limits of flora seem to be set by physiological tolerances of desiccation and thermal stress while the lower distribution limit is set by biological factors such as competition and predation. He found strong corelations between species distribution with physical factors (temperature, wave action and upwelling).

Lüning (1990) described an adaptive criterion to emersion where, seaweeds lose their water content like a ‘gelatin covered glass plate’ and that species with a small specific-surface to volume ratio (specific-surface), such as *Chondrus* sp. experience a reduction in desiccation damage as water loss is slow. *P. capensis* has monostromatic thalli and as such high specific-surface, meaning it can lose its water very fast. As an adaptation to conserve moisture it exists in densely layered clumps where one thallus provides cover for several thalli during emersion to reduce dessication (Appendix 2). Water loses beyond species’ specific critical water content during emersion lead to non-reversible damage to photosynthetic apparatus, but within the critical water content values, photosynthesis is restored with submergence (Lüning, 1990). Other algae reduce drying under desiccation and carry out photosynthesis during emersion by having a central cavity filled with seawater (Oates, 1985). Distribution of flora, therefore, depends on the inert mechanisms possessed to adapt to or escape desiccation at different zones. Other factors that influence distribution of flora along the shore are described below.

2.1.7.1 Effect of light

Light as an energy source, is another variable that comes to play in aquatic systems affecting the distribution of flora on intertidal shores. Light limits the depth to which algae can grow and it is also a signal for seasonality in seaweeds (Lüning, 1990). The amount of photon fluence light (irradiance) available to seaweeds at different depths varies and as such species are adapted to the spectral light available. Algae possess different photosynthetic and accessory pigments to facilitate photosynthesis at different ocean depths. Red algae contain Chlorophyls a & c; β-carotenes; xanthophylls as well as the phycobiliproteins namely; phycoerythrin, phycocyanin and allophycocyanin.

Phycoerythrin containins the red phycoerythrobilin as the chromophoric group; phycocyanin containing the bluish phycocyanobilin as the chromophoric group and allophycocyanin also contains phycocyanobilin. Red algae of the sublittoral zone however, have more phycoerythrin than phycocyanins while eulittoral red algae have more phycocyanins giving eulittoral red algae thalli like *Porphyra* species a more brownish-violet colour. The conchocelis stage of *Porphyra* species, found in shells within the sublittoral zone is pink in colour and as such use the dim bluish-green light dominating that zone using their phycoerythrobilin light receptor. Inappropriate irradiances with respect to zonation, cause photoinhibition which lowers photosynthetic rate and can cause photo damage to the photosynthetic system seen as bleached seaweed.

Light triggers the different developmental phases and changes in morphology experienced by higher plants in a phenomenon known as photoperiodism which means dependence on day length (Lüning, 1990). Day length varies with seasons in temperate regions. Photoperiodic reactions are induced in short day plants when day length falls below a certain value (critical day length) as perceived by phytochromes and in long day plants as soon as a critical day length is passed. Photoperiodism signals in seasonally induced physiological processes like gametogenesis. Laboratory induced photoperiodic response was demonstrated in *P. tenera* to show that phytochrome in the red alga mediates similar photoperiodic responses as in flowering plants (Dring, 1967b). Photoperiodism in seaweeds has been observed in species with heteromorphic life cycles where there is a seasonal switch between macroscopic or microscopic forms. This was observed in the alga *Bonnemaisonia hamifera* Hariot where in late autumn the filamentous phase produces tetraspores which then develop into the gametophyte (Lüning, 1990). The same author gives a list of photoperiodic responses in other

seaweeds (p 312). In vitro cultures of *Porphyra* species in the laboratory follow the photoperiodic inductions observed in vivo. The appearance and disappearance of the different development phases of seaweed flora at different sites along the shore, is an adaptive mechanism to variations in spectral quality and quantity.

2.1.7.2 Effect of temperature

High temperatures on the supralittoral zone and beyond, limit the diversity of fauna and flora to those adapted to heat stress since high temperatures denature proteins and cause damage to heat sensitive enzymes. Lower temperatures reduce the metabolic rate of life significant processes such as photosynthesis that requires a minimum quantum of energy. Freezing temperatures on the other hand lead to formation of ice crystals which rupture cellular membranes. Some organisms are adapted to extremes of temperature or have developed a mechanism of escaping extremes of temperatures (stress evasion). Organisms with a wide range of temperature tolerance are said to be eurythermal and are widely distributed; where as those with a narrow range are referred to as stenothermal species and are found only in specific regions (Lüning, 1990). There is temperature gradient between the suppralittoral zone and the sublittoral caused by light attenuation with depth.

Growth and reproduction in seaweeds are temperature sensitive processes (Lüning, 1990). Bolton & Luning (1983) reported on *Laminaria digitata* (Hudson) J. V. Lamouroux, *L. saccharina* and *L. heyperborea* as having an optimal growth and reproduction temperature of 15^{o}C. Mshigeni (1976) reported that *Hypnea cervicornis* J. Agardh has an optimal growth and reproduction temperature of 28^{o}C; where-as McLachlan & Bird (1984) reported that *Gracilaria coronopifolia* J. Agardh have an

optimal growth and temperature range of between 20 – 28^{o}C. Amosu, Robertson-Anderson, Kean et al. (2014) reported that *Ulva lactuta* Linnaeus could be cultured between 15 and 20°C. *Pyropia vietnamensis* Tanaka et P. –H. Ho tolerated a temperature range of between 10 ^{o}C and 30 ^{o}C depending on the developmental phase as well as other environmental parameters of irradiance, salinity and photoperiod (Ruangchuay & Notoya, 2002). The *Porphyra* gametophyte generally occupies the eulittoral and supralittoral zones and avoids heat damage during emersion by local shading of its thalli through folding and layering.

2.1.7.3 Competition and grazing pressure

Seaweeds tend to occupy sites within the shore where they have a competitive advantage. Stress tolerant species at the upper eulittoral zone, are confined to their stressful habitat with ample substratum for attachment, as well as light and nutrient flow during submergence. Such species could occupy less stressful zones but they would experience competition for space, light and nutrients from the majority of less stress tolerant seaweed species. Barnacles occupy the upper eulittoral zone as they can withstand desiccation and wave action unlike the mussels which occupy a lower zone as their desiccation tolerance is lower. Predators like limpets that avoid wave exposed areas would prey on barnacles and mussels in the intertidal zone, to maintain a population size of optimum carrying capacity. Faunal distribution also relies on their ability to withstand adverse abiotic conditions.

Porphyra species usually forms monospecific stands in the eulittoral and splash zones, where predators avoid as it is a stressful environment (Stegenga et al,. 1997). Sometimes however, predators like *Patella granularis* Linnaeus that occupy the littoral zone,

migrate to the splash zone to graze on microalgae and germlings of macroalgae such as *Porphyra* spp. to avoid competition in the littoral zone, as reported by Jernakoff (1985). Predator/prey population dynamics in marine coastal ecosystems are similar to terrestrial ecosystems.

2.1.8. Phenological patterns in seaweeds

Phenological patterns observed in living organisms are as a result of adaptations to seasonal changes of weather and climate as influenced by latitude or altitude (Smith, 1996). Temperature, rainfall, wind direction, tidal amplitude and photoperiod are variables that are affected by the seasons. Mathieson (1989) established that species richness of some seaweed communities within the Gulf of Maine exhibited seasonal variations, peaking in summer. Another phenomenon that followed seasonal patterns according to the author was gametophytic or sporophytic dominance. Similarly, there were reproductive, growth and longevity patterns following seasonal changes. In *Chondrus verrucosus* Mikami propagule release was reported to be correlated to tidal rhythms, which were influenced by seasons (Alecia, & Masakazu, 2006). Ang Jr. (2006) studied the phenology of *Sargassum* spp. from Hong Kong and reported that plant length reached maximum between January and March, the peak reproductive season was between February and March, plant die back after spore release peaked between March and May and rapid growth phase of laterals was observed in November.

The phenology and seasonal growth of *Porphyra linearis* Greville was investigated and it was observed that growth rate and reproduction cycles followed a seasonal pattern governed by varying climatic factors with seasons (Valera–Alvares et al., 2007). Understanding the phenology of a species is important in providing the optimum

conditions of temperature, salinity, photoperiod and irradiance for the various growth phases in the establishment of mariculture farms.

2.1.9 Seaweed productivity and growth rates

The annual primary productivity (carbon fixed per square meter per year) for most seaweeds is higher than their biomass (fresh/dry mass per meter squared of rocky area) as some of the carbon is lost when tissues erode as reported by Kirkmann (1984) where 20 kg m^{-2} in wet fresh weight was lost annually in the standing crop of *Ecklonia radiata* (C.Agardh) J.Agardh. Thallus material in other seaweeds is similarly lost by wave action, through grazing, or disintegration with age after spore release (personal observation). The standing crop biomass therefore does not give a 100% true reflection of productivity but it is a fair representation of material available for the next trophic level. The primary productivity of some seaweeds could be linked to that of dense terrestrial vegetation, where self-shading in dense vegetation such as that of sublittoral *Laminaria* species, leads to a decrease in standing crop values with increase in depth Lüning (1969b), Jupp & Drew (1974). This is similar to shading caused by canopy leaf area of forest communities (Waring, 1983). *Porphyra* thalli grow in layers on exposed rocks as an adaptation to reduce desiccation stress during emersion but this could affect the standing crop values of individual thalli, as a result of lowered photosynthetic rates of shaded thalli.

Several models have been proposed in an attempt to explain population dynamics with respect to available habitat resources. These models are true for terrestrial as well as marine ecosystems. Pianka (1970) proposed the r – K continuum model, which is a density dependent model where natural selection favours plants/seaweeds with traits that

enable them to occupy specific habitats during specific periods of population growth. In this model, natural selection favours different sets of adaptations when resources are limited and when in plenty. The r-selected species are pioneer species that have high intrinsic rate of growth, are small in size, have high rates of reproduction, high number of offsprings, (from high numbers of spores/seeds), short life cycles (annuals) and successful dispersal mechanisms; and these are adaptations to limited resources and unfavourable or fluctuating habitats.

K-selected species on the other hand have slow growth rates, are large in size (incorporate a large portion of their carbon production into their body biomass), produce fewer offsprings, make maximum use of the available resources and their population size is within the carrying capacity of the habitat. Such species are mainly perennials, such as kelp. The ratio of annual primary production to actual biomass is higher in r- strategists than K- strategists. Biomass of r- strategists is variable as environmental conditions vary unlike K-strategists that are well adapted to environmental fluctuations, build up their thalli and dominate the canopy (Lüning, 1990). *Porphyra* species on the other-hand can be considered r-strategists as they start to occupy bare rocks on the splash zone. *Porphyra* species are annuals; a lot of energy is channeled towards production of gametes and have both sexual and asexual life cycles (Blouin, Xiugeng, Peng, Yarish & Brawley, 2007) to ensure reproduction success and perpetuity of species.

Grime (1977) came up with the CSR model which stands for competition, stress and ruderal (disturbance), where plants/seaweeds have evolved adaptations for continued existence in a variety of habitats. Successful plants/seaweeds have evolved coping mechanisms in environments where conditions are so stressfull that they restrict

production (shortages of light, nutrients, sub-optimal temperatures); or perturbed habitats where biomass is destroyed by herbivores, pathogens or man's influence. In this model survival depends on having a competitive edge as well as resistance or tolerance to stressful conditions. Any of the above strategies can be used to explain the presence of seaweed (or a phase in a seaweed life cycle) along the coastal ecosystem.The presence of *Porphyra* thalli on the rocks throughout the year means that their strategy for a viable population is to produce several generations in a year.

The relative growth rate for seaweeds (*R*), assuming no loss of plant material, can be calculated using the formula below which measures change in weight over time (Evans 1972 as cited in Lüning 1990 p 363).

$$R = \frac{lnW2 - lnW1}{T2 - T1}; \qquad \text{[Equation 1]}$$

Where: W1=Weight at time T1

W2 = weight at time T2

The fact that annuals have higher growth rates, shorter turnovers and higher production rates than perennials is important when planning which seaweed should be promoted for mariculture.

2.1.9.1 Abundance assessments

Species abundance, assessed from cover, density, frequency or biomass of individual species is a reflection of how well the environment meets the species' needs. A decline in abundance of a particular species in a community is an indication of environmental degradation which could lead to species extinction if not well managed.

Terrestrial plant biomass assessment generally involves clipping, weighing and drying of 'harvested material'. This is a more accurate method of estimating production. Cover is sometimes used as a substitute measure for biomass even though the correlation between cover and biomass is perceived to be low, it is fast and non-destructive (Chen, Shiyomi, Bonham et al., 2008 & Chiarucci, Bastow, Anderson et al., 1999). A relationship between biomass and cover will be investigated in the current study.

2.1.10 Seaweed harvesting

Harvesting natural resources is a common human activity throughout the world. The ecological challenge however is sustaining a viable population of the natural resource in the wild while meeting needs. For r-selected species, harvesting loses can be rapidly replaced if conditions are favourable, because of their rapid population growth. It is however different for K-selected species as they take longer to recover from harvesting loses due to slow growth rate. Harvesting levels for individual species should be carried out at sustainable yields determined by prior ecological studies on turnover and carrying capacity. Maintaining a sustainable yield of a resource requires making allowances for unexpected disturbances. A resource can be driven to extinction if management strategies do not consider ecological information on growth rates and regeneration capacity.

In some Asian traditions seaweeds are harvested from wild populations for local consumption and trade. In Hawaii, 18 species of seaweeds worth US$ 38,849 were collected in the year 2002, for home consumption (McDermid, & Stuerke, 2003). The effect of harvesting *Porphyra* thalli from wild populations was studied (Nelson, & Conroy, 1989; Roland & Coon, 1984; Griffin, Bolton, & Anderson, 1999) and

recommendations were made on how to maintain sustainable populations in the areas where these investigations took place. Harvesting of seaweeds for local or commercial use has not been reported in Namibia, but this study looked at the effect of harvesting frequency to annual yield in anticipation of its future exploitation.

2.1.11 Phylogenetics

Phylogenetics is a science that makes inferences on evolutionary relationships among groups of organisms. This relationship is described in terms of relative recency of common ancestry (Harrison, & Langdale, 2006). In molecular phylogenetics, molecular sequencing data (DNA or proteins) are used to explore such relationships and the information is portrayed in phylogenetic trees (Baldauf, 2003). Phylogenetic trees are pictorial illustrations (in the form of branching trees) of the historical order in which evolutionary events are supposed to have taken place within species or populations of interest. Hereditary materials such as DNA naturally and spontaneously can undergo variations due to mutations and these variations are retained and passed on through generations. Mutations can be detected in sequences during phylogenetic analyses. Such variations can lead to intraspecific diversity, speciation or extinctions in a population. Phylogenetic information is thus important in taxonomic evaluations.

The main phylogenetic relationships as explained in Harrison and Langdale (2006) are monophyly, paraphyly and polyphyly. They describe monophyletic group members as descending from a single ancestor whereas polyphyletic members as being grouped together as a result of convergent evolution, not sharing a unique common ancestry since each has a different most recent ancestor. Paraphletic groups are similar to the monophletic group in that they descend from a single ancestor but a lineage (some

descendants) is excluded. In a phylogenetic tree, branches meet at nodes where a node represents the last common ancestor of the branches arising from it such that monophyletic groups will share one node and the whole group is referred to as a clade (Baldauf, 2003). In producing a phylogenetic tree a multisequence analysis (MSA) of taxa/specimens in question is carried out, and homologous sequences in the alignment would mean they have evolved from a common ancestor and will form a clade. The order Bangiales is reported to be monophyletic (Milstein, & Oliveira, 2005) but the genus *Porphyra* is polyphyletic (Nelson, Farr & Broom, 2006).

Ribosomal DNA of eukaryotes, consists of a tandem repeat unit segments (clusters of repeated sequences) where 18S (SSUrDNA), 5-8S and 28S genes (coding) are separated by internal transcribed spacers (ITS1 & ITS2 that are non-coding) and an intergenic spacer (IGS) which is also non coding (see Figure 2.1), (Zagoskin, Lazateva, Grishanin & Mukha (2014). In their article they refer to the coding regions as being highly conserved among species and that comparison of their sequences could allow an estimation of evolutionary distances between generic and higher taxonomic ranks. ITS regions are however considered highly variable due to indels and point mutations such that they only infer phylogenetic relationships at species and population levels. In their study, the phylogenetic relationships among Copepods at higher taxa were resolved by 18S and 28S genes whereas the ITS2 gene resolved relationships at species level.

The nuclear small subunit (SSUrDNA) is thus a conservative gene that accumulates mutations quite slowly and similarity in sequences would definitely infer common ancestry as recounted in Broom et al. (1999) as well as Zagoskin et al. (2014). Similarly, the ITS region, is considered to evolve faster than the SSUrDNA and the presence of

areas of high variability are analyzed to discriminate closely related taxa as discussed likewise by Ding, Wang, H. Xu, L. Xu & Zhou (2000) and Milstein & Oliveira (2005). These two regions have been explored in phylogenetic studies of *Porphyra* species (Broom et al., 1999; Broom et al., 2002; Jones et al., 2004; Kunimoto et al., 1999a; Li, Shen, He, Xu, & Wang, 2009; Milstein, & Oliveira, 2005; Muller, Oliveira, Sheath, Bhattcharya, 2001; Oliviera, & Ragan, 1994).

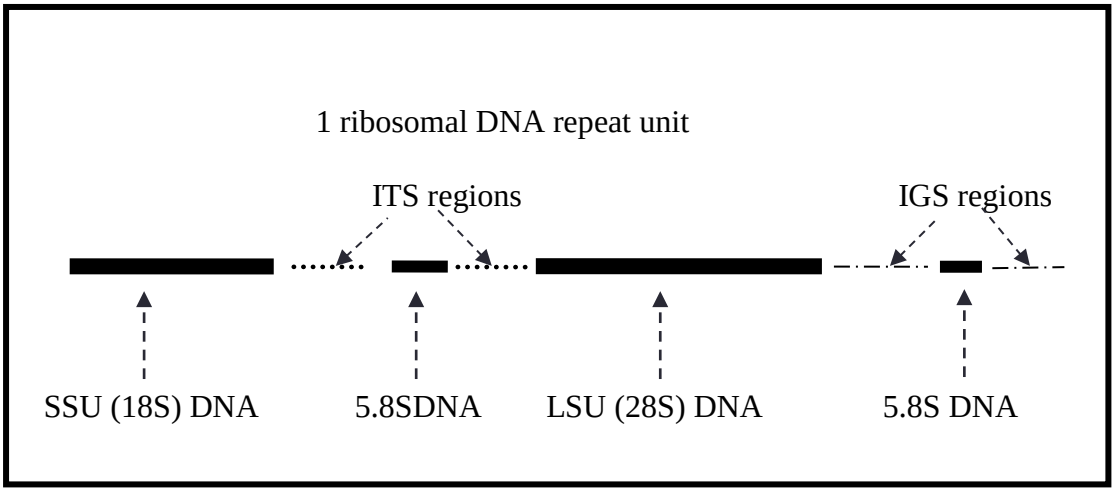

Figure 2.1 Nuclear ribosomal DNA repeat unit showing the coding regions (18S, 5.8S & 28S) with non-coding ITS and IGS regions (modified from Li et al., 2009 & Zagoskin et al., 2014).

2.2 The genus *Porphyra* C. Agardh

2.2.1 General description

The genus *Porphyra* C. Agardh as described in Agardh (1824) consists of membranous monostromatic or distromatic thalloid marine plants, whose cells have one or two stellate

chloroplasts and a large central pyrenoid. The *Porphyra* 'plant' is the thallus (the haploid gametophyte) where plants are either monoecious or dioecious depending on the species. In dioecious plants the mature female plants' margin is intense red because of the presence of the pigmented zygotospores within the zygotosporangia (carposporangia). The mature male plants' margins are light in colour (yellowish) as the spermatia within the spermatangia are colourless due to loss of chlorophyll during spermatogenesis (Hoek van den, Mann & Jahns 1995). The distribution of spermatangia and zygotosporangia within the thallus in monoecious plants vary among species.

Most *Porphyra* species have similar simple morphological characters making identification very difficult (Blouin et al., 2011). The thallus morphology varies among its species ranging from lanceolate, cordate, linear, reniform, laciniate, ovate and umbilicate. Sometimes morphologies within a species vary as a result of individuals' position on the intertidal zone, shade, as well as the effect of water motion (personal observations 21st April 2009, Swakopmund). Individual thalli growing on the rock platform or boulder surface, exposed to high illumination and subjected to long emersion periods are tough, more rosette like or umbilicate. Their holdfasts are strong and firm. Individuals growing within the intertidal range where water motion is high tend to have thalli that are more linear, laciniate or lanceolate. Individuals growing in the shade, within crevices, where water action is gentle, have thalli that are large; cordate or reniform.

The thallus margins for the genus are entire, ruffled or dentate. Thallus colour varies, depending on type of species, position on the intertidal zone, and maturity; where, juveniles are more olive green while adults have brownish/ purplish hues as described by

Barsanti & Gualtieri (2006) as well as Hoek van den, Mann & Jahns (1995). The size of thalli in this genus ranges from a few mm to 3m in length and 20 µm to 150 µm in thickness (Stegenga et al., 1997). Guiry & Guiry (2006) recorded 267 species of *Porphyra* C. Agardh in the 'www.AlgaeBase.org' species database.

The taxonomy of the genus *Porphyra* C. Agardh is challenging because of its phenotypic plasticity. Graves (1969), described the different morphological forms of the species *P. capensis* from South Africa as well as the type, size and number of male and female reproductive cells which seemed to be species specific. It has since then become apparent that what was described then as *P. capensis* was in fact several *Porphyra* species namely, *P. capensis, P. saldanhae, P. gardneri*, and *P. sp*. indet (Stegenga et al., 1997). Dlaza (2011) described new *Porphyra* species from the South African West Coast and these are *P. ramiculata*, *P. aurifolia*, *P. gravesiae*, and *P. chameleona.* The epiphytic *P. aeodis* N. J. Griffin, Bolton, & Anderson on the other hand was described by Griffin, Bolton, & Anderson, (1999c). *Porphyra* is described as dioecious, with thalli up to 50 cm long and between 100-150 µm thick (Stegenga et al., 1997). The order Bangiales has recently been revised where foliose taxa previously placed in the genus *Porphyra* are now placed in eight genera including *Porphyra* and a number of filamentous genera are now recognized (Sutherland, Lindstrom, Nelson, Brodie, Lynch et al. 2011). *P. saldanhae* and *P. aeodis* now belong to the genus *Pyropia.*

2.2.2 Life history and reproduction in *Porphyra* C. Agardh

The life history of the genus *Porphyra* C. Agardh has been well documented by many authors including; Abdel-Rahman (2005); Cole & Conway (1980); Drew (1949); Drew (1954); Tseng & Chang (1955a) & Tseng & Sun (1989). It mainly involves a

heteromorphic alternation of generations where the gametophytic phase also referred to as blade, thallus or foliose phase alternates with the microscopic, filamentous, conchocelis phase; the sporophyte. This was first established by Drew (1949) in *P. umbilicalus.* The foliose phase is haploid whereas the conchocelis phase is diploid as established by Tseng & Sun (1989) reporting that, the chromosome number in the vegetative thallus cells and in spermatia was haploid whereas zygotospores, conchocelis filaments and concho-sporangial cells were diploid. Meiotic stages were observed in the germinating conchospores.

Reproduction in *Porphyra* species is either asexual, sexual or both (Blouin et al., 2007; Hawkes 1978; Nelson et al., 1998; Nelson & Knight, 1995; Nelson et al., 1999; Notoya, 1999; Tseng & Chang, 1954; 1955b). Sexual reproduction was reported in *P. umbilicalis* (Drew, 1949, 1954), *P. gardneri* (Hawkes 1978), *P. leucostica* (Yarish et al., 1998), *P. purpurea* (Nelson et al., 1998) and *P. suborculata* (Nelson et al., 1998). Hawkes (1978) gave an interesting account of sexual reproduction in *P. gardneri* where a spermatium was seen attaching on a prototrichogyne, followed by emptying of its contents into a carpogonium via a fertilization canal. Resulting zygotospores and conchocelis had diploid chromosomes confirming sexual reproduction. Asexual reproduction takes place in some *Porphyra* species where, a vegetative cell differentiates to release a spore like product referred to as archeospores in Blouin et al. (2011); neutral spores in (Blouin et al., 2007).

Blouin et al. (2011), while studying *P. yezoensis*, reported that archeospores responsible for asexual reproduction develop from juvenile thalli. Other names given to asexual spores in literature include agamospores (Kornmann, 1994) and endospores (Nelson &

Knight, 1995). Asexual spores directly regenerate the foliose phase. Kornmann (1994) used life histories (sexual and asexual) together with characteristics of reproductive features (spores) as a basis of taxonomy and classification of *Porphyra* species from Helgoland.

Asexual reproduction through vegetative propagation by tissue culture was demonstrated in Notoya (1999) where tissues from mature thalli, as well as tissues from juvenile thalli developed into the foliose phase. While *Eucheuma, Kappaphycus* and *Gracilaria* can be propagated vegetatively (one–step farming), *Porphyra, Ulva, Laminaria* and *Undaria* cultivation has to start from spores (Pereira, & Yarish, 2008). *Pyropia yezoensis* **(**Ueda) *comb nov.* is capable of reproducing via sexual and asexual pathways (Notoya, 1999). The evolution of parthenogenesis has been linked to 'male' limitation (Schwander, Vuilleumier, Dubman & Crespi (2010). This could imply that, the reduction in the number of male thalli in populations of dioecious *Porphyra* species could have led to the unique reproductive biology of native *P. umbilicalis* from the gulf of Maine, which reproduces solely by asexual (neutral) spores (Blouin, 2007).

The sexual reproduction phases of *Porphyra* species can be summarized as reported in Dlaza, (2011) & Graves, (1969). Spermatia (male gametes) are produced in the spermatangial area of the blade in male sori. Differentiation of the vegetative cells in the female gametangial area of the blade produces female sori (zygotosporangia). The zygotosporangia are made up of female gamete cells (each referred to as a carpgonium). The non- motile spermatia released from the blade by dissolution of its margin, move randomly in the water. When a spermatium settles on a female gamete cell it fuses with the tip of the trichogyne (Appendix 3) where a channel is enzymatically opened to allow

the spermatium's nucleus to enter and fuse with the nucleus of the female gamete producing a zygote. The zygote divides mitotically to form diploid zygotospores (see Plate 2. 1). Zygotospores are released by dissolution of the blade margin and on germination become the conchocelis. With further development the conchocelis filaments become "fat filaments" the conchosporangia. The conchosporangia are septate and each cell forms a spore, the conchospore. Upon maturity the conchospores are released and each develops into a haploid gametophyte (thallus).

In the review carried out by Nelson et al. (1999) on the reproduction of *Porphyra spp.* the existence of a blade reproduction phase from either asexual or sexual spores was confirmed. It was reported in this review, that blade archaespores and neutral spores, germinate directly into the blade phase. Other types of asexual spores described were haploid agamospores that are formed by the blade cells, but on germination form haploid conchocelis filaments.

The different growth and developmental phases in the life history of *Porphyra* species in culture require different environmental conditions depending on the species under investigation (Dlaza, 2011; Lopez-Vivas, Pacheco-Ruiz, Riosmena-Rodriguez, & Yarish, 2011; Stekoll, Lin, & Lindstrom, 1999). The current study adopted some of the optimum conditions established by (Dlaza, 2011; Pereira, Pinto & Yarish, 2004; as well as Stekoll, et al., 1999), to culture Namibian populations of P. capensis from zygotospores.

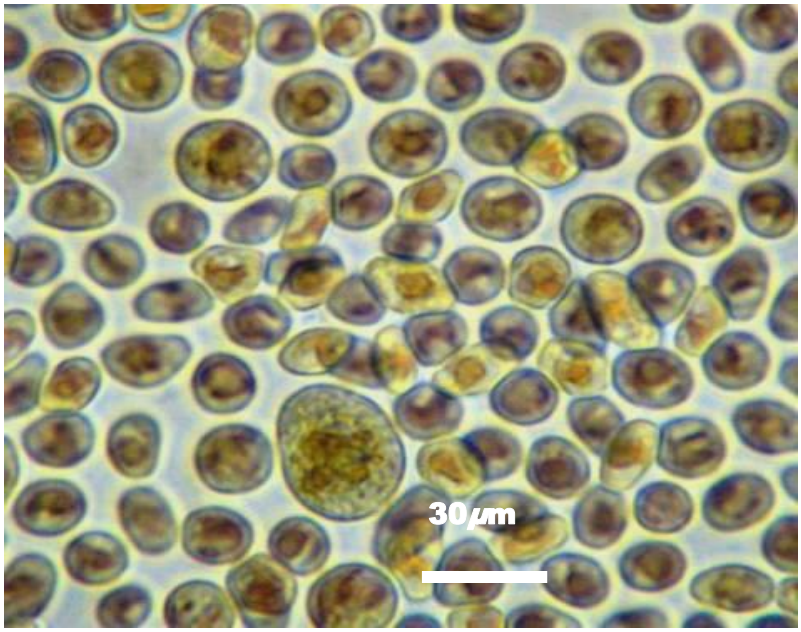

Plate 2.1 Different sizes of *Porphyra capensis* spores released from fertile thalli; zygotospores (small) and archaespores (large) (Dlaza, 2011)

It has been reported that conchosporangia cells, undergo meiosis, producing haploid conchospores (Hawkes, 1978). Sometimes diploid conchocelis filaments produce diploid plantlets that release conchocelis diploid spores (conchocelis archaespores) which later develop into more diploid conchocelis filaments (Nelson, et al., 1999). Understanding the life history of a *Porphyra* species is valuable information for breeders and farmers (Blouin, et al., 2011).

2.2.2.1 Mariculture

Mariculture is the cultivation of marine organisms in seawater within tanks, ponds, ocean bays and ocean lagoons, by artificial manipulation of their life cycles. It is a multi-billion dollar industry in Japan, Korea & China. Optimum conditions of light, temperature, nutrients and photoperiod as well as management of diseases and weeds are important

factors that are addressed in successful mariculture. Availability of appropriate substrates is crucial for organisms that require firm attachment.

The history of *Porphyra* farming in Japan began in the 17th century where farmers would place bamboo branches under mature thalli to collect spores. The seeded bamboo branches (substrate) were then placed strategically in nutrient rich estuaries for blade growth. This method was later improved by tying seeded synthetic nets onto bamboo poles to increase the substrate surface area. The later method increased production substantially. In China, *Porphyra* thalli were plucked off the rocks to clear the rocks for attachment of new spores and growth of new blades. As the demand for this seaweed and its products outstripped supply, mariculture became the alternative way to increase production.

The discovery of the conchocelis phases in *P. umbilicalis* by Dr. Kathleen Drew in Drew (1949), allowed researchers to manipulate the life cycle for high scale commercial cultivation of *Porphyra* under controlled conditions. In general the mariculture of *Porphyra* species starts indoors within laboratories where zygotospores are released from fertile female thalli. The zygotospores germinate into conchocelis filaments and the latter are reared in special nursery rooms until conchospores are produced within the fat filaments; the conchosporangia. On release the conchospores are seeded on to nets that are then out planted, stored or sold. In the laboratory seawater enriched with nutrients, trace elements and vitamins is used to rear spores, the conchocelis and the conchospores. A variety of media are available in literature but the most popular is the Provasoli enrichment seawater medium (PES) in Provasoli (1968); as well as other formulations such as the West and McBride (1999) which is a modification of the PES (Appendix 4).

In all the media formulations, seawater is enriched with macronutrients (nitrates and phosphates), trace metals (zinc, cobalt, manganese, iron as well as with vitamins B12, thiamine and biotin). The addition of germanium dioxide (GeO_2) at optimum concentrations is sometimes added into working media to prevent the growth of opportunistic diatoms (Dlaza, 2011, Markham, & Hagmeier, 1982, Stekoll et al., 1999). Sometimes antibiotics are added into media to control bacterial contamination (Iwasaki, 1961).

The conchocelis can also be multiplied vegetatively for mass production of conchospores. Iwasaki (1961) grew conchocelis in liquid media as well as solid media enriched with $CaCO_3$ and agar. Good growth of conchocelis took place in both types of media implying that $CaCO_3$ was not necessary in mass production of conchocelis. Currently breeding experiments are taking place to come up with fast growing and disease resistant cultivars as well as cultivars with preferred flavour and texture (Blouin et al., 2011). The conchocelis of desired strains are mass produced and then cryopreserved until needed (Taylor & Fletcher 1999; Wenjun, Yun & Jixun 2007). The conchocelis phase is sub cultured by transferring pieces of filament into new media every two months, to refresh stock as carried out by Iwasaki (1961)

Cultivation of *Porphyra* for "nori" in North America began in the 1970's through technology transfer from Japan and Korea (Mumford, 1990). The current study used the West and McBride (1999) nutrient media formulation for Namibian *Porphyra* culture trials, adding Germanium dioxide to prevent the growth of diatoms and Penicillin as an antibiotic.

2.2.3 Distribution of *Porphyra C. Agardh*

Most *Porphyra* species grow epilithically from within the eulittoral zone to the supralittoral zone of temperate coastal waters. A few are epiphytic on other seaweeds between the sublittoral and eulittoral zones or epizoic on mussels and oysters (Stegenga et al., 1997). The natural distribution and abundance of *P.capensis* in Namibia is constrained by unavailability of suitable rocky substrates. Presence of grazers like limpets that feed on *Porphyra* germlings also limits its local distribution. In South Africa *P. capensis* grows more prolifically on the west coast due to the cooler waters of the Benguela Current System (Graves, 1969). The Namibian coast likewise lies within the cool Benguela Current System whose sea surface temperature is within the optimum range for *Porphyra* growth.

The distribution of *Porphyra* spp. in Angola was described by John, Van-Reine, Lawson, Kostermans & Price (2004); and in South Africa by (Anderson, Bolton & Stegenga (2005); Bolton & Anderson (1990); Graves (1969); John, Price, Maggs & Lawson (1979); Silva, Bason & Moe (1996) and Stegenga et al. (1997). In Namibia the distribution of *Porphyra* species was described by (Engledow & Bolton (2003); Lawson, Simmons & Isaac (1990); Rull-Lluch (2002). The current distribution of *Porphyra* species in Namibia is not the subject of discussion in this study but a limited survey was carried out prior to setting up the study sites.

2.2.4 Nutritional content of some *Porphyra* species

Porphyra species have thin monostomatic blades that present a high surface area to volume ratio for easy diffusion of elements from the seawater. There are also anionic

functional groups within the cell walls of seaweeds in general, that offer binding sites for mineral elements accounting for the high mineral content in seaweeds (Davis, Volesky, & Mucci, 2003). Some trace elements such as iron, iodine, manganese and copper, have been determined in edible seaweeds (De la Rocha, Sanchez-Muniz, Gomez-Juaristi, & Larea-Marin, 2009). *Porphyra* species are considered among the most nutritious seaweeds, containing proteins, vitamins, minerals, fibre as well as trace elements (Barsanti, & Gualtieri, 2006; Chen, 1999; Dlaza, 2011).

Some *Porphyra spp.* have been studied and nutritionally characterized by different authors over the years, (Darcy-Vrillon, 1993; Lahaye, 1991; McDermid & Stuercke, 2003; Turner, 2003). Darcy-Vrillon (1993) reported that *Porphyra spp.* have low levels of saturated fats. *Porphyra* ('nori') contain a high percentage of Vitamin B12 (Watanabe et al., 1999). Lahaye (1991) investigated soluble and insoluble dietary fibre content in seven edible seaweeds including *Pyropia tenera* (Kjellman) comb nov. and concluded that they are rich in dietary fibre (32.7 – 74.6% dry weight). The nutritional composition of 22 edible Hawaiian seaweeds was assessed and *Pyropia vietnamensis* (Tak. Tanaka et P.H.Ho) J. E.Sutherl.et Monotila comb. Nov. had a 16.5% protein content relative to total dry mass, 4.4% crude lipid and an energy content of 2770 cal per gram dry mass (McDermid, & Stuercke, 2003). Turner (2003) carried out the nutritional analysis of some *Porphyra* species and hailed the high amounts of proteins, vitamins and mineral salts present in their thalli.

Porphyra proteins are made up of 17 types of amino acids (Sánchez-Machado, López-Cervantes, López-Hernández, & Paseiro-Losada, 2004) and this is comparable to soybean proteins. High levels of the amino acids: alanine, glutamic acid and glycine are

responsible for the unique flavor of 'nori' (Barsanti & Gualtieri, 2006). *Porphyra* is also rich in iron (Shaw, & Liu, 2000) and other trace elements (Noda, 1993). Mumford & Miura (1988) reported that *Porphyra* contain high levels of Vitamin A (as beta-carotene), Vitamin B group vitamins and high levels of Vitamin C in fresh samples. The nutritional content of some South African *Porphyra* species were studied (Dlaza, 2011) and *P. capensis* was found to contain 4.55% N, 37.8% C, 0.24% Ca, 0.42% Mg and 0.73% P of dry matter, in addition to 13.87mg /100gDM of Fe. The quantities of some nutritional elements in Namibian *Porphyra* thalli were established in this study and compared to that of lettuce. Lettuce was chosen as it is leafy like *Porphyra* spp, and it is popular in Namibia such that when *Porphyra* is promoted as a health food, people will have a familiar food item to make reference to.

CHAPTER 3

3.0 MATERIALS AND METHODS

3.1 Description of the study area

3.1.1 Location and geology

Figure 3.1 Map of Southern Africa showing the study sites in Namibia and South Africa (Appendix 5a)

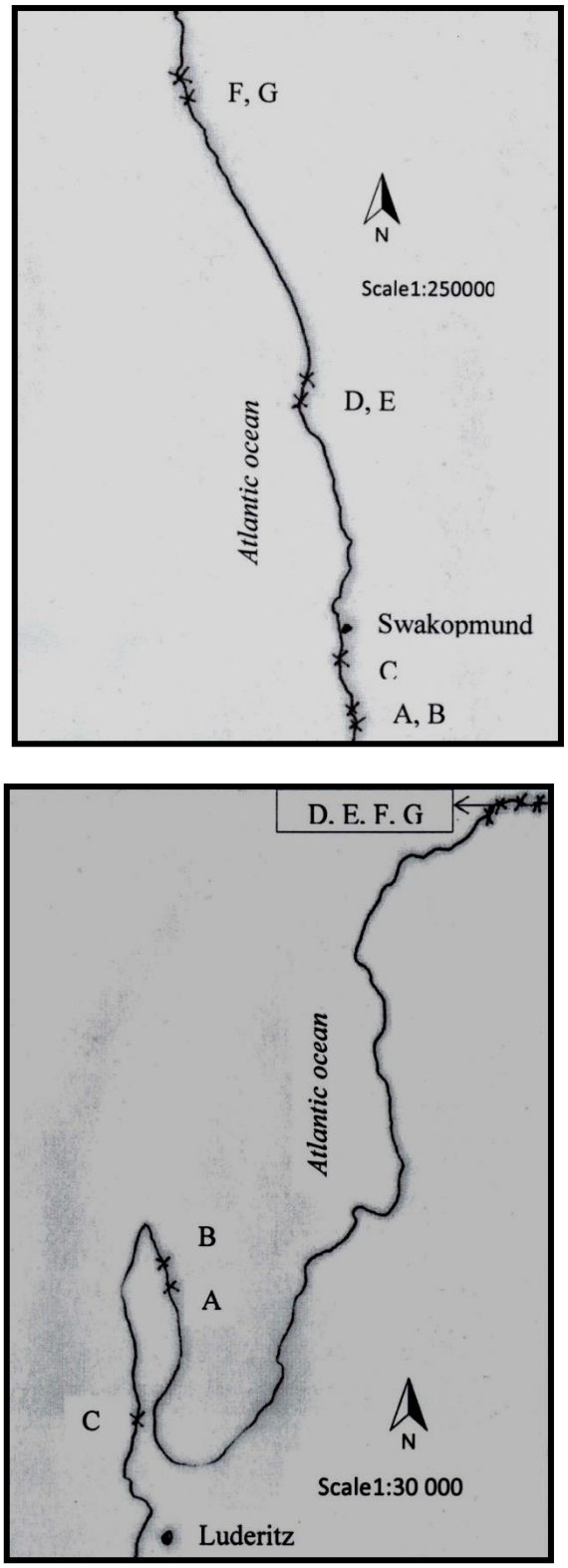

Figure 3.2 Detailed sketch map of field sampling blocks (A, B, C, D, E, F & G) along Swakopmund and Luderitz shores (Appendix 5b)

Samples for phylogenetic studies were obtained from sites within Namibia and South Africa (Figure 3.1). Ecological field studies were set up at some accessible sites along the shores of Lüderitz town (S26° 38′ 01.5″, E015° 09′ 09.9″ (blocks A, B, C, D, E, F & G) and near Swakopmund town (S 22° 49′ 53.9″, E014° 32′ 26.6″(blocks A, B, C, D, E, F, & G) in Namibia (Figure 3.2). *Porphyra* thalli for phylogenetic studies were obtained from the above blocks as well as Tora bay (S20° 19′ 37.0″, E013° 17′ 18.0″), Ugab (S21° 04′ 59.3″E 013° 33′ 24.2″) and Cape Cross (S21° 45′ 51.7″ E013° 57′ 4.7″) in Namibia; in addition to specimens collected from Cape Town (S33° 54′ 45.8″, E 018° 23′ 16.8″) in South Africa.

Some small rock pieces from the different sites were sampled for X-ray diffraction analysis to identify the basic rock types that make up *P. capensis* substrate.

3.1.2 Temperature and seasonality.

The sea surface temperature (SST) at Swakopmund ranges from 14^{o}C during winter to 20^{o}C in summer while around Lüderitz it ranges from 10^{o}C to 14^{o}C in winter and 14^{o}C to 15^{o}C in summer (Mendelson et al., 2002). The mean monthly sea surface temperature and ambient temperature values (for Lüderitz and Swakopmund) calculated over a period of at least 10 years are presented in Figures 3.3 and 3.4.

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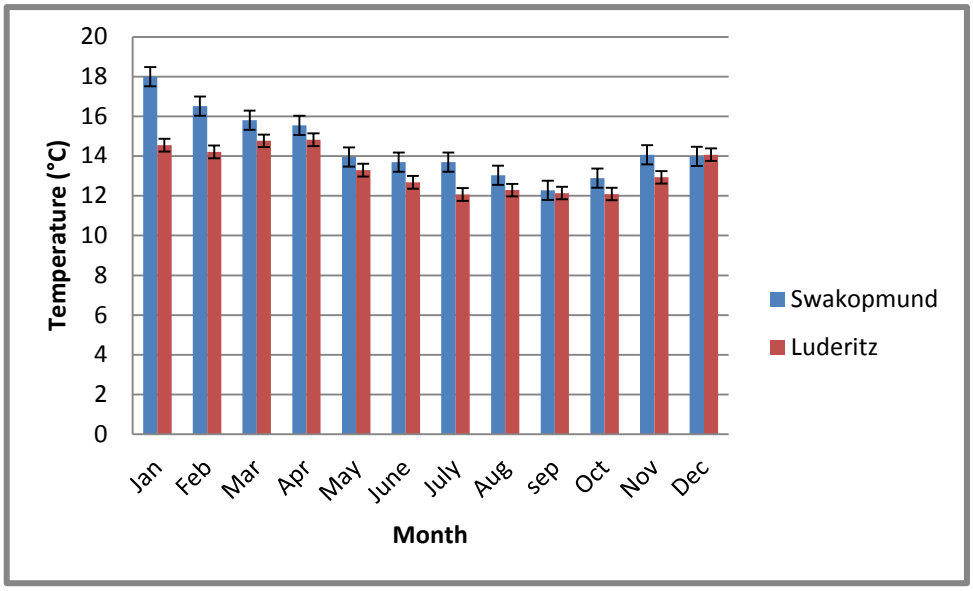


Figure 3.3 Mean monthly sea surface temperatures (SST) for Lüderitz and Swakopmund (10 years average). Source: Ministry of Fisheries and Marine Research Centre: Swakopmund

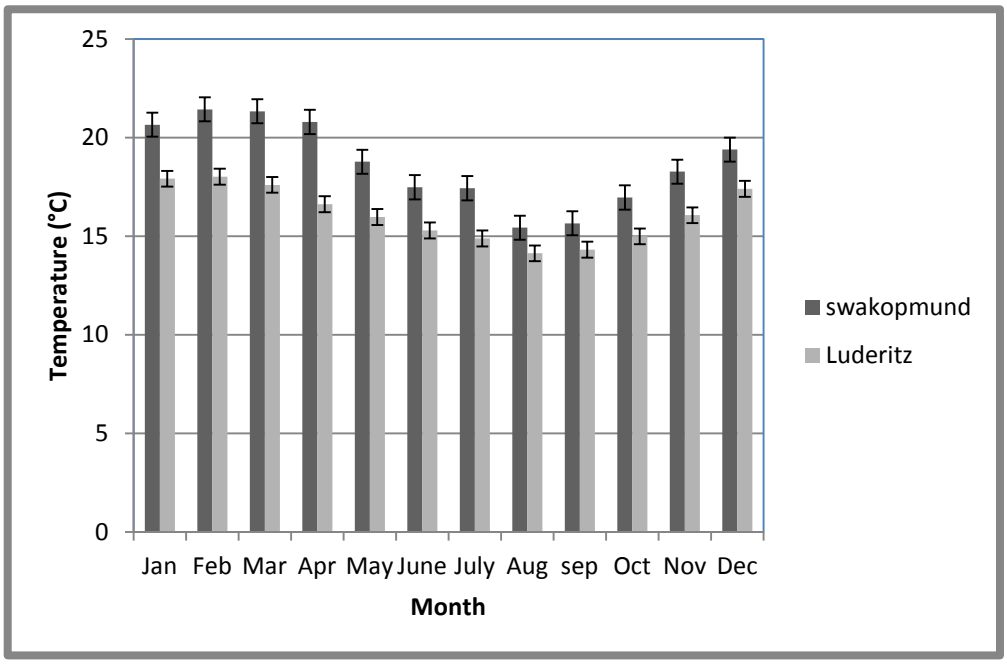


Figure 3.4 Mean monthly ambient temperatures (10 years average). Source: Meteorological Department (Windhoek)

Namibia is characterized by the four temperate seasons namely; summer (December-February), autumn (March-May), winter (June–August) and spring (September-November). Resulting from this, day-lengths are not the same throughout the year as winter has less hours of sunlight than summer (Figure 3.5).

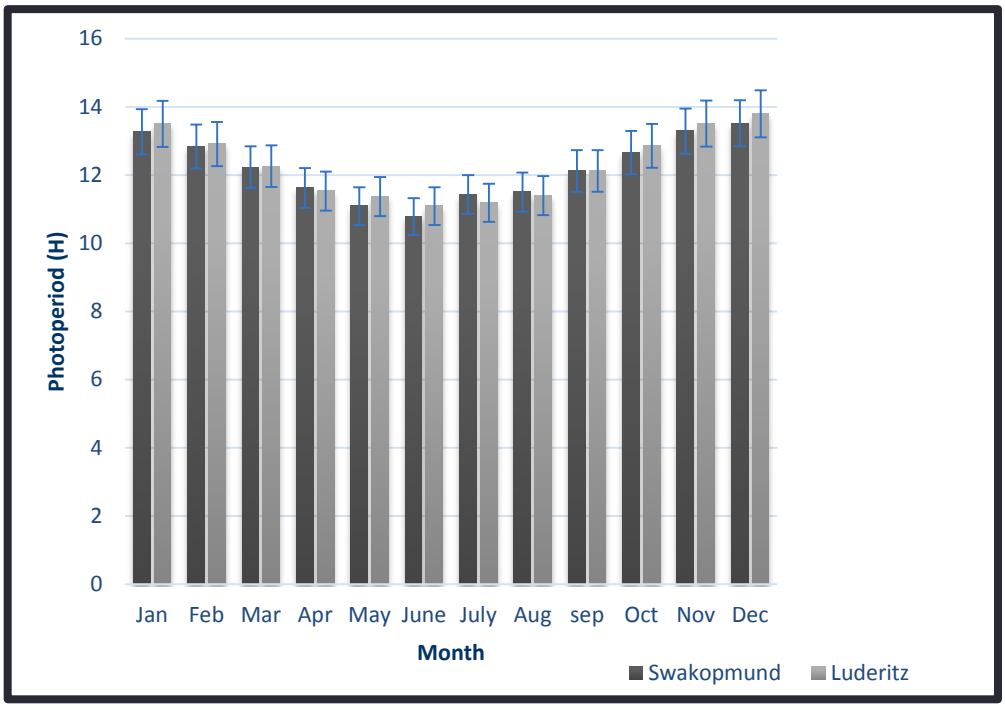


Figure 3.5 Mean annual variation in photoperiod (H) in Luderitz and Swakopmund for years 2011 and 2012. (Data source: Cell-Stop Namibia)

3.1.3. Influential oceanographic conditions along the Namibian coast

The Namibian coast experiences semi-diurnal tides and the highest tidal ranges are experienced in autumn and spring (Figure 3.6). The coast microclimate is highly influenced by the Benguela Current System with major upwelling nodes at Lüderitz and Cape Frio. The nutrient load along the Namibian coast fluctuates with upwelling forces, being high during major upwelling events (Figure 3.7).

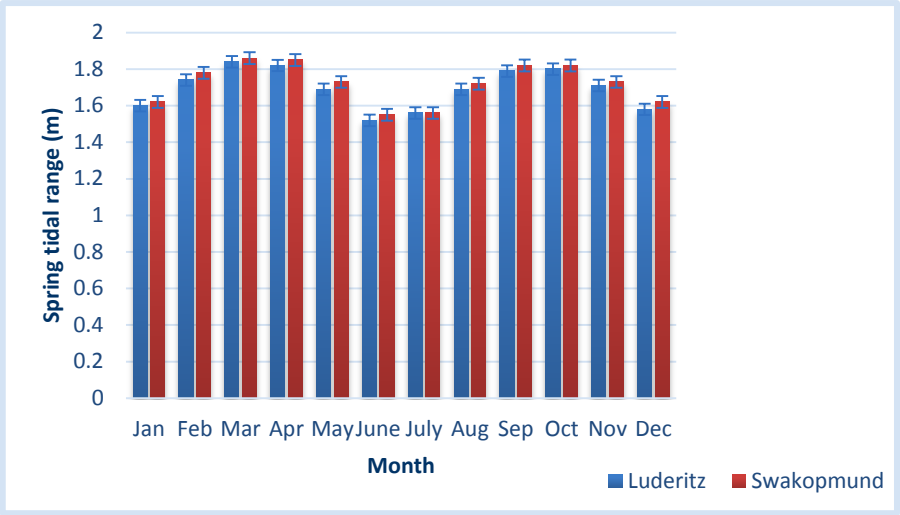


Figure 3.6 Mean annual variation in spring tidal ranges along Luderitz and Swakopmund shores for years 2011 & 2012 (Data from Cell-Stop Namibia)

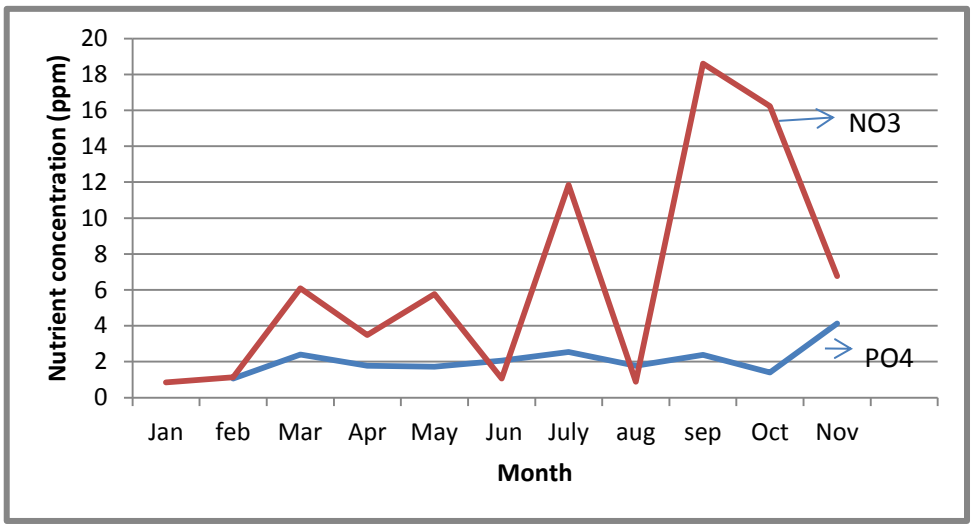


Figure 3.7 Mean annual levels of nitrates and phosphates (in parts per million) along the shores of Swakopmund. Data source: Ministry of Fisheries and Marine Research Centre, Swakopmund (1999-2004).

3.2 Characterization of Namibian populations of P. capensis

3.2.1 Phylogenetic characterization

Thalli were collected wearing surgical gloves, during random walks (in a completely randomized design) along the shores of Lüderitz, Swakopmund, Cape Cross, Ugab and Tora Bay and the GPS locations were recorded. Several thalli from adjacent holdfasts (forming a clump) were kept in a labeled freezer bag (the label showed serial number of specimens, location and date) and transported in a cool box from the field to a hotel (in Lüderitz or Swakopmund) for storage in a freezer at -18℃. The frozen thalli were later transported from either Lüderitz or Swakopmund in a coolbox packed with ice cubes to the research laboratory in Windhoek. The contents of each freezer bag were emptied in a basin of water and cleaned to remove any epiphytes and the general shape of wholesome specimens was recorded. Each selected specimen was then preserved individually (within a labeled freezer bag) in a freezer at -20^{O}C awaiting DNA extraction. The specimens were handled wearing surgical gloves all the time.

Six silica gel preserved thalli of *P.capensis* were obtained from the University of Cape Town, collected from Western Cape area for DNA extraction. These were hydrated and later frozen like the other Namibian thalli. One hundred (100) mg of each thallus was ground in liquid Nitrogen and total genomic DNA was extracted using the standard DNeasy Plant mini kit protocol (Qiagen, 2006). The rest of the thalli were placed in freezer bags individually, and then stored in the freezer for further studies.

The quantity of DNA obtained was estimated following the Ethidium Bromide-stained gel method (Stephenson, 2010). The genomic DNA obtained from the various

specimens, was run on a 1% agarose gel electrophoresis incorporating a 1kb DNA ladder (Fermentas Co) in one of the wells. At the end of the run the ladder had separated itself along the length of the gel according to the concentration and size of its fragments. It was assumed that if the DNA concentration from sample equals that of DNA on a ladder fragment, they will have the same intensity, since Ethidium Bromide stains DNA in a concentration-dependent manner. The more the DNA in a band on a gel, the more intensely it was stained and the actual concentration was calculated from a standard equation (Stephenson, 2010).

The DNA was used as a template for PCR (polymerase chain reaction) amplifications of the ITS and 18S regions of the nuclear rDNA gene using appropriate primers. The following primers were obtained from Inqaba Biotec Laboratories in Pretoria South Africa. Primers ITS1; F 5' TCC GTA GGT GAA CCT TGC GG 3' and ITS1; R 5'GCT ACG TTC TTC ATC GAT GC 3' (White, Bruns, Lee, & Taylor, 1990) and were used to amplify the ITS region. Primers GO1; F 5'CAC CTG GTT GAT CCT GCC AG 3' and G14; R 5'CCT TGG CAG ACG CTT TCG GAG 3' (Saunders, & Kraft, 1994) were used to amplify the 18S region.

Polymerase chain reactions (PCR) were carried out in 3 different cyclers; Bio-Rad My-cycler PCR machine, Labnet cycler and ESCO- Swift Max Pro machines. The reactions for PCR amplifications were performed in a final volume of 25µL. The reactions mixtures contained 12.5µL Top Taq Master Mix (Qiagen), 2µL genomic DNA, 0.5µL of each primer and 9.5µL RNA free water (Fermentas Co). The thermal cycling program for ITS, started with 5 min denaturation at 94^{O}C, followed by 35 cycles of 1 min

denaturation at 94^{O}C, 1 min annealing at 57^{O}C, and 1.5 min extension at 72^{O}C and finally 10 min final extension at 72^{O}C. The amplicons were held at 4^{O}C.

The reaction mixtures for 18S amplifications were the same but the thermal cycling program started with 2 min denaturation at 94^{O}C followed by 35 cycles of 15 sec denaturation at 94^{O}C, 30 sec annealing at 59^{O}C and 2 min extension at 72^{O}C. The final extension took place at 72^{O}C for 10 min and the products were held at 4^{O}C.

To confirm the presence of amplicons, the PCR products were electrophoresized on 1.5% agarose gel stained with 5µL Ethidium Bromide. The annealing temperatures were optimized by first calculating the melting temperatures (T_m) of the primer-template pairs, followed by gradient PCR protocols (Roux, 1995, Lopez, & Prezioso, 2001). The fragments obtained were sequenced by the Inqaba Biotec laboratory in Pretoria, South Africa. The resulting sequences were edited by Bio-edit program (Hall, 1999), alignment was carried out by Clustal X2 program (Thompson, Gibson, Plewiniak, Jeanmougin, & Higgins, 1997). The phylogenetic trees were obtained using the molecular evolutionary genetic analysis (MEGA) software version 5.1 (Tamura, Peterson, D, Peterson, N, Stecher, Nei, & Kumar, 2011).

The sequences aligned using Clustal X2 were converted and uploaded into the MEGA 5.1 format. To construct the tree in MEGA 5.1, the Jukes-Cantor model was used and the Neighbour-joining method was used for statistical analysis. To test phylogeny, 100 Bootstrap replicates were carried out and the Bootstap values appeared on the nodes as percentages. Bootstrap values greater than 70% inferred the reliability of nodes. Nodes with a Bootstrap value greater than 50% were shown on the topology.

3.2.2 Morphological diversity and anatomical characterization of thalli.

3.2.2.1 General morphology of thalli

One thallus from each bunch collected in section 3.2.1, was pressed on herbarium sheets for reference. Information about the specimens was written on the label. Photographs of the various forms were taken.

3.2.2.2 Anatomical characterization

Very thin transverse sections (TS) were made of some of the specimens used for phylogenetic analysis and the thickness compared as this characteristic is used in *Porphyra* species taxonomic delimitation. Transverse sections of the vegetative and fertile areas for the Namibian *Porphyra* and the typical South African *P. capensis* type specimens were also made for comparisons (GPS readings of collection locations: Appendix 5a). The surface views (SV) of vegetative areas of thalli were also examined under a compound microscope. Photographs were taken showing arrangement of spermatangia and zygotosporangia packets for comparison. Sizes were measured using an ocular graticule for the Olympus microscope model CX21 which had been calibrated by a stage micrometer (model OBM 1/100).

3.3 Ecological field studies on Namibian populations of *P. capensis*.

3.3.1 Assessment of abundance from percentage cover

A systematic sampling procedure was followed to assess percent cover of foliage (thalli). Sections of a line transect were set according to the shape of the shore, accessibility and presence of thalli in each study site (Lüderitz and Swakopmund), from the upper growth limit of *P. capensis* along the eulittoral zone. The total length of each section was 100m.

In total there were seven sections in Lüderitz and seven sections in Swakopmund. These sections were within homogenous patches of thalli roughly 150m long, along the eulittoral zone. There was one line transect per patch. Estimation of percent cover of the thalli was done visually following the cover quadrat method (Smith, 1996) using 50cm x 50cm quadrat frames placed 5m apart. A total of 20 quadrats were assessed within the 100m sections, making 140 quadrats in Lüderitz and 140 in Swakopmund. The starting points were randomly picked using random numbers (1 – 10). The random numbers indicated a distance in meters from a point where one was standing within the line transect but outside the sampling area of interest. The starting point was marked with antifouling paint and GPS location recorded. The assessments took place during low tide.

3.3.2 A predictive model for biomass from cover assessments

To evaluate the relationship between cover and biomass, the systematic sampling procedure was followed. Before the assessment of cover along the 100m sections within the patches (section 3.3.1), 5 numbers (1 - 20) were randomly picked. Suppose numbers 5, 9, 13, 16 and 18 were picked, during assessment of cover, on reaching quadrat 5, after recording the cover, the thalli within the 50cm x 50cm quadrat frame were harvested (hand-picked as close to the holdfast as possible) and placed in a pre-labeled freezer bag. The assessment of cover proceeded and on reaching quadrat 9, the thalli were similarly harvested, until 5 quadrats were harvested from each 100 m sections.

Later, in the Marine Research Institute laboratory, the thalli from each freezer bag were individually emptied in a bucket of tap water and cleaned to remove sand and epiphytes. The clean thalli were placed in a nylon mesh; excess water was squeezed out and then returned into the labeled freezer bag and weighed. This was recorded as wet mass. To

obtain the dry mass, the samples were air-dried in brown paper bags in a room at 45°C at the Main Campus of the University of Namibia in Windhoek. Both wet and dry mass were measured in grams to one decimal place. Biomass was presented as grams of dry matter (DM) per unit area and cover was recorded in percentages (%). The biomass data (from 50cm x50cm quadrat frame) was multiplied by a factor of 4 to be expressed as gDM m^{-2}. The estimation of cover and biomass per quadrat data were carried out in spring (September, 2011). There were 35 quadrats from Lüderitz and 35 from Swakopmund with % cover and respective biomass data.

3.3.3 Assessment of seasonal variation in standing crop biomass

The assessment of seasonal variation in standing crop biomass was carried out the following year (2012); summer (January 2012), autumn (April 2012), winter (July 2012) and finally spring (September/October 2012) following the stratified random sampling design. Seven areas with homogenous stands of thalli were identified from Lüderitz and Swakopmund. These areas were referred to as blocks A, B, C, D, E, F & G for Lüderitz; A, B, C, D, E, F & G for Swakopmund (Appendix 5b). Five (5) quadrats frame (50cm x50cm) were thrown randomly within each of the sampling blocks avoiding areas that had been harvested before by referring to GPS records. The thalli within the quadrats were hand-picked and placed in labeled freezer bags and later washed to remove sand and epiphytes. Clean thalli were weighed at the Marine Research Institute laboratory. The samples were dried, re-weighed and calculations were made to obtain standing crop biomass expressed in grams dry matter per m^{-2} ($gDMm^{-2}$). The dry mass obtained from an area of $0.25m^2$ and was multiplied 4x to get a value for an area of $1m^2$.

Standing crop biomass = gDM / $1m^2$ = $gDMm^{-2}$.

3.3.4 Effect of different harvesting frequencies on annual yield (biomass)

To assess the effect of different harvesting frequencies on annual yield (biomass), experimental plots were set up in a stratified randomized design. Lüderitz and Swakopmund were considered as two sites. Three permanent plots (2m x 1m) representing the three treatments (T1, T2, & T3), were randomly replicated seven (7) times in purposefully chosen areas where *P. capensis* populations were more or less homogenously distributed (strata) and each stratum was recorded as; a, b, c, d, e, f, and g, such that each site (Lüderitz and Swakopmund) had 7 replicates for treatments 1, 2 and 3 set up along the shores. Stratum 'a' had 3 permanent plots each for T1, T2 & T3 respectively. The sequence 1, 2, 3 in Table 3.1, was randomly generated by picking the numbers from a bag. The plots were arranged in the following manner; T1a, T2a, T3a; T3b,T1b, T2b; T1c, T2c, T3c up toT1g in a south- north direction. The detailed sampling strata are represented in Figure 3.2; where, (blocks A-G) for section 3.3.3.were set up in the same homogenous areas as (strata a-g) in section 3.3.4.

Table 3.1 Harvesting treatments sampling design for replicates (a - g) for treatments (T1, T2 & T3). The replicates were between 50 - 300m to several kilometers apart depending on accessibility.

PLOTS						
T1a	T3b	T1c	T1d	T3e	T2f	T2g
T2a	T1b	T2c	T3d	T2e	T1f	T3g
T3a	T2b	T3c	T2d	T1e	T3f	T1g

The GPS location for each plot was recorded (Appendix 5c). The corners were painted with sea guardian antifouling marine paint (Nova Marina- Lüderitz) and photographs were taken for future reference. The three harvesting treatments were marked on the rock, with numbers 1, 2 and 3 indicating the type of treatment. Harvesting was done at 3 months' intervals for treatment 1; 6 months' interval for treatment 2, while for treatment 3 harvesting was done once, 12 months after initial clearing of plot.

At the beginning of the experiment (September 2011), a 50cm x 50cm quadrat frame was placed at the centre of each plot and thalli were hand-picked about as close as possible to the holdfasts. This was taken as the initial biomass sample (covariate) and was placed in a pre-labeled freezer bag for cleaning, weighing, drying and re-weighing to obtain biomass value. Another quadrat frame (50cm x 50cm) was placed at the right hand corner of each plot and its thalli were not harvested. This was the control. The rest of the plot was cleared of all thalli. Photographs showing the position of the control were taken for future reference. The first harvesting assessment started 3 months later in January 2012 for treatment1.

All plots marked treatment 1 in each of the 7 replicates, had a quadrat frame 50cm x 50cm placed at the centre of the plot and harvested in January 2012, April 2012, July 2012 and September/October 2012, during spring low tide. The harvested thalli were placed in pre-labeled freezer bags, cleaned and weighed. They were later dried, re-weighed and data recorded. The rest of the thalli on the plot were removed except for the control.

The second harvesting treatment (treatment 2) involved harvesting an area 50cm x 50 cm at the centre of plot 2 in each replicate, six months after the original harvesting treatment

(April 2012), during spring low tide. The harvested thalli were placed in pre-labeled freezer bags and processed as per samples for treatment 1 and the biomass data was recorded. An area 50cm x 50cm at the right hand corner of the plot was preserved as a control while the rest of the plot was cleared of all thalli.

The third harvesting treatment (treatment 3) involved harvesting an area 50cm x 50cm from the centre of plot 3 for each replicate, twelve months after the initial harvesting treatment (September 2011-October 2012). The samples were processed as the samples for treatment 1.

All the controls in all the plots were harvested at the end of the field study in October 2012. The samples from the controls were processed in the same manner as for the treatment samples and their dry mass was recorded.

The annual cumulative biomass data for each treatment per plot was pooled together. In the case of treatment 1, the biomass harvested from plots 1a, 1b, 1c, 1d, 1e, 1f & 1g for both sites (Lüderitz and Swakopmund) was added up and mean calculated. This was biomass T1. The same was done for treatment 2 where the biomass from plots 2a to 2g was obtained, added together and the mean calculated (biomass T2). In the case of treatment 3 the biomass from each plot (3a – 3g) was added together and the mean calculated (biomass T3). Biomass T1, T2 and T3 represented the cumulative biomass for the three treatments. The initial biomass (covariate) from each treatment plot was obtained and the mean was calculated. The mean biomass for the controls of each treatment plot was obtained at the completion of field study. The initial biomass and control biomass data was needed to gauge the existence of plot or habitat differences.

The growth rate was estimated from data obtained from T1 harvesting frequencies, as the rate of biomass accumulation within seasons. The data from Lüderitz and Swakopmund were pooled together for blocks A-G to underscore seasonal variations.

3.4 Nutritional content of Namibian populations of P. capensis

3.4.1 Collection of thalli and sample preparation

The letters A, B, C, D, E, F & G, representing the study sampling blocks (section 3.3.3) were placed in a bag and two letters were picked randomly to identify the places from which thalli would be picked for nutritional analyses. Area D and G in Lüderitz as well as areas A and G in Swakopmund were picked. Twenty thalli were randomly collected from each of the four areas above and placed in pre-labeled freezer bags. The thalli were thoroughly cleaned in a bucket at the Marine Research Institute laboratories and excess water was squeezed out through a nylon wire mesh bag.

The thalli from the representative areas were placed back in their respective freezer bags and weighed using a top loading balance. This was the wet mass after deducting the mass of the empty freezer bag. The samples were later placed in labeled brown paper bags and placed on tables in a room at 45℃. Within a week the samples had dried to constant weight and were re-weighed. The assumption was that, drying the thalli at that temperature would not alter their chemical composition but would remove most of the water. Four (4) lettuce heads (Appendix 6) were purchased from 4 different local super-markets; the leaves were removed and washed separately. The leaves from 2 heads were mixed, weighed together and dried together in a brown paper bag at 45℃. The dry matter was weighed and the mass recorded. Lettuce was chosen as it is a popular leafy salad

vegetable in Namibia. *Porphyra* thallus has a leafy appearance and could be eaten as a salad. Namibian seaweeds are not eaten yet and comparing *Porphyra* to lettuce is a way of popularizing it.

The dry *Porphyra* thalli and lettuce leaves were ground separately into a powder using a Retsch & Mellerware grinding machine model 38635 and stored in pre-labeled air-tight jars in a dry dark cupboard at 18^{O}C, awaiting chemical analyses. There were a total of 6 samples; 2 samples of *Porphyra sp* from Lüderitz, 2 samples of *Porphyra sp* from Swakopmund and 2 of lettuce for nutritional analyses. Part of the 6 samples were sent to the Ministry of Agriculture, water and forestry; agriculture laboratory in Windhoek, Namibia for the analyses of ash, fat, calcium, phosphorus, magnesium, iron, crude protein, (for nitrates), neutral detergent fibre and carbon content. Other portions from the 6 samples were taken to the Government Chemist Laboratories in Dar es Salaam Tanzania for determination of Iodine and β-Carotene. Adequate sample replication could not be done due to cost constraints. For data analysis the mean values (obtained were re-sampled 1000 times through bootstrapping.

3.4.2 Sample analyses

Water content of freshly harvested thalli and lettuce was calculated as a percentage of the total fresh mass as follows:

Water content % = {(fresh mass- dry mass)/fresh mass} x 100.

For assessment of total ash the AOAC (1995) analytical protocol was followed where, 2 g of ground sample was combusted in a muffle furnace at $550^{O}C$ for 4 hours after 1 hour pre-treatment in the same furnace set at $250^{O}C$. Ash content was calculated as follows: Ash% = (change in mass after ashing / original mass) x 100.

Total Nitrogen (N) was determined by the Kjeldahl oxidation method (AOAC 1995). Crude protein % = N% x Factor (the standard correction factor used was 6.25).

Crude fat content was assessed following the Soxhlet method (AOAC 1995).

For estimation of neutral detergent fibre, the protocol by Robertson & Van Soest (1981) was followed, which determines the percentage of insoluble cell wall components in plant material. One (1) g of ground sample was placed on a crucible in a fibre-tec system, and treated with the neutral detergent solution at pH 7.0 (to remove proteins) followed by the enzyme alpha-amylase, which converts starch into soluble sugars. The extracted fibre was then ashed and the percentage of the ashed fibre residue in the original sample was determined.

Total phosphorus was determined spectrophotometically as the yellow phospho-vanado-molybdate complex at 400nm following the colorimetric method in Cavell (1955).

Calcium (Ca), magnesium (Mg) and iron (Fe), content in the samples was determined by atomic absorption flame spectroscopy method in AOAC (1995).

Quantification of **β**- carotene (a Vitamin A precursor in humans) was carried out by spectrophotometric method (www cyanotech.com 2002). **β**- carotene was obtained from *Porphyra sp.* after methanol and heptane extractions. Absorbance was read at 436 nm vis a heptane blank and quantity calculated using the formula;

β- carotene % = {[Absorbance /196 x sample wt (mg) x dry wt.] x 25ml x1.25 x 100 x 0.84}, where 0.84 is an adjustment factor for other carotenoids.

Determination of Iodine was carried out by the 'iodometric titrations' method (Maeyer, Lowenstein, & Thilly, 1979), where iodine could be calculated as;

I_2 (mg/Kg) = V x 0.1058 x 100; where, V= volume of 0.005N sodium thiosulphate solution used in the titration and 0.1058 is the coefficient of variation.

3.5 Laboratory initiated Namibian *P. capensis* cultures

3.5.1 Enriched seawater media preparation

Natural seawater was enriched with nitrates, phosphates, trace metals and vitamins following the recipe by West and McBride (1999). Constituents of the enrichment stock solutions were prepared in de-ionized water and autoclaved. Vitamin stock solutions were similarly prepared after-which the appropriate volumes (measured with sterile surgi + plus latex free syringes) were filtered using millipore filters (Millipore Millex-GV with a pore size of 0.22µm), into the enrichment stock solution. The rest of the vitamin stock solutions were refrigerated at 4^oC for storage. Filtered and UV sterilized seawater was autoclaved and salinity assessed using a refractometer (ATAGO hand refractometer

model “S/Mill - E”). The salinity of seawater was brought to 30 PSU using sterile de-ionized water. To prepare enriched seawater working medium, 20 mL of the enrichment stock solution was added to 980 mL of filtered and UV-sterilized and autoclaved natural seawater of 30PSU salinity. The pH of the working nutrient medium was set at 7.8 using 32% HCL and IN NaOH as was required (pH meter model “Ecoscan pH 5”). The salinity of working medium was checked after addition of nutrients and maintained between 32 - 38 PSU in culture petri dishes. Schott-Duran petri dishes of 9.5cm diameter and a height of 5.5 cm were used, covered by a $10.5cm^2$ glass plates. To prevent the growth of diatoms and other microalgae $50mgl^{-1}$ of GeO_2 was added to the enriched seawater working medium (Dlaza, 2011). To prevent the growth of bacteria and other microalgae, 100µL Penicillin ($1gl^{-1}$ w/v) was added to each culture petri dish. Sterile conditions were maintained at all work stations (Appendix 7)

3.5.2 Field collection and spore isolation

The protocol on spore isolation and laboratory management of cultures were personalized adaptations of protocol in (Appendix 7), and Dlaza (2011) depending on facilities available and after several attempts in the laboratory. Mature and fertile female thalli were collected randomly from the field two hours after high tide when they were still wet. Both male and female thalli were collected. They were held overnight in a shallow tank containing seawater and supplied with air in the mariculture lab. A few male and female thalli were selected, washed in sterile seawater and fertile areas scrubbed using a fine painter’s brush to remove surface contamination. Pieces of fertile regions approximately 1cm x 2cm were excised and rinsed in deionized water. The pieces were blotted dry in between sterile Kleenex tissue. The clean dry pieces were

placed in petri dishes with sterile slides at the bottom; and allowed to air dry on the lab bench for 2 hours after which 40 ml of sterile seawater was added. After 6 hours the petri dishes were placed in the growth chamber (Model EKOCHL 700) set at 15^{o}C, 16L: 8D photoperiod and irradiance of about 3270 Lux. The excised pieces were removed after spore release. Released spores settled on the glass slides as well as the bottom of petri dishes. To refresh the media, most of the seawater medium was pipetted out of culture dishes and 40 ml of half strength working medium containing 50gl-^{1} GeO_2 was added. Penicillin antibiotic (1% w/v) was added into each of the culturespetri dishes at 100µL per petri dish.

3.5.3 Conchocelis development and mass production

Zygotospores were incubated in the growth chamber at 15^{o}C, 16L: 8D photoperiod and 3270 Lux irradiance in 40ml of half strength modified PES media, removing 20ml and adding fresh 20ml of media weekly. Zygotospores germinated forming conchocelis filaments with conchocelis archaespores. The archaespores formed more conchocelis filaments and eventually colonies or tufts of conchocelis could be seen floating in the media. A few filaments were put in petri dishes containing half strength media with sterilized oyster shells as substrates and placed in the growth chamber. A known amount of conchocelis filaments were vegetatively propagated in a bubble culture medium in the mariculture lab set at 21^{o}C 16L: 8D and irradiance of about 450 Lux.

3.5.4 Conchosporangia development and conchospore release.

Some conchosporangia formed from the culture containing oyster shells were ground using a pestle and mortar and the extract was poured into a petri dish containing fresh

medium and placed back into the growth chamber. The conchospores released were maintained under the same conditions of the growth chamber and were monitored for thalli development.

3.6 Data analyses

3.6.1 Statistical analyses

All statistical analyses were performed using the SPSS software package (IBM statistics 21). Most of the charts were processed in either Microsoft excel or SPSS 21. Data were tested for normality using the Shapiro-Wilk test, after which appropriate parametric or non-parametric statistical tests were carried out for further analysis of the data. Differences in data were considered significant at $p < 0.05$.

3.6.2 Phylogenetic analysis

The evolutionary history of the 59 nucleotide sequences (ITS) and 66 nucleotide sequences (18SrDNA) was inferred using the Neighbor-Joining method (Saitou, & Nei, 1987). The evolutionary distances were computed using the Jukes-Cantor method (Jukes, & Cantor, 1969) while the evolutionary analyses and tree production were conducted in MEGA5.1 (Tamura et al., 2011).The bootstrap confidence intervals (Felsenstein, 1985) of the trees were based on 100 replicates. There were a total of 502 positions in the final ITS dataset and 1654 positions in the 18SrDNA dataset. Percent (%) sequence divergencies for all sequences were computed from pairwise evolutionary distances (MEGA 5.1) for comparison.

3.6.3 Anatomical characterisation

Descriptive statistics of the data on thallus thickness were carried out and difference in the means was tested statistically using one way analysis of variance (ANOVA).

3.6.4 Analysis of nutritional content

The data on nutritional content was summarized in descriptive statistics. Independent samples t-test was carried out to compare the nutritional content of *P. capensis* to that of lettuce after bootstrapping values obtained from the analyses. One sample t-tests for equality of means were also carried out to compare the quantities of some nutritional elements of Namibian *Porphyra* to other *Porphyra* species from elsewhere as recorded in literature.

3.7 Ecological field studies on *P. capensis*

3.7.1 Abundance assessment

3.7.1.1 Percent cover

The distribution of % cover data within quadrat frames at both sites Lüderitz and Swakopmund were compared using a non parametric test. The Mann–Whitney U test for 2 independent samples was carried out. The data was also summarized in descriptive statistics.

3.7.1.2 Standing crop biomass

Descriptive statistics on the data was followed by the Kruskal- Wallis statistical test which tested whether the hypothesis that the standing crop biomass for Lüderitz was equal to that of Swakopmund.

3.7.1.3 Seasonal variation of environmental parameters and standing crop biomass.

The descriptive statistics for the seasonal standing crop biomass were recorded. The mean rank biomass per season was compared using the K independent sample analysis test, (Kruskal-Wallis test). The mean seasonal values for the different environmental parameters (photoperiod, sea surface temperature, ambient temperature and tidal range) between Lüderitz and Swakopmund were calculated and presented in a report. They were analysed using Kruskal Wallis test. Bonferroni pairwise multiple comparison Post-hoc test was done to compare their variations between seasons.

3.7.1.4 Abundance prediction models

Descriptive statistics for cover and standing crop biomass data were carried out. Equation models for predicting biomass from percent cover values were stipulated from regression analyses between the mass/cover data. The relationships beween the variables (mass =Y g and cover = x %) were explored graphically in scatter plots to assess how the dependent variable (mass = Y g) could be explained knowing the value of the independent variable (cover = x %). The linear, power, exponential and polynomial equations were compared, to find out which one had a higher coefficient of determination.

3.7.1.5 Effect of harvesting treatments

The Kruskal-Wallis test was carried out to find out whether harvesting frequency (treatments) had an overall effect on the annual cumulative biomass. The Bonferroni Post hoc test was carried out for pairwise comparison between the seasons. The Friedman's test evaluated whether the medians of the controls and the initial biomass, for the different treatments were significantly different from each other.

3.7.1.6 Growth rate of thalli after harvesting

The growth rate was calculated as the harvested weight gained divided by the number of days between harvests (see Equation 1; section 2.1.9).

3.8 Laboratory culture of Namibian *Porphyra sp*.

The time taken by each developmental phase of the Namibian *Porphyra* capensis laboratory cultures was recorded. Cumulative percent germination of zygotospores was calculated. The conditions under which the various phases operated were also recorded.

CHAPTER 4

4.0 RESULTS

4.1 Species characterization

4.1.1 Phylogenetic characterization

Extraction of DNA from 70 *Porphyra C. Agardh* specimens following the DNeasy kit protocol yielded low amounts of DNA, which ranged from 1ng/mL to 9ng/mL. These amounts were however, sufficient for PCR amplifications (Plate. 4.1a).

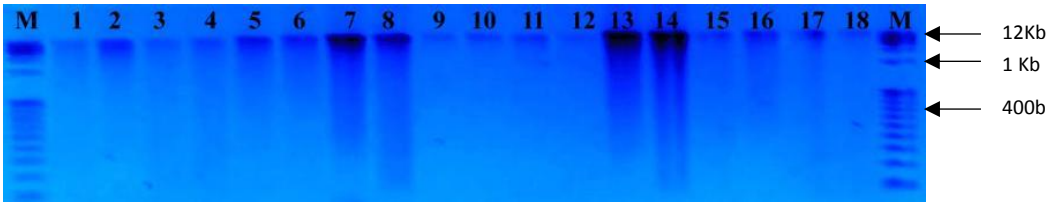


Plate 4.1a Electropherogram of extracted genomic DNA on 1.5% agarose gel stained with Ethidium bromide. Lanes ‘M’ showing the sizes of Tandem ladder (marker) fragments.

The sizes of PCR amplicons from samples 13, 33, 72, and 73 (collected from Swakopmund) when run on 1.5% agarose gel stained with Ethidium bromide revealed that the sizes of ITS fragments were about 400 base pairs while those of 18S fragments measured approximately 1500 base pairs (Plate 4.1b).

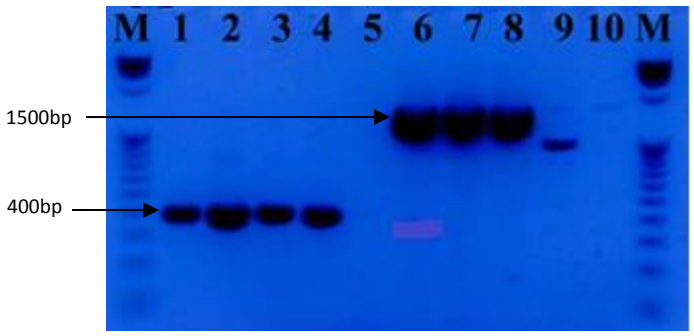


Plate 4.1b Electropherogram of 1.5% agarose gel stained with Ethidium bromide showing the sizes of PCR fragments (amplicons).

Key; Lane 'M'= tandem ladder. Lanes 1, 2, 3 & 4 show the size of ITS fragments of samples 13, 33, 72 and 73 respectively. Lanes 6, 7, 8 & 9 show the sizes of the 18S fragments from samples 13, 33, 72 and 73 respectively. Lanes 5 & 10 are the negative controls.

Most of the amplicons were successfully sequenced. Amplicons whose sequences were very short were excluded from further analysis. Amplicons whose DNA showed similar sequencing pattern were brought together after multi-sequencing alignment (MSA). The MSA showed that the sequences from Namibian specimens from different sites together with the sequences of South African *P. capensis,* including GenBank sequences share conserved areas where the base alignment is 100% (Figure 4.1). It is such areas that reveal a shared ancestry for all the specimens see bp 74-158 & 1723-1803. There exists however regions of variability within the MSA such as between bp 833-901 and bp 1518-1547 (Figure 4.2) where nucleotides present within the sequences from the South African *P. capensis* are absent in the Namibian sequences.

Pairwise-distances between sequences in the alignment were computed using the program MEGA 5.1. The pairwise distance methods analysis estimated the genetic distance for all pairs of sequences, where the genetic distance reflected the number of

nucleotide changes that had taken place per site since the two sequences diverged from their common ancestor which then gave a value of percentage divergence between sequences (Van, de Peer, 2009). A total of 2146 comparisons were made on the 18SrDNA gene studied and the results showed that % divergence within the Namibian *P. capensis* sequences ranged from 0 % to 1.8%. Some Namibian *P. capensis* sequence pairs were similar, with a divergence percentage of 0%. These include numbers 44 & 22; 37 & 61; 62 & 45; 43 & 56; 44 & 15; 30 & 40; 35 & 25; 1& 7; 61 & 31. Within the S. African specimen's sequences including the GenBank sequences AY766361 and AY292929, the divergence was between 0.1 and 0.2%. The divergence between the sequences belonging to the South African *P. capensis* and and the Namibian *P. capensis* specimens's alignment ranged from 0.6 to 2.1%. The divergence between the Namibian *P. capensis* sequences and the out-group (*Bangia fuscopurpurea* (Dillwyn) Lyngbye AF342745) ranged between 12.9% and 14.7%.

Phylogenetic trees were constructed by taking into account the relationship between the pair-wise distances. The phylogenetic tree resulting from the analysis of the 18S region of ribosomal DNA (Figure 4.3) indicates that the *P. capensis* samples from Cape Town in S Africa (49, 50, 51, 52, 53 & 54) share a common ancestry with the specimens from various sites along the Namibian coast (Bootstrap 100%). However, none of the sequences from the Namibian coast (B, C and the other un-clustered sequences) became part of that unique South African clade A.

The GenBank sequences AY766361 (Milstein, & Oliveira, 2005) as well as AY292629 (Jones et al., 2004) became part of the South African *P. capensis* clade. The clusters

formed by the Namibian specimens do not follow any local geographical pattern but information within the multisequence alignments (MSA) revealed the presence of indels.

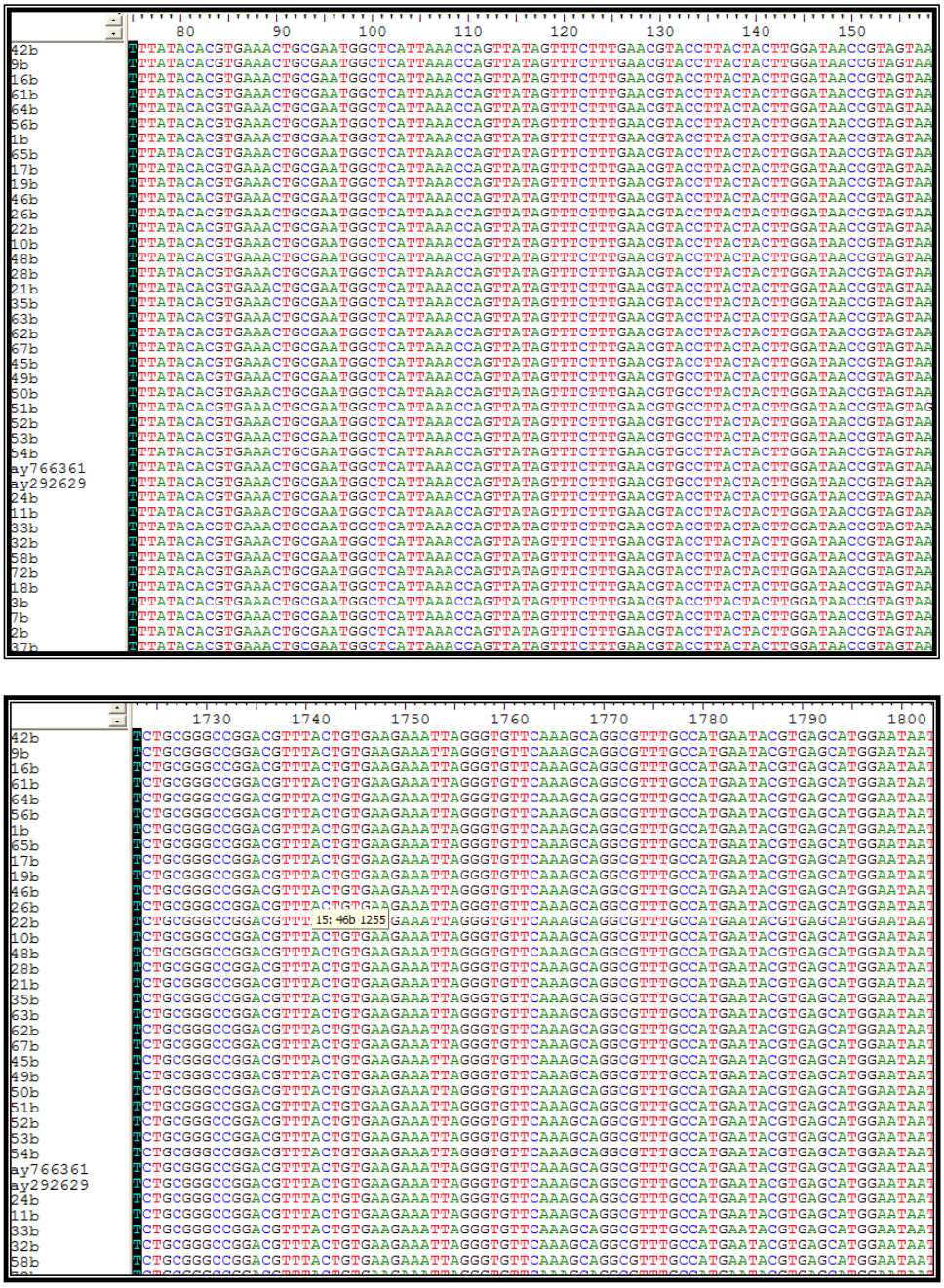


Figure 4.1 Portions of conserved areas for Namibian and South African *Porphyra* sequences based on the 18SrDNA gene studied. South African *P. capensis* sequences which were part of this study are No: 49-54 with AY766361and AY292629 as GenBank sequences for morphologically typical *P. capensis* from S. Africa.

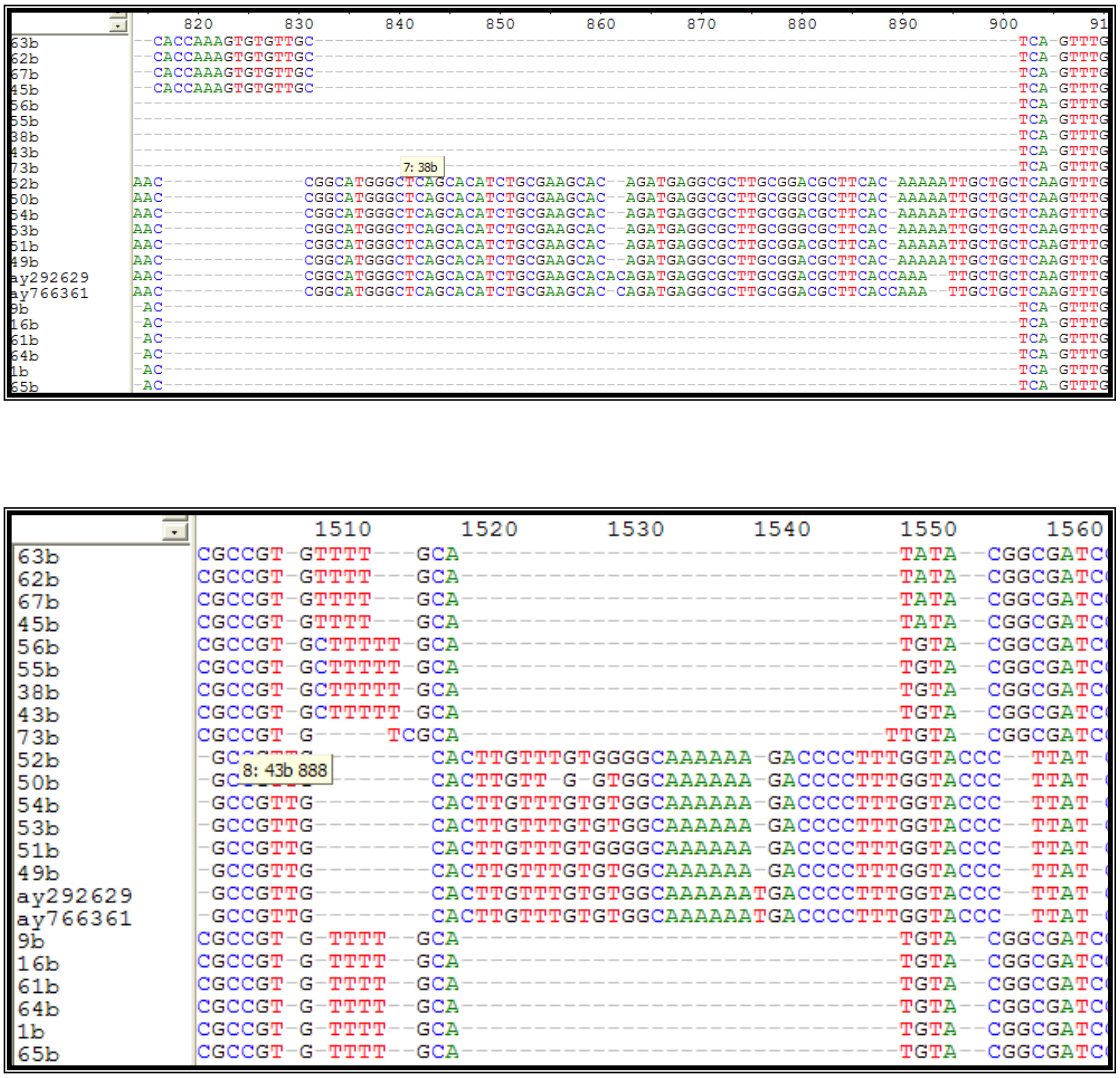


Figure 4.2 A section of the 18SrDNA gene multisequence analysis results showing a large segment of base nucleotides present in the *P. capensis* specimens from South Africa (49 - 54), as well as sequences AY766361 and AY292629 from GenBank, but absent in all Namibian specimens (a few sequences shown here).

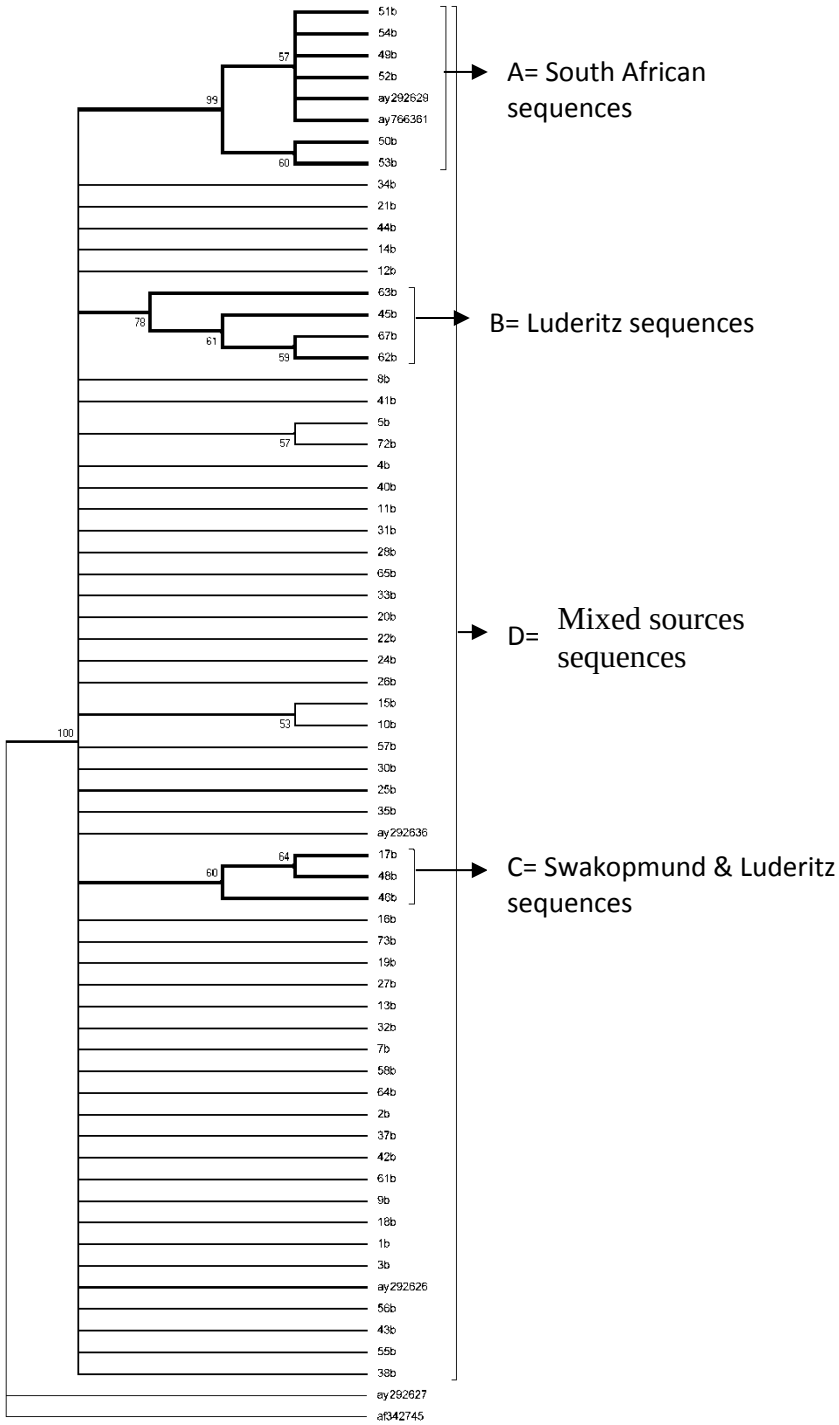


Figure 4.3 Consensus tree resulting from neighbor joining analysis of 18SrDNA sequence data for Namibian and South Africa thalli of *Porphyra* sp. Bootstrap value of 50% and above are shown at nodes. About 1450 base pairs compared.

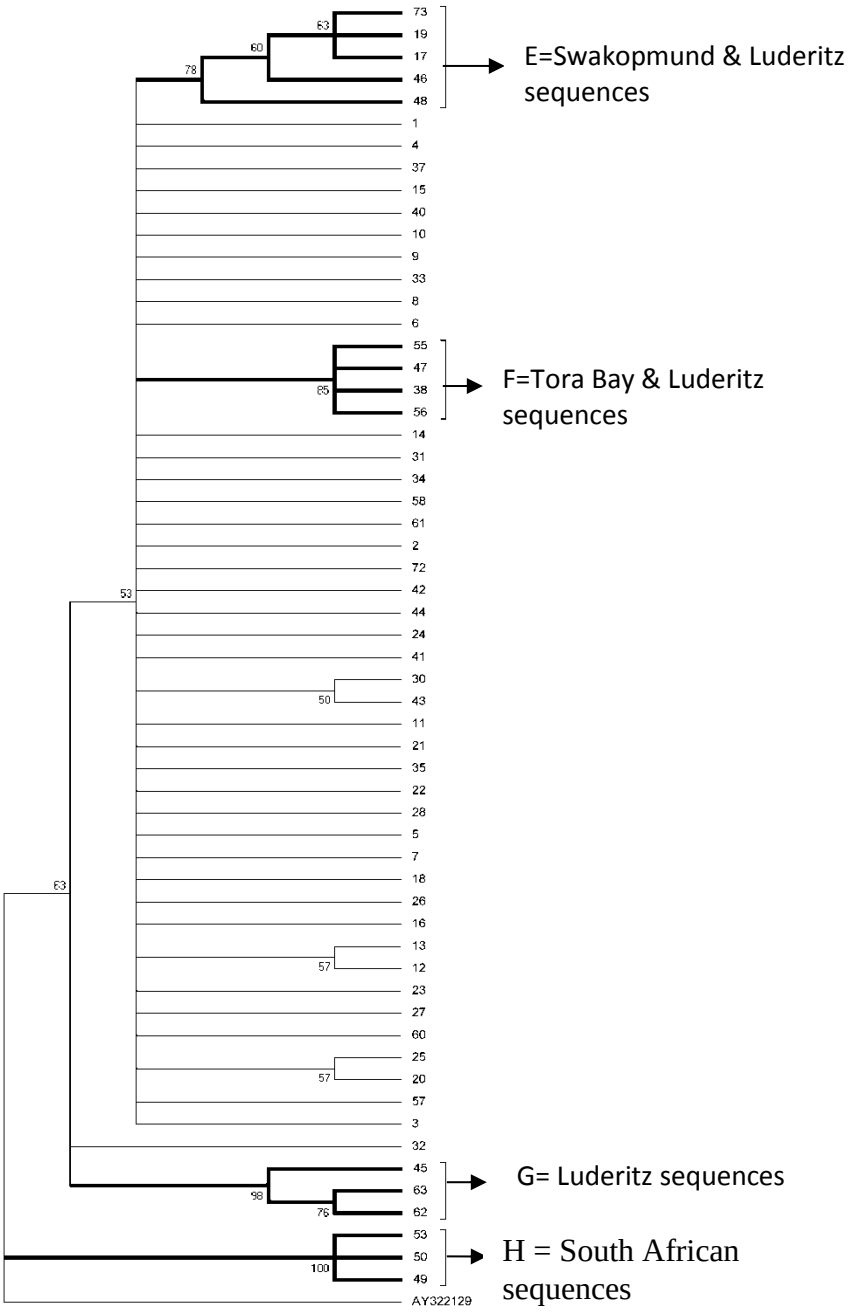


Figure 4.4 Consensus tree resulting from neighbor joining analysis of ITS sequence data for Namibian and South African thalli of *Porphyra sp.* Bootstrap value of 50% and above are shown at nodes. About 502 base pairs compared.

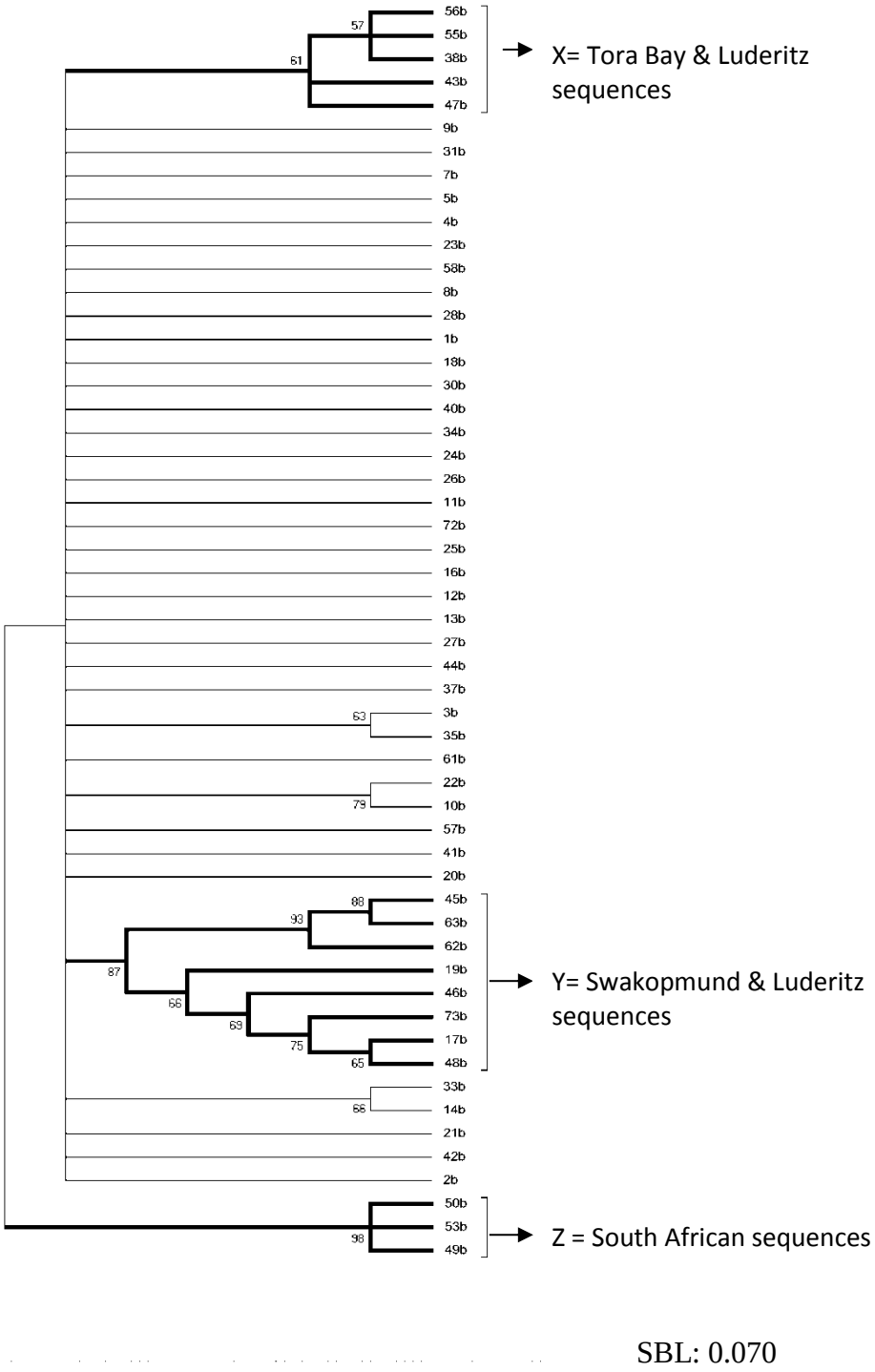


Figure 4.5 A consensus tree resulting from neighbour joining analysis of a combination of ITS and 18SrDNA sequences of Namibian specimens and S. African *P. capensis* type specimens. Bootstrap value of 50% and above are shown at nodes. About 2220 base pairs compared.

The Lüderitz cluster on Figure 4.3 made up of sequences 63, 45, 67 & 62 however, had a divergence of 0.1% among themselves but a higher divergence with sequences outside the cluster, such as between 2 & 63 where the divergence was 0.6%; 45 & 17 had 0.6% divergence; 67 & 26 showed 0.7% divergence and 1 & 62 had a 0.7% divergence. The other cluster made up of 17, 46 & 48 had a 0. 1% divergence within them but between 17 and 65 which was outside the cluster the divergence was 1.5 %.

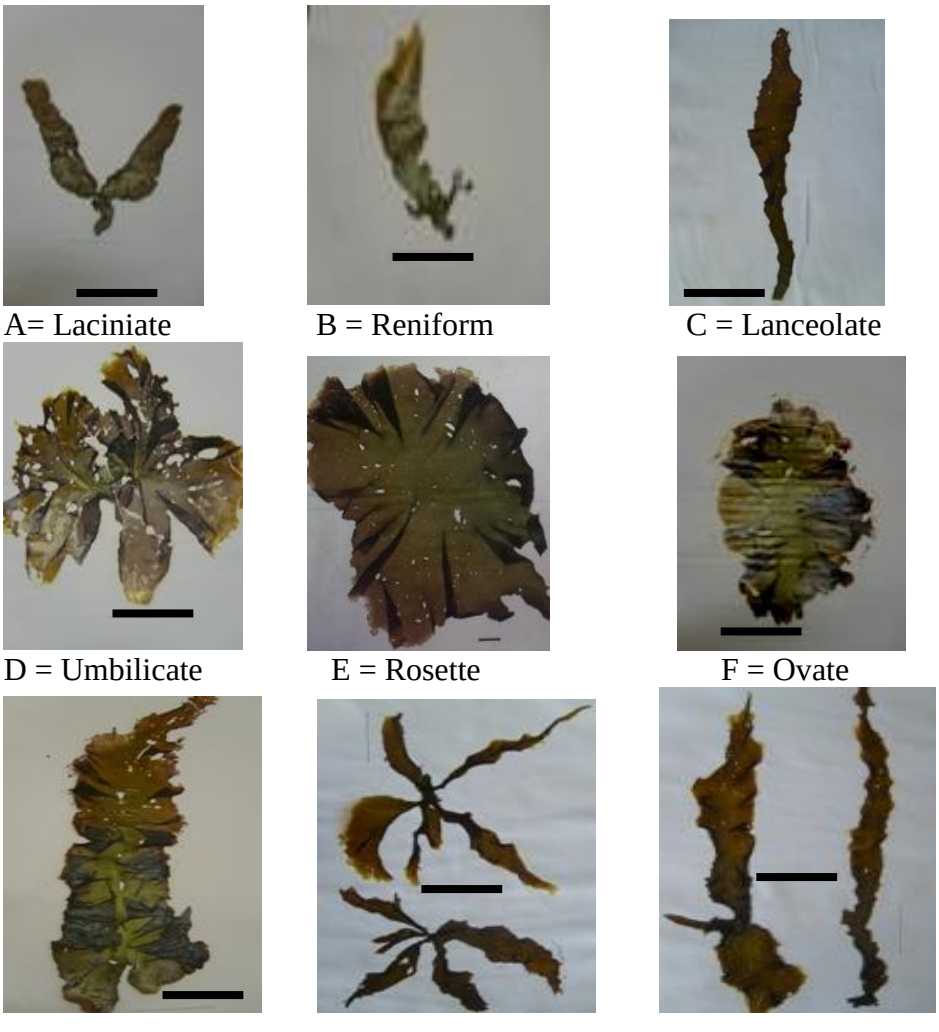
The three phylogenetic trees (Figures 4.3, 4.4 & 4.5) exhibited similar topologies where the S. African specimens and GenBank sequences AY766361 as well as AY292629, formed a unique clade with strong bootstrap values. In the 18SrDNA phylogenetic tree, a strong bootstrap value of 100% links the Namibian sequences with the South African *P. capensis* sequences revealing a shared ancestry. With the ITS tree the bootstrap value that link the two groups is only 63% implying that there is weak link between the two groups. The tree formed after combining 18SrDNA sequences and ITS showed no bootstrap values at all making the link weak.

The phylogenetic tree resulting from the analysis of sequences from the ITS region (Figure 4.4) still showed that *P. capensis* from Cape Town form a unique clade (H). The sequences from Namibian specimens formed several sub clusters but they all shared a common origin (clusters E, F, G). The phylogenetic tree resulting from combining ITS and 18S sequences from Namibian specimens and South African *P. capensis* specimens from S. Africa (Figure 4.5), similarly revealed the unique clustering of the specimens from Cape Town.

4.1.2 Morphological diversity and anatomical characterization.

4.2.2.1 Morphological diversity

Several authors have reported on the morphological diversity in members of the genus *Porphyra* C. Agardh (1824) such as Brodie et al. (1996), Broom et al. (1999) & Jones et al. (2004). The authors above have explained that this variability is mainly caused by differences in the environment as explained in section 2.2.1 (see plate 4.2; A-I).



A= Laciniate B = Reniform C = Lanceolate

D = Umbilicate E = Rosette F = Ovate

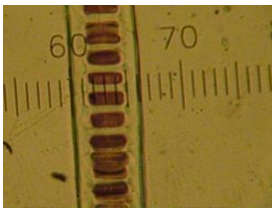
G = Cordate H = Laciniate I = Linear

Plate 4.2 Morphological diversity of some Namibian populations of *P. capensis* thalli (A – I)

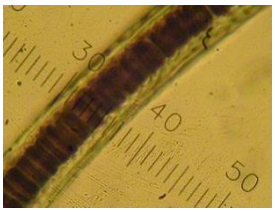
Scale bar = 5cm, except E where scale bar = 1cm. Data generated in this study.

4.1.2.2 Thallus thickness

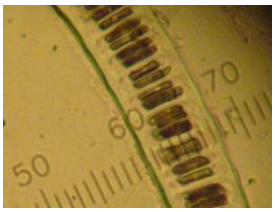
The thallus thickness of some of the collected specimens from Lüderitz and Swakopmund ranged from 60µm to 150µm, whereas those from Cape Town ranged from 110 µm to 130 µm (Plate 4.3).



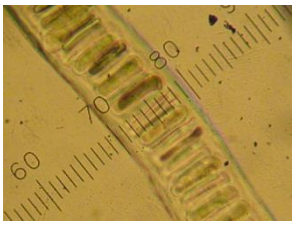
A, (40, Lud) = 60µm



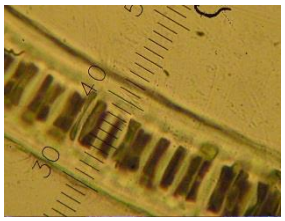
B, (57, Ugab) = 70µm



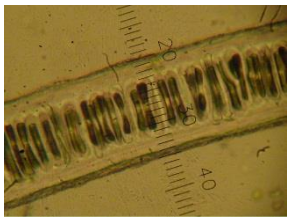
C, (25, Swak) = 80µm



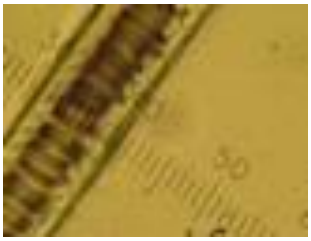
D, (43, Lud) = 100µm



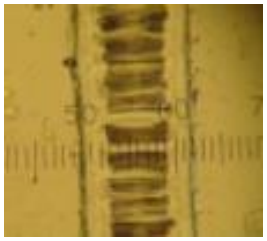
E, (5, Swak) = 120µm



F, (9, Swak) = 150µm



G, (52, CT) = 110 µm



H, (54, CT) = 120 µm

Plate 4.3 (A-H, Scale 1=10µm) Thallus thickness of some representative specimens (Namibian *P. capensis* specimens; 40, 57, 25, 43, 5 & 9; and specimens 52 and 54 from Cape Town).

These sections were made from the vegetative parts of the thallus. Key: Lud = Lüderitz; Swak = Swakopmund; CT = Cape Town. Data generated from this study (summary data; Appendix 8).

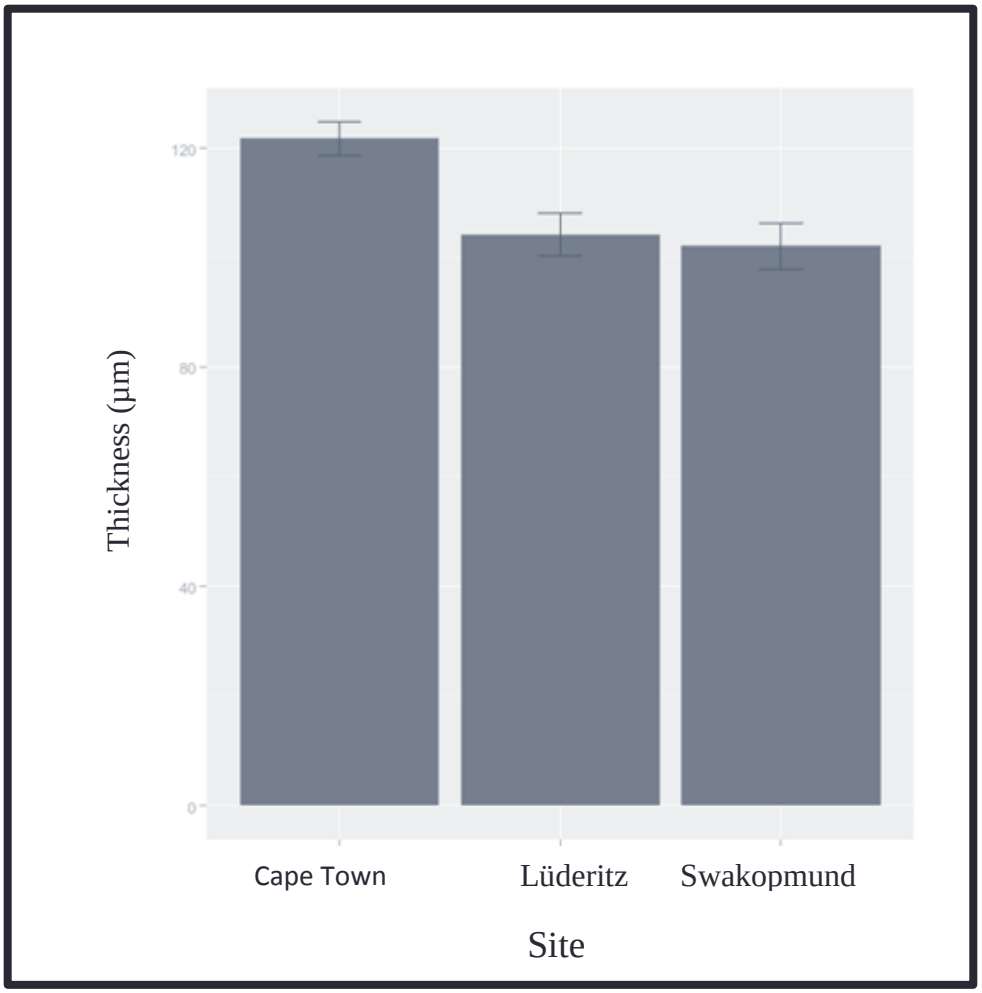
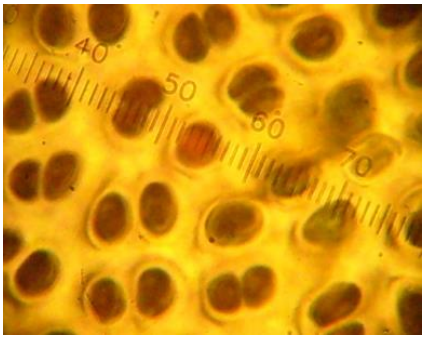


Figure 4.6 Mean thallus thickness (µm) for specimens collected from Lüderitz, Swakopmund and Cape Town.

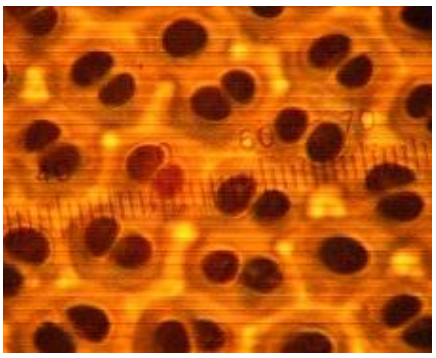
The results of the One Way ANOVA showed that there was no significant difference between the mean thallus thickness from the three sites ($F = 2.612$, $df = 52$, $p = 0.82$) (see Figure 4.6).

4.1.2.2 Tissue anatomy

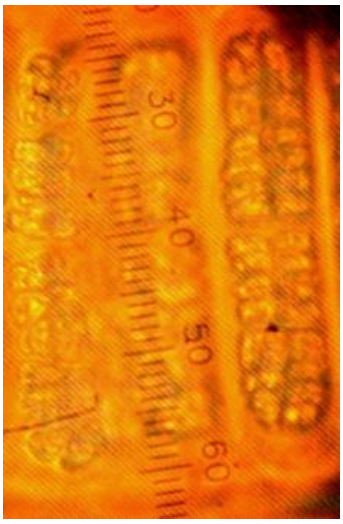
Surface view (SV) and transverse sections (TS) of South African & Namibian *P. capensis* gametophytes are shown (see Plate 4.4; A-J)



A = SV of S. African male vegetative part
Scale:1division = 2.5µm

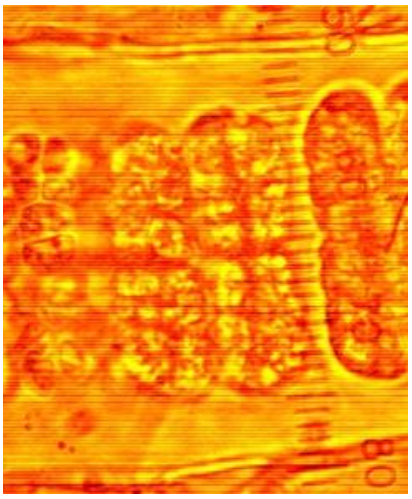


B = SV of Namibian male vegetative part.
Scale: 1division = 2.5µm

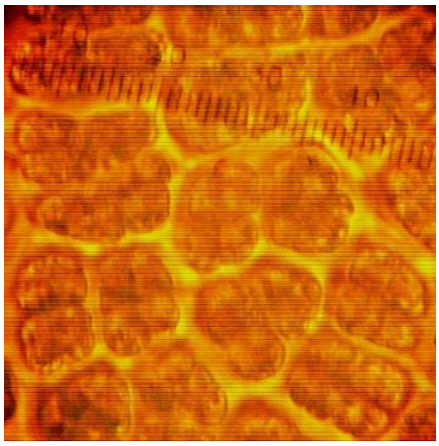


C = TS of S. African male spermatangia 128 pkts of spermatia.

Scale: 1division = 2.5µm

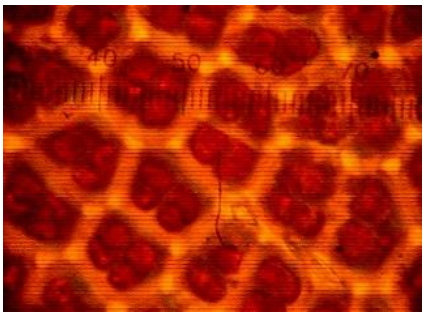


D = TS of Namibian male spermatangia; 64 pkts of spermatia.
Scale 1division = 2.5µm

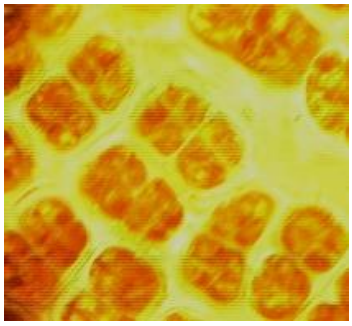


E = SV of S African male spermatangia.
Scale: 1division = 2.5µm

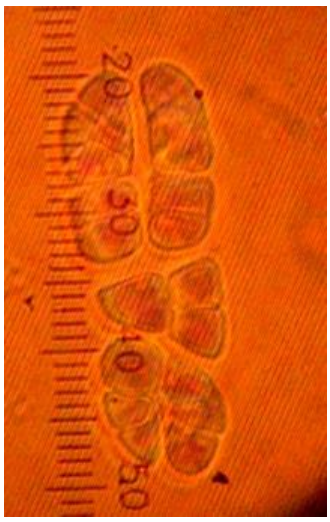
F = SV of Namibian male spermatangia Scale 1division = 2.5µm



G = SV of S. African female zygotosporangia
Scale 1division = 2.5µm



H = SV of Namibian female zygotosporangia
Scale 1division = 2.5µm



I = TS of S. African female Zygotosporangium 32 pkts of zygotospores
Scale 1division = 2.5µm

J = TS of Namibian female zygotosporangium 32 pkts of zygotospores
Scale 1division = 2.5µm

Plate 4.4 (A-J) Tissue sections of South African and Namibian thalli specimens.

The vegetative cells from male South African and Namibian thalli look similar. The transverse sections of the South African male spermatangia were having more tiers of spermatia packets (16) than to the Namibian male spermatangia which had 8 tiers. The SV of the S. African male spermatangia showed 4 x 2 packets of spermatia whereas the SV of the Namibian male spermatangiai had 4 x 1 packets resulting in 128 and 32 packets of spermatia, respectively.

The height of the S. African male spermatangia was approximately 90µm but the Namibian male spermatangia measured between 45 - 50µm. The S. African male vegetative cells were more or less the same size as the Namibian vegetative cells measuring between 18 – 20µm. Both S. African and Namibian female zygotospores had

8 tiers of 4 spores (32 spores) each and were approximately 75µm high. The SV of the S. African zygotosporangia were similar to SV of the Namibian zygotosporangia.

4.2 Ecological field studies on *P. capensis*.

4.2.1 Abundance assessments

4.2.1.1 Percent cover of *Porphyra* thalli

Foliage cover which is often used to assess abundance was not significantly different between the two sites of Lüderitz and Swakopmund (n = 280 for combined data, Mann U = 8663, Z = -1.687 and p = 0.092); (see Figure 4.7).

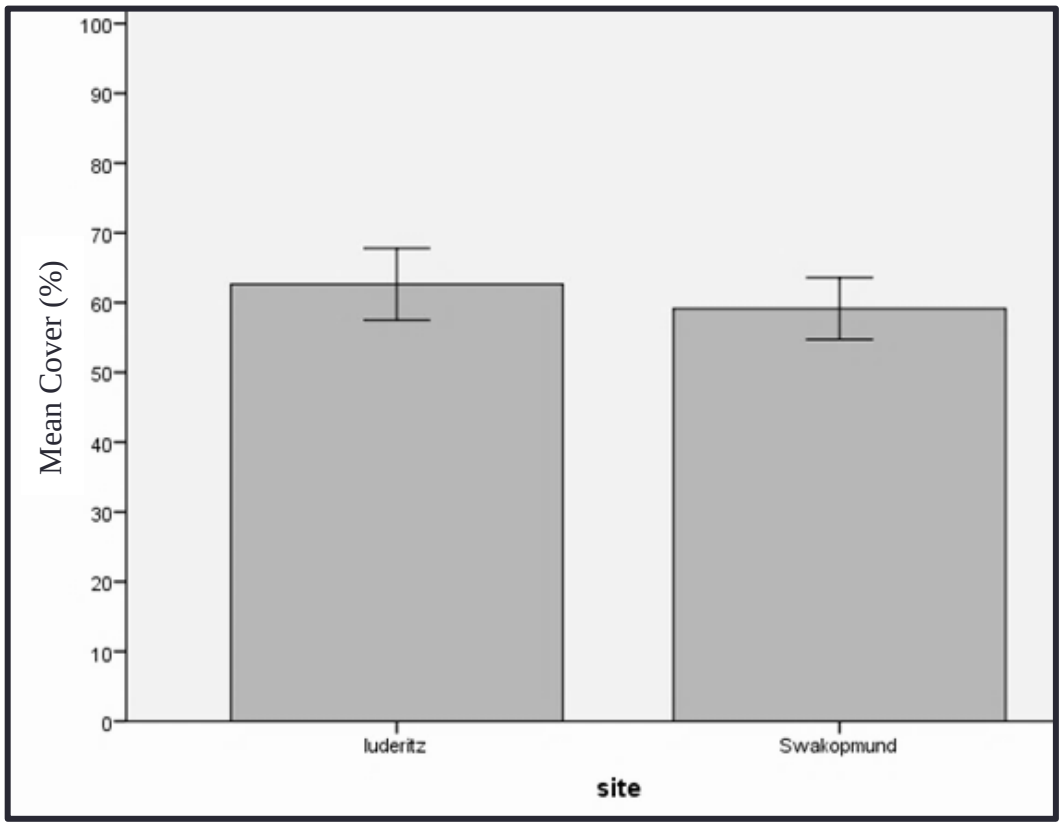


Figure.4.7 Comparison of mean percent (%) cover (± SE) for of *P. capensis* thalli within $1m^2$ quadrats along Lüderitz and Swakopmund shores (summary data: Appendix 9).

4.2.1.2 Standing crop biomass

In this study standing crop biomass of *Porphyra* thalli was considered to be the total amount of accumulated mass harvested from an area of $1m^2$ at a particular time. The Mann Whitney U test for independent samples showed that there was no significant difference in the mean standing crop biomass between Lüderitz and Swakopmund (U = 484.5, Z = -1.504, df = 70, p = 0.133); (see Figure 4.8). Summary data on the relationship between mean standing crop biomass and cover is presented in Appendix 9b

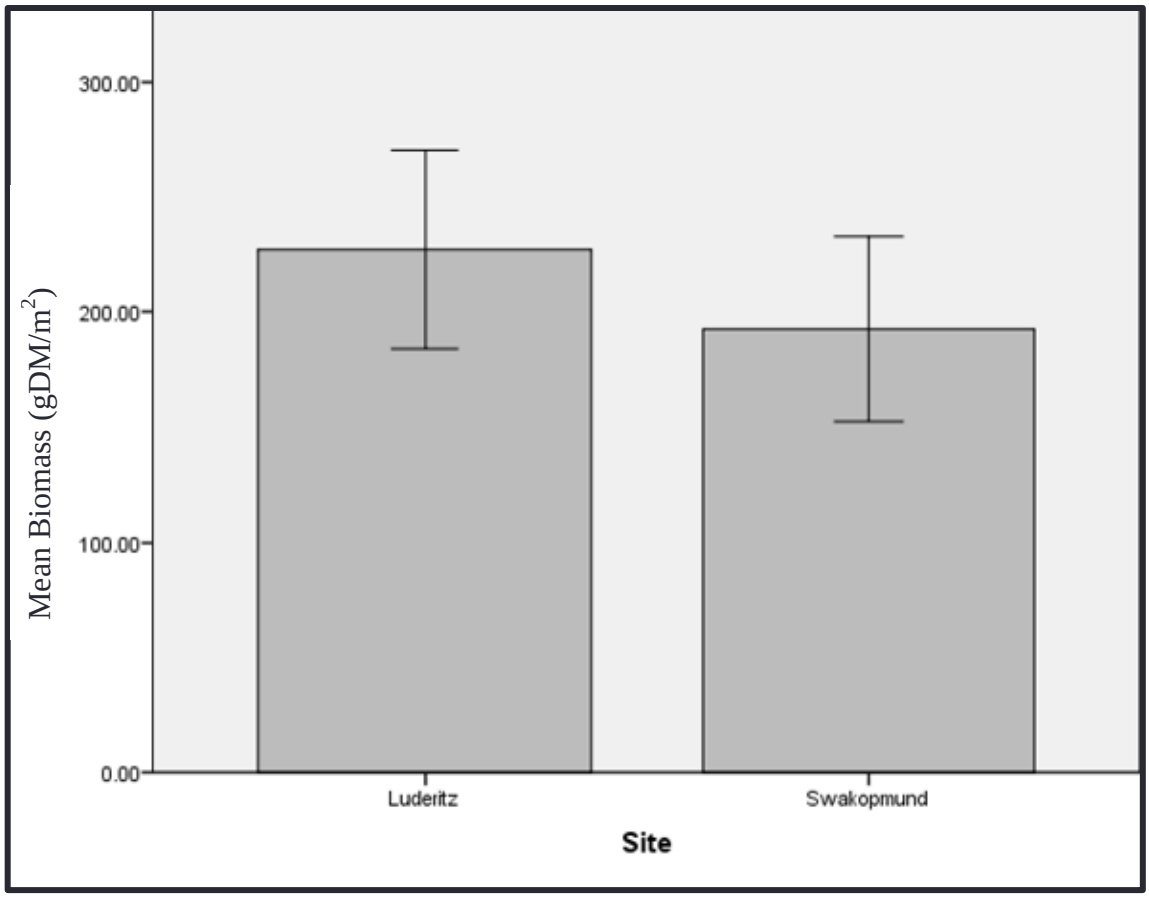


Figure 4.8 Mean standing crop biomass of *P. capensis* thalli within $1m^2$ quadrats along Lüderitz and Swakopmund shores (summary data: Appendix 10)

4.2.1.3 Seasonal variation of environmental variables and standing crop biomass

The Namibian Coast experiences a temperate climate, influenced by the four seasons of summer, autumn, winter and spring. Environmental conditions thus are expected to fluctuate with seasonal cycles. The cool Benguela Curent System however causes modifications to local conditions.

Table 4.1 Summary data on seasonal variation of some environmental variables (SST, ambient temperature, tidal range, and photoperiod) for Lüderitz and Swakopmund combined. (Data source: See Figures 3.3 to 3.7)

Season	**Summer**	**Autumn**	**Winter**	**Spring**	
Variables	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	K/W test
SST (°C)	15.48 ± 0.96	14.695 ± 0.573	12.91 ± 0.785	12.725 ± 0.488	p=0.139
Ambient Temp (°C)	19.13 ± 1.92	18.52 ± 2.524	15.78 ± 1.421	16.04 ± 1.301	p=0.280
Tidal Range (m)	1.69 ± 0.02	1.795 ± 0.021	1.60 ± 0.014	1.78 ± 0.014	p=0.104
Photo-Period (d)	13.12 ± 0.13	11.69 ± 0.049	11.24 ± 0.007	12.76 ± 0.099	p=0.083

The Kruskal Wallis tests on environmental variables showed that there were no significant differences in the various environmental conditions between seasons along the Lüderitz and Swakopmund shores as indicated in the last column of the table above, ($p > 0.05$). However, the Bonferroni multiple comparison Post hoc test revealed that

photoperiod is significantly low in autumn/winter, p = 0.056, same as tidal range which is low between summer/winter, p = 0.055, where as temperature does not vary significantly with seasons. This explains the seasonality in biomass.

Factors that affect the rate of photosynthesis such as light quality and quantity, temperature, nutrients, water and concentration of Carbon dioxide, will affect plant production rate and biomass accumulation. When these factors change in the environment due to seasonality, biomass accumulation will also vary (see Figure 4.9).

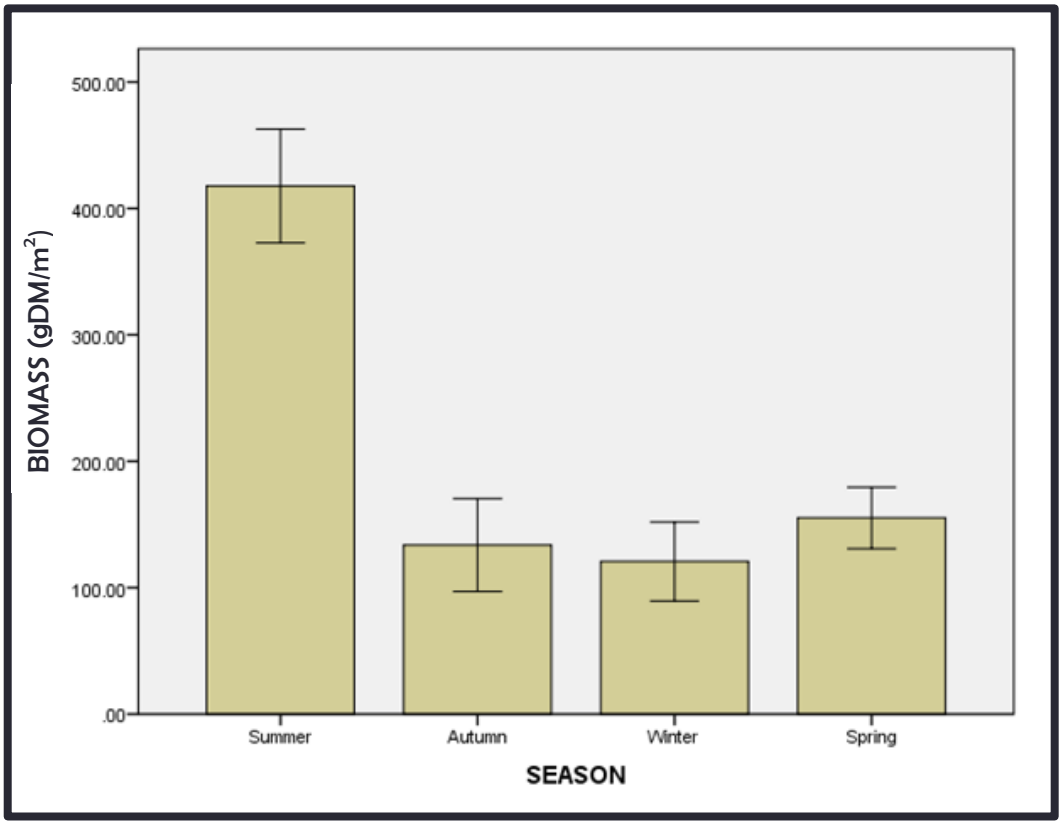


Figure 4.9 Seasonal variation of mean standing crop biomass for Namibian populations of *P. capensis* along Luderitz and Swakopmund shores (summary data: Appendix 11).

The results of statistical analysis showed that standing crop biomass was significantly higher in summer along the Namibian coast as confirmed by the K- Independent

samples, Kruskal Wallis test (X^2 = 102.517, df = 70 p = 0.001). The Bonferroni Post hoc multiple comparison test on the means however revealed that biomass between autumn and winter was not significantly different, p = 1.000; similarly between autumn and spring, p = 1.000 as well as between winter and spring, p = 1.000 Comparison of biomass between summer and autumn, summer and winter as well as between summer and spring revealed a level of significance p = 0.001.

4.2.1.4 Prediction model of thallus mass from percentage cover

The distribution of the data in the chart below (Figure 4.10) shows how standing crop biomass varied within the percent cover values of data collected in September 2011.

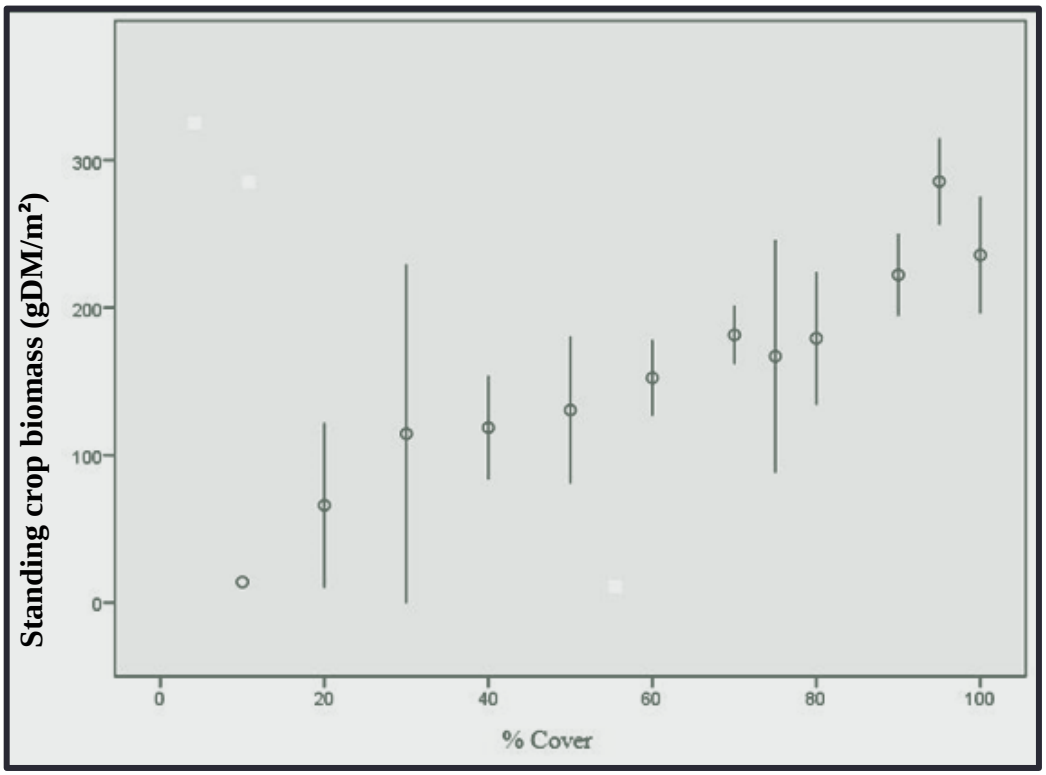


Figure 4.10 The relationship between the percent cover of thalli within 1m^2 quadrats and their corresponding standing crop biomass.

The regression analyses models describing the relationship between standing crop biomass and cover are presented in Figures 4.11 to 4.14 and Appendix 9b. The linear model (Figure 4.11) revealed that there exists a linear relationship between standing crop biomass and percentage cover with a coefficient of determination, R^2= 0. 5653. This implies that 56% of the variation in standing crop biomass can be explained by the variation in percent cover. The subsequent equation is $Y = 2.2779\ x + 19.213$, where Y is the standing crop biomass of *Porphyra* thalli in (grams per square meter of substrate), and x is its visual foliar cover expressed as a percentage.

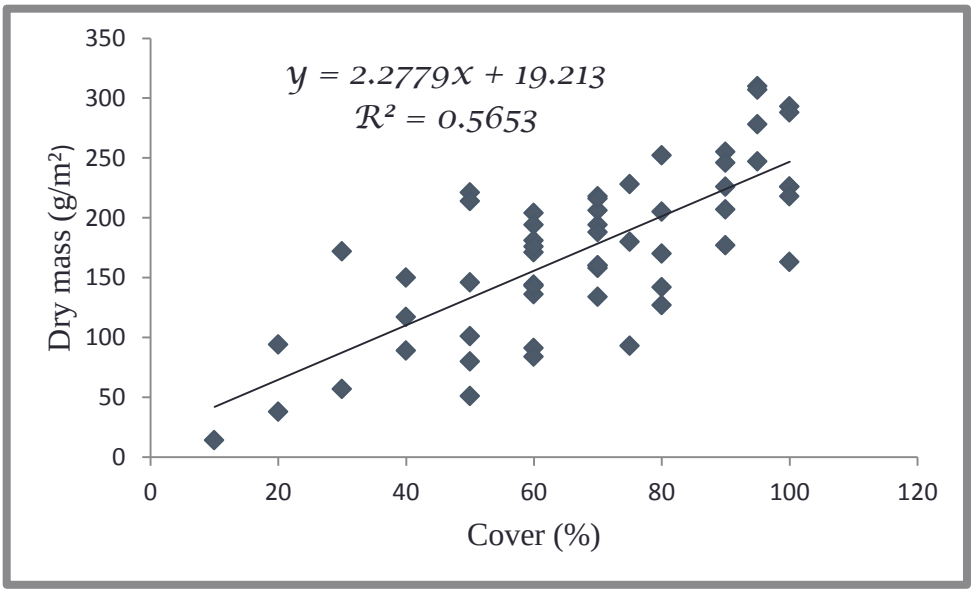


Figure 4.11 Linear regression model of the relationship between percentage cover of thalli and its standing crop biomass.

The second model is the power model (Figure 4.12) represented by the equation:

$Y = 2.5429x^{0.9932}$

Where, Y is the standing crop biomass in grammes per square meter of substrate and x is its visual foliar cover expressed as a percentage. The power model has a higher

coefficient of determination; R^2 = 0.649, implying that 65% of time, biomass can be explained by the corresponding folliar cover at Lüderitz and Swakopmund shores along the Namibian coast.

The coefficient of determination for the exponential model (Figure 4.13) is R^2 =0.5498 which is lower than that of the power model giving it a lower reliability. The polynomial model (Figures 4.14) has a coefficient of determination, R^2 = 0.566. This is similarly lower than the power model making it less reliable to infer standing crop from percentage cover values.

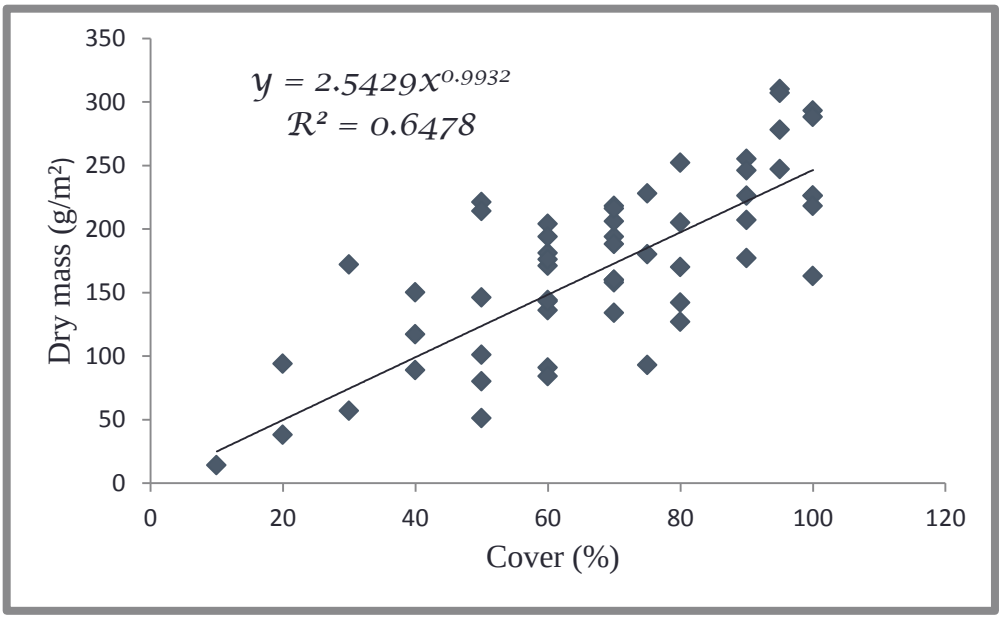


Figure 4.12 Power model of the relationship between percentage cover of thalli and its standing crop biomass.

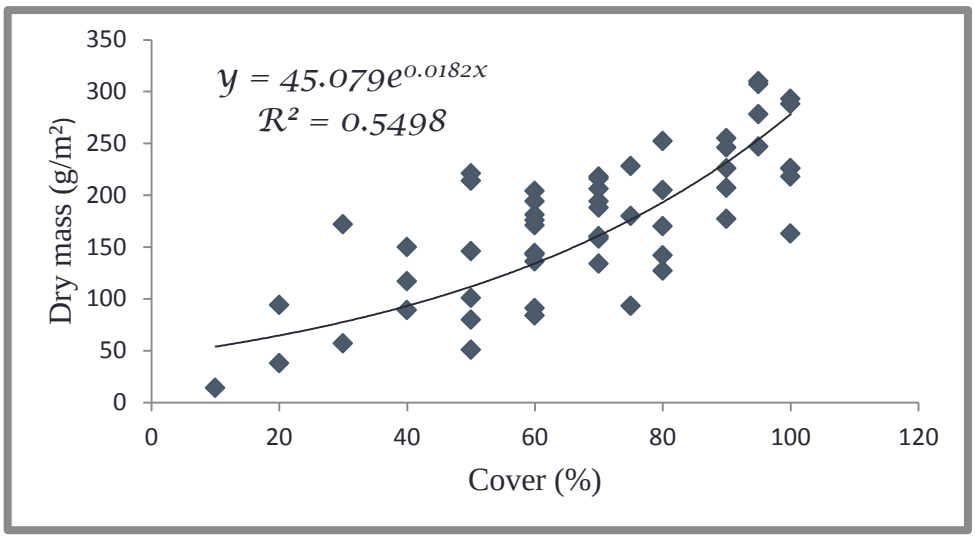


Figure 4.13 Exponential model of the relationship between percentage cover and its standing crop biomass

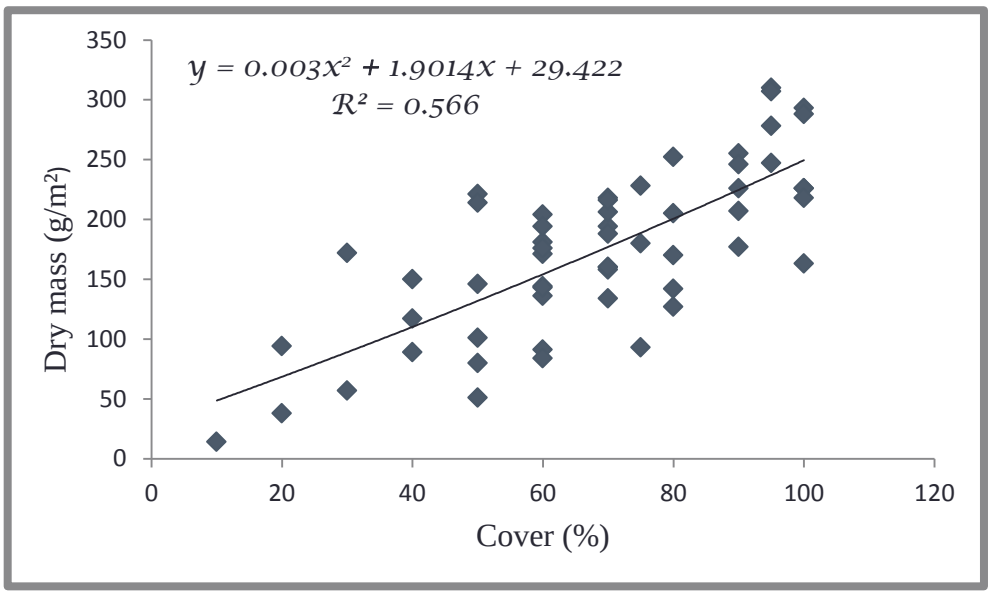


Figure 4.14 The polynomial model of the relationship between percentage cover and its standing crop biomass

4.2.1.5 The effect of harvesting frequency on annual cumulative biomass

Appropriate harvesting regimes are established by bioresource managers to maximise yield while sustaining viable gene pools in the field. This study established a harvesting

frequency of *P. capensis* thalli in the field that would maximise annual yield, and results are presented in Table 4.2 followed by statistical inference. The results showed that regular harvesting frequency (T1) had a positive significant effect on the cumulative annual biomass (Kruskal- Wallis, $\chi^2 = 9.617$, $p = 0.008$), as annual yield increased with frequency of harvesting. Using the Bonferroni Posthoc test on the means the following were the results. There was no significant difference between cummulative biomass of T1& T2 with $p = 0.058$; and between T2 & T3, with $p = 0.992$.

Table 4.2 A summary of mean (initial, control and cummulative) biomass ($gDMm^{-2} \pm$ SE) of *P.capensis* thalli, harvested from experimental plots, T1,T2 and T3.

Treatment	Initial biomass (mean ± SE)	Biomass of controls (mean ± SE)	Cumulative biomass (mean ± SE)
T1	391.54±52.2	170.26±37.6	363.11±74.9
T2	324.00±30.9	175.46±49.5	159.46±62.5
T3	411.89±71.9	185.09±40.9	72.94±31.3

NB: Degrees of freedom N = 14 throughout.

There was no significant difference in the initial mean biomass from the plots representing the 3 treatments obtained from the related samples Friedman's 2 way Anova test by ranks with $\chi^2 = 0.691$ and $p = 0.708$. Similarly, there was no significant difference in the mean biomass collected from the controls at the different plots representing the 3 treatments, as the repeated sample Friedmans test gave $\chi^2 = 3.200$ and $p = 0.202$. The difference between the cumulative biomass between T1 & T3, was significant $p = 0.004$. Regular harvesting T1, (4 times a year) gave a significantly higher

cummulative annual yield than harvesting once a year, T3. Cumulative biomass for T1 was significantly higher compared to its control biomass (t = 2.5777 df 13 p = 0.023), and cumulative biomass for T3 was significantly lower than its control biomass (t = -3.583 df 13 p = 0.003). There was no significant difference between the cumulative biomass and control biomass for treatment T2 (t = -0.256, df 13 p = 0.802)

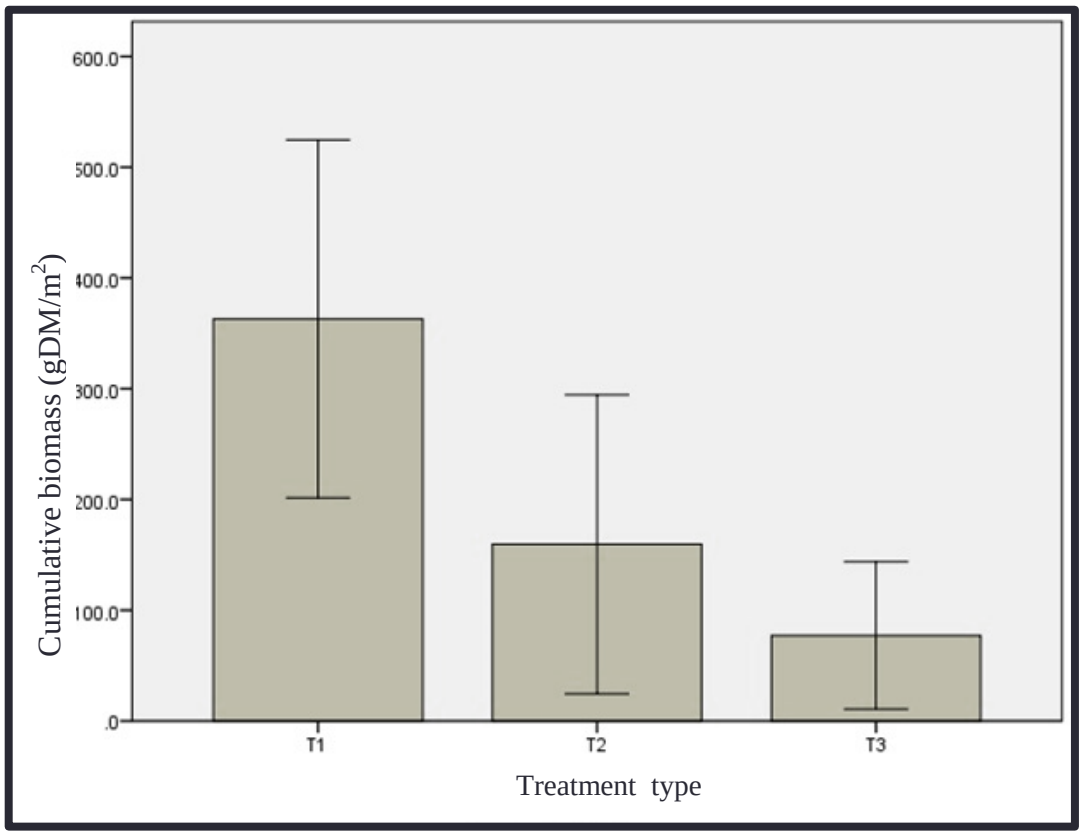


Figure 4.15 The annual cumulative biomass for the three treatments; T1 representing harvesting at three months intervals, T2 representing harvesting at six months interval and T3, harvesting after an interval of twelve months (Data: Table 4.2).

4.2.1.6 The relative growth rate of *Porphyra* thalli

The relative growth rate was calculated as the rate of biomass accumulation between harvesting periods from the regular harvesting treatment (T1) biomass data using Equation1 in section 2.1.9. Harvesting followed a seasonal pattern therefore the relative growth rates could infer seasonal growth patterns (Table 4.3). The KruskalWallis test showed that the variations in growth rate between the seasons were not statistically significant (χ^2 =5.5000, p = 0.139).

Table 4.3 Seasonal variation in the mean growth rate at milligrams per day. Pooled data from blocks a to g for Lüderitz and Swakopmund combined.

Season	Mean growth rate (mgd^{-1}) ± Standard Error
Summer	57.2 ±0.0013
Autumn	46.7± 0.0004
Winter	43.0 ± 0.0002
Spring	53.3 ± 0.0004

NB: Degrees of freedom N = 70 throughout.

4.3 Comparison of nutritional content of *P. capensis* and lettuce

The evaluation of the nutritional content between *Porphyra* and lettuce provided significant data that might encourage Namibians to eat *Porphyra* as a salad or try out different recipes. The results for Iodine are not included since the concentration could not be determined by the analytical method used.

Table 4.4 Mean nutrient composition of *Porphyra* thalli and lettuce from Namibia.

Sample	***P. capensis***	**Lettuce**	**Significance**
Moisture	82.42 ± 2.760	95.6 ± 0.150	*
NDF	29.8 ± 0.850	23.48 ± 0.480	*
Ash	15.41 ± 2.200	10.00 ± 0.270	NS
Ca	0.279 ± 0.010	0.23 ± 0.004	*
Mg	0.23 ± 0.033	0.09 ± 0.002	NS
P	0.096 ± 0.007	0.071 ± 0.002	NS
N	3.17 ± 0.167	2.52 ± 0.045	NS
CP	19.82 ± 1.040	15.77 ± 0.230	NS
Fat	0.495 ± 0.060	2.215 ± 0.041	**
C	34.98 ± 3.630	47.08 ± 0.015	NS
Fe	73.37 ± 10.300	38.05 ± 0.050	NS
β-carotene	277.3 ± 35.510	174.1 ± 26.510	NS

NB: Degrees of freedom N = 6 throughout.

Key: for Table 4.6, (NDF = Neutral Detergent Fibre; Ca = calcium; Mg = Magnesium; P = Phosphorus; N = Total Nitrogen; CP = Crude Protein; C= Carbon; all expressed in % grams Dry Matter (% gDM). Fe =expressed mg/Kg Dry Matter.

β- Carotene expressed in mg/100gDry Matter.

Levels of statistical significance:

NS = $p > 0.05$; * = $p \leq 0.05$; ** = $p \leq 0.01$; *** = $p \leq 0.001$

The results established that fibre was significantly higher in *Porphyra* than in lettuce (p = 0.016) as well as calcium (p = 0.036), whereas fat, was significantly higher in lettuce than *Porphyra* (p = 0.0026). Moisture content was also higher in lettuce than in *Porphyra*, p = 0.021. The other nutrients were not statistically significantly different. When compared to Namibian *P. capensis* however, crude protein was significantly higher in South African *P. capensis* (p = 0.006).

Seaweeds thalli accumulate minerals from the seawater. Most of these minerals have dietary value to humans. Both Lüderitz and Swakopmund shores have rocks made up of muscovite schists and gneisses (metamorphic rocks) as well as granites and aplites (igneus rocks) as analysed by X- ray diffraction for this study. The bedrock along the coast was described by Robertson et al. (2012) as made up of metamorphic complexes and meta-sediments. This means that the main rock constituents are either quartz and / or micas along both shores. These solid rocks provide a stable substratum for seaweeds. None of the rock constituents however contained elements in a water soluble form that could leach out into the sea increasing localized concentrations of mineral elements, affecting the composition of the various elements in the thalli.

4.4 Laboratory initiated cultures of Namibian populations of *P. capensis.*

Mariculture of *Porphyra* species is routine in most Asian countries as well as in other parts of the world, where cultures are initiated in the laboratory, followed by outplanting the resulting miniature blades (thalli) in large ponds or out into the sea. The success in completing the life cycle depends on optimizing growth conditions of temperature, photoperiod, irradiance, nutrients and salinity for each developmental phase in the laboratory as well as outside. This is the first study that has initiated laboratory cultures

for the Namibian populations of *P. capensis* and the different growth phases as observed in laboratory maintained cultures are shown in Figure 4.15. The different phases are explained in sections 4.4.1 – 4.4.4 below.

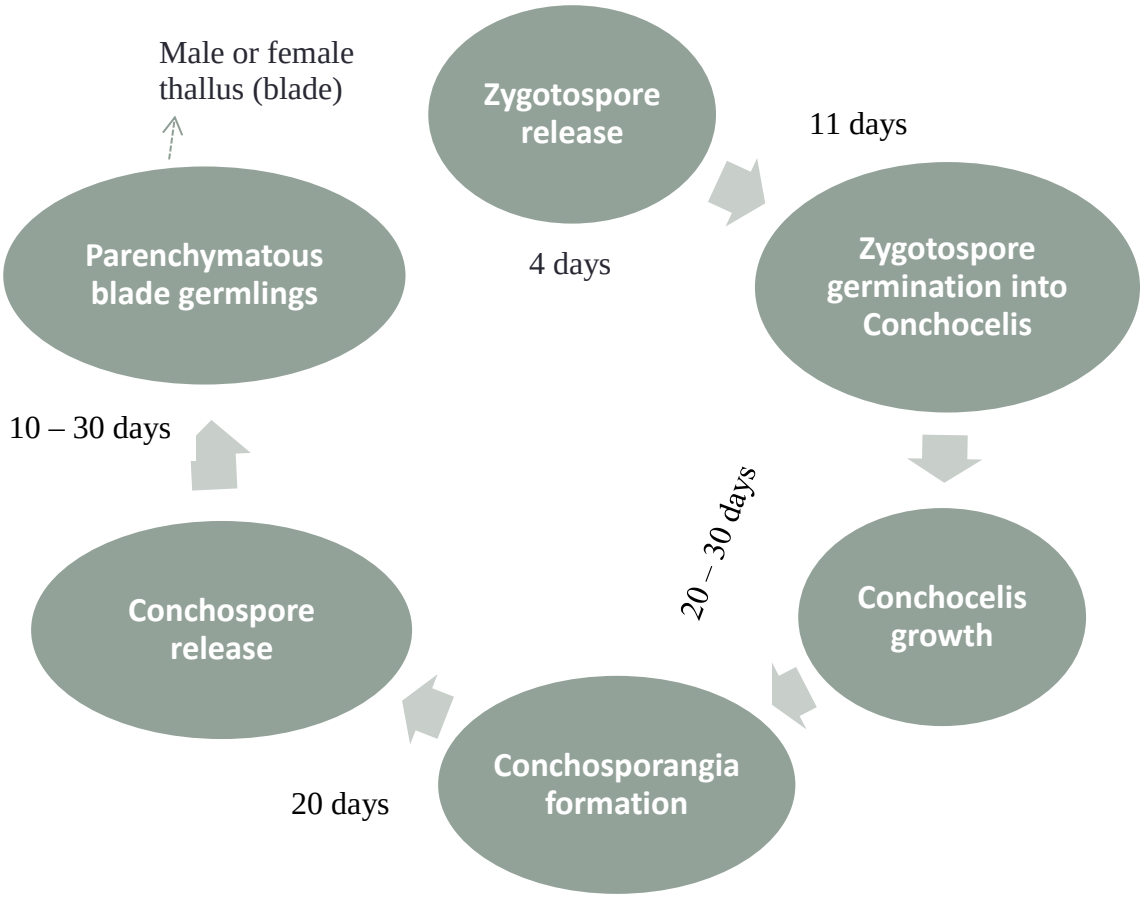


Figure 4.15 Growth cycle and approximate time taken between the growth phases for the Namibian *P. capensis*, in the laboratory maintained cultures at SANUMARC.

4.4.1 Spore release

Spores from mature female thalli were released from strips starting from 4 hours up to 48 hours. Stellate chromatophores, characteristic of *P. capensis* were visible within the spores.

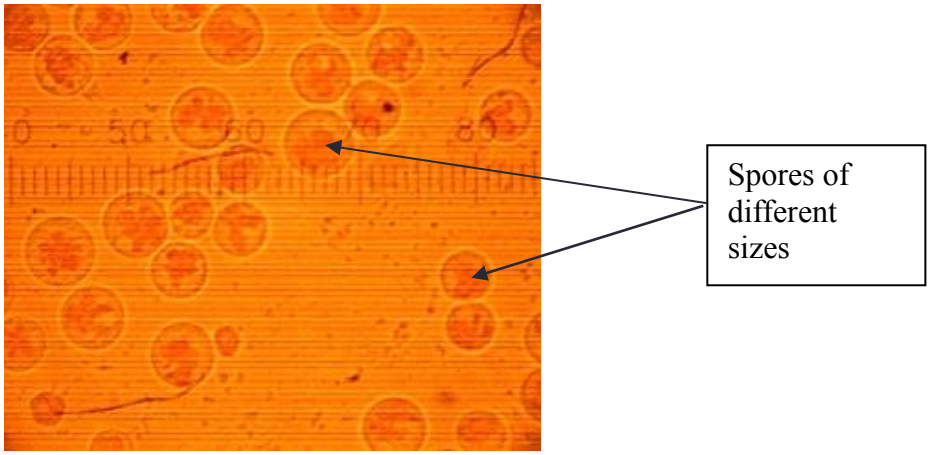


Plate 4.5 Different sizes of spores released from mature female thalli. *P. capensis* usually release spores of varying sizes. Scale: 1division = 2.5µm.

Larger spores measured between 13.75-16.25µm, while the smaller spores measured between 8.75-11.25µm. Some spores were released after 4 hours while still on the laboratory bench at 22°C, 155 Lux in sterile seawater of salinity of 30 PSU. Spore release continued when the cultures were moved into the growth chamber set at 15°C, irradiance of 3268 Lux from fluorescent lights and half strength PES medium at 35 PSU salinity.

4.4.2 Spore germination into conchocelis

The germination of zygotospores, recognized by the presence of protruding germtubes started from day 10 to day 11 in most cultures (Table 4.5), after the zygotospores were released from the thalli strips. Germination was unipolar (Plate 4.6). Germination was minimal to start with (about 10- 12%) but 4 days from the start of germination, most zygotospores (55-70%) in the microscopic field of view had germinated. While more zygotospores continued to germinate, the germtubes started growing to form thin filaments. Within a week of germination almost all the zygotospores in the field of view in most cultures had germinated. Germination tubes developed into thin branching filaments forming different type of spores (oval shaped). These were the the conchocelis filaments bearing conchocelis archaespores.

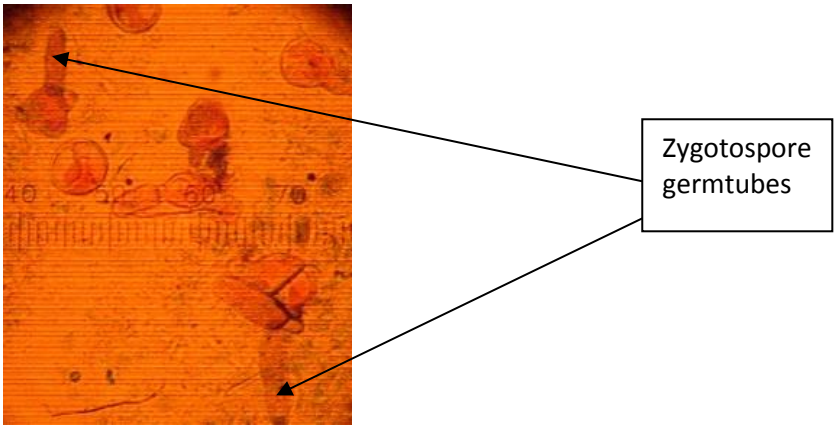


Plate 4.6 Germinating zygotospores exhibiting unipolar germination. Scale: 1division = 2.5µm.

Table 4.5 Mean cumulative percent germination of released zygotospores in modified PES culture medium in a growth chamber at 15°C, 16L: 8D, 3270 irradiance (Lux).

Days after spore release	Mean cumulative germination (%) ±SE
11	11.75 ± 0.84
13	19.25 ± 1.11
15	30.00 ± 4.24
17	58.25 ± 3.32
19	86.50 ± 2.53
21	99.5 ± 0.5

Spores that failed to germinate in some of the cultures, disintegrated (Plate 4.7), releasing their protoplasm into the extracellular matrix. Most spores had germinated within 3 weeks. Spores disintegrated when the culture medium salinity increased beyond 38 PSU due to evaporation (salinity of sea water is around 34-35PSU).

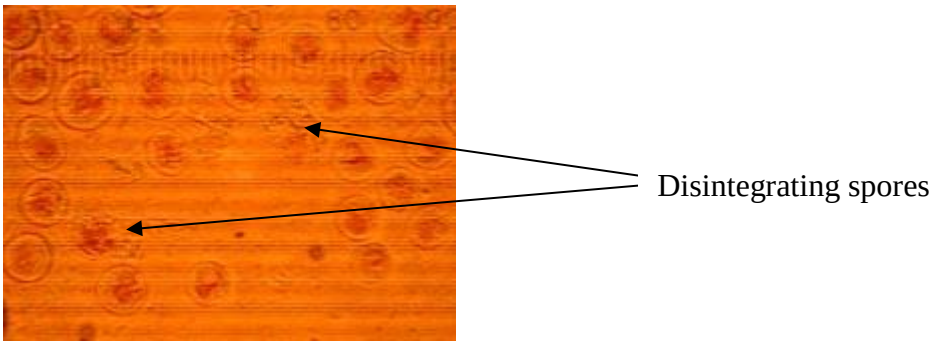
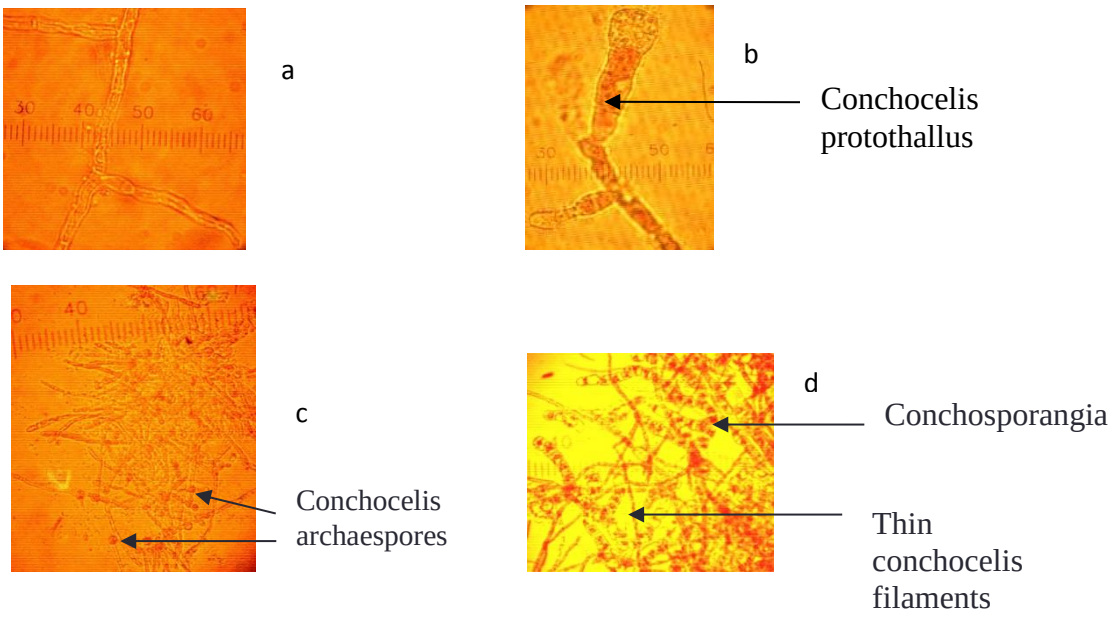


Plate 4.7 Disintegrating spores. Scale: 1division = 2.5µm.

4.4.3 Conchocelis development and mass production

About 30 days after spore release conchocelis filaments tufts (colonies) were seen in most of the petridishes (see Plate 4.8). The fillaments were pinkish-red in colour and septate. The conchocelis archaespores produced more conchocelis filaments creating tufts of colonies. Conchocelis prothallus is diagnostic of *P. capensis* as well (Dlaza 2011). Mature conchocelis filaments become dense and fatter as conchospores developed within them.



Plates 4.8 (a-d); Young conchocelis filaments showing the alternate branching of filaments as is typical of *P. capensis.* (b); Conchocelis protothallus on a filament, typical of *P. capensis*. (c); Colony (tuft) of conchocelis filaments bearing conchocelis archaespores. (d); Thin filaments (conchocelis) and fat filaments (conchosporangia) in a mature conchocelis culture. Scale: 1division = 2.5µm.

4.4.3.1 Mass production of conchocelis.

About 0.1674g of conchocelis was inoculated into a bubble culture medium in the mariculture laboratory as indicated in section 3.5.3 and after 15 days the weight had increased 6 times (see plate 4.9).

Plates 4.9 (a-b). (a) The bubble culture containing innoculum, as set up in the mariculture laboratory. (b) Free floating conchocelis colonies 15 days after innoculation.

4.4.4 Conchosporangia development and conchospore formation

Conchosporangia (fat filaments) were formed in the growth chamber at 15°C, 16L: 8D, 3270 Lux in petri dishes containing oyster shells and as free floating cultures in petri dishes. Conchosporangia formed on oyster shells had deep orange colour conchospores (Plate 4. 10 & 4.11). Conchosporangia from free floating conchocelis in the petri dishes were yelowish in colour (see Plate 4.12).

Plate 4.10 Oyster shells penetrated by conchocelis filaments (reddish spots on underside ofshell); followed by conchosporangia filaments being released from the shells as seen in the mass at the arrow head

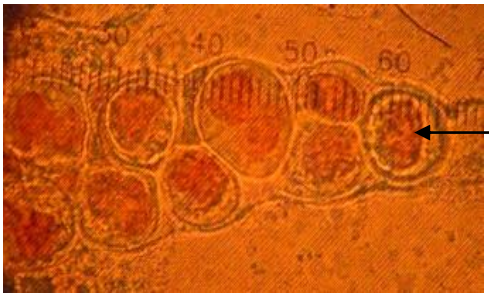


Plate 4.11 Details of conchosporangia (fat filament) with conchospores from culture containing oyster shells. Scale: 1division = 2.5µm.

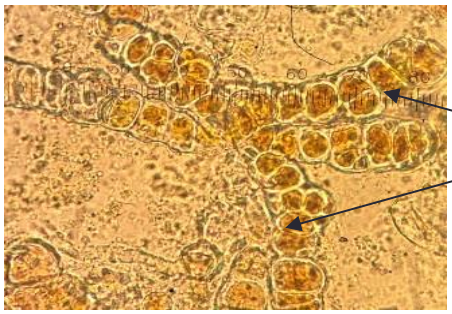


Plate 4.12 Conchosporangia filaments bearing conchospore (from culture dishes of free floating conchocelis). Picture taken from a Zeiss dissecting microscope,

4.4.5 Thallus development

Conchospores released from ground conchosporangia and distributed into petri dishes containing fresh media in the growth chamber, started dividing and after 11 days when some rhiziods were visible (see plate 4.13). Dividing conchospores developed into parenchymatous thallus germlings 26 days after conchosporangia were ground to release conchospores. These were dividing in a sheet like manner (see plate 4.14) as well as in random projections.

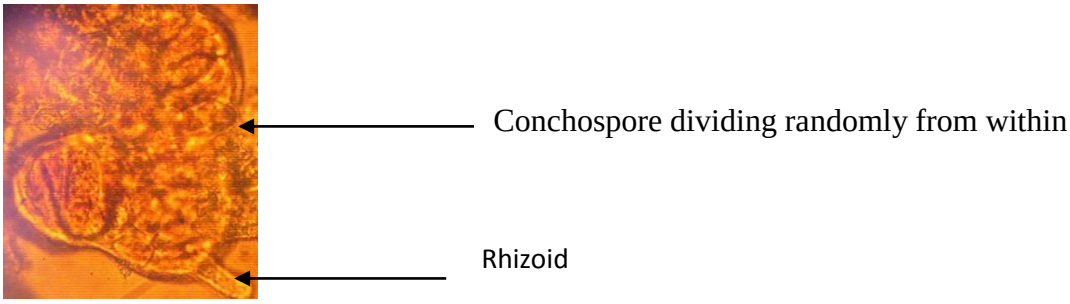


Plate 4.13 Germinating conchospore, 11 days after conchospore release

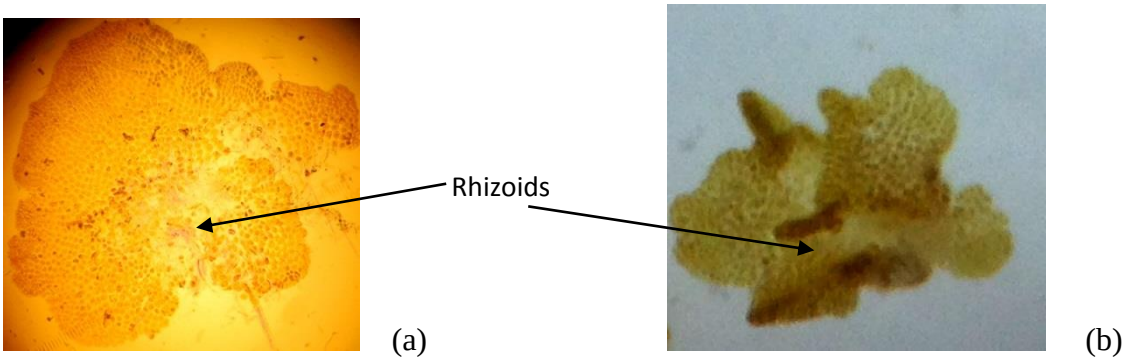


Plate 4.14 Parenchymatous phase of thallus development (germlings a & b). Pictures taken from a Zeiss dissecting microscope 100 X.

Pictures without a scale were taken from a dissecting microscope where the ocular graticule could not be inserted (see plates 4.9, 4.11, 4.13 and 4.14; a & b).

CHAPTER 5

5.0 DISCUSSION

5.1 Species characterization resulting from morphological and molecular data

The Namibian *Porphyra* species from the upper eulittoral zone has been referred to as *P. capensis* as it morphologically resembles the S. African *P. capensis* as described by Graves (1969). The 18SrDNA gene had also been used to reassess the taxonomic status of the genus in S. Africa (Griffin, 2003; Jones et al., 2004; Milstein & Oliveira, 2005). In the current study the Namibian *P. capensis* and the S. African *P. capensis* were phylogenetically compared using the same gene. The ITS gene was also used to compare the Namibian sequences with the typical South African *P. capensis* sequences. Similar results were obtained; that is the sequences from the South African specimens formed their own monophyletic group. Both results confirm that South African *P. capensis* belong to its own clade despite sharing a common ancestor with the Namibian *P. capensis.* This study therefore proposes the Namibian *P. capensis* as a sub-species of the South African species.

The other reason why the Namibian *P.capensis* should attain a sub-species status is the degree of dissimilarity among the multisequence alignments (MSA), where there is a divergence of 2.1% between the South African *P. capensis* sequences and the sequences from the Namibian populations of *P. capensis*. The difference is caused by indels as seen in Figure 4.2 where, nucleotides present in the type S. African specimens' sequences are totaly missing in all the Namibian specimens' sequences. The divergences between the sequences belonging to the South African *P. capensis* clade from the current study are

between 0.1 & 0.2%. Brodie et al. (1996) delimited *P. purpurea* from *P. laciniata* on the strength of a single base substitution in their alignment with a percentage difference of 1.3% using the rubisco spacers of *P. purpurea* and *P. laciniata*.

Griffin (2003) in an investigation of variation within the nuclear SSUrDNA gene on the genus *Porphyra* from South Africa, reported that pairwise distances between sequences from S. African *Porphyra* resulted in a disimmilarity ranging from 0% - 8.0%, while the S. African *P. capensis* 'complex' shared divergence of 1.8%. That study revealed a high level of variation within the genus and recommended further taxonomic evaluation of the S. African *P. capensis* complex to verify its taxonomic status. This implied that within what was referred to as S. African *P. capensis* complex, there could be sub-species and further studies were recommended to thoroughly characterize the group (Jones et al. 2004).

In a *Champia* Desvaux delimitation study, Koh, Cho, & Kim, (2013) delimited species with 3.2 - 4.4% divergences. *Champia sp* and *Champia recta* Noda were delimited as different species as their divergence ranged from 1.7 to 13.6%. The South African *P. capensis* and the Namibian *P. capensis* showed a divergence of 2.1% and this warants at least subspecies recognition.

There are no marked differences between the general morphology of the typical South African *P. capensis* specimens and the Namibian specimens because of the phenotypic plasticity observed in *Porphyra* species in general. Stegenga et al. (1997) had used thallus thickness differences to delimit S. African *Porphyra* species. Some Namibian specimens shared the same thickness to the typical *P. capensis* from S. Africa but their sequences were different. The South African *P. capensis* specimens' thickness ranged

from 110 – 130µm whereas specimens from the Namibian populations of *P. capensis,* thickness ranged from 60 – 150µm. Specimen # 40 which had a thallus thickness of 60µm, is similar in the multisequence alignment to specimens 1 with a thickness of 100µm; specimen 9 with a thickness of 150µm and specimen 14 with a thickness of 80µm. This means that they belonged to the same group even though their thallus thickness was different. The mean thallus thickness of the Namibian specimens (Figure 4.6) was not significantly different from the typical *P. capensis* specimens from S. Africa proving some degree of similarity in the morphology and anatomy of the two entities. The observed differences are part of *Porphyra's* phenotypic plasticity and, besides, thallus thickness is not a reliable character to delimit species within the *P. capensis* complex.

Further anatomical comparisons of thalli revealed some differences between South African specimens' male spermatangia and the Namibian (Plate 4.4, C & D). The South African specimens had at least16 tiers of 8 spermatia packets (128) as was similarly presented in Graves, (1969). Stegenga et al. (1997) described the South African *P. capensis* as having up to 24 tiers of spermatia packets. The Namibian specimens' spermatangia were however, made up of 8 tiers of 8 spermatia packets (64). The zygotosporangia had 8 tiers of 4 zygotospores (32) in both the S. Africa and Namibian specimens. In view of these differences and similarities in the anatomical sections as well as the sequence dissimilarities, this study considers the Namibian populations of *P. capensis* belonging to a sub-species of the *P. capensis* from South Africa.

The differences between the two entities could have resulted from adaptations to differences in sea surface temperature. The Benguela Current system which runs from

Cape Agulhas on the west coast of S Africa to Southern Angola has been divided into two ecological provinces with the centre at the strong upwelling cell aound Lüderitz (Engledow & Bolton, 2003, Engledow et al., 1992; Hutchings et al., 2008; Kreiner, Van der Lingen & Freon, 2001), forming the northern and southern Benguela regions. The southern half of the Benguela region's waters are cooler (SST range between 11.5 – 14.0 °C mean monthly temperature) while the waters along the northern half of the Benguela region are warmer (12 -18.4°C around Walvis bay) as reported in Engledow et al. (2003). One of the effects of the regional separation is the differences in seaweed species diversity as reported by the same authors. Teske, Fronema, Barker et al. (2007) have attributed the presence of intraspecific genetic variants of the caridean shrimp (*Palaemon peringueyi* Stebbing 1915) to biogeographic boundaries caused by temperature differences. Isolation by distance was also imputed for the low level of gene flow between the shrimp regions.

Observations on the distribution of sardines (*Sardinops sagax* Mann) revealed that there appears to be a northern stock extending from southern Angola to Lüderitz, and a southern stock extending from the Orange river to Kwazulu- Natal, and the two do not migrate into the other's territory (Kreiner, Van der Lingen & Freon, 2001). If this has been the case one can speculate that Namibian *P.capensis* has been geographically isolated from the S. African species and even though they may have shared a common ancestry, they have developed some genetic variations with time.

5.2 Field studies on *P. capensis* abundance and harvesting treatments

The standing crop biomass of *P. capensis* in Lüderitz was similar to that of Swakopmund. This part of the Namibian coast is in the northern part of the Benguela

Current System that experiences regular upwelling with Lüderitz being its epicenter (Engeldow, & Bolton, 2003; Griffin, Bolton, & Anderson, 1999 & Sakko, 1998). Upwelling brings with it nutrients from the continental shelf. The upwelled water travels northwards distributing nutrients along the coast (see Figure 1.2). Upwelling mixes the water and this explains the minimal temperature difference between Lüderitz and Swakopmund shores (Figure 3.3). Engledow & Bolton (2003) investigated the factors that affect seaweed trends along the Namibian coast and made the conclusion that factors like temperature differences, sand inundation and availability of suitable substrates along these shores are responsible for the variations observed in species diversity and abundance. This study established that mean annual temperature differences are however not statistically different between the two sites explaining the similarity in standing crop biomass.

P. capensis gametophytes occupy the upper eulitorral zone and changes in sea surface temperature would only affect the thalli during submergence. This is true for both Lüderitz and Swakopmund shores as they experience similar tidal patterns with very little time lag between the two sites. The duration of emergence and submergence is the same since tidal amplitude as a factor was found not to be significantly different between the two shores in this study (Figure 4.9). The effect tidal amplitude would have on the conchocelis filaments burrowing within moluscan shell in the sublittoral zone would thus be similar along both shores and resultant temperature variations would be similar for Lüderitz and Swakopmund shores. This means *P. capensis* in the gametophyte or sporophyte phase experiences similar temperature variations along the two shores resulting in similar biomass patterns.

There is however, more sand inundation along the Central Namibia coast (Swakopmund area) than Lüderitz making the area more heterogenous but *Porphyra* populations always occur in patches upon the eulittoral zone and above, and these are similar for both Lüderitz and Swakopmund areas. The few sites that have experienced sand inundation have not caused a statistically significant difference in the biomass most probably because the sample size was not very large.The similarity in environmental parameters make both sites suitable for mariculture farms which will not be affected by heterogenousity of habitats and substrates.There is therefore potential for *Porphyra* mariculture farms to be successfully established at both sites given the similarity in environmental factors discussed above.

This study showed that standing crop biomass was highest in summer compared to other seasons. The data on seasonal variation of abiotic variables of temperature, photoperiod and tidal range, showed that there was no significant difference in the variables in between. The synergistic effect of increase in sea surface temperature together with increase in photoperiod in summer could be the reason why summer biomass was higher than the other seasons as there is more time and energy for photosynthesis.

McQuaid (1985) reported that there were seasonal variations of algal species biomass (including *Porphyra*) along the rocky shores of Cape of Good Hope. The author also found out that there were correlations between biomass and fluctuations in abiotic factors of radiation, SST and tidal regime, where biomass was higher in summer when radiation & sea surface temperature were elevated and day length increased, and reported that raised high tides led to greater biomass. The levels of nitrates in the seawater along the Namibian shores peak in September and seem to be low in December (Fig 3.7). This

coincides with an upwelling surge along the central Namibian coast promoting growth and biomass accumulation resulting in a peak summer biomass. Nitrogen levels in seawater were reported to directly control the biomass of macroalgae increasing biomass when levels in seawater increased (Fong et al., 1993).

Even though seasonality is not as pronounced in Namibia as it is in South Africa where Griffin et al. (1999) reported dense summer seaweed populations, Namibia does experience an elevation in sea surface temperature during summer and may be this amount meets the threshold to stimulate increased productivity. Molloy (1990) concluded that intertidal seaweeds on the Namibian coast showed marked seasonality despite small variations in temperature and light, and that autumn showed maximum cover for most seaweeds tested but the maxima spanned through summer and early winter.

This study showed that regular harvesting (T1) led to a high cumulative annual yield compared to harvesting twice (T2) or once a year (T3), (see section 4.3.1.5). This can be explained by the fact that harvesting removed mature and less productive individual plants (*Porphyra* thalli erode after spore release; personal observation) that were providing shade for unharvested sporelings allowing light penetration leading to growth. New growth from sporelings and regrowth from holdfast leads to increased biomass accumulation as turnover increased.

Griffin et al. (1999) similarly reported increased growth in harvested quadrats and this was explained as resulting from increased recruitment of sporelings or regrowth from holdfasts. The same authors suggested that as long as harvesting was not so extensive as to reduce thalli that produce spores for continuous recruitment, *Porphyra* populations

were capable of providing sustainable yield in the wild. It has been reported that in land based plants and under favourable conditions, vigorous regrowth or overcompensation takes place in response to herbivory (Belsky, Carson, Jensen et al. 1993). The authors describe overcompensation as increased vegetative productivity as a consequence of herbivory or any other physical damage to above ground vegetation. Knapp et al. (2012) similarly reported that grazing altered the canopy structure by allowing light to reach the ground layer, enabling growth from tillers and dormant seeds that needed light to germinate, increasing primary production.

Turner (2003) reported that when *Porphyra abbotae* was harvested by hand it regenerated itself so quickly that within a month it had regenerated enough for repeat picking. Further-more two pickings from the same site in a year were considered sustainable along the Pacific Coast of Canada. In this study, the control plots were not harvested regularly so as to supply spores for further recruitment of harvested patches.

This study showed that relative growth rates did not vary significantly with seasons along Lüderitz and Swakopmund shores. As discussed earlier, seasonality is not pronounced along the Namibian coast because of the effect of the cool Benguela Current System cooling the waters in summer and intrusion of some warm waters from the Angolan border warming the waters in winter (Engledow, & Bolton, 2003). This implies that mariculture farms can be established at both sites.

Griffin et al. (1999) argued that poor recruitment of *Porphyra* was caused mainly by grazers that fed on sporelings rather than modest fluctuations in environmental parameters. *Porphyra* is adapted to the extremes of dessication and high temperature as well as irradiance which is typical of upper littoral zones, meaning mariculture farming

in areas where grazers are monitored and controlled will lead to high annual yield. The factors that have been reported as being limiting to recruitment, distribution and productivity in seaweeds are sand inundation, presence of grazers and availability of suitable substrates (Engeldow, & Bolton, 2003), and these will not affect mariculture farms.

Four models were presented in this study to predict standing crop biomass from percentage cover (Figures 4. 11 - 4. 14). The power model (Figure 4. 12) with the highest coefficient of determination (0.6478) compared to the other models, can explain the variation of biomass from percentage cover values 65% of the time only. This low coefficient of determination can be explained by the patchy distributions of *Porphyra* populations along the shore (Appendix 2). Biomass is either very high within a small area as a result of clumping or very low as a result of patchy distribution. The growth form and state of thalli accounts for the inability to get higher coefficients of determination as juveniles and thin laciniate thalli will not have the same biomass within a standard area compared to fully grown reniform and cordate thalli.

The Power model equation in Figure 4.12 can be used as a rough estimate of biomass when percentage cover is known, as a quick assessment of standing crop biomass in the field for management purposes. Mariculture however, will supply thalli of uniform size and will not suffer from patchy distributions as the artificial substrates (nets) are placed in such a way as to avoid shading of thali.

5.3 Nutritional content of Namibian *P. capensis.*

This study showed that there are a number of dietary mineral elements in the Namibian populations of *P. capensis* and lettuce (Table 4.5). Crude protein was significantly higher in *Porphyra* sp. from Japan (p = 0.004) but not significantly different from Hawaian *Porphyra sp.* (p = 0.138). Magnesium (Mg) was significantly lower in Namibian *Porphyra* when compared to South African *P. cap*ensis and Hawaiian *Porphyra* with (p = 0.016) and (p = 0.008) respectively. These differences are probably due to diffences in the concentration of the elements in their respective waters.

The nutritional content of specimens from the Namibian populations of *P. capensis* is similar to that of lettuce in many aspects. When compared to lettuce the quantities of fiber are higher in *Porphyra* than in lettuce. MacArtain, Gill, Brooks, Campbell & Rowland (2007) discussed the advantages of including fiber in a diet and indicated that eating about 24g of seaweed could provide 12.5% of a person's daily fiber needs without increasing one's glycemic load that usually accompanies whole grain fiber intake. Reduction of fecal transit time which is facilitated by high fibre diets can prevent colon cancer. Most high fibre content foods such as whole grains increase glycemic load which could cause complications with obesity and Diabetis. Lettuce is a very popular salad in Namibia but its fibre content is not as high as that of *Porphyra*.

Porphyra is currently not consumed in Namibia but its taste can be acquired with frequent consumption of its products. *Porphyra* snacks can be sold in local health shops for promotion with information on the nutritional content on the packet (see Appendix 1b). *P. capensis* can be dried and stored or packaged in different forms without spoilage or loss of nutritional content (Turner, 2003) unlike lettuce which can only be consumed

fresh. Fresh produce on market shelves get spoiled fast because of the high ambient temperatures in Namibia. Dried *Porphyra* can have a long shelf life and will not require refrigeration. The amount of fat in *Porphyra* is lower than in lettuce, which is good for consumers interested in weight management. The lipids present in seaweeds are made up of poly unsaturated fatty acids (PUFA) such as OMEGA 3 and OMEGA 6 fatty acids (MacArtain et al., 2007). These essential fatty acids must be incorporated in one's balanced diet and these can be resourced from different types of seaweeds. Dickinson (2002) summarized the importance of some vitamins and mineral elements to the human body. UNICEF Namibia (2012) lamented that Namibians suffer from 'hidden hunger' a deficiency of important micronutrients. Supplementing peoples' diets with dried seaweeds would provide a balanced diet composed of proteins, fats and a variety of vitamins and mineral elements, without the need to supplement each individual nutrient separately.

This study showed that there are a number of dietary mineral elements in *P. capensis.* The bioaccumulation of mineral elements varies with species and with seasons (Dlaza, 2011) and as such promotion of mariculture will ensure continuous supply nutrient rich *Porphyra* thalli.

5.4 Laboratory initiated cultures of *P. capensis*

The main purpose of initiating Namibian *Porphyra* culture was to find out if the life cycle could be completed under existing laboratory conditions at Sam Nujoma Marine and Coastal Resources Research Centre (SANUMARC) of the University of Namibia in Henties Bay. Laboratory cultures produce the 'seeds' needed for large scale mariculture

of *Porphyra*. In the current study, the growth cycle of Namibian *Porphyra* was successfully completed *in vitro* as it was reported by Iwasaki (1961).

In this study, conchocelis were multiplied free floating in media as well as in molluscan shells. Molluscan shell conchocelis cultures were produced for the incubation and mass production of conchospores for seeding nets in *Porphyra* mariculture (Blouin et al., 2001). Studies have shown that the covering calcareous material from moluscan shells in which conchochelis bore protected the conchocelis from ultra violet radiation (UVR) damage and this could be an evolutionary defensive mechanism that *Porphyra* conchocelis gained for the success of its species in the wild (Jiang, Gao, & Helbling, 2009). The above authors demonstrated that, the photosynthetic machinery of *P. haitanensis* conchocelis could be protected from harmful UVR by an artificial covering of calcareous materials. Free floating laboratory cultures of conchocelis likewise produce conchospores for seeding nets (He, & Yarish, 2006).

Iwasaki (1961) had already reported that calcareous substrates were not necessary in the growth of conchocelis colonies and that appropriate liquid media and optimum conditions of temperature irradiance and photoperiod were sufficient. Free floating conchocelis cultures are preferred in laboratory breeding experiments for molecular studies and mass production of favoured strains (Pereira & Yarish (2008). The boring of the conchocelis phase of the *Porphyra sp* life cycle within molluscan shells in the sublittoral zone in the wild is an adaptation that reduces herbivory, protection against UV light destruction, as well as a way of mass production of conchospores (Jiang, et al., 2009). In the current study, conchosporangia were mass produced in cultures with shell

substrates. They were also vegetatively propagated as free floating conchocelis colonies in a bubble culture set up.

While studying the development of conchospore germlings of *P. yezoensis*, *P. oligosprmatangia* and *P. haitanensis*, it was reported that the basal cell of the 4 cell meiotic product of conchospore division, developed into a rhizoid(s) while the other 3 divided to form a linearly arranged blade (Wang et al., 2010). In this study, conchospore successfully germinated into the parenchymatous stage of blade development and rhizoids could be seen after the initial divisions of the conchospores (see Plate 4.12).

This was the first time Namibian *P. capensis* was grown in culture at the research centre and the whole of Namibia at large. Laboratory cultures of *Laminaria* sp had been initiated at the research centre but the work is yet to be completed. The main challenges encountered had to do with maintaining optimum conditions for the different stages in the *Porphyra* life cycle. Obtaining the various ingredients for the special growth media took time since they had to be ordered from outside the country.

In future, more trials should be carried out as this study experienced some limitations. Special equipment (light meter) and glassware set aside for this particular project were not immediately available and this caused some delays. A growth chamber was available which worked perfectly but when variables needed to be changed to optimize an aspect of growth, an alternative growth chamber or room whose temperature and photoperiod could be set was not available meaning trials could not be run in paralel to establish which conditions worked faster or better. This study focused on initiating growth from zygotospores. The cycle takes a long time to reach maturity. Blade archeospores and other blade spores (Nelson et al., 1999) have been used before to reproduce thalli without

going through the conchocelis phase (Blouin et al., 2007). This can be attempted at the research center.

CHAPTER 6

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Namibian *Porphyra C. Agardh* from the eulittoral zone was referred to as *P. capensis* by early seaweed collectors as it morphologically resembled the South African *P. capensis*. This study concludes that despite the fact that they share a common ancestry, the Namibian *Porphyra capensis* is a subspecies of the South African *P. capensis* because of the observed genetic variability in its sequences. This conclusion has been reached on the strength of the 2.1% sequence divergence obtained after the multisequence analysis as well as the similarity in the three tree topologies that clearly separated the typical *P. capensis* specimens' cluster from the cluster constituting the Namibian specimens. There was a difference in the tissue anatomy of the male thalli where the South African male thalli had considerably more spermatia tiers compared to the Namibian specimens. This characteristic supports the subspecies decree as size of reproductive units is used to delimit species.

Regular harvesting of *P. capensis* thalli improved its annual yield. Removal of mature thalli exposed young thalli and sporelings to sunlight and nutrients increasing productivity as observed in overcompensation of above ground primary production as a result of grazing. Upweling forces and other water movements transport spores, sporophytes, nutrients and gasses to harvested sites for recolonisation.

For natural resources management purposes, the amount of resource available at any particular time should be quantifiable. The proposed power model equation can be used

to estimate standing crop biomass of *Porphyra* growing in the field. This is the first time such a model has been proposed.

The nutritional composition of the Namibian populations of P. capensis was analysed for the first time in Namibia and compared to that of lettuce. They had similar quantities of nutrients except for fibre and fat. *Porphyra* had higher levels of fibre and lower amounts of fat. This is a good organic food which needs to be promoted to be consumed as a salad the way lettuce is. Fortification of cereals with vitamins and minerals is encouraged in some countries. Namibia mills could add *Porphyra* powder into wheat and maize flower as a means of fortification, to give consumers a healthier choice. *Eucheuma spp*. powder is added to jams, cakes, honey and other products as seen in Plate 1.1b to add nutritional value to ordinary products.

P. capensis could be successfully raised from spores in a laboratory according to the current study. These are the initial stages in mariculture production of the seaweed. This had not been accomplished in Namibia before, and therefore a very important achievement as a prerequisite to mariculture production in order to conserve wild populations, while mass producing in mariculture for consumption.

6.2 Recommendations

This study has contributed knowledge especially in the field of Phycology and seaweed culture as it is the first study conducted on the Namibian populations of P. capensis. These recommendations arise from the study.

- Phylogenetic studies should be done on Namibian populations of P. capensis from different areas along the coast and different zones along the shore to appreciate its molecular diversity using different genes such as the tubulin genes used in the study of Arabidopsis or Rubisco genes of the large sub-unit, as has been tried on Eubacteria before. The use of the conchocelis in phylogenetics rather than the gametophyte should be carried out too.
- Thorough anatomical descriptions of all *Porphyra* like species present along the Namibian coast should be undertaken, complete with taxonomic keys. This is important in order to characterize the species/ subspecies more. These should be carried out hand in hand with molecular studies. The above two studies will lead to the naming of the subspecies, evident from this study.
- Mariculture field trials should take place to complete the whole life cycle and to work with different subspecies of *Porphyra* if they exist in Namibia. This will identify subspecies of high taste and texture qualities. It will involve growing out the germlings produced in the laboratory (after molecular verification and selections), into tanks and eventually into specific sites along the seashore probably in lagoons to conserve wild populations.
- Trials on mariculture production are recommended, to guarantee sustainable biomass productivity

- Partnerships should be sought with potential stakeholders both locally, and abroad for product development, value addition and marketing.

REFERENCES

Abdel-Rahman, M. H. M. (2005). Control of conchospores formation in *Pyropia leucostica (Thur) in le Joliset* Thuret, a genuine short-day response. *International Journal of Agriculture Biology, 7* (1), 1 – 4.

Agardh, C. A. (1824). *Systema algarum. 3*, 1-312, Lund. Berlin

Alecia, B., & Masakazu, A. (2006). Small-scale temporal variation in propagule supply of an intertidal red alga, *Phycologia, 45*, (4), 458-464

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.

Anderson, R. J., Bolton, J. J. & Stegenga, H. (2005). Using the biogeographical distribution and diversity of seaweed species to test the efficacy of marine protected areas in the warm-temperate Agulhas Marine Province, South Africa. *Diversity and Distribution*.15, 1017 – 1027.

Andrews, W.R.H., Hutchings, L. (1980). Upwelling in the southern Benguela current. *Progress in Oceanography, 9*, 1-81

Ang, P. O., Jr. (2006). Phenology of *Sargassum spp* in Tung Ping Chan Marine Park, Hong Kong SAR, China. *Journal of Applied Phycology 18* (3-5) 629-636.

AOAC (1995) In: Horwitz W (Ed.), *Official Methods of the Association of Analytical Chemists*. (16^{th} ed.). Washington, DC, USA.

Baldauf, S. L. (2003). Phylogeny for the faint of heart: a turtorial. *Trends in genetics.19* (6), 345-351

Barsanti, L., & Gualtieri, P. (2006). *Algae: Anatomy, Biochemistry and Biotechnology*. Taylor & Francis Group. Boca Raton.

Bartholomae, C. H., & Van Der Plas, A.K., (2007). Towards the development of environmental indices for the Namibian shelf, with particular reference to fisheries management. *African Journal of Marine Science, 29*(1), 25-35.

Belsky, A. J., Carson, W. P., Jensen, C. L. & Fox, G. A. (1993). Overcompensation by plants: herbivore optimization or red herring? *Evolutionary Ecology, 7*, 109-121

Bianchi, G., Carpenter, K. E., Roux, J. P., Molloy, F.J., Boyer, D., Boyer, H. J. (1999). *FAO species identification guide for fishery purposes. Field guide to the living marine resources of Namibia*. FAO, Rome.

Bhatia, S., Sharma, A., Sharma, K., Kavale, M., Chaugule, B. B., Dhalwal. K. et al. (2008). Novel algal polysaccharides from marine resources: Porphyra C. Agardhn. *Journal of pharmacognosy reviews. 2*(4), 271-276

Blouin, N. A., Brodie, J. A., Grossman, A.C., Xu, P. & Brawley, H.S. (2011). *Porphyra C. Agardh*: a marine crop shaped by stress. *Trends in Plant science*. *16* (1), 29-37.

Blouin, N. A., Xiugeng, F., Peng, Jiang., Yarish, C. & Brawley, S.H. (2007). Seeding nets with neutral spores of the red alga *Porphyra umbili*calis (L.) Kutzing for use in intergrated multitropic aquaculture (IMTA). *Aquaculture 270,* 77 - 91

Bolton, J.J. & Anderson, R.J. (1990).Correlations between intertidal seaweed community composition and seawater temperature patterns on a geographical scale. *Botanica Marina 33*, 447 – 457.

Bolton, J. J. & Lüning, K. (1983). Optimal growth and maximal survival temperatures of Atlantic *Laminaria* species (Phaeophyta) in culture. *Marine Biology 66*, 89-94

Boyd, A.J. (1987). *The oceanography of the Namibian region*. Ph D thesis, University of Cape Town, South Africa.

Branch, G. M., & Branch, M. (1981). *The living shores of Southern Africa*. Struik publishers. Cape Town.

Braune, W., & Guiry, M.D. (2011). *Seaweeds: A colour guide to common benthic green, brown and red algae of the world's oceans*. A. R. G. Gantner Verlag KG. Ruggell.

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

Brodie, J. & Lewis, J., (Eds) (2007). *Unravelling the Algae: the Past, Present and Future of Algal Systematics.* CRC Press, Boca Raton, USA.

Brodie, J., Mortensen, A. M., Ramirez, M.E., Russell, S., & Rinkel, B. (2008) Making the links: towards a global taxonomy for the red algal genus *Porphyra C. Agardh* (Bangiales, Rhodophyta). *Journal of Applied Phycology, 20,* 939 – 949.

Broom, J. E., Jones, W. A., Hill, D. F., Knight, G. A., & Nelson, W. A. (1999). Species recognition in New Zealand *Porphyra C. Agardh* using 18SrDNA 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* Kjellman, *Porphyra carolinensis Coll et Cox & Porphyra lilliputiana* W. A. Nelson, G. A. Knight et W.W Hawkes (Bangiales, Rhodophyta) based on molecular and morphological data. *European Journal of Phycology, 37*, 227-235.

Burke, A. (2004). *Wild flowers of the Central Namib*. Namibian Scientific Society. Windhoek. Namibia.

Bustamante, R. H., Branch, G. M., & Eekhout, S. (1997). The influence of physical factors on the distribution and zonation patterns of South Africa rocky shore communities. *South African Journal of Marine Science 18*, 119-136

Cavell, A. J. (1955). The calorimetric determination of phosphorus in plant materials. *Journal of the Science of Food & Agriculture*, *6*(8), 479-480.

Cervin, G., Undergarth, M., Vlejo, R. M., & Abeg, P. (2004). Effects of small scale disturbances of canopy and grazing on intertidal assemblages on the Swedish Coast. *Journal of Experimental Marine Biology & Ecology*, *302*(1), 35-49.

Chapman, V. J., & Chapman, D. J. (1980). Seaweeds and their uses. (3rd ed.). Chapman and Hall, New York.

Chen, J., Shiyomi, M., Bonham, C. D., Yasuda, T., Hori, Y., Yamamura, Y. (2008). Plant cover estimation based on the beta distribution in grassland vegetation. *Ecological Research,* 23(5) 813-819

Chen, Lisa. (1999). Porphyra C. Agardh; Human consumption; retrieved June 25, 2009 from http://www.mbari.org/staff/conn/botany/reds/lisa/consume.htm

Chiarucci, A., Wilson, J. B., Anderson, B.J., & De Dominicis, V. (1999). Cover versus biomass as an estimate of species abundance; does it make a difference to the conclusions? *Journal of Vegetation Science, 10* (1). 35-42.

Cole, K., & Conway, E. (1980). Studies in the Bangiaceae: Reproductive modes. *Botanica Marina,* 23, 545–553.

Critchley, A.T. & Rotmann, K.W. G. (1992). Industrial Processing of seaweeds in Africa: the South African experience. In *Proceedings of the First International Workshop on Sustainable Seaweed Resource Development in Sub-Saharan Africa.* Mshigeni K, E., Bolton, J, J., Critchley, A., & Kiangi, G. (Eds.), March 22-29, 1992, Windhoek, Namibia. pp. 85-97.

Darcy-Vrillon, B. (1993) Nutritional aspects of the developing use of marine macroalgae for the human food industry. *International Journal of Food Science and Nutrition, 44*, 23-35.

Davis, T.A., Volesky, B., Mucci, A. (2003). A review of the biochemistry of heavy metal biosorption by brown algae. *Water Research, 37*, 4311-4330

De la Rocha, S. R., Sanchez-Muniz, F. J., Gomez-Juaristi, M., Larrea-Marin, M. T. (2009). Trace elements determination in edible seaweeds by an optimized and validated ICP-MS Method. *Journal of Food Composition and Analysis, 22,* 330-336.

Diaz, H. F. & Markgraf, V. (1992). El Niño: *Historical and paleoclimatic aspects of the Southern Oscilation*. Cambridge University Press. Great Britain.

Dickinson, A. (2002). Recommended intakes of vitamins and essential minerals; *In Benefits of Nutritional Supplements:* Council for responsible nutrition (CRN). Washington. USA.

Ding, X.Y., Wang, Z.T., Xu H., Xu, L.S. Zhou, K.Y. (2000). Database establishment of the whole rDNA ITS region of *Dendrobium* species of "Feng-dou" and authentication by analysis of their sequences. *Acta Pharmacologica Sinica, 37*(7), 567-573

Dlaza, T.S., Maneveldt, G.W., Viljoen, C, (2008). Growth of post-weaning abalone *Haliotis midae* fed commercially available formulated feeds supplemented with fresh wild seaweed. *African Journal of Marine Science, 30,* 199 – 203.

Dlaza, T. S. (2011). *Development in culture, ecophysiology and nutritional content of three South African Porphyra C. Agardh (Rhodophyta, Bangiales) species*. PhD thesis. University of Cape Town.

Drew, K. M. (1949). Conchocelis – phase in the life history of *P. umbilicalis* (L) kutz. *Nature,* 164, 748 - 749

Drew, K.M. (1954). Studies on Bangioideae 111. The Life history of *P. umbilicalis* (L) Kutz var *laciniata* (Lightf.). *Journal of Agriculture Annals of Botany New Series 18,* 183 –211

Dring, M.J. (1967b). Phytochrome in red alga; *Pyropia tenera comb nov.* Kjellman. *Nature 215,* 1411-1412

Dos Santos, C. E., & Mshigeni, K. E. (1992). Seaweeds and their uses in Angola. In: *Proceedings of the First International Workshop on Sustainable Seaweed Resource Development in Sub-Saharan Africa.* Mshigeni K, E., Bolton, J, J., Critchley, A., & Kiangi, G. (Eds.), March 22-29, 1992, Windhoek, Namibia. pp.111-115.

Engledow, H.R., Bolton, J.J., & Stegenga, H. (1992). The biogeography of the seaweed flora of Namibia. In: *Proceedings of the First International Workshop on Sustainable Seaweed Resource Development in Sub-Saharan Africa.* Mshigeni, K, E., Bolton, J, J., Critchley, A., & Kiangi, G. (Eds.), March 22-29, 1992, Windhoek, Namibia. pp.117-130

Engledow, H. R. & Bolton, J. J. (2003). Factors affecting seaweed biogeographical and ecological trends along the Namibian coast. In: *Proceedings of the 17th International Seaweed Symposium*. Chapman, A.R.O., Anderson, R.J., Vreeland, V.J & Davison, I. R. (Eds.). Oxford: Oxford University Press. pp. 285-291

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

Fong, P., Zedler, J.B. & Donohoe, R. M. (1993). Nitrogen vs phosphorus limitation of algal biomass in shallow coastal lagoons. *Limnology Oceanograpy 38* (5).906 – 923.

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

Griffin, N. J. (2003). Harvest ecology and biodiversity of South African *Porphyra* C. Agardh. PhD thesis. University of Cape Town. Cape Town.

Griffin, N.J., Bolton, J.J., & Anderson R. J. (1999). The effects of a simulated harvest on *Porphyra C. Agardh* (Bangiales, Rhodophyta). *Hydrobiologia,* (398/399), 183-189.

Griffin, N. J., Bolton, J.J., & Anderson, R. J. (1999c). *Pyropia aeodis (N. J. Griffin. J.J. Bolton et R. J. Anderson) J. E. Sutherl. comb. nov.* sp. nov. (Bangiales, Rhodophyta), an epiphyte on *Aeodis orbitosa* (Suhr) Schmitz from South Africa. *European Journal of Phycology*, *34*, 505 – 512.

Grime, J. P. (1977). Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory.*The American Naturalist 3,* 1169-1194.

Guiry, M. D., & Blunden, G. (Eds). (1992). *Seaweed Resources in Europe; Uses and Potential*. Chichester: John Wiley.

Guiry, M. D., & Guiry, G. M. (2006). *AlgaeBase.* World-wide electronic publication, National University of Ireland, Galway. http://www.algaebase.org.

Hall, T. A. (1999). A user friendly biological sequence alignment editor (BioEdit) and analysis program for Windows. *Nucleic Acid Symposium Series 41*, 95-98

Harrison C. J., & Langdale, J. A. (2006). A step by step guide to phylogeny reconstruction. *The Plant Journal, 45,* 561-572

Hawkes, M. W. (1978). Sexual reproduction in *Porphyra gardneri* (Smith *et* Hollenberg) Hawkes (Bangiales, Rhodophyta). *Phycologia 17,* (3), 329-353.

He, P., & Yarish, C. (2006). The developmental regulation of mass cultures of free-living conchocelis for commercial net seeding of *Porphyra leucostica* Thuret from Northeast America. *Aquaculture, 257,* (1-4), 373-381.

Hoek, C. van Den., Mann, D. G., & Jahns, H. M. (1995). *Algae. An introduction to Phycology*. Cambridge University Press. Cambridge. Great Britain.

Hutchings, L., van der Lingen, C.D., Shannon, L.J., Crawford, R.J.M., Verheye, H. M. S., Bartholomae, C. H., . . . Monteiro, P M. S. (2009). The Benguela current: An Ecosystem of four components. *Progress in Oceanography, 83,* 15-32.

Indergaard, M., (1983). The aquatic resource I. The wild marine plants: a global bioresource. *In Biomass Utilization.* W. A. Cote (Ed.). Plenum Publishing Corporation, N.Y. pp. 137 – 168.

Iwasaki, H. (1961). The life cycle of *Porphyra tenera* Kjellman in vitro. *Biological Bulletin, 121,* 173 – 187.

Jensen, A. (1992). Seaweeds in the livestock feed industry. In *Proceedings of the First International Workshop on Sustainable Seaweed Resource Development in Sub-Saharan Africa* Mshigeni K, E., Bolton, J, J., Critchley, A., & Kiangi, G. (Eds.), March 22-29, 1992, Windhoek, Namibia, pp.131- 140

Jernakoff, P. (1985). Interactions between the limpet *Patelloida latistrigata* (Angas 1865) and algae on an intertidal rock platform. *Marine Ecology Progress Series 23*, 71-78.

Jiang, H., Gao, K. & Helbling, E.W. (2009). The conchocelis of *Pyropia haitanensis T.J.Chang & B.F.Zheng* T.J. Chang & B. F. Zheng (Rhodophyta) is protected from harmful UV radiation by the covering calcareus matrix. *Journal of Phycology, 45*, 1270-1277.

Jiang, Z., Hama., Yamaguchi, K. & Oda, T. (2012). Inhibitory effect of sulphated polysaccharide *Porphyra* C. Agardh on nitric oxide production in lipopolysaccharide-stimulated RAW264.7 macrophages. *Journal of Biochemistry, 151* (1):65-74.

John, D.M., Price, J.H., Maggs, C.A. & Lawson, G.W. (1979). Seaweeds of the western coast of tropical Africa and adjacent islands: a critical assessment. III. Rhodophyta (Bangiophyceae). *Bulletin of the British Museum (Natural History) Botany, 7*, 69-82.

John, D.M., Prud'homme van Reine, W.F., Lawson, G.W., Kostermans, T.B. & Price, J.H. (2004). A taxonomic and geographical catalogue of the seaweeds of the

western coast of Africa and adjacent islands. *Beihefte zur Nova Hedwigia*, *127*, 1-339,

Jones, W. A., Griffin, N.J., Jones, D. T., Nelson, W.A., Farr, T. J., & Broom, J. E. (2004). Phylogenetic diversity in South African *Porphyra* C. Agardh (bangiales, Rhodophyta) determined by nuclear SSU sequence analyses. *European Journal of Phycology, 39*, 197-211.

Jukes, T. H., & Cantor, C.R. (1969). Evolution of protein molecules. In Munro, H. N. (Ed), *Mammalian Protein Metabolism*, pp. 21-132, Academic Press, New York.

Jupp, B., P. & Drew, E., A. (1974). Studies on the growth of *Laminaria hyperborean* (Gunn.) Fosl. 1. Biomass and productivity. *Journal of Experimental Marine Biology and Ecology*, *15*, 185-196

Kiangi, E. (1992). Medicinal uses of seaweeds. In: *Proceedings of the First International Workshop on Sustainable Seaweed Resource Development in Sub-Saharan Africa* Mshigeni K, E., Bolton, J, J., Critchley, A., & Kiangi, G. (Eds.), March 22-29, 1992, Windhoek, Namibia, pp. 141-146

Kirkman, H. (1984). Standing stock and production of *Ecklonia radiata* (C. Agardh) *J.* Agardh. *Journal of Experimental Marine Biology and Ecology, 76*, 119-130

Knapp, A. K., Hoover, D. L., Blair, J. M., Buis, G., Burkepile, D. E., Chamberlain, A., Collins, S.L…….Zinn, A. (2012). A test of two mechanisms proposed to optimize grassland aboveground primary productivity in response to grazing. *Journal of Plant Ecology, 5* (4) 357 – 365.

Kuetzing, F, T. (1843). *Phycologia Generalis*. F. A. Brockhaus. Leipzig, 1 – 458.

Koh, Y.H., Cho, G.Y., & Kim, M. S. (2013). Species delimitation of the Genus *Champia* (Rhodymeniales, Rhodophyta) from Korea using DNA bar coding. *Journal of Ecology & Environment, 36* (4), 449-463.

Kornmann, P. (1994). Life histories of monostromatic *Porphyra* C. Agardh species as a basis for taxonomy and classification, *European Journal of Phycology, 29*, (2), 69-71,

Kreiner, A., Van der Lingen, C. D., & Freon, P (2001). A comparison of condition factor and gonadosomatic index of sardine *Sardinops sagax* Mann in the northern and southern Benguela upwelling ecosystems 1984-1999. Payne, A. I. L., Pillar, S. C & Crawford, R.J. M. (Eds). *South African Journal of Marine Science, 23*, 123-134.

Kunimoto, M., Kito, H.,Yamamoto, Y., Cheney, D. P., Kaminishi, Y., & Mizukani, Y. (1999a). Discrimanation of *Porphyra C. Agardh* species based on small subunit ribosomal RNA gene sequence. *Journal of Applied Phycology, 11*, 203-209

Lahaye, M. (1991). Marine algae as sources of fibres: Determination of soluble and insoluble dietary fibre contents in some ‘sea vegetables’. *Journal of the Science of Food and Agriculture*, *54*, 587-594

Lawson, G. W., Simmons, R. H. & Isaac, W.E. (1990).The marine algal flora of Namibia: its distribution and affinities. *Bulletin of the British Museum (Natural History)* Botany, *20*, 153-168

Li, Y., Shen, S., He, L., Xu, P., & Wang, G. (2009). Sequence analysis of the ITS region and 5.8S rDNA of *PPyropia haitanensis (T. J. Chang et B. F. Zheng) Zheng N. Kikuchi et M. Miyata comb. nov.* T.J. Chang & B. F. Zheng. *Chinese Journal of Oceanology and Limnology, 27,* (3) 493-501

Lopez, J., Prezioso, V. (2001). A better way to optimize: Two-Step Gradient PCR. *Biosystems Laboratory*. Brinkmann instruments, Inc. Westbury, NY, U.S.A: Retrieved on 22nd Nov 2010.

Lopez-Vivas, J.M., Pacheco-Ruiz, Isai., Riosmena-Rodriguez, R. & Yarish, C. (2011). Life history of *Pyropia hollenbergii E. Y. Dawson* Dawson (Bangiales, Rhodophyta) from the Gulf of California, Mexico. *Phycologia, 50,* (5) 520-529.

Lüning, K. (1969b). Standing crop and leaf area index of the sublittoral *Laminaria* species near Helgoland. *Marine Biology, 3,* 282-286

Lüning, K. (1990). *Seaweeds: Their Environment, Biogeography, and Ecophysiology*. John Wiley & Sons Inc. New York

Maeyer, E. M., Lowenstein, F.W., & Thilly, C.H. (1979). *The Control of Endemic Goiter.*World Health Organization, Geneva

MacArtain, P., Gill, C. I. R., Brooks, M., Campbell, R. & Rowland, I. R. (2007). Nutritional value of edible seaweeds. *Nutritional Reviews, 65,* (12) 535 – 543.

Mathieson, A. C. (1989). Phenological patterns on Northern New England seaweeds. *Botanica Marina, 32,* 419-438.

Markham, J. W. & Hagmeier, E. (1982). Observations on the effects of germanium dioxide on the growth of macro-algae and diatoms. *Phycologia,* 21: 125-130

McDermid, K. J., & Stuercke, B. (2003). Nutritional composition of edible Hawaiian seaweeds. *Journal of Applied Phycology*, *15*, 513 – 524

McLachlan, J. & Bird, C. J. (1984). Geographical and experimental assessment of the distribution of species of *Gracilaria* in relation to temperature. *Helgoländer Meeresunters, 38*, 319-334

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,* 28, 539-544

Mendelson, J., Jarvis, A., Roberts, C., & Robertson, T. (2009). *Atlas of Namibia: A Portrait of the Land and its People*. (3rd ed) Cape Town, South Africa: David Philip Publishers.

Milstein, D., & Oliveira, M. C. (2005). Molecular phylogeny of Bangiales (Rhodophyta) based on small subunit rDNA sequencing: emphasis on Brazilian *Porphyra C. Agardh* species. *Phycologia, 44*(2). 212-221

Molloy, F. J. (1990). Utilized and potentially utilizable seaweeds on the Namibian Coast; Biogeography and accessibility. *Hydrobiologia*, *204-205*, 293-299.

Muller, K.M., Oliveira, M. C., Sheath, R.G., Bhattcharya, D. (2001). Ribosomal DNA phylogeny of the Bangiophycidae (Rhodophyta) and the origin of secondary plastids. *American Journal of Botany*, *88*(8), 1390-1400.

Mumford, T. F., Jr. (1990). Nori cultivation in North America: growth of the industry. *Hydrobiologia 204 ⁄ 205,* 89–98.

Mumford, T.F., Jr., & Miura, A. (1988). *Porphyra* C. Agardh as food: cultivation and economics. In Lembi, C.A and Waaland, J.R. (Eds.) *Algae and Human Affairs.* Cambridge University Press, Cambridge, pp. 87 –117.

Mshigeni, K. E. (1976). Effects of environment on developmental rates of sporelings of two *Hypnea* species (Rhodophyta: Gigartinales). *Marine Biology, 36,* 99-103.

Mshigeni, K.E., (1992). Seaweeds: an overview, with special reference to their biodiversity and economic potential. In: *Proceedings of the First International Workshop on Sustainable Seaweed Resource Development in Sub-Saharan Africa* Mshigeni K, E., Bolton, J, J., Critchley, A., & Kiangi, G. (Eds.), March 22-29, 1992, Windhoek, Namibia.

Mshigeni, K. E., (2001). *The cost of scientific and technological ignorance; with reference to Africa's rich biodiversity.* The University of Namibia. Windhoek.

Msuya, F. E., (2011). The impact of seaweed farming on the socioeconomic status of coastal communities in Zanzibar, Tanzania. *World Aquaculture, 42* (3), 45-48

Msuya, F. E., Buriyo, A., Omar, I., Pascal, B., Narrain, K., Ravina, J. J. M., Mrabu, E., Wakibia, J. G. (2014). Cultivation and utilization of red seaweeds in the Western Indian Ocean (WIO) Region. *Journal of Applied Phycology, 26,* 699-705

Nelson, G., Hutchings, L., (1983). The Benguela upwelling area. *Progress in Oceanography, 12*, 333-356

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. (1995). Endosporangia- a new form of reproduction in the genus *Porphyra* C. Agardh (Bangiales, Rhodophyta). *Botanica Marina, 38*, 17-20.

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

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

Nelson, W. A., Farr, T. J., & Broom, J. E. S. (2006) Phycologenetic relationships and generic concepts in the red order Bangiales: challenges ahead. *Phycologia, 45*, 249 – 259.

Noda, H. (1993). Health benefits and nutritional properties of nori. *Journal of Applied Phycology, 5*, 255–258.

Notoya, M. (1999). 'Seed' production of *Porphyra spp*. by tissue culture. *Journal of Applied Phycology, 11*, 105 – 110.

Nshubemuki, L. & Mshigeni, K. E. (1992). The potential of seaweeds in agroforestry. In: *Proceedings of the First International Workshop on Sustainable Seaweed*

Resource Development in Sub-Saharan Africa Mshigeni, K, E., Bolton, J, J., Critchley, A., & Kiangi, G. (Eds.), March 22-29, 1992, Windhoek, Namibia. pp. 275-281

Oates, B. R. (1985). Photosynthesis and amelioration of desiccation in the intertidal saccate alga *Colpomenia peregrina* Sauvageau. *Marine Biology 89,*109-119

Oliviera, M.C., Ragan, M. A. (1994). Variant forms of a group 1 Intron in nuclear small-subunit rRNA genes of the marine red alga *Porphyra spiralis var. amplifolia* Oliveira Filho & Coll. *Journal of Molecular Biology and Evolution, 11,*(2),195-207

Oliveira, E.C. (1992). Seaweed exploitation and cultivation in Brazil. In: *Proceedings of the First International Workshop on Sustainable Seaweed Resource Development in Sub-Saharan Africa* Mshigeni, K, E., Bolton, J, J., Critchley, A., & Kiangi, G. (Eds.), March 22-29, 1992, Windhoek, Namibia. pp. 283-294

Pereira, R., Sousa-Pinto, I., & Yarish, C. (2004). Field and culture studies of the life history of *Porphyra dioica* Brodie & L. M. Irvine (Bangiales, Rhodophyta) from Portugal. *Phycologia, 43,* 6 756-767

Pereira, R., & Yarish, C. (2008). *Mass Production of Marine Macroalgae*. Ecological Engineering. Elsevier B.V. pp. 2236-2246

Pianka, E. R., (1970). On r and K selection. *American Naturalist. 104*, 592- 597

Plaschke, R. & Morgan, P. P. (1999). *Measuring salinity of seawater samples*. CSIRO. Division of Oceanography. Hobart, Tasmania. Australia

Provasoli, L. (1968). Media and prospects for cultivation of marine algae. In *Cultures and Collections of Algae*. Watanabe, A & Hattori, A. (Eds.) Proceedings of the USA-Japan Conference. Hakone, September 1966, Japan Society Plant Physiologist pp. 63-75

Qiagen, (2006). *DNeasy Plant Handbook.* New York. Quiagen.

Rabesandratana, H. D., Rabesandratana R. N. (1992). Seaweeds and their uses in Madagascar. In: *Proceedings of the First International Workshop on Sustainable Seaweed Resource Development in Sub-Saharan Africa* Mshigeni, K, E., Bolton, J, J., Critchley, A., & Kiangi, G. (Eds.), March 22-29, 1992, Windhoek, Namibia. pp. 297-307

Robertson, J. B., & Van Soest, P. J. (1981). *The Analysis of Dietery Fibre in Food.* James, W.P.T. & Theander, O. (Eds.). Dekker, New York.

Robertson, T., Jarvis, A., Mendelsohn, J & Swart, R. (2012). *Namibia's Coast; ocean riches and desert treasures*. Directorate of Environmental Affairs. Ministry of Environment and Tourism. Namibia.

Roland, W.G. & Coon, L.M. (1984). Post harvest recovery of beds of edible red alga, *P. perforate*. *Canadian Journal of Botany, 62*, 1968-1970.

Rotmann, K. W. G., (1992). The marketing of seaweed from Africa with special reference to the South African experience. In: *Proceedings of the First International Workshop on Sustainable Seaweed Resource Development in Sub-*

Saharan Africa, Mshigeni, K, E., Bolton, J, J., Critchley, A., & Kiangi, G. (Eds.), March 22-29, 1992, Windhoek, Namibia. pp. 321-335

Roux, K. H. (1995). Optimization and troubleshooting in PCR. *Genome Research,* 4, 185-194. Cold Spring Harbour Laboratory. Retrieved 22nd Nov 2010

Ruangchuay, R. & Notoya, M. (2003). Physiological responses of blade and Conchocelis of *Porphyra vietnamensis* Tanaka et Pham-Hoang Ho (Bangiales, Rhodophyta) from Thailand in culture.*Algae*, 18, (1), 21-28.

Rull Lluch, J. (2002). Marine benthic algae of Namibia. *Scientia Marina,* (66), (Suppl.): 5-256.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution, 4*, 406-425.

Sakko, A. L. (1998). The influence of the Benguela Upwelling System on Namibian marine biodiversity. *Biodiversity and Conservation, 7*, (4) 419 – 433.

Sánchez-Machado, D. I., López-Cervantes, J., López-Hernández, J., & Paseiro-Losada, P. (2004). Fatty acids, total lipid, protein and ash contents of processed edible seaweeds. *Food. Chemistry. 85*, 439-444.

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.

Schwander, T., Vuilleumier, S., Dubman, J. & Crespi, B. J. (2010). Positive feedback in the transition from sexual reproduction to parthenogenesis. In: *Proceedings of the Royal Society of Biological Sciences. (277),* pp.1435-1442

Shannon, L.V. (1985). The Benguela ecosystem, Part1. Evolution of the Benguela, physical features and processes. *Oceanography and Marine Biology: An Annual Review, 23*, 105-182.

Shaw N, and Liu Y. 2000. Bioavailability of iron from purple laver (*Porphyra* spp.) estimated in a rat haemoglobin regeneration bioassay. *Journal of Agriculture and Food Chemistry, 48*, (5), 1734 – 1737.

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*, 1-1259.

Sloan, A. E. (2003). What, when, and where Americans eat. *Food Technology 57,* 48 – 66.

Smith, R. L. (1996). *Ecology and Field Biology*. (5th ed.). Harper Collins College Publishers. New York.

Stekoll, W. S., Lin, R. & Lindstrom, S.C. (1999). *Porphyra C. Agardh* cultivation in Alaska: conchocelis growth of three indigenous species. *Hydrobiologia* 398/399, 291 - 297

Stephenson, F. H. (2010). *Calculations for Molecular Biology and Biotechnology: A guide to mathematics in the laboratory*. (2nd ed). Elsevier Inc. London.

Stephenson, T. A., & Stephenson, A. (1949). The universal features of zonation between tidemarks on rocky coasts. *Journal of Ecology 37*, 289-305

Stegenga, H., Bolton, J. J., & Anderson, R.J. (1997). *Seaweeds of South African West Coast.* Bolus Herbarium. University of Cape Town, Cape Town.

Stiller, J. W., and Waaland, J. R. (1993) Molecular analysis reveals cryptic diversity in *Porphyra* C. Agardh (Rhodophyta). *Journal of Phycology, 29*, 506 – 517.

Sutherland, J. E., Lindstrom, S.C., Nelson, W. A., Brodie, J., Lynch, M. D. J., Hwang, M.S. et al. (2011). A new look at an ancient order: Generic revision of the Bangiales. *Journal of Phycology, 47*, 1131-1151

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., & Kumar, S. (2011). Molecular Evolutionary Genetics Analysis (MEGA5.1): using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution*.

Taylor, R & Fletcher,R. L. (1999). Cryopreservation of eukaryotic algae – a review of methodologies. *Journal of Applied Phycology, 10*, 481-501.

Teas, J., Pino, S., Critchley, A., & Braverman, L.E. (2004). Variability of Iodine content in common commercially available edible seaweeds. *Thyroid, 14*, (10), 836-841

Teske, P. R., Fronema, P. W., Barker, N, P., & McQuaid, C. D. (2007). Phylogeographic structure of the caridean shrimp (*Palaemon peringueyi* Stebbing 1915) in South Africa: further evidence for intraspecific genetic units associated with marine biogeographic provinces. *African Journal of Marine Sciences, 29,* (2), 253-255.

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

Tseng, C. K. & Chang, T. J. (1954). Studies on *Porphyra* C. Agardh 1. Life history of *Pyropia tenera comb nov.* Kjellman. *Acta Botanica Sinica, 3*: 287 – 302

Tseng, C. K. & Chang, T. J. (1955a). Studies on the life history of *Porphyra* C. Agardh *tenera* Kjellman. *Scientia, Sinnica, 4*: 375 – 398

Tseng, C. K. & Chang, T. J. (1955b). Studies on *Porphyra* C. Agardh 111. Sexual reproduction of *Porphyra* C. Agardh *Acta Botanica. Sinica. 4*: 153-166.

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

Turner, N. J. (2003). The ethnobotany of edible seaweed (*Pyropia abbottiiae (V. Krishnam.) S. C. Lindstrom comb nov.* Krishnamurthy and related species; Rhodophyta: Bangiales) and its use by First Nation on the Pacific Coast of Canada. *Canadian Journal of Botany, 81*, 283-293

UNICEF Namibia. (2012). Malnutrition in Namibia, summary. Retrieved from http://www.slideshare.net/UnicefNamibia/malnutrition-in-namibia-summary.

Van de Peer, Y. (2009). Phylogenetic inference based on distance methods: Theory. In Lemey, P., Salemi, M., & Van Damme, A-M. (Eds.). *The phylogenetic*

handbook: a pratical approach to phylogenetic analysis and hypothesis testing, pp142-160. Cambridge University Press.

Varela-Alvarez, E., Stengeli, D. B. & Guiry, M. D. (2007). Seasonal growth and phenotypic variation in *Porphyra linearis* Greville (Rhodophyta) populations on the West Coast of Ireland. *Journal of Phycology*, (43), 90-100.

Vencatasamy, D. R. (1992). Seaweeds and their uses in Mauritius. In: *Proceedings of the First International Workshop on Sustainable Seaweed Resource Development in Sub-Saharan Africa* Mshigeni, K, E., Bolton, J, J., Critchley, A., & Kiangi, G. (Eds.), March 22-29, 1992, Windhoek, Namibia. pp. 353-357

Wamukoya, G. M., (1992).Seaweeds and their uses in Kenya. In: *Proceedings of the First International Workshop on Sustainable Seaweed Resource Development in Sub-Saharan Africa* Mshigeni, K, E., Bolton, J, J., Critchley, A., & Kiangi, G. (Eds.), March 22-29, 1992, Windhoek, Namibia. pp. 375-381

Wang, J., Zhu, J., Zhou, W., Jiang, P., Qin, S. & Xu, P. (2010). Early development patterns and morphogenesis of blades in four species of *Porphyra C. Agardh* (Bangiales, Rhodophyta). *Journal of Applied Phycology, 22*, 297 – 303.

Waring, R. H., (1983). Estimating forest growth and efficiency in relation to canopy leaf area. *Advances in Ecologial Research 13*, 327 – 354.

Watanabe, F., Takenaka, S., Katsura, H., Zakir, H., Masumder, S. A. M., Abe, K., Tamura, Y. & Nakano, Y. (1999). Dried green and purple lavers (nori) contain substantial amounts of biologically active vitamin B_{12} but less of dietary iodine

relative to other edible seaweeds. *Journal of Agricultural and Food Chemistry*, *47*, 2341 – 2343.

Wayne, M.J. (1986). Report on a collection of benthic marine algae from the Namibian Coast. *Nova Hedwigia,* 43, 311-355.

Wenjun, Z., Yun, L. & Jixun, D. (2007). Study on cryopreservation of *Pyropia yezoensis* (Ueda) M.S Hwang & H.G. Choi 2011 conchocelis. *Journal of Ocean University of China*, *6*, (3) 299-302.

West. J.A. & McBride, D. L. (1999). Long term diurnal carpospores discharge patterns in the Ceramiaceae, Rhodomelaceae and Delesseriaceae (Rhodophyta). *Hydrobiologia, 298/299.* 101 – 113.

White, T. J., Bruns, T., Lee, S., & Taylor, J (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In Innis, M. A., Gelfand, D. H., Sninsky, J.J., & White, T.J (Eds.), *PCR protocols: A Guide to Methods and Applications,* pp. 315-322. Academic Press, San Diego, California, USA.

Yamamotu, I., Maruyama, H., and Takahashi, M. 1987. The effects of dietary or intraperitoneally injected seaweed preparations on the growth of sarcom-180 cells subcutaneously implanted into mice. *Cancer Letters, 30* (2): 125-131.

Yarish, C., Wilkes, R., Chopin, T., Fei, X. G., Mathieson, A. C., Klein A. S., Neefus, C. D., Mitman, G.G. & Levine, I. (1998). Domestication of indigenous *Porphyra* C. Agardh (nori) species for commercial cultivation in Northeast America. *World Aquaculture. 29,* 26 – 30.

Zagoskin, M. V., Lazareva, V. I., Grishanin, A.K & Mukha, D. V. (2014). Phylogenetic information content of Copepoda ribosomal DNA repeat units: ITS1 & ITS2 impact. *Biomedical Research International 2014,* article ID 926342,

APPENDICES

APPENDIX 1a:

A recipe for mushroom – nori soup

Ingredients;

3 tablespoons nori, cut in strips

2 tablespoons olive oil

1 medium onion, minced

1/4 cup sweet red pepper, finely chopped

1/4 cup green pepper, finely chopped

2 cloves garlic, minced

1 medium carrot, chopped

3 cups oyster mushrooms, cut in strips

4 cups strong vegetable broth

1 teaspoon miso paste, optional

Method:

1. Place nori strips in a bowl and pour warm water over to cover, to rehydrate. Heat oil in soup pot and sauté onion, peppers, garlic and carrot.

2. Add mushroom strips and sauté until they collapse. Strain excess water from nori and add to pot. Add broth to pot, bring to boil.

3. Cover pot and reduce heat to a simmer. Simmer for about 20 minutes. At the table, place miso in soup bowl and top with hot soup. Stir well and enjoy.

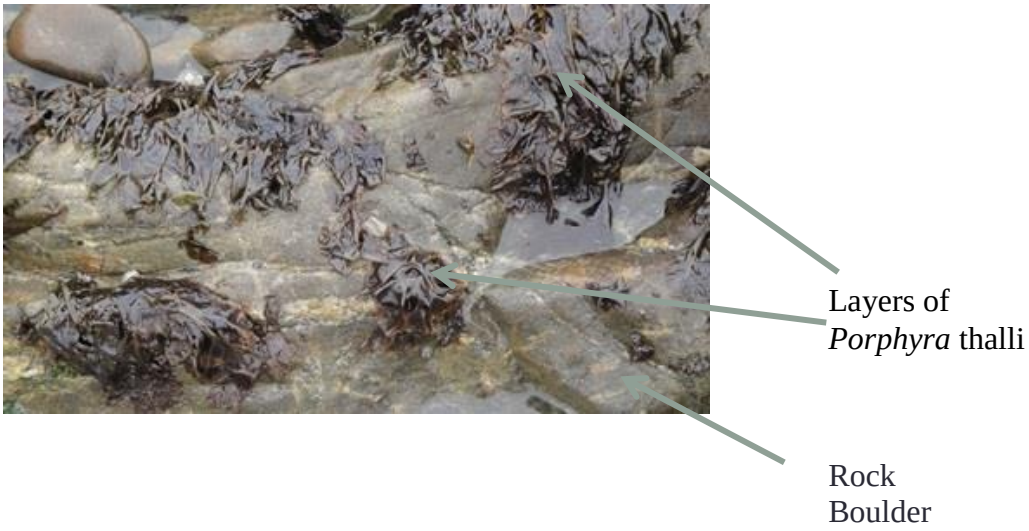
http://www.vegweb.com/recipes/mushroom-nori-soup

APPENDIX 1b:

An example of packaged dried seaweed for sale, to be used as per instructions at the back of the packaging.

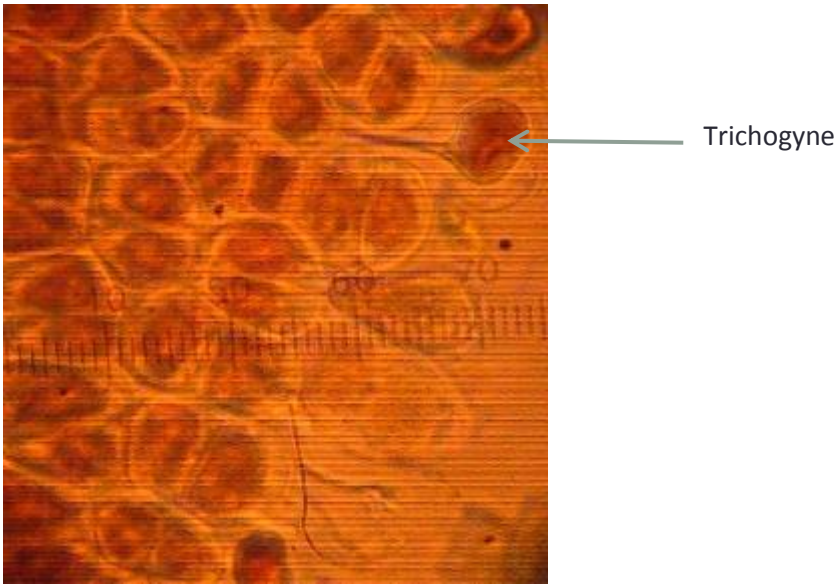
APPENDIX 2:

A picture showing a patchy distribution of thalli on the rocky substrates. Layering of thalli, to escape desiccation stress of covered thalli.



APPENDIX 3:

Differentiated carpogonia cells showing the presence of a trichogyne.



APPENDIX 4:

Enriched Seawater Medium; A Modified Provasoli medium by West & McBride (1999)

Preparation of 2-L Stock

Stock Solutions Glass distilled or MilliQ water	Amount in each stock	Amount of each stock solution to add to make 2-L
Disodium DL-$^{\beta}$ - glycerophosphate pentahydrate	50g/L	16mL
$NaNO_3$	35g/L	220mL
Iron-EDTA (1: 1 molar) $Fe(NH_4)_2(SO_4)_2{}^{*}6H_2O$ Plus Na2EDTA	 700mg/L 660mg/L	 200mL
Tris buffer Trizma 7.7 pre-set pH crystals	50mg/L	160mL
Vitamin B12	25mg/L	7mL
Thiamine	500mg/L	16mL
Biotin	50mg/L	16mL
PII Trace Metals Mix	See below	400ml

Add all solutions to 2- litre flask; bring to volume with glass distilled or Milli Q water.

Steam sterilize (not autoclave) and store at 4^{O}C.

Vitamins stock solutions to be frozen for storage.

P II Trace Metals Mix (1 –litre stock)

Na2EDTA		1.0 g/L
Boron	Boric Acid (H_3BO_3)	1.12g/L
Iron	Ferric chloride ($FeCL_3*6H_2O$)	48mg/L
Manganese	Manganese sulphate ($MnSO_4*H_2O$)	120mg/L
Zinc	Zinc sulphate ($ZnSO_4*7H_2O$)	22mg/L
Cobalt	Cobalt sulphate ($CoSO_4*7H_2O$)	5mg/L

Combine in the above order to 500 ml glass distilled or MilliQ water. Mix. Bring to 1 litre. Steam sterilize. Store at 4^{O}C. To make ES media; add 20mls PES stock to 980 ml sterile seawater (check pH and salinity)

References:

West, J. A. & McBride, D. L. 1999. Long-term and diurnal carpospore discharge patterns in the Ceramiaceae, Rhodomelaceae and Delesseriaceae (Rhodophyta). *Hydrobiologia* 298/299: 101-113.

APPENDIX 5a: The global positioning system (GPS) locations of *Porphyra C. Agardh capensis* specimens collected from Lüderitz, Swakopmund and Cape Town used in the thallus thickness comparison.

Sample #	Locality	S	E	Sample #	Locality	S	E
1	Swakopmund	22°49′ 53.9″	14° 32′ 26.6″	47	Luderitz	26° 36′ 30.8″	15° 10′ 29.4″
3	Swakopmund	22°49′ 45.3″	14° 32′ 27.1″	48	Luderitz	26° 36′ 30.9″	15° 10′ 28.2″
5	Swakopmund	22° 49′ 31.5″	14° 32′ 30.0″	49	Cape Town	33° 54′ 45.8″	18° 23′ 16.8″
7	Swakopmund	22° 49′ 17.6″	14° 32′ 28.9″	50	Cape town	33° 54′ 45.8″	18° 23′ 16.8″
9	Swakopmund	22° 41′ 41.9″	14° 31′ 24.7″	51	Cape Town	33° 54′ 45.8″	18° 23′ 16.8″
10	Swakopmund	22° 41′ 41.4″	14° 31′ 24.9″	52	Cape Town	33° 54′ 45.8″	18° 23′ 16.8″
14	Swakopmund	22° 27′ 05.7″	14° 27′ 29.9″	53	Cape Town	33° 54′ 45.8″	18° 23′ 16.8″
15	Swakopmund	22° 27′ 05.2″	14° 27′ 29.9″	54	Cape Town	33° 54′ 45.8″	18° 23′ 16.8″
17	Swakopmund	22° 35′ 09.8″	14° 30′ 19.6″	55	Torra bay	20° 19′ 37.0″	13° 17′ 18.0″
18	Swakopmund	22° 35′ 08.8″	14° 30′ 19.4″	57	Ugab	21° 04′ 59.3″	13° 33′ 24.2″
19	Swakopmund	22° 27′ 07.2″	14° 27′ 29.4″	61	Cape Cross	21° 45′ 51.7″	13° 57′ 44.7″
21	Swakopmund	22° 27′ 04.6″	14° 27′ 29.7″	62	Luderitz	26° 36′ 30.9″	15° 10′ 28.2″
25	Swakopmund	22° 35′ 05.9″	14° 30′ 20.7″				
26	Swakopmund	22° 35′ 05.3″	14° 30′ 20.4″				
30	Luderitz	26° 38′ 01.5″	15° 09′ 09.9″				
31	Luderitz	26° 36′ 31.4″	15° 10′ 25.2″				
32	Luderitz	26° 38′ 01.1″	15° 09′ 08.9″				
35	Luderitz	26° 37′ 57.7″	15° 09′ 08.6″				
38	Luderitz	26° 37′ 55.8″	15° 09′ 07.3″				
40	Luderitz	26° 38′ 28.5″	15° 09′ 04.3″				
41	Luderitz	26° 36′ 31.4″	15° 10′ 24.7″				
42	Luderitz	26° 36′ 32.3″	15° 10′ 24.2″				
43	Luderitz	26° 38′ 03.0″	15° 09′ 12.6″				
45	Luderitz	26 °36′ 31.1″	15° 10′ 31.8″				
46	Luderitz	26° 36′ 31.2″	15° 10′ 30.1″				

APPENDIX 5b: The global positioning system (GPS) coordinates of field blocks A – G along Swakopmund and Lüderitz shores.

Site	Block	S	E
Swakopmund	A	22° 49 ′ 48.6 ″	014° 32′ 27.0 ″
	B	22° 49 ′ 12.6 ″	014° 32 ′ 29.0 ″
	C	22° 41 ′ 43.2 ″	014° 31 ′ 24.5 ″
	D	22° 27 ′ 07.2 ″	014° 27 ′ 29.4 ″
	E	22° 27 ′ 04.6 ″	014° 27 ′ 29.7 ″
	F	22° 35 ′ 08.4 ″	014° 30 ′ 19.7 ″
	G	22° 35 ′ 08.8 ″	014° 30 ′ 19.6 ″
Lüderitz	A	26° 38′ 01.1″	015° 0.9 ′ 08.9 ″
	B	26° 37 ′ 56.3 ″	015° 09 ′ 09.0 ″
	C	26° 38 ′ 28.5 ″	015° 09 ′ 04.3 ″
	D	26° 36 ′ 31.0 ″	015° 10 ′ 30.5 ″
	E	26° 36 ′ 30.5 ″	015° 10 ′ 28.4 ″
	F	26° 36 ′ 31.4 ″	015° 10 ′ 25.2 ″
	G	26° 36 ′ 32.6 ″	015° 10 ′ 22.7 ″

APPENDIX 5c: The global positioning system (GPS) coordinates of treatments plots T1 T2 & T3 for blocks (a – g) along Swakopmund and Lüderitz shores.

Plots	Swakopmund	Lüderitz
T1a	S22 49 48.6 E14 32 27.0	S 26 38 01.1 E 15 0.9 08.1
T2a	S 22 49 48.7 E 14 32 27.1	S 26 38 01.1 E 15 0.9 08.2
T3a	S 22 49 41.2 E 14 32 26.8	S 26 38 01.0 E 15 0.9 08.0
T1b	S 22 49 17.2 E 14 32 28.5	S 26 37 56.3 E 15 0.9 09.0
T2b	S 22 49 12.6 E14 32 29.0	S 26 37 56.1 E 15 0.9 09.0
T3b	S 22 49 17.7 E 14 32 28.9	S 26 37 56.2 E 15 0.9 09.0
T1c	S 22 41 43.2 E 14 31 24.5	S 26 38 28.5 E 15 09 04.3
T2c	S 22 41 43.6 E 14 31 23.7	S 26 38 28.4 E 15 09 04.2
T3c	S 22 41 47.2 E 14 31 21.7	S 26 38 28.3 E 15 09 04.3
T1d	S 22 27 07.2 E 14 27 29.4	S 26 36 31.0 E 15 10 30.5
T2d	S 22 27 07.6 E 14 27 29.5	S 26 36 31.0 E 15 10 30.4
T3d	S 22 27 08.1 E 14 27 29.1	S 26 36 31.0 E 15 10 30.3
T1e	S 22 27 05.2 E 14 27 29.9	S 26 36 30.4 E 15 10 28.9
T2e	S 22 27 04.5 E 14 27 29.6	S 26 36 30.5 E 15 10 28.9
T3e	S 22 27 04.6 E 14 27 29.7	S 26 36 30.6 E 15 10 28.8
T1f	S 22 35 08.4 E 14 30 19.7	S 26 36 31.4 E 15 10 25.1
T2f	S 22 35 08.4 E 14 30 19.8	S 26 36 31.4 E 15 10 25.2
T3f	S 22 35 08.4 E 14 30 19.8	S 26 36 31.5 E 15 10 25.4
T1g	S 22 35 08.8 E 14 30 19.4	S 26 36 32.7 E 15 10 22.7
T2g	S 22 35 09.8 E 14 30 19.6	S 26 36 32.6 E 15 10 22.7
T3g	S 22 35 09.9 E 14 30 19.7	S 26 36 32.8 E 15 10 22.5

APPENDIX 6:

Halved lettuce head.

APPENDIX 7:

Porphyra spp laboratory culture techniques (Prof. Stekoll, W. S, personal communication, June 2014).

1. Sterile techniques

- Wash culture vessels, glassware etc. with detergent.
- Rinse with hot running tap water several times.
- Rinse with distilled water several times.
- Air dry on dish rack.

Once dry, seal open surfaces with aluminum foil and place piece of *autoclave tape* on the object. Make sure the object is autoclavable. PP is autoclavable and so are the square, clear Tupperware containers. Some types of "plastic" will melt in the autoclave. Consult with someone if you are unsure whether the object is autoclavable.

- Autoclave for 20 minutes at 121 degrees Celsius.
- Dry the autoclaved glassware, etc. in a drying oven

To destroy old and/or contaminated cultures, add bleach to cultures and let stand overnight before disposing. Another method of destroying contaminated cultures is to autoclave them before they are disposed. Always use sterilized glassware, pipettes (etc.) when making new media, creating new cultures and when checking and changing the media in existing cultures.

2. Protocol for obtaining zygotospores release from fertile *Porphyra C. Agardh* blades.

Soak *Porphyra C. Agardh* blade in 1% betadine solution (1 mL betadine per 100 mL sterile seawater) for approximately two minutes. Rinse in sterile seawater. Lay blade on herbarium paper. Cut out fertile piece near margin. Try to avoid the extreme outer edges since most of the diatoms and contamination resides there. Place fertile piece of blade on top of a petri dish. Dry in refrigerator for 20 minutes - 2 hours; avoid over drying. Put the dry, fertile piece of blade into a small petri dish with 10 ml of autoclaved and filtered seawater and add 50 μL of GeO_2 to inhibit diatom growth. Label the specimen remaining on the herbarium paper as follows:

Genus

Area found

Location

Date

Name of collector

Dry and press for future reference. Check the petri dish for spore release which will occur within 24 hours to several days, depending upon the species. Once spores have been released, transfer spores to new petri dishes with fresh media and 50 μL GeO_2 and 100 μL antibiotic solution (1 gram each of penicillin and streptomycin sulfate per 1 L autoclaved seawater; sterile filtered). Monitor the dishes every one to two weeks for conchocelis growth and development. Change the media monthly. Once tufts get large enough to pick up with sterile forceps, select tufts from as many individual thalli as are clean enough to start stock cultures.

APPENDIX 8

Summary data for mean thallus thickness (µm) for specimens collected from Swakopmund, Luderitz and Cape Town.

Site	Mean ± SE	N
Swakopmund	104.07 ± 3.859	27
Lüderitz	102.00 ± 4.329	20
Cape Town	121.67 ± 3.073	6

APPENDIX 9a

Summary data showing the comparison between mean % cover for the Namibian population of *P. capensis* within $1m^2$ quadrats along Lüderitz and Swakopmund shores.

Site	Mean cover % ± SE
Lüderitz	62.646 ± 2.584
Swakopmund	59.156 ± 2.221

NB: Degrees of freedom N = 140 throughout.

APPENDIX 9b

Summary data showing mean % cover and mean standing crop biomass (gDM/m^2) for combined Luderitz and Swakopmund data.

Variable	Mean ± SE
% cover	68.51 ± 2.996
Standing crop biomass (gDM/m^2)	172.98 ± 9.110

NB: Degrees of freedom N = 57 throughout.

APPENDIX 10

Summary data comparing mean standing crop biomass (gDM/m^2) between Lüderitz and Swakopmund.

Site	Mean biomass ± SE
Lüderitz	227.063 ± 21.636
Swakopmund	192.366 ± 20.166

NB: Degrees of freedom N = 35 throughout.

APPENDIX 11

Summarized data on seasonal variation of mean standing crop biomass ($gDMm^{-2}$) for the Lüderitz and Swakopmund combined data.

Season	Mean biomass ± SE
Summer (Dec-Feb)	417.77 ± 22.58
Autumn(Mar-May)	133.75 ± 18.41
Winter (June-Aug)	120.71 ± 15.64
Spring (Sept-Nov)	155.21 ± 12.17

NB: Degrees of freedom N = 70 throughout.