Epiphytic diatom assemblages associated with South African kelps: *Ecklonia maxima* and *Laminaria pallida*

By

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Declaration

I hereby state that "**Epiphytic diatom assemblages associated with South African kelps: *Ecklonia maxima* and *Laminaria pallida***" is my own, unaided work and has not been submitted in this or any form for any degree or examination at any other university. All the sources I have used or quoted have been indicated and duly acknowledged by complete references.

Work discussed in this thesis was carried out under the supervision of Prof. Albertus J. Smit of the Department of Biodiversity and Conservation Biology of the University of the Western Cape and Dr. Roksana Majewska of the Unit for Environmental Sciences and Management, North-West University.

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To my late father, Matthias Ntambwe Mayombo

Thank you for leaving the education as a legacy in the family.

To my family

Thank you for your unconditional love, support and encouragement.

Table of contents

List of tables

List of figures

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Abstract

Kelp forests are dynamic and productive ecosystems which host large biodiversity of sessile fauna and flora, including diatoms. These microalgae occur at the base of coastal marine food webs and contribute substantially to the productivity of marine ecosystems. Diatoms constitute one of the most common and species-rich groups of both phytoplankton and phytobenthos. Possessing a unique silica cell wall, diatoms play a key role in the global carbon and silicon cycles. As the changes in species composition of diatom communities are a direct reaction to the combination of environmental factors prevailing in their ecosystems, diatom analysis is widely and successfully used in biomonitoring of various environmental conditions and paleoecological reconstructions. Although diatoms have been long studied in South Africa and used for biomonitoring, particularly in freshwater ecosystems, very little is known about their community structures and composition in marine environments, especially those associated with macroalgae.

This MSc thesis studied the ecology and biodiversity of the epiphytic diatoms associated with the kelp forests in False Bay, around Miller's Point, off the west coast of South Africa. Diatom community structure and abundances on different parts of adult and juvenile specimens of two common South African kelp species *Ecklonia maxima* and *Laminaria pallida* were examined. A floristic study of all diatom taxa found in 96 samples (288 subsamples) was also conducted based on morphological observations of the frustules using light (LM) and scanning electron microscopy (SEM). Advanced multivariate statistical analyses were performed to draw the links between diatoms and the biotic factors: a) host species, b) host age, and c) thallus part, possibly affecting the epiphytic diatom community composition and biofilm abundances.

A total of 22 diatom genera were found during the SEM observations and density assessments of the diatom biofilm still attached to the kelp surface. A thorough analysis of cleaned (i.e. acid-digested) diatom material allowed for a documentation and identification of 48 taxa belonging to 28 diatom genera. *Gomphoseptatum* Medlin, *Nagumoea* Witkowski and Kociolek, *Cocconeis* Ehrenberg, and *Navicula* Bory were the most frequently occurring diatom genera, being present in 84%, 65%, 62.5% and 45% of the analysed subsamples respectively. Among these, *Cocconeis* and *Gomphoseptatum* were the most abundant, contributing 50% and 27% of total diatom cells counted collectively across all subsamples. PERMANOVA revealed that all investigated main factors (kelp species and age and thallus part), as well as their two- and three-way interactions, except for the interaction between the host species and age, were significant. The high residual variance (72%) suggested that the sum of other unexamined factors contributed the largest component of the variation observed in the kelp-associated diatom communities. Grazing and possible defence strategies utilised by kelps are suggested as processes playing an important role in the structuring of epiphytic diatom communities. Moreover, the possible endophytism of tissue-boring diatoms colonizing both kelp species is briefly discussed.

Keywords: Epiphytic diatoms, Bacillariophyceae, diatom abundances, *Cocconeis*, *Gomphoseptatum*, *Nagumoea*, kelp, Phaeophyceae, macroalgae, *Ecklonia maxima*, *Laminaria pallida*, thallus part, Multivariate analyses, False Bay, Southern Africa.

Chapter 1

Introduction

1.1. Kelp forest ecosystems

Kelps are large macroalgae of the order Laminariales within the class Phaeophyceae. Being associated with cold-water upwelling systems throughout the world, they form dense stands along rocky coastlines in temperate to polar regions (Bustamante and Branch 1996; Bengtsson and Øvreås 2010; Arnold *et al.* 2016; Krumhansl *et al.* 2016). Marine areas with a high density of kelps are called kelp forests, whereas smaller areas of anchored seaweeds are termed kelp beds. These kelp-based habitats constitute one of the most complex, dynamic and productive coastal ecosystems providing shelter, food and/or growth substratum for a multitude of other organisms, such as marine mammals, fishes, crabs, sea urchins, molluscs, and other algae (Allen and Griffiths 1981; Steneck *et al.* 2002; Fredriksen 2003; Teagle *et al.* 2017). A single kelp thallus is stratified into various microhabitats providing a growth substratum for a multitude of other organisms (Leclerc *et al.* 2016). These formations are considered to be sentinel biotopes because they are highly responsive to human-induced environmental changes (Wernberg *et al.* 2013), and they are directly exposed to many anthropogenic activities that impact the coastal zones (*e.g.*, harvesting, pollution, sedimentation, invasive species, overfishing, recreation) (Blamey *et al.* 2015; Krumhansl *et al.* 2016). In recent years, it was shown that kelp forests declined in many regions, *e.g.* Australia, in response to a variety of stressors (Steneck *et al.* 2002; Wernberg *et al.* 2013; Krumhansl *et al.* 2016), while kelp abundances have been stable or even increasing in other areas, such as South Africa (Bolton *et al.* 2012; Blamey *et al.* 2015).

In the past, southern African kelp bed ecosystems occurred from the Cape Agulhas (around the southwest coast of South Africa) to 60 km north of Lüderitz (Namibia) along the southern African west coast; this rocky infratidal zone is largely influenced by the cold waters of Benguela Current (Velimirov *et al.* 1977; Field *et al.* 1980; Allen & Griffiths 1981; Bolton 1986; Bustamante & Branch 1996, Anderson *et al.* 2007). Recently Bolton *et al.* (2012) reported that South African kelp forests have been expanding. The authors observed that the eastern limit of the major South African kelp forest formed by a single macroalgal species *Ecklonia maxima* (Osbeck) Papenfuss had expanded 73 km eastward along the south coast of the country since 2006, after remaining unchanged for almost 70 years (Bolton *et al.* 2012). Currently, four different kelp species form the southern African kelp forest: *Ecklonia maxima*, *Ecklonia radiata* (C. Agardh) J. Agardh, *Laminaria pallida* Greville, and *Macrocystis pyrifera* (Linnaeus) C. Agardh (previously known in South Africa as *Macrocystis angustifolia* Bory de Saint-Vincent). *Ecklonia maxima* and *L. pallida*, having by far the highest biomass in the inshore rocky environment, are exploited commercially in South Africa (Rothman *et al.* 2017). These two species are investigated in the present study.

1.2. Marine epiphytism: complexity of interactions

Marine plants are susceptible to colonisation by usually smaller sessile and motile biota (epibionts), including algae and invertebrates. Marine epiphytism is a common phenomenon, especially within rocky coastal habitats, where macro- and micro- algae settle and grow on submerged host macroalgal thalli. The term ‘epiphyton’ encompasses both the ‘true epiphyton’ attached to the host plant as well as the ‘metaphyton’ thriving in the mucilaginous matrix surrounding it (Snoeijs 1994; Medlin and Juggins 2018). Each of the microalgal organisms comprising epi- and metaphyton generates the so-called phycosphere – a region in the direct vicinity of the living algal cell enriched with carbon compounds excreted by the cell, which may be used as a source of nutrients by other microorganisms. The phycosphere is comparable to the rhizosphere in terrestrial plants and likely plays a crucial role in biofilm formation (Ashworth and Morris 2016; Seymour *et al.* 2017; Johansson *et al.* 2019).

Do host macroalgae influence the community composition and densities of their associated epiphytic assemblages? Do the host species and age and the part of the colonised thallus matter? These questions have received considerable interest and have been widely debated, although no unanimous consensus has been reached. Epiphyton growing on the host thalli interact biologically, chemically and physically with their host macroalgae. These interactions may either favour or inhibit the epiphytic biofilm development (Medlin *et al.* 1985; Mutinova 2015; Medlin and Juggins 2018). Cattaneo and Kalff (1979) found host organisms to be neutral substrata for their associated microalgae as no significant differences were observed among epiphytic assemblages on *Potamogeton richardsonii* (A. Bennett) Rydberg and other morphologically similar macroalgae. Gough and Gough (1981) showed that, while some host substrata are neutral to their epiphyton, others affect significantly the colonisation processes, and the epiphytic community composition may vary greatly from a host species to another. These variations are due either to host morphology or mechanisms independent of morphology. Similar observations were also made by Gough and Woelkerling (1976), who reported that some host macroalgae species are neutral substrata for epiphytic microorganisms, while others actively influence their associated epiphytic community through biological (*e.g*. host species and morphology) and chemical (*e.g*. nutrient assimilation, secretion of photosynthates and inorganic nutrients, and depletion of substances by rapid uptake) interactions. The composition and density of epiphyton on different host species with comparable morphology, even within the same sampling site, were significantly different (Blindow 1987), suggesting that not only the host morphology matters. Other factors contribute as well in driving the community composition and density of epiphytic assemblages on host macroalgae substrata.

Host-epiphytes interactions may be positive in various ways. Some macroalgae release inorganic nutrients through their surface, which may be used by their associated epiphytic microalgae, particularly in oligotrophic waters (Burkholder *et al.* 1990). Nutrients released on the surface of the macroalgae may favour the development of a mutualistic relationship between the host seaweed and epiphytic microalgae. The host macroalgae benefit from this mutualistic relationship by being protected from grazing by the layer of epiphytes, because grazers would prefer to feed on epiphytic microalgae over the macroalgal tissues (Amsler *et al.* 2019). Furthermore, growth enhancing metabolites such as auxin compounds secreted by some host macroalgae may potentially have positive effects on associated epiphytes. Le Bail *et al.* (2010) reported the occurrence of auxin-mediated, long-range control of the developmental patterning in the brown alga *Ectocarpus siliculosus* (Dillwyn) Lyngbye*.* Although the auxin concentration varied in different parts of the thallus (Le Bail *et al.* 2010), the possible effect of the growth enhancing compounds on the associated epiphyton has yet to be documented.

Marine epiphytism is sometimes seen as a deleterious phenomenon for the host macroalga. It was shown that epiphytes decreased the growth rate of the intertidal red alga *Rhodomela larix* (Turner) C. Agardh, thus increasing the probability of axis breakage and decreasing the host reproductive output (D'Antonio 1985). Host thalli and their epiphytic microalgal assemblages may compete for nutrient and sunlight (Hudon and Bourget 1983; Gosselain *et al.* 2005). Therefore, the host macroalgae may develop mechanisms to deter the growth of an undesirable epiphytic community through, for example, production of allelopathic substances. Chemical defences against epiphytes are common and relatively well-known in marine macroalgae. Macroalgal chemical defence strategies may include secretion of chemically active metabolites, which act as repellent or antifouling agents (Helio *et al.* 2001; Kubanek *et al.* 2003). Kubanek *et al.* (2003) isolated lobophorolide, a compound responsible for targeted antifungal activities in the small thalloid brown alga *Lobophora variegata* (J. V. Lamouroux) Womersley ex E. C. Oliveira. Amsler *et al.* (2005) studied the potential chemical defence strategies of Antarctic macroalgae against diatom fouling. The authors found extracts of two species collected from McMurdo Sound to be toxic to diatoms at/or below concentrations originally present in the macroalgal thalli (the ''natural concentration''). Lam *et al.* (2008) described specific activity patterns in the extracts from the North Sea macroalgae, suggesting targeted effects on individual fouling species of four benthic diatoms (*Nitzschia* sp., *Navicula phyllepta* Kützing, *Navicula arenaria* Donkin and *Amphora* sp.). Wang *et al.* (2017a, 2017b) found that at least part of the defence against fouling organisms in the red alga *Gracilaria vermiculophylla* (Ohmi) Papenfuss is based on extractable secondary metabolites, such as oxylipins produced from arachidonic acid after wounding or damaging the macroalga tissue.

On the other hand, water-soluble phlorotannin compounds exuded by the sublittoral kelp *Ecklonia radiata* (C. Agardh) J. Agardh previously thought to play a role in the chemical defence against epiphytes (Sieburth and Conover 1965; Langlois 1975), were found to have negligible effect on microalgal colonisation (Jennings and Steinberg 1997). This study reported that some other factors such as the relative age of different parts of the thallus (e.g. exposure time of the macroalgal host tissue to the surrounding water and other growth enhancing factors), depth, and tissue texture and rugosity have influenced epiphytes colonisation on the host macroalgae surface (Jennings and Steinberg 1997). High levels of polyphenols, as defence against grazers, were recorded in the thin outer meristoderm tissues of the holdfasts, stipes and blades of *E. maxima* and *L. pallida* (Tugwell and Branch 1989). Antagonistic effects of these polyphenolic substances on the growth of the associated epiphytes were documented by several studies (McLachlan and Craigie 1964; Cundell *et al.* 1977; Rönnberg *et al.* 1986).

Mechanical defence strategies, such as sloughing of the outer layer of the seaweed epidermis, also known as epidermal shedding, constitute another mechanism employed by some long-lived marine macroalgae to effectively control the epiphytic growth on their surfaces (Moss 1982; Yamamoto *et al.* 2013; Halat *et al.* 2015). Nylund and Pavia (2005) reported that the low degree of fouling found naturally on the red alga *Dilsea carnosa* (Schmidel) Kuntze was likely a consequence of the mechanical defence, *e.g.* sloughing off the outermost cell layer to remove associated epibiota. Halat *et al.* (2015) quantified the epidermal shedding on a weekly basis over a period of one year in a population of the brown alga *Ascophyllum nodosum* (Linnaeus) Le Jolis in Nova Scotia. The authors estimated that ca. 25% of the frond epidermis was shed every week and for at least 9 months of the year. The shedding occurred at the population level and cycles of skin shedding were followed by re-accumulation of epiphytes (Halat *et al.* 2015).

Hydrodynamic forces may dislodge the epiphyton. It was shown that host plants such as *Odonthalia floccosa* (Esper) Falkenberg fouled with diatoms experienced twice as much drag in the field as this same species without fouling epiphytes, due to increased cross-sectional area resulting from the epiphytic diatom cover (Ruesink 1998). Similarly, the host macroalgae may benefit the attached epiphytes by reducing the amount of drag experienced by them and allowing them to thrive in more wave exposed areas (Anderson and Martone 2014). Biomechanical interactions between algal epiphytes and their host macroalgae are complex and not necessarily negative, which may partially justify the evolution and persistence of the host-epiphyte relationships in wave exposed coastal ecosystems (Anderson and Martone 2014). The host plant provides support to the epiphytes in wave exposed environments whereas the epiphytes feed the grazers, thus preventing the host macroalgae from being grazed (Reynolds *et al.* 2014).

Moreover, antimicrobial effects of the host-associated microorganisms in the epiphytic biofilm (such as fungi and bacteria) contribute to controlling the community composition and epiphyton densities (Jenkins *et al.* 1998; Amstrong *et al.* 2001; Dobretsov and Qian 2002, Lam *et al.* 2008). For example, solanapyrones isolated from unidentified marine fungi showed toxicity towards the marine unicellular alga *Dunaliella* sp*.* Theodoresco at concentrations as low as 100 µg.ml^{-1} (Jenkins *et al.* 1998). Amstrong *et al.* (2001) suggested that epibiotic bacteria strains isolated from the surface of the marine macroalga *Codium fragile* (Suringar) Hariot released antimicrobial compound into the surrounding water, thus controlling the microbial communities on their host. Dobretsov and Qian (2002) investigated the effect of epibiotic bacteria associated with the green alga *Ulva reticulata* Forsskål on marine micro- and macrofouling. They found that the antifouling mechanism of *U. reticulata* depended not only on the compounds produced by the macroalga itself but also on those excreted by the epibiotic bacteria developing on the algal surface (Dobretsov and Qian 2002).

Grazing may significantly contribute to reducing the abundance of epiphytes on macroalgal host surfaces. It was indicated as the most important factor determining the temporal variability of early growth of individual microalgal species colonisation in a sublittoral *Ecklonia radiata* forest off Sydney, Australia (Kennelly 2006). Reynolds *et al.* (2014) described the macroalgae-epiphytes-mesograzers interactions based on their observations on the small invertebrates controlling the accumulation of epiphytic algae, and thus buffering the eutrophication effects and facilitating eelgrass dominance in Chesapeake Bay. The same kind of mutualistic relationship was documented along the Western Antarctic Peninsula, where the natural abundances of gastropods on the brown alga *Himantothallus grandifolius* (A. Gepp & E. S. Gepp) Zinova maintained in mesocosms significantly reduced fouling by microscopic algae, primarily diatoms (Amsler *et al.* 2019).

In conclusion, marcoalgae use a range of mechanisms that aid them to eliminate or reduce the extent of marine epiphytism. These defense mechanisms were widely reported for many species of macroalgae, but the information regarding the interactions between the epibiont and its host in Laminarian species is generally less well represented in the scientific literature.

1.3. Epiphytic diatoms

Diatoms form a major and abundant group of benthic microalgae in most shallow water habitats. Diatom assemblages develop wherever there is adequate water, enough sunlight for photosynthesis and nutrients, especially silica which is the major constituent of their frustules (Round *et al.* 1990). One important feature of sessile epiphytic diatom species is their adherence to the host plant substrata. Adhesive strength is also thought to be one of the remarkable ecological characteristics of epiphytic diatoms, driving their micro-distribution and dispersion along the host plant thallus (Tanaka 1986). Epiphytic diatom communities are categorised into different growth forms based on their mode of adhesion to their host substrata (Tanaka 1986; Costa *et al.* 2016). Growth form describes the architecture of an organism and its autecology. Diatom growth forms refer to the set of adaptations developed by different taxa for a particular mode of life, thus allowing taxonomically unrelated species to grow in similar habitat, and to compete for available resources (Hudon and Legendre 1987). According to Hudon and Bourget (1983), epiphytic diatom growth forms are determined using three characteristics, *e.g.* form, posture and mobility, combined in different ways. Several diatom growth forms can be distinguished, which mainly differ in their attachment mode and degree of motility (Barnett *et al.* 2015). They may range from simple spheroid unicells to complex three- dimensional colonies comprised of hundreds of cells and reaching macroscopic sizes (Nakov *et al.* 2015).

Epiphytic diatom growth forms include: motile forms comprising mostly large biraphid taxa, freely moving on their host surface (*e.g.* taxa belonging to genus *Navicula* Bori de Saint-Vincent, *Trachyneis* P. T. Cleve, *Diploneis* Ehrenberg ex Cleve). Tube dwelling forms including naviculoid or nitzschoid taxa living in mucilaginous tubes, within which they are highly motile (Totti *et al.* 2009). Adnate forms encompass biraphid and monoraphid taxa with flattened cell shape that allows them to closely contact their host substrata (*e.g.* taxa belonging to *Amphora* Ehrenberg and *Cocconeis* Ehrenberg genera). Adnate diatoms are rarely colonial (Totti *et al.* 2009). Erect forms comprise cells attached to the substrata by mucilaginous stalks or pads (*e.g.* taxa belonging to the genera *Gomphoseptatum* Medlin, *Rhoicosphenia* Grunow, *Grammatophora* Ehrenberg, *Licmophora* C. A. Agardh) (Totti *et al.* 2009; Costa *et al.* 2016; Majewska *et al.* 2016). Planktonic forms include truly planktonic diatom species (e.g. *Cylindrotheca* L. Rabenhorst) which are allochtonous to the benthic epiphytic communities, as well as tychoplanktonic diatoms (e.g. *Cyclotella* (F. T. Kützing) A. de Brébisson, *Thalassiosira* P. T. Cleve) which are non-motile species found mainly in benthic environments, but may be temporarily re-suspended into the water column (Ribeiro 2010). The growth forms of epiphytic diatoms depend on morphological differences on the thalli of host macroalgae (Costa *et al.* 2016).

Biofilm development on any substratum occur in four phases constituting an overlapping time sequence: biochemical conditioning, bacterial colonisation, unicellular and, finally, multicellular eukaryont fouling (Wahl 1989). Diatom colonisation is very often preceded by bacterial fouling. Small- celled species that grow attached to a substratum *via* a mucilaginous pad, *e.g.* small adnate and motile forms, are early colonisers of host substrata and are adapted for resistance to scouring from water currents (Nakov *et al.* 2015). On the other hand, long filamentous or branched colonies forming erects diatoms tend to establish later in the succession, when the substratum is crowded and cells improve access to nutrients and light by rising above the boundary layer (Nakov *et al.* 2015). Grazers were found to prefer arborescent and filamentous diatom colonies over smaller species growing closely attached to their substratum (Hudon and Legendre 1987).

Numerous studies investigated epiphytic diatom assemblages on macroalgae in different regions across the globe. The distribution of epiphytic microalgae on host thalli and, more specifically, the composition of benthic diatom communities in terms of growth form and cell abundance is affected by a number of biotic and abiotic factors. Some authors have suggested the existence of host effects on associated epiphytic diatom assemblages because significant differences in community compositions and population densities were found on different macroalgal host species (Medlin *et al.* 1985; Snoeijs 1994; Al-Handal and Wulff 2008; Chunk and Lee 2008; Totti *et al.* 2009; Majewska *et al.* 2013b, 2016; Medlin and Juggins 2018), while others found no significant differences among host species (Majewska *et al.* 2013a).

Surface texture and morphology of the host macroalga seem to strongly affect both the community composition and density of the associated diatom assemblages (Medlin *et al.* 1985, Chung and Lee 2008; Majewska *et al.* 2013a, 2013b, 2016; Shams El-Din *et al.* 2015; Costa *et al.* 2016). According to Tanaka (1986), the dominant diatom growth form that would show a certain adhesive strength would be a result of both the biotic factors from the host as well as the abiotic factors from the environment. As these factors may differ along the macroalgal thallus, the structure and composition of the diatom communities associated with different parts of the host would vary too (Tanaka 1986).

Moreover, during the development of periphyton communities, the density and biomass of attached microalgae increase over time (Blindow 1987). Therefore, high densities of epiphytic diatoms and more mature communities should occur on older host plant tissues compared to younger ones. The species composition of epiphytic diatom assemblages correlated closely to the lifespan of examined seagrass species in the southern coast of Korea (Chung and Lee 2008). Early colonisers such as the genus *Cocconeis* Ehrenberg were more abundant on the seagrass *Z. japonica* which blades have a shorter lifespan of 21-22 days on average, while the late colonising diatoms such as *Navicula* Bory and *Nitzschia* Hassall dominated the assemblages on *Z. marina* which blades have a longer lifespan averaging 36-48 days (Chung and Lee 2008).

On the other hand, Sterrenberg *et al.* (1995) observed no major differences in epiphytic diatom flora on young and older leaves of the seagrasses *Enhalus acoroides* (Lf.) Royle and *Thalassia hemprichii* (Ehrenberg) Ascherson in South Sulawesi, Indonesia. Similarly, no significant differences were found between epiphyte assemblages associated with different thallus regions (upper, lateral and basal) of the macroalgae *Leathesia marina* (Lyngbye) Decaisne (Phaeophyceae) on the Patagonian Atlantic coast, Argentina (Poza *et al.* 2018).

Several studies have reported that epiphytic diatom community composition and abundances were also influenced by abiotic or environmental changes. Temperature rise was the most important factor influencing epiphyte density on the giant kelp *Macrocystis pyrifera* (Linnaeus) C. Agardh blades under aquarium conditions in Taiwan (Tew *et al.* 2017). The authors demonstrated that as the temperature in the tank increased because the experiment was conducted in a subtropical region where water temperature is always above 20˚C, diatom density and diversity increased as well (Tew *et al.* 2017). Salinity was the overriding factor affecting the distribution and abundance of epiphytic diatoms in the Baltic Sea (Snoeijs 1995). Epiphytic diatom flora along the Swedish coast were affected by temperature, salinity, light, tide, geographical position and water current (Edsbagge 1966). Nutrient availability may also influence the community composition of associated diatoms. In an experimental field manipulation, Frankovich *et al.* (2009) found that the effects of nitrogen (N) and phosphorous (P) additions on epiphyte communities in Florida Bay were generally smaller, mostly site and time specific. These effects were mainly due to P additions, which favoured macroalgal epiphytes and decreased diatom abundance particularly in summer. Within diatom communities, responses to P additions, though evident, were not as strong (Frankovich *et al.* 2009). In a laboratory competition experiment conducted with attached natural diatom assemblages from Dona Paula Bay, west coast of India, Mitbavkar and Anil (2007) observed that excess nutrients favoured those diatom species with comparatively higher growth rates, thereby suppressing the growth of other co-existing species and reducing species diversity within a fouling diatom community.

Sampling depth was reported to explain about 10% of the variation in epiphytic diatom growth forms, such as tube dwelling diatoms dominated in shallowest depths, and erect and metaphytic forms were more abundant in deepest waters of the Ross Sea, Antactica (Majewska *et al.* 2016). A decrease in diatom species diversity was observed as depth below the sea ice increased at Cape Evans, Antarctica (Sutherland 2008). Depth, sunlight, wind action, water currents are also among the environmental factors driving the community structures of epiphytic diatom assemblages in exposed coastal ecosystems (Chunk and Lee 2008; Costa *et al.* 2014). An increase in diatom cell density from apical portions towards the basal parts of *Galaxaura rugosa* (L. Ellis & Solander) J. V. Lamouroux (Nemaliales: Rhodophyta) from Fernando de Noronha archipelago in north-eastern Brazil was rather attributed to the influence of strong currents and water movements on the surface of the ocean, favouring the settlement of taxa with larger adhesive strength on the apical parts of the host plants (Costa *et al.* 2014). Tanaka (1986) showed that adhesive forces of some diatom taxa (*e.g. Cocconeis* Ehrenberg, *Pseudogomphonema* Medlin) are sufficiently strong to allow them to stay attached to the biotic substratum even in the areas subjected to large hydrodynamic forces like those present on the west coast of South Africa (Field *et al.* 1980).

Studies reporting epiphytic diatom assemblages associated with kelps are very scarce. Siqueiros-Beltrones *et al.* (2002) studied epiphytic diatom assemblages on blades of *Macrocystis pyrifera* (Linnaeus) C. Agardh and other kelp species from the Baja California Peninsula, Mexico. They noted a high dominance of *Cocconeis* cf. *britannica* Naegeli ex Kützing in the diatom communities. Other important epiphytic diatom species recorded in this study included *Cocconeis speciosa* W. Gregory*, Gomphonemopsis pseudoexigua* (Simonsen) Medlin*,* and *Climacosphenia moniligera* Ehrenberg which was associated to a colonial *Navicula* sp. These species were restricted to *M. pyrifera* since none of them was observed on the other sampled kelp species or substrata (rock and sediments) (Siqueiros-Beltrones *et al.* 2002). Although no diatoms were found on the blades of *Eisenia arborea* Areschoug collected from the same region during the aforementioned study, Siqueiros-Beltrones and Argumedo-Hernández (2014b) found a quasi-monospecific community of *Pteroncola inane* (Giffen) Round (Fragilariales, Bacillariophyceae) on blades of *E. arborea*. The authors concluded that high abundance of *P. inane* on the investigated kelps may suggest that the *E. arborea* thallus provides an optimal substratum for this diatom species (Siqueiros-Beltrones and Argumedo-Hernández 2014b). The following study (Siqueiros-Beltrones *et al.* 2016) presented the first floristic list of diatoms thriving on *E. arborea* from the Baja California. Although the list included 99 diatom taxa, most of the examined communities were each time dominated by *P. inane*, showing generally a very low compositional diversity.

1.4. Diatoms as bioindicators

1.4.1. Bioindicators and biomonitoring

Monitoring environmental conditions in water bodies can be done by means of different approaches, including physicochemical monitoring and biomonitoring. Physicochemical monitoring involves the determination of the physical and chemical status of the water based on the presence and content of specific physical and chemical variables (DWAF 1996). These traditional monitoring methods using water physicochemical measurements target only water quality variables and may also fail to capture a spike or bolus of pollutant that will impact populations of organisms and/or to capture changing concentrations of a particular pollutant (Taylor *et al.* 2007a, 2007b). Such analyses are expensive, time consuming, and reflect only momentarily conditions occurring at the time of sampling (Day 2000). Furthermore, the most toxic and dangerous pollutants in the environment occur in minute concentrations, below the detection limits of even the most sophisticated analytical methods (DWAF 1996).

On the other hand, biomonitoring investigates the behavioural and physiological response of living organisms to their environment, and it is a good alternative to monitor the integrity ('health') of ecosystems (Todd and Roux 2000). Biomonitoring assesses changes in aquatic biota (flora or fauna), establishes the relationships between the biological features and the overall chemical status and the reactions of individual species to specific nutrients and/or pollutants (Round 1991). A good bio-indicator must have a short life span to integrate short-term environmental variability, be abundant and easy to sample, be sessile to reflect conditions at one site, and be response-specific to identify a particular impact on the ecosystem (Desrosiers *et al.* 2013). Resident marine communities (macroinvertebrates, fish, macrophytes, benthic algae) were found to better reflect the overall ecological integrity ('health') of their environment because their structure and composition are the results of past and current impacts of physical and chemical disturbances (Todd and Roux, 2000). Thus, they can be used as bioindicators to determine impaired waters, identify marine or aquatic life stressors, set pollution load reductions, and indicate improvement. Biomonitoring methods were proposed as good alternative to the shortcomings of physicochemical monitoring.

One of the major limitations of biomonitoring is that although a change to aquatic ecosystem 'health' may be detected, identifying the cause of that change may be challenging. However, the use of a range of metrics developed to detect specific types of impact coupled with expert knowledge of the ecology of the indicator taxa will improve the ability of users to determine causes of environmental degradation (Todd and Roux 2000). According to

Desianti *et al.* (2017), some of the major difficulties associated with developing biomonitoring protocols include calibrating protocol sensitivity to different pollution types and interpreting outputs in the context of often high natural variability of assemblage structure (patchy distribution of species). Although some training is required to plan and execute sampling protocols and then to identify and interpret output data, the protocols require relatively little and inexpensive equipment and are rapid (Todd and Roux 2000). Macroinvertebrates, fish, riparian vegetation (macrophytes), bacteria, protozoa, non-diatom algae, and diatoms have been widely used as tools of biomonitoring. Macroinvertebrates are useful as short-term indicators, while fish, riparian vegetation and algae are good long-term indicators of ecosystem health (Todd and Roux, 2000). Because diatoms are microalgae found abundantly in almost all aquatic ecosystems throughout the world, they have been proposed to be used as bioindicators. Diatom-based biomonitoring techniques are rapid, reliable, and much more affordable tools of water quality assessment, particularly in freshwater ecosystems where they have been extensively investigated (Kelly *et al.* 2009).

1.4.2. Diatom analysis

Since 2000, the European Framework Directive has made it mandatory to use diatoms for assessing the ecological status of watercourses (Kelly *et al.* 2009). Therefore, diatoms are now widely used as easily accessible, and well-understood bioindicators. Diatom-based analysis is widely used for water quality assessment in rivers in Europe (Rimet *et al.* 2009; Pace *et al.* 2012; Jüttner *et al.* 2012), North America (Lavoie *et al.* 2004, 2009; Stevenson *et al.* 2008), Latin America (Lobo *et al.* 2010; Bere and Tundisi 2011), Asia (Di *et al.* 2013; Tan *et al.* 2013), and Oceania (Chessman *et al.* 1999). Diatom community structure reflects better the impact of various physical, chemical, and biological factors such as temperature (Elias *et al.* 2012), nutrient concentrations (Cejudo-Figueiras *et al.* 2010; Rimet 2012), acid mine drainage (Gray and Vis 2013), and grazing (McCormick and Stevenson 1989). Diatom-based indices developed for water quality monitoring in temperate river systems (USA, Europe, Japan) were successfully tested in South Africa (De la Rey *et al.* 2004, 2008; Taylor *et al.* 2005a, 2007a, 2007b; Walsh and Wepener 2009), Kenya (Ndiritu e al. 2003, 2006; Bellinger *et al.* 2006) and Zimbabwe (Bere *et al.* 2013; Bere and Mangadze 2014; Mangadze *et al.* 2015). However, the occurrence of many taxa endemic to tropical and subtropical systems makes it necessary to develop local diatom indices that should include the autecology of all the local taxa.

1.4.3. Marine diatoms

It is widely recognised that benthic diatoms (Bacillariophyceae) are good biological indicators for the monitoring of freshwater environment quality, because of: (i) their short life span as unicellular microalgae, that can capture short-term environmental fluctuations at sampling sites; (ii) their sensitivity to numerous environmental parameters and to organic and nutrients enrichment, which allows classification of species according to functional categories such as pH; salinity, nitrogen uptake metabolism, oxygen requirements and trophic state (Van Dam *et al.* 1994; Desrosiers *et al.* 2015). However, opposite to freshwater ecosystems, relatively little is known about diatom community composition and its relationships to environmental conditions in marine environments (Potapova *et al.* 2016). As one may expect, this is also true for all the coastal habitats in Africa. Most applied studies of diatoms in marine environments exploit their sensitivity to water salinity, which allows using the composition of fossil diatom assemblages to reconstruct marine paleoenvironmental conditions (Desianti *et al.* 2017). Such studies do not necessarily require fine-resolution species-level diatom taxonomy, as many diatom genera are specific to either fresh or saline waters (Desianti *et al.* 2017). However, responses of diatom assemblages to anthropogenic pollution, on the other hand, require species-level characterization, which is often challenging because of the exceptionally high diversity and poorly understood species boundaries of many coastal diatoms (Desianti *et al.* 2017).

Benthic diatoms of marine environments were used as bioindicators to infer past environmental conditions and to assess present ecosystem ‘status’ of coastal habitats; *e.g.* surface sediment diatoms communities from New York and New Jersey coastal lagoons correlated to several metals and total polycyclic aromatic hydrocarbons (PAHs) (Potapova *et al.* 2016). Diatom inference models for salinity and sediment nitrogen content were developed by Desianti *et al.* (2017). They concluded that diatom species with relatively high optima for sediment nitrogen may be used as indicators of nutrient enrichment in studied New Jersey coastal lagoons (Desianti *et al.* 2017). In the Mid-Atlantic coastal wetlands, benthic and marsh soil diatom assemblages were jointly controlled by tidal elevation, salinity, sediment texture, and nutrient content, while the relative importance of these factors varied among datasets targeting different environmental gradients (Desianti *et al.* 2019). The authors demonstrated that despite inherent challenges, such as tidal redistribution of diatom frustules across the intertidal zone, diatoms can be successfully used as an independent source of evidence in paleoreconstructions of sea-level change and nitrogen enrichment, as well as for monitoring current nutrient pollution in Mid-Atlantic wetlands (Desianti *et al.* 2019).

Several studies have also reported that marine epiphytic diatoms are sensitive to various environmental conditions, including salinity, temperature, light, tide, geographical position, and current (*e.g.* snoeijs 1994, 1995; Edsbagge 1966), nutrients (Mitbavkar and Anil 2007; Frankovich *et al.* 2009), sampling depth (Sutherland 2008; Majewska *et al.* 2016), sunlight (Hudon and Bourget 1983), wind action and water currents (Costa *et al.* 2014). Therefore, there is a possibility to use diatoms as bioindicators of coastal ecosystems ‘health’ as they have been used in freshwater systems. However, the successful determination of any aquatic ecosystem conditions using diatoms as indicators is primarily dependent on the correct identification of the diatom taxa (Schoeman 1982).

1.5. Marine diatoms of Southern Africa

In South Africa, diatoms have been studied extensively for several decades (Boden and Day 1949; Cholnoky 1963; Giffen 1963, 1966, 1970, 1973; Schoeman 1982). The pioneer diatom studies were mostly taxonomic. These early studies indicated that although some diatom species are endemic to the region, the majority of species are common in other parts of the world (Taylor *et al.* 2005, 2007a, 2007b; Dalu *et al.* 2016). Thus, the taxonomic value of the contributions by South African diatom scholars has been tremendous (Cholnoky, 1963; Giffen, 1963, 1966, 1970, 1973, 1975, 1976; Schoeman, 1982; Dalu *et al.* 2016). Almost all the previous South African studies on marine diatoms focused on the taxonomic identification and classification of the diatom species. A few studies, *e.g.* Bate and Smailes (2008), Gordon *et al.* (2008), Bate *et al.* (2013) and Dalu *et al.* (2016), investigated the relationships between physicochemical variables and diatoms in estuaries.

Diatom research in South Africa has received much more interest in freshwater ecosystems, particularly for water quality monitoring in rivers (De la Rey *et al.* 2004, 2008; Taylor *et al.,* 2005a, 2007a, 2007b; Walsh & Wepener, 2009). However, marine diatoms are very poorly studied in the African continent as a whole, particularly in Southern Africa. Considerably less work has focused on epiphytic diatom assemblages associated with kelps worldwide. In South African coastal waters, marine diatom studies based either on their taxonomy, including floristics, their ecology, biogeography or eco-physiological interactions with their host substrate or environment, *etc.* are lacking, especially for the past couple of decades. Epiphytic diatom communities growing on common South African kelp species, *Laminaria pallida* and *Ecklonia maxima,* have not been documented.

1.6. Aims and structure of the thesis

The aim of the present Master’s thesis was to:

1) characterize the diatom communities associated with the two common South African kelp species, *Ecklonia maxima* and *Laminaria pallida*, in terms of their taxonomic composition and abundances;
2) determine whether and how the host species, age and different parts of the thallus affect the associated diatoms.

This Master's thesis was completed by achieving the following objectives:

1) the identification of diatoms associated with different parts of the thalli of multiple juvenile and adult specimens belonging to two common kelp species using light (LM) and scanning electron microscopy (SEM);

2) the analysis of the diatom community composition and measurements of the diatom biofilm densities through the counting of diatom cells still attached to the original host kelp substratum (using SEM);

3) the application of advanced multivariate statistical analyses to link diatom community characteristics with the biotic factors, such as a) host species, b) age and c) thallus part, which were proposed to serve as affecting the epiphytic diatom communities. All the main effects of the three factors as well as their interactions on the community structure of epiphytic diatoms were investigated simultaneously, which allowed to reliably estimate the relative influence of the factors linked to the host biology on the associated epiphytes.

This thesis is structured in five chapters:

Chapter 1 is an introduction (this Chapter), where the justification and rationale of the research are exposed and made clear.

Chapter 2 deals with the taxonomy of diatoms associated with *E. maxima* and *L. pallida*. The main objective of this Chapter was to provide the first illustrated checklist of the epiphytic diatoms thriving on these two common Laminariales from False Bay, South Africa.

Chapter 3 has been published in (Mayombo *et al.* 2019), and as such is included verbatim in published form, prefixed by a short discource that explains how the paper fit into the greater scheme of the Master's thesis. The subject of this Chapter is to provide an overview and baseline descriptions of epiphytic diatom assemblages associated with the two most common southern African kelp species, *E. maxima* and *L. pallida*, at different growth stages. It is the first report presenting and discussing local epiphytic diatom abundances and structural and compositional differences between communities growing on adult and juvenile specimens.

Chapter 4 was prepared as manuscript to be submitted for consideration for publication in the journal *Diversity*, and as such is also reproduced in paper format. As with Chapter 2, it is also accompanied by a short preamble section that positions the paper into the overall thesis context. The aims and objectives of this Chapter is to examine epiphytic diatom assemblages on different thallus parts of adult and juvenile specimens of *E. maxima* and *L. pallida*.

Chapter 5 concludes this dissertation with a synthesis of all the findings covered in Chapters 2–4, reviewing key results and evaluating how the findings improve understanding of the ecology of South African kelp associated diatom assemblages. It also proposes potential paramenters to investigate in future research.

Chapter 2

Contribution to the floristic study of diatoms associated with South African kelps

2.1. Preamble

I assumed that kelp thallus provides a large substratum on which various diatom taxa can settle and grow. Thefrefore, in order to identify diatom species associated with South African kelps *Ecklonia maxima* and *Laminaria pallida*, four adult and four juvenile specimens of each kelp species were randomly sampled by scuba diving, around Miller's Point in winter (24 July) 2017. The samples were completely digested by boiling in concentrated nitric and sulphuric acids, at a ratio of 2:1 in order to collect all the diatom frustules attached to their surfaces. The resulting cleaned diatom material was used to prepare both permanent slides for light microscopy (LM) observations and specimen stubs for scanning electron microscopy (SEM). Diatom identification was done based on valve morphological observations. Basic morphometric data, such as length of the valve, its width, striation density, shape, striation pattern, and other features visible under LM or SEM were gathered and used for diatom identification. Diatom species listed in this Chapter were identified using taxonomic references that dealt with taxa from various regions, obviously with different environmental conditions, because there is lack of updated diatom flora for Southern Africa. Thus, the correct identification of epiphytic diatom taxa associated with South African kelp specimens is vital for floristic and comparative studies in the region.

2.2. Introduction

Diatom taxonomy is never static because existing taxonomic and nomenclatural concepts are frequently modified to represent a better understanding of their biology (Joynt and Wolfe 1999). Floristically, diatoms (Bacillariophycaea) form a very diverse group of microalgae, consisting of an estimated 200,000 diferrent species (Armbrust 2009). Moreover, the total number of taxa remains elusive since new genera and species are described on a regular basis (Schoefs *et al.* 2019). Despite continuous advances in studies investigating marine diatom biodiversity, many habitats remain poorly explored. For example, little is known about epiphytic diatom assemblages on kelp thalli worldwide. The exploration of various localities and different substrata for diatom analysis are expected to add many new records to floristics everywhere, may be even with the decriptions of new species (Siqueiros-Beltrones and Argumedo-Fernándes 2014). Epiphytic diatoms associated with kelps from Baja California Peninsula, Mexico, were documented (Siqueiros-Beltrones *et al.* 2002; Siqueiros-Beltrones and Argumedo-Hernández 2014b; Siqueros-Beltrones *et al.* 2016). However, considerably less work has focused on kelp-associated diatom community composition in orther regions around the world.

In Sub-Saharan Africa, particularly in South African coastal waters, marine diatom studies based either on their taxonomy, including floristics, their ecology, biogeography or eco-physiological interactions with their host substratum or environment, *etc.* have received much less attention. Although some pioneer studies on the floristics of marine diatoms were conducted in South African coastal waters in the 1960s and 1970s, there is still a lot of work to be done, specifically with the floristics to better understand diatom community structures and compositions on different substrata along our shorelines, their ecology and physiology, as well as the mechanisms that may drive them. Among pioneering studies on diatom floristics along South African coastal waters available in the literature, one can find the work of Boden and Day (1949) who made an interesting contribution to the marine planktonic diatom flora from the west coast of South Africa. There is also several studies by Malcom H. Giffen who dealt with the taxonomy of diatoms of the marine littoral regions of South Africa in a series of investigations at different sites from east to west coasts, specifically the Gulu River complex along salinity gradients (1963), the vicinity of Kidd’s Beach (1966), around Sea Point, near Cape Town (1970), at Steenberg's Cove in St. Helena Bay (1973), at Langebaan, Saldanha Bay (1975), and at Saldanha Bay Lagoon (1976).

Several studies, *e.g.* Bate and Smailes (2008), Gordon *et al.* (2008), Bate *et al.* (2013) and Dalu *et al.* (2016), investigated the relationships between physicochemical variables and diatom assemblages in estuarine ecosystems. However, there is no recent work dealing with epiphytic diatom communities on macroalgal hosts in the marine littoral ecosystems of South

Africa. Among other habitats, diatoms associated with South African kelps have never been documented. Therefore, the main objective of this Chapter was to provide the first illustrated checklist of the epiphytic diatoms thriving on two common South African kelp species *Ecklonia maxima* and *Laminaria pallida* from False Bay.

2.3. Materials and methods

2.3.1. Study site

Kelp specimens used in this study were collected on 24 July 2017 by scuba diving around Miller's Point (34.219833° S, 18.639997° E), within False Bay. False Bay is a 33 km indentation of the coast, 30 km wide at its mouth which lies within approximately 200 km of the coast between Cape Point and Cape Agulhas, making part of the warmest south/west coast transition zone (Bolton 1986). This coastal transition zone supports some large kelp beds, but the understorey and intertidal seaweed flora is intermediate between those of the cool west coast and the warmer south coast (Anderson *et al.* 1997). Temperatures within the bay are high because of solar heating of entrained water. At Miller's Point specifically, where our specimens were collected, the sea bottom was mainly rocky, with patches of sand present between the kelp clusters and some pavements of encrusting coralline red algae covering the hard surfaces. The maximum depth at the site was 10 m and the water temperature recorded *in situ* at the time of collection was 15 °C (with maximal and minimal temperatures recorded during winter 2017 being 15.2 °C and 12.2 °C, respectively).

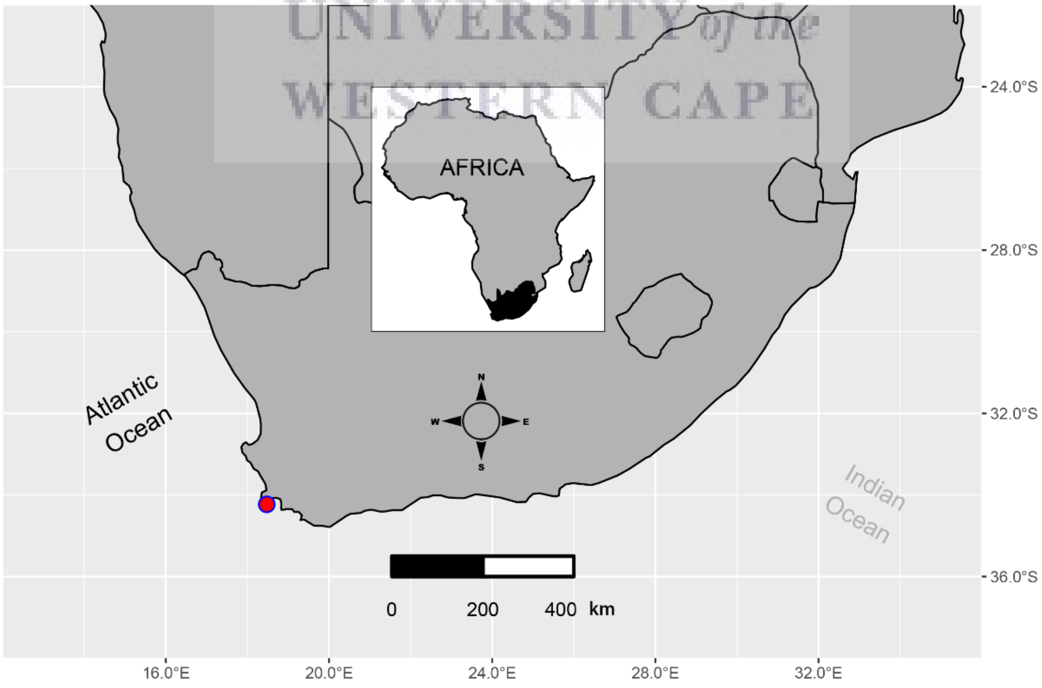

Figure 2.1: **Map of South Africa showing the location of Miller´s Point, False Bay, South Africa (red point).**

2.3.2. Material collection

Four juvenile (<1.5 m long) and four adult (>3 m long) specimens of each kelp species were collected from randomly chosen sites within a sampling area of *ca.* 10m^2. Each thallus was cut in six different parts such as holdfast, lower stipe, upper stipe, primary blade, lower blade, and upper blade as shown in Figure 2. Each part was kept in separate plastic containers and fixed in a 4% formaldehyde solution in filtered seawater immediately after collection. The samples were subsequently processed in the laboratories of the Department of Biodiversity and Conservation Biology of the University of the Western Cape (UWC, Cape Town, South Africa) and the Unit for Environmental Sciences and Management at North-West University (NWU, Potchefstroom, South Africa).

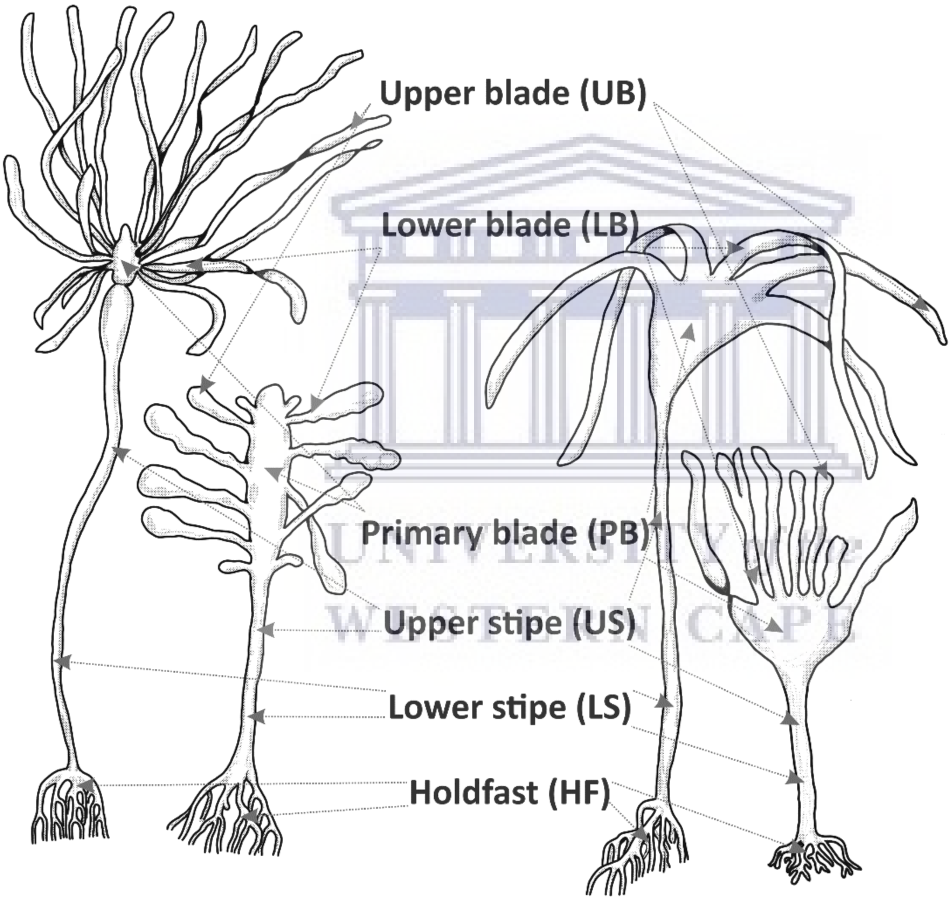

Figure 2.2: Schematic representation of adult and juvenile specimens of *Ecklonia maxima* and *Laminaria pallida* showing the six sampled parts.

2.3.3. Sample processing

For taxonomic diatom identification, two or three sections of about 2 cm^2 of the kelp subsamples were digested by cooking in a concentrated nitric and sulphuric acids solution at ratio of 2:1. The resulting solutions were repetitively centrifuged and rinsed with distilled water until an approximately neutral pH was reached. The cleaned diatom material was used to prepare both permanent slides for light microscopy (LM) and specimen stubs for scanning electron microscopy (SEM) observations. The permanent slides and stubs are stored at the Department of Biodiversity and Conservation Biology, UWC. All diatom frustules were identified up to the highest possible taxonomic level, following the available literature (*e.g.* Giffen 1963, 1970, 1973, 1975, 1976; Medlin and Round 1986; Al-Handal and Wulff 2008a, 2008b; Navarro and Lobban 2009; Witkowski *et al.* 2011; Lobban *et al.* 2012; Al-Handal *et al.* 2016; Siqueiros-Beltrones *et al.* 2002, 2016; Siqueiros-Beltrones and Argumedo-Hernández 2014a, 2014b; Park *et al.* 2018).

2.4. Results

2.4.1. Microscopical observations of cleaned materials

Up to 48 diatom taxa belonging to 28 genera were identified by examining the clean materials of South African kelp specimens. Most of these taxa were rare on different parts of the kelp macrothalli. The most abundant and frequently occurring diatom species in the subsamples included *Gomphoseptatum pseudoseptatum* A. Witkowski, H. Lange-Bertalot & D. Metzeltin and taxa belonging to the *Cocconeis* genus, particularly *Cocconeis costata* Gregory and *Cocconeis scutellum* Ehrenberg, which sometimes formed large single species colonies. *Cocconeis* frustules accounted for more than 50% of the total number of diatom observed in the analysed cleaned subsamples. Other relatively frequent taxa included *Nagumoea* sp., *Navicula* spp., *Rhoicosphenia genuflexa* (Kützing) Medlin and *R. flexa* Giffen.

2.4.2. Floristic list of kelp associated diatom taxa

***Actinoptychus* cf. *aster* J. J. Brun (Plate 4, Figure 41)**

Reference: Siqueiros-Beltrones *et al.* 2017, p. 23, Fig. 2A.

***Asteromphalus* sp. Ehrenberg (Plate 4, Figure 38)**

***Achnanthes* sp. (cf. *A. brevipes* var. *intermedia* (Kützing) Cleve) (Plate 1, Figure 9)**

Reference: Al-Handal and Wulff (2008a), p. 423, Fig. 109.

***Amphora bigibba* Grunow (Plate 4, Figure 37)**

References: Siqueiros-Beltrones and Yuriko (2017), p. 46, Fig. 57; Giffen (1973), p. 33, Fig. 6.

***Amphora helenensis* Giffen (Plate 4, Figures 36, 39)**

Reference: Giffen (1973), p. 33, pl. 1, Figs. 7–9.

***Amphora proteoides* Hustedt (Plate 4, Figure 35)**

Reference: Witon and Witkowsi (2006), p. 210, Figs 191, 192.

***Campyloneis grevillea* var. *grevillei* (W. Smith) Grunow (Plate 4, Figures 44)**

References: De Stefano *et al.* 2003, p. 2, Fig. 1.

***Cocconeis californica* Grunow (Plate 1, Figures 2)**

Reference: Siqueiros-Beltrones and Argumedo-Hernández (2014a), p. 21, Figs. 59, 63.

***Cocconeis costata* Gregory var. *costata* (Plate 5, Figures 57)**

Reference: Romero and Rivera (1996), p. 321, Figs 2–16, Table 2.

***Cocconeis costata* Gregory var. *hexagona* Grunow in van Heurck (plate 5, Figures 61)**

Reference: Romero and Rivera (1996), p. 327, Figs 17–34, Table 2.

***Cocconeis pseudomarginata* Gregory (Plate 2, Figures 22–23)**

References: Giffen 1970, p. 90, Fig. 12; Romero and Navarro 1999, p. 2, Figs. 1–6, 13–15, 20–31. Riaux-Gobin et al. (2014), p. 17, Figs. 33–34.

Description: Apical axis, 36.5 µm; transapical axis, 28.5 µm.

***Cocconeis scutellum* Ehrenberg (Plate 1, Figures 3)**

References: Gordon *et al.* (2008), Pl. 1, Fig. d; Al-Handal *et al.* (2016), p. 22, Pl. 7, Fig. 6; Lobban *et al.* (2012), p. 288, Pl. 41, Figs. 6, 7.

Description: Apical axis, 10–22 µm; transapical axis, 5–14 µm; striae, 14–22 in 10 µm.

***Cocconeis* sp. (Plate 4, Figure 33)**

***Craspedostauros* sp. E. J. Cox (Plate 5, Figure 45, 46)**

References: Cox (1999), Figs. 11–23 (LM), 24–38 & 40-53 (SEM); Ashworth *et al.* (2017), Figs. 2 (LM) & 3–5 (SEM), Table 2.

Description: Apical axis, 87.5–108 µm; striae, 14–16 in 10 µm.

***Craspedostauros* sp.**

***Delphineis* sp. (Plate 4, Figure 43)**

***Diploneis crabro* (Ehrenberg) Ehrenberg (Plate 1, Figure 8)**

References: Park *et al.* (2018), p. 116, Fig. 64; Lobban *et al.* (2012), p. 290, pl. 44, Fig. 5, pl. 45, Figs. 1, 2. López-Fuerte *et al.* (2010), pl. 19, Figs. 1–4, pl. 20, Fig. 1.

***Diploneis papula* (A. Schmidt) Cleve (Plate 2, Figure 17)**

References: Pennesi *et al.* 2017, p. 205, Figs. 24–31.

***Diploneis littoralis* (Donkin) Cleve (Plate 2, Figures 16, 21)**

References: Pennesi *et al.* 2017, p. 205, Figs. 17–23.

***Fragilariopsis* sp. (Plate 3, Figure 29)**

***Gomphoseptatum pseudoseptatum* (Giffen) A. Witkowski, H. Lange-Bertalot & D. Metzeltin (Plate 5, Figures 53–56)**

References: Witkowski *et al.* (2000), p. 222, pl. 60, Figs. 22–26.

Description: Apical axis, 7–26.5 µm; transapical axis, 1.5–3 µm; straie, 18–25 in 10 µm.

***Gomphoseptatum* sp.**

***Grammatophora angulosa* Ehenberg (Plate 3, Figure 26)**

Reference: Lobban *et al.* (2012), p. 262, pl. 19, Figs. 1, 2.

***Grammatophora angulosa* var. *islandica* (Ehrenberg) Grunow (Plate 3, Figure 31, 32)**

Reference: Witon and Witkowski (2006), p. 205, Figs. 96, 97, 100.

***Grammatophora marina* (Lyngbye) Kützing (Plate 3, Figure 28)**

References: Witon and Witkowski (2006), p. 205, Figs. 103, 104.

***Grammatophora oceanica* Ehrenberg (Plate 3, Figure 27)**

References: Siqueiros-Beltrones and Agumedo-Hernández (2015), p. 118, Figs. 29, 34, 38.

***Halamphora luciae* (Cholnoky) Levkov (Plate 4, Figure 40)**

Reference: Daglio *et al.* (2016), Figs. 1–30.

***Hantschia marina* (Donk.) Grunow (Plate 5, Figure 52)**

Reference: Giffen (1963), p. 233, pl. IV, Fig. 58.

***Licmophora* sp. (Plate 3, Figure 34)**

***Mastogloia manokwariensis* Cholnoky (Plate 5, Figure 47)**

References: Lobban *et al.* (2012), p. 277, pl. 32, Figs. 1–3; Park *et al.* (2018), p. 115, Figs. 60a, b.

***Nagumoea africana* Kociolek, Witkowski & C. Archibald (Plate 5, Figure 62, 63)**

References: Witkowski *et al.* (2011), p. 172, Figs. 40–57, 67–86 (LM) & 58–66, 87–94 (SEM); Van De Vijver and Kociolek (2018), Figs. 1–9.

***Nagumoea* sp.**

***Navicula* cf. *johanrossii* Giffen (Plate 2, Figure 12, 18)**

References: Giffen (1966), p. 268, Plate 3, Figs. 63–64; Siqueiros-Beltrones and Argumedo-Hernández (2015), Fig. 105.

***Navicula ramosissima* (C. Agardh) Cleve (Plate 2, Figure 5, 19)**

References: Gordon *et al.* 2008, p. 72, pl. 1, Fig. e; Cox 1999, p. 212, Fig. 8.

***Nitzschia* sp. 1 (Plate 5, Figure 49)**

***Nitzschia* sp. 2 (Plate 5, Figure 50)**

***Nitzschia* sp. 3 (Plate 5, Figure 51)**

***Opephora pacifica* (Grunow) Petit (Plate 5, Figure 58)**

Reference: Giffen (1973), p. 43, Figs. 66, 67.

***Parlibellus* sp.**

***Pinnularia* cf. *rectangulata* (W. Gregory) Rabenhorst (Plate 1, Figure 6)**

Reference: Giffen (1976), p. 387, Figs. 56–57; Siqueiros-Beltrones *et al.* (2017), p. 31, Table 1, Figs. 5 (G–H).

***Planothidium* sp. (Plate 5, Figure 48)**

***Pleurosigma intermedium* W. Smith (Plate 1, Figure 7)**

Reference: Sar *et al.* (2009), Figs. 1–12, Table 1.

***Pleurosigma marinum* Donkin (Plate 1, Figure 4)**

References: Giffen (1966), pl. 5, Fig. 111.

***Pseudogomphonema kamtschaticum* (Grunow) Medlin (Plate 2, Figures 20)**

References: Medlin and Round (1986), p. 216, Figs. 23–30 (LM), 64–70 (SEM); Al-Handal and Wulff (2008a), p. 423, Figs. 95–100.

Description: Apical axis, 21.5–43.6 µm; transapical axis, 4–6 µm; straie, 16 in 10 µm.

***Rhoicosphenia flexa* Giffen (Plate 2, Figure 13)**

References: Giffen 1970, p.96, p. 96, Figs. 2: 55–58; Levkov *et al.* (2010), p. 157, Figs. 18a–w, 19a–f, 20a–g.

***Rhoicosphenia genuflexa* (Kützing) Medlin (Plate 2, Figure 14, 15)**

References: Medlin and Fryxell 1984, p. 257, Figs. 1–5; Levkov *et al.* 2010, p. 158, Figs. 21a–ab.

***Tabularia investiens* (W. Smith) D. M. Williams & Round (Plate 2, Figure 11)**

References: Siqueiros-Beltrones and Yuriko (2017), Fig. 22.

***Trachyneis aspera* (Ehrenberg) Cleve (Plate 3, Figures 24, 25 and 30)**

References: Giffen (1975), p. 94; Park *et al.* (2018), p. 121, Fig. 85; Lobban *et al.* (2012), p. 293, pl. 48, Figs. 4–9; Al-Handal *et al.* (2016), p. 30, pl. 8, Fig. 15.

2.5. Discussion

Overall 48 diatom taxa belonging to 28 genera were identified by analysing the cleaned materials of South African kelp specimens. The most abundant and frequently occurring diatom species in the samples included *Gomphoseptatum pseudoseptatum* and taxa belonging to the *Cocconeis* genus, such as *Cocconeis costata* and *Cocconeis scutellum*, which sometimes formed large single species colonies. *Cocconeis* cells accounted for more that 50% of the total diatom frustules observed in the analysed samples. Other relatively abundant taxa found on the examined kelp specimens included *Nagumoea* sp., *Navicula* spp., *Rhoicosphenia genuflexa* and *R. flexa*. Siqueiros-Beltrones *et al.* (2002) observed a high dominance of *Cocconeis cf. britannica* on the kelp *Macrocystis pyrifera* blades, which covered large areas of the blades and apparently served as substratum for the other diatom species, specifically *Cocconeis speciosa*, *Gomphonemopsis* arborea *pseudoexigua*, and *Climacosphenia moniligera*.

The kelp *Eisinia arborea* Areschoug was initially reported to be completely devoid of epiphytic diatoms (Siqueiros-Beltrones *et al.* 2002). However, a later examination of the blade of *E. arborea* specimens revealed a quasi-monospecific proliferation of the diatom *Pteroncola inane* (Giffen) Round (Fragilariales, Bacillariophyceae) (Siqueiros-beltrones and Argumedo-Hernández 2014b). More recently, Siqueiros-Beltrones *et al.* (2016) identified 99 diatom taxa associated with the kelp *E. arborea*, with unusually lower values of diversity and an extremely higher dominance of only one diatom species, *P. inane*. The results of the present study suggest that kelps are not favourable substrata for diatom biofilm settlement and development. The dominance of just few epiphytic diatom taxa while all others remain rare as observed in this study, confirm that kelps are relatively unstable substrata with efficient antifouling mechanisms that prevent the development of diatom biofilm into climax stage. This is a proof that diatom communities on kelp surfaces were still at their early successional stage. However, the apparently highly successful antifouling strategies employed by kelp species against epiphytes in general may operate selectively in favour of some epiphytic species that have evolved the means to elude their effects. This may justify the dominance of just few diatom taxa, such as those belonging to *Cocconeis* genus, observed in the examined kelp specimens. Further investigations are required in order to better understand the stages of successional development of epiphytic diatoms on kelp macrothallus and other factors, *e.g.* abiotic, that may drive them.

2.6. Conclusion

This Chapter is the first floristic report of the epiphytic diatom assemblages associated with two South African kelp species, *Ecklonia maxima* and *Laminaria pallida*. Marine diatoms are very poorly studied in Southern Africa. Thus, the set objective in this Chapter was to identify all the diatom taxa occurring on these macroalgae samples collected in False Bay, South Africa. The main aim was primarily to document the epiphytic diatoms growing attached on the two common South African kelps, which are believed to harbour a variety of epiphytic macro- and microalgae. Overall 48 taxa of epiphytic diatoms belonging to 28 genera were identified. The most abundant and frequently occurring diatom taxa included *Gomphoseptatum pseudoseptatum*, *Cocconeis costata*, *Cocconeis scutellum*, *Nagumoea* sp., *Rhoicosphenia genuflexa*, *Rhoicosphenia flexa* and *Navicula* spp. These baseline floristic data may serve to investigate the different biotic, as well as abiotic, factors that drive the community composition and structures of kelp associated diatom assemblages off the west coast of South Africa in future studies.

Chapter 3

Diatoms associated with two South African kelp species: *Ecklonia maxima* and *Laminaria pallida*

3.1. Preamble

After I completed the taxonomic study of kelp-associated diatom assemblages using a light microscope, I decided to estimate their abundance per unit area of the host macroalgal blades under SEM and to assess the influence of biotic factors, *e.g.* host species and age, on associated epiphytic microalgal communities. To avoid possible biases due to differences in substratum morphology, only primary blade (the primary axis and growth zone) subsamples were analysed in this Chapter. Each subsample was prepared in three replicates, making up a total of 48 (16 × 3) analysed kelp specimens for estimating the epiphytic diatom abundance and assessing the host species and age effects. The subsamples were prepared by progressive dehydration in ethoanol solutions at increasing concentrations, and then treated by critical point drying. Finally, subsamples (~1cm^2 each) were mounted on specimen aluminum stubs using adhesive carbon tapes and sputter-coated with gold palladium. Diatom observations and counting were performed directly on the surface of the host macroalgae, using a SEM. The identification of the attached frustules was done up to genus level, because most of the cells were laying on girdle view on the SEM, making the determination of species names very challenging. Epiphytic diatom density was estimated over a surface area of ~1 mm^2. Advanced multivariate statistical analyses were performed to relate the epiphytic diatom assemblages to the influence of the investigated biotic factors. The aim of this published Chapter was to present the first report of epiphytic diatom abundances on adult and juvenile specimens of *Ecklonia maxima* and *Laminaria pallida*, two common and commercially valuable South African kelp species, discussing structural and compositional differences between communities based on host species and age effects.

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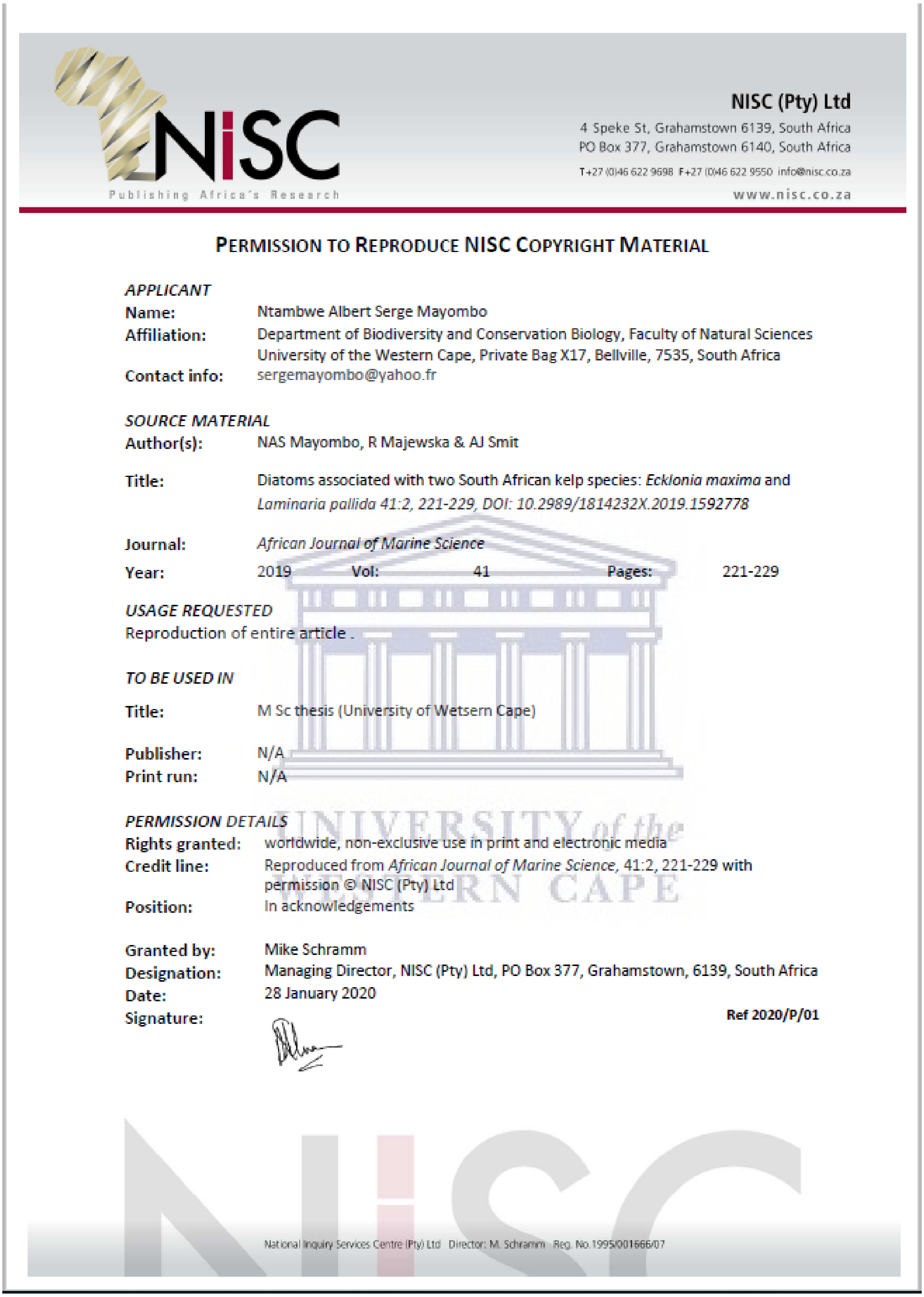

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Short communication

Diatoms associated with two South African kelp species: *Ecklonia maxima* and *Laminaria pallida*

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Kelp forests are believed to host a large biomass of epiphytic fauna and flora, including diatoms, which constitute the base of aquatic food webs and play an important role in the transfer of energy to higher trophic levels. Epiphytic diatom assemblages associated with two common species of South African kelps, *Ecklonia maxima* and *Laminaria pallida*, were investigated in this study. Primary blades of adult and juvenile thalli of both kelp species were sampled at False Bay in July 2017 and analysed using scanning electron microscopy. Our findings showed that both kelp species hosted relatively low densities of diatoms (ranging from 7 [SD 5] cells mm^{-2} on adult specimens of *L. pallida* to 43 [SD 66] cells mm^{-2} on blades of juvenile *E. maxima*), with *Amphora* and *Gomphoseptatum* reaching the highest absolute abundances. Although non-metric multidimensional scaling showed overlapping and largely scattered sample sets, a significant relationship between the diatom communities and the species and age of the host macroalga was detected by two-way PERMANOVA. In general, more abundant and diverse diatom communities were observed on juvenile thalli than on adult thalli, with species belonging to *Navicula* and *Rhoicosphenia* contributing significantly to the observed dissimilarity. Due to a significant interaction between species and age effects, however, the overall ability of kelp species, their age, and their interaction to explain the variation in diatom community structure was limited. We suggest that the low densities of epiphytic diatoms were directly related to the sloughing of epithelial cells observed in both kelp species. We further speculate that on such unstable substrata some diatom taxa might adapt to an endophytic life to avoid the antifouling mechanisms developed by their hosts.

Keywords: Bacillariophyceae, diatom abundance, endophytic diatoms, epiphytes, False Bay, macroalgae, Phaeophyceae, seaweeds

Introduction

Kelps, the large brown macroalgae (Phaeophyceae, Laminariales), colonise almost all temperate to polar rocky shorelines throughout the world (Arnold et al. 2016; Krumhansl et al. 2016) and constitute highly complex, dynamic and multifunctional coastal benthic ecosystems (Leclerc et al. 2016). Kelp forests provide distinct macro- and micro-habitats for numerous associated biota, which make them one of the most-productive marine environments (Steneck et al. 2002). In southern Africa, kelp forests occur from Cape Agulhas (South Africa) to Lüderitz (Namibia), within the rocky shorelines influenced by the cold waters of the Benguela upwelling system (Velimirov et al. 1977; Allen and Griffiths 1981; Bustamante and Branch 1996). *Ecklonia maxima* (Osbeck) Papenfuss and *Laminaria pallida* Greville ex. J. Agardh are the dominant kelp species in this region, with *Macrocystis angustifolia* Bory sometimes present in sheltered bays. The dynamics, composition, distribution patterns and standing crops of various macroalgal and animal communities living within South African kelp beds have been extensively investigated and are relatively well documented (Velimirov et al. 1977; Bustamante et al. 1995; Browne et al. 2013). However, studies investigating epiphytic diatom assemblages associated with local seaweeds are sparse, with most being purely floristic surveys dating back to the 1970s (Giffen 1971, 1973, 1976). In many marine ecosystems, epiphytic diatoms, rather than the basiphyte with which they are associated, constitute the basis for much of the primary

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consumer productivity (Daume et al. 1997; Kasim and Mukai 2006), and in order to establish the trophic importance of diatoms in kelp ecosystems, an assessment of the diversity, abundance and distribution of these microalgae is essential.

Diatoms thrive in all aquatic, semi-aquatic, and even moderately humid or semi-arid ecosystems throughout the world, flourishing on muddy and sandy sediments, rocks, aquatic plants and animals, and all non-toxic immersed artificial objects (Round et al. 1990; Tiffany 2011), and they are known to be sensitive and reliable bioindicators of various environmental conditions (Smol and Stoermer 2010). Species composition of sessile epiphytic diatom communities is a direct result of the particular combination of long-term environmental factors prevailing in their ecosystems. Therefore, it is likely that they could be used effectively as proxies for reconstructing past climatic conditions (Kirsten et al. 2018), and for monitoring present multidimensional changes recently detected in South African coastal waters (Schlegel et al. 2017a, 2017b).

Several studies have investigated epiphytic diatom assemblages on macroalgae in different regions across the globe (Takano 1962; Snoeijs 1994; Chung and Lee 2008; Totti et al. 2009; Majewska et al. 2013a, 2013b, 2015, 2016; Costa et al. 2014, 2016). Yet, similar reports that focus on kelp-associated diatoms are generally very scarce, descriptive, and geographically limited (SiqueirosBeltrones et al. 2002, 2014, 2016). This article aims to provide an overview and baseline descriptions of epiphytic diatom assemblages associated with the two most common southern African kelp species, *E. maxima* and *L. pallida*, at different growth stages. It is the first report presenting and discussing local epiphytic diatom abundances and the structural and compositional differences between communities growing on adult and juvenile specimens.

Materials and methods

Sample collection

Kelp thalli used in this study were collected by SCUBA diving in False Bay, South Africa, east of the Cape Peninsula, around Miller's Point (34.219833° S, 18.639997° E), on 24 July 2017. The sea bottom was mainly rocky, with patches of sand present between the kelp clusters and encrusting coralline algae covering the hard surfaces. The maximum depth at the site was 10 m and the water temperature recorded *in situ* at the time of collection was 15 °C (with the maximal and minimal temperatures recorded during winter 2017 being 15.2 °C and 12.2 °C, respectively). Four juvenile (<1.5 m long) and four adult (>3 m long) specimens of each kelp species were collected. Immediately after collection, each thallus was cut, fixed in a 4% formalin solution made up with filtered seawater, and subsequently processed in the laboratories of the Department of Biodiversity and Conservation Biology at the University of the Western Cape (UWC, Cape Town, South Africa) and the Unit for Environmental Sciences and Management at North-West University (NWU, Potchefstroom, South Africa). To avoid possible biases due to differences in substrate morphology, only primary blades (the primary axis and growth zone) were chosen for further analyses.

Three pieces of approximately 1 cm^2 of each specimen were dehydrated by immersion in ethanol solutions at increasing concentrations (30%, 50%, 60%, 70%, 80%, 90%, 95%, and absolute ethanol), followed by critical-point drying (HCP-2 critical point dryer, Hitachi, Japan), before being mounted on 13-mm aluminum stubs with adhesive carbon tape, and sputter-coated with gold/palladium (Au/Pd) using a Q150T ES (Quorum Technologies Ltd, UK) sputter coater. Diatom observation and counting were carried out at high magnification using an Auriga field emission scanning electron microscope and a Leo 1450 scanning electron microscope (Carl Zeiss Group, Germany). Diatom cells, still attached to the dehydrated kelp thalli, were counted over a surface area of at least 1 mm^2 on each of the three replicates prepared.

For taxonomic identification of diatoms, two or three sections of about 2 cm^2 of the analysed thalli were digested with boiling concentrated nitric and sulphuric acids, at a ratio of 2:1. The resulting solutions were repetitively centrifuged and rinsed with distilled water until an approximately neutral pH was reached. The cleaned diatom material was used to prepare both permanent slides for light microscopy observations and specimen stubs for scanning electron microscopy. The slides are stored at the Department of Biodiversity and Conservation Biology, UWC. All diatom frustules were identified to at least genus level, following the available literature (e.g. Giffen 1971, 1973, 1976; Medlin and Round 1986; Witkowski et al. 2011; Lobban et al. 2012; Al-Handal et al. 2016).

Data analysis

All statistical analyses were performed using R 3.5.1 (R Core Team 2018), the 'vegan' package (version 2.5-3; Oksanen et al. 2018), and the 'mvabund' package (version 3.13.1; Wang et al. 2018). Species-abundance data were subjected to a logarithmic standardisation ($\log_2 x + 1$ for $x > 0$, where x is the number of cells per 1 mm^2), which gives less weight to taxa present at a greater abundance relative to those that are less abundant (Anderson et al. 2006). The multivariate homogeneity of group dispersions (variances) was assessed using the 'vegan' 'betadisper' function, prior to undertaking a permutational multivariate ANOVA (PERMANOVA) using the 'vegan' 'adonis2' function to test for significant differences between diatom assemblages associated with different kelp species and age groups (999 permutations for both 'betadisper' and 'adonis2'). The 'manyglm' function was used (Warton et al. 2012; Benesh and Kalbe 2016) to examine the question of which of the diatom taxa were responsible for differences in the main effects, and this was achieved by examining species one at a time (i.e. multiple univariate general linear models [GLMs]) and adjusting the *p*-values for multiple comparisons in order to minimise the chance of committing Type I errors (Wang et al. 2012). This latter approach was used instead of the well-known SIMPER analysis, which is unable to detect taxa that differ among groups because of ambiguities that stem from betweengroup (differences in mean) and within-group (dispersion) effects (Warton et al. 2012). Non-metric multidimensional scaling (nMDS) was then applied using the Bray–Curtis similarity index, with 999 permutations to visualise the group differences.

Results

The SEM images of both seaweed species revealed highly homogeneous surfaces harbouring sparse diatom assemblages (Figure 1). The average diatom cell densities on *Laminaria pallida* were 7 (SD 5) cells mm^{-2} and 38

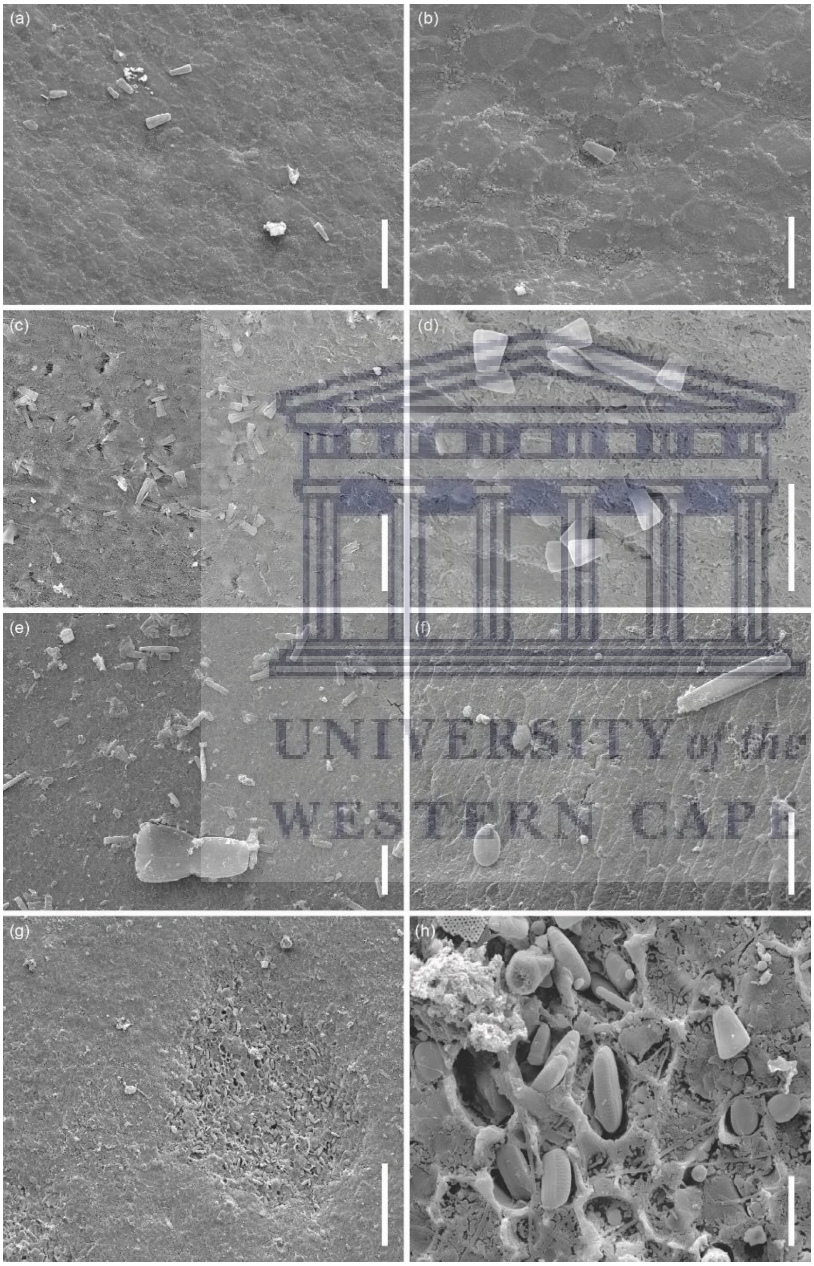

Figure 1: Epiphytic diatoms associated with *Laminaria pallida* (a–d) and *Ecklonia maxima* (e–h) collected in False Bay, South Africa: (a, b) *Gomphoseptatum* sp. on the surface of an adult thallus of *L. pallida*; (c) diatoms growing on juvenile *L. pallida*; (d) *Gomphoseptatum* sp. growing on juvenile *L. pallida*; (e, f) diatoms growing on adult *E. maxima*; (g, h) diatoms dwelling in tissue of juvenile *E. maxima*. Scale bars = 10 µm (h); 20 µm (b, d, f); 40 µm (e); 50 µm (a, c); 100 µm (g)

(SD 77) cells mm^{-2} on adult and juvenile specimens, respectively. On *Ecklonia maxima*, average absolute diatom abundances amounted to 21 (SD 15) cells mm^{-2} and 43 (SD 66) cells mm^{-2} on adult and juvenile blades, respectively (Figure 2). Six subsamples, including five replicates of *L. pallida* and one of *E. maxima*, contained no epiphytic diatoms and were excluded from further analyses. In total, 18 diatom genera were found associated with the two host kelp species.

Differences in homogeneity of group dispersions were not significant for either the host species or age effects (df = 1, SS = 0.059, F = 2.609, p = 0.114 and df = 1, SS = 0.001, F = 0.060, p = 0.807, respectively) (Figure 3a, b). The nMDS graphs showed overlapping and largely scattered sample sets (Figure 3d, e). The analysis indicated a strong correlation between the observed dissimilarity and the ordination distance (R^2 = 0.96), with a low stress of 0.19 (Figure 3c). Given the PERMANOVA showing significant interaction between the species and age effects (df = 1, SS = 0.573, F = 4.544, R^2 = 0.073, p = 0.001), a large between-plant variation interacting with kelp species and age (df = 12, SS = 3.317, F = 2.193, R^2 = 0.423, p = 0.001), and the high residual variance (df = 26, SS = 3.277, R^2 = 0.418), we find weak support for asserting that the host-kelp species or age affect diatom community structure even though the main effects were both significant (host species: df = 1, SS = 0.299, F = 2.373, R^2 = 0.038, p = 0.029; age: df = 1, SS = 0.373, F = 2.957, R^2 = 0.0475, p = 0.005).

Although overall diatom community structure did not differ between kelp species and age, the 'manyglm' function revealed that the abundances of seven diatom genera (*Rhoicosphenia*, *Navicula*, *Grammatophora*, *Nitzschia*, *Amphora*, *Cocconeis* and *Nagumoea*) were responsible for ~75% of the limited variance observed between the adult and juvenile thalli, with the general trend of higher diatom abundances for the juveniles. The taxa whose abundances differed significantly between adult and juvenile kelps were *Navicula* spp. (Wald = 3.66, p = 0.017) and *Rhoicosphenia* spp. (Wald = 4.06, p = 0.004) (Figure 4), and in both instances their number was greatest on the juvenile kelp thalli.

Discussion

The observed epiphytic diatom abundances, ranging from 7 (SD 5) cells mm^{-2} to 43 (SD 66) cells mm^{-2}, were generally low, although comparable to those recorded for other seaweed species growing in various parts of the world (Snoeijs 1994; Totti et al. 2009; Majewska et al. 2013a, 2013b, 2015, 2016; Majewska and De Stefano 2015; Siqueiros-Beltrones et al. 2002, 2016; Costa et al. 2014, 2016). Totti et al. (2009) found from 7 (SD 5) to 7 524 (SD 3 491) diatom cells mm^{-2} on 10 species of brown, red and green macroalgae from coastal waters of Iceland, with the lowest and the highest values observed on two Ochrophyta species, *Laminaria saccharina* (Linnaeus) J.V. Lamouroux and *Fucus vesiculosus* Linnaeus, respectively. Majewska et al. (2013a, 2016) recorded a mean total diatom cell density ranging from 21 (SD 13) cells mm^{-2} to >8 000 cells mm^{-2} on three red algae (Rhodophyta) species from the Ross Sea (Antarctica), and from 2 951 (SD 78) cells mm^{-2} to 10 919 (SD 2 260) cells mm^{-2} on *Plocamium cartilagineum* (Linnaeus) P.S. Dixon (Rhodophyta) from Admiralty Bay (King George Island, Antarctica; Majewska et al. 2015). Thomas and Jiang (1986), who analysed 15 species of brown, red and green macroalgae from another Antarctic region (Prydz Bay), reported that ubiquitous *Cocconeis* spp. reached densities approaching 105 cells cm^{-2} (i.e. ~1 000 cells mm^{-2}), whereas *Nitzschia* spp. were twice as abundant.

Similarly, the number of diatom genera (18) found growing epiphytically on the examined blades of *Ecklonia maxima* and *Laminaria pallida* was relatively low, suggesting low diatom diversity. Totti et al. (2009) found 19 diatom genera in seaweed samples from Reykjanes Peninsula (Iceland), whereas Majewska et al. (2016) observed 44 diatom genera associated with rhodophytes from the Ross Sea and 21 associated with *P. cartilagineum* from Admiralty Bay. Al-Handal and Wulff (2008) recorded 29 diatom genera in samples of 19 seaweed species from Potter Cove (Antarctica), with 11 genera present in samples of seven Phaeophyceae species. Furthermore, 29 diatom genera were found in an analysis of three seaweed species from northeastern Brazil (Costa et al. 2016).

It was suggested (Majewska et al. 2013a, 2013b, 2016; Costa et al. 2016) that macroalgal thalli with highly homogeneous surfaces (micro-topography) and uniform morphology, as in both *E. maxima* and *L. pallida*, would host less-diverse epiphytic diatom communities due to high competition among epiphytic organisms with overlapping niches. This may partly explain the low numbers of diatom taxa found in the present study. Nevertheless, it does not explain the very low diatom abundances.

Seaweeds, including brown macroalgae, defend themselves against both biofouling and grazers using a range of strategies (Hellio et al. 2001; Yamamoto et al. 2013). These may involve secretion of chemically active metabolites, which act as antifouling or repelling agents (Kubanek et al. 2003; Wikström and Pavia 2004), or shedding of the outer layer of the thallus (epithallus) (Moss 1982; Yamamoto et al. 2013; Halat et al. 2015). The SEM observations confirmed that surface-cell sloughing occurs in both kelp species examined and appears to be a particularly efficient antifouling mechanism. We speculate that this mechanical defence against surface colonisation is one of the major factors that affect epiphytic diatom communities, preventing their development into the climax stage, which would explain the observed low similarity levels within the examined groups of samples (classified according to the host seaweed species and age) and would obscure the patterns and differences in diatom composition detected between the groups.

However, despite the apparently highly successful antifouling strategies employed by both kelp species, we found indications of endophytic diatom growth in areas of presumably damaged or growing surface tissue and natural superficial breaks (Russell and Veltkamp 1984). This phenomenon was described by Klochkova et al. (2014), who found an unknown endophytic species of *Pseudogomphonema* (Naviculaceae, Bacillariophyceae),

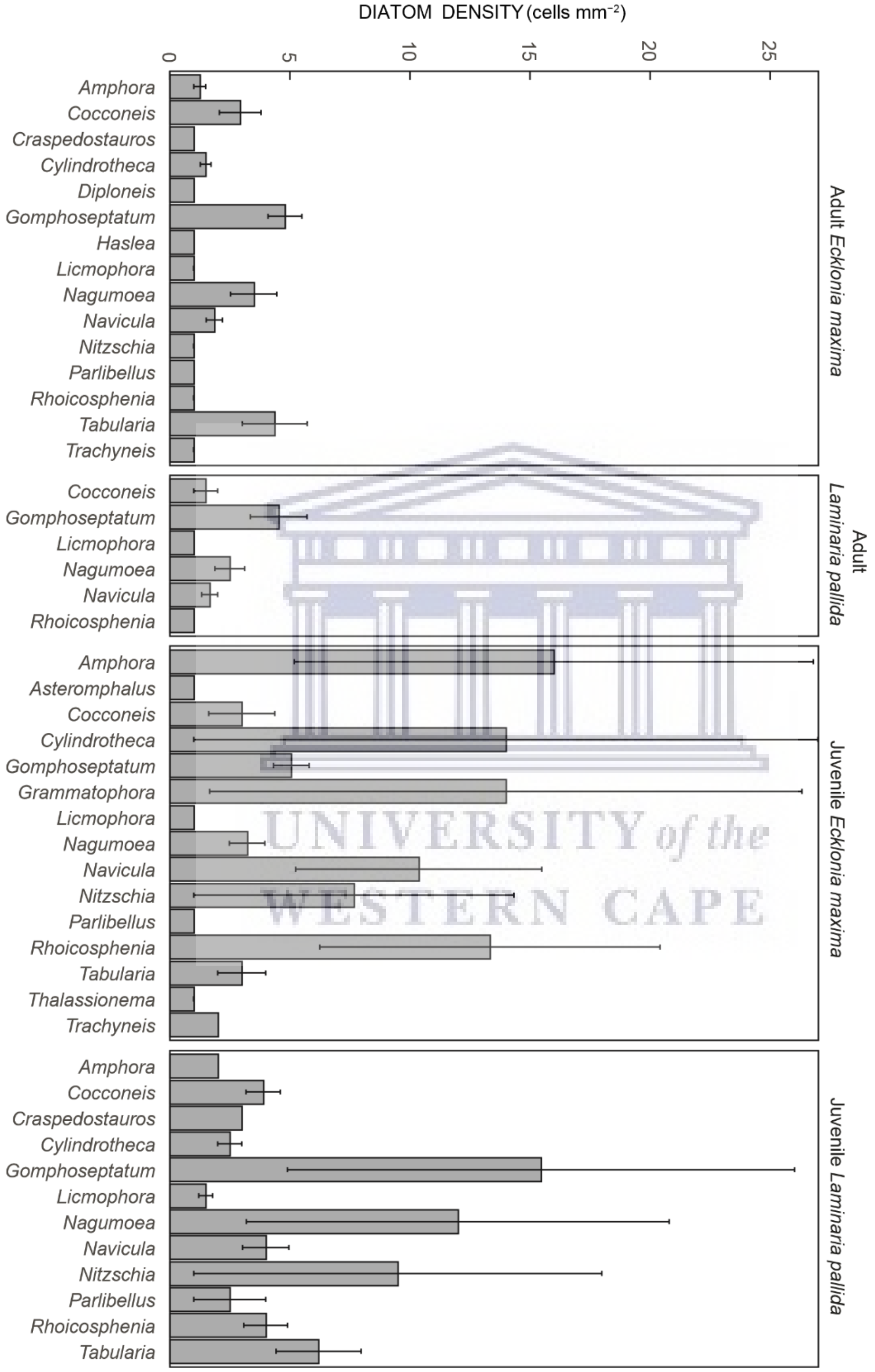

Figure 2: Mean abundances of diatom taxa on adult and juvenile thalli of *Ecklonia maxima* and *Laminaria pallida* sampled in False Bay, South Africa, July 2017. Error bars represent standard error (SE)

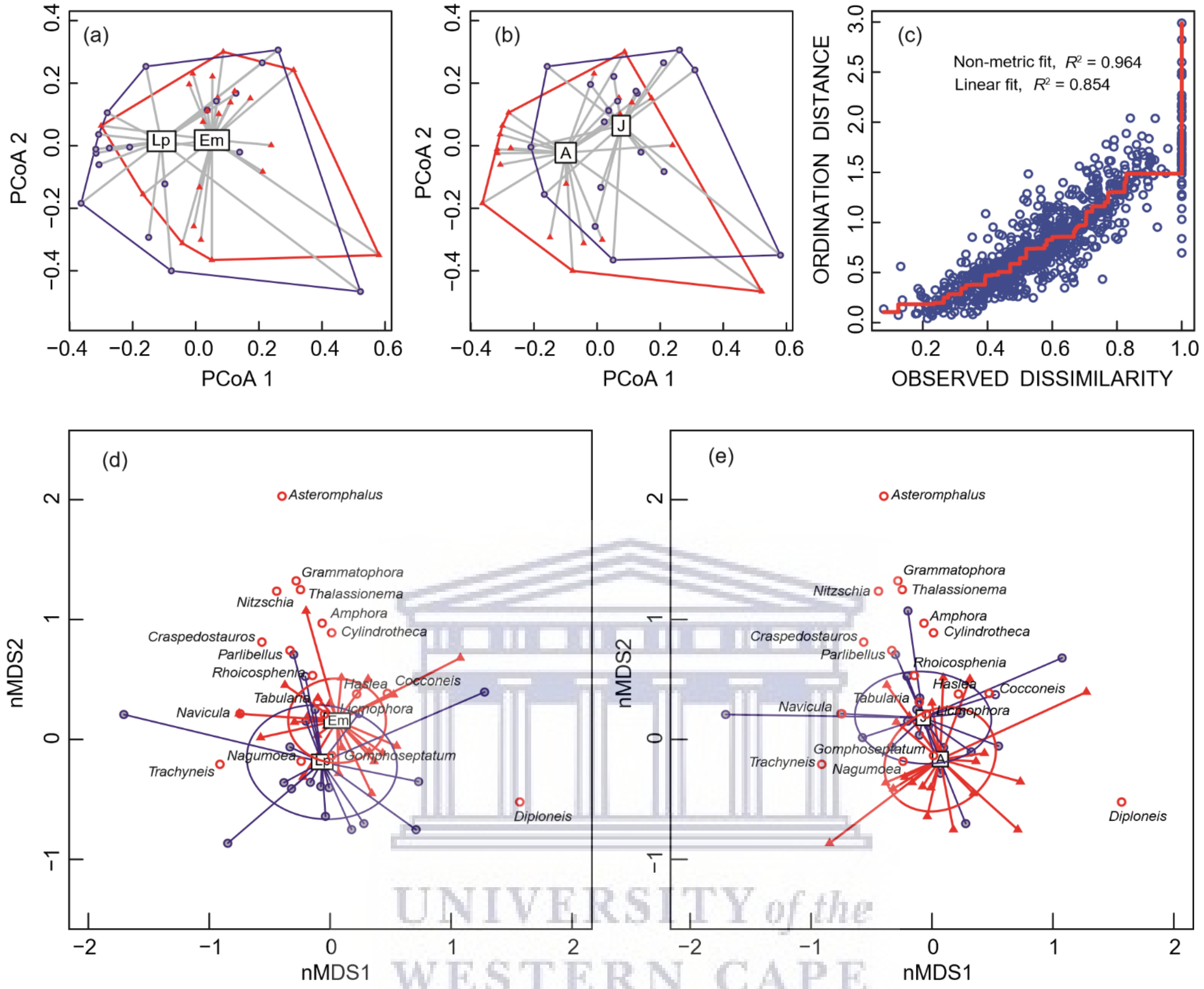

Figure 3: Unconstrained ordination diagrams. Plots produced by principal coordinates analysis (PCoA), showing the diatom assemblages on the two host species of kelp (a) and by host age (b). (c) Shepard plot of the non-metric multidimensional scaling (nMDS) results. nMDS graphs based on diatom taxa abundances, showing the relationship between the diatom communities growing on both host species (d) and by host age (e). A = adult; J = juvenile; Em = *Ecklonia maxima*; Lp = *Laminaria pallida*

which was reproducing within the medullar layer of the host alga thallus, to be responsible for significant and widespread tissue damage in the red alga *Neoabbottiella* Perestenko (Halymeniaceae, Rhodophyta). Species of endophytic diatoms were previously described by Hasle (1968), who found *Navicula endophytica* living in the intercellular substance of *Ascophyllum nodosum* (L.) Le Jolis (Ochrophyta), and by Okamoto et al. (2003), who described *Gyrosigma coelophilum* from thalli of *Coelarthrum opuntia* (Endlicher) Børgesen (Rhodophyta). Although all aspects of diatom endobiosis are either very poorly studied or unknown, this kind of survival strategy and development mode may be not uncommon in marine habitats.

According to the results of the current study, epiphytic diatom communities were influenced by their hosts' age, with generally both more abundant and diverse diatom communities found on juvenile thalli, whereas the species of algal host seemed to be of less importance. Nevertheless, due to a significant interaction between the two factors tested, the overall ability of both kelp age and species to explain the variation in diatom community structure was low. Future investigations will aim to determine whether the same patterns prevail on other parts of the kelp thallus, such as the secondary blade, stipe and holdfast.

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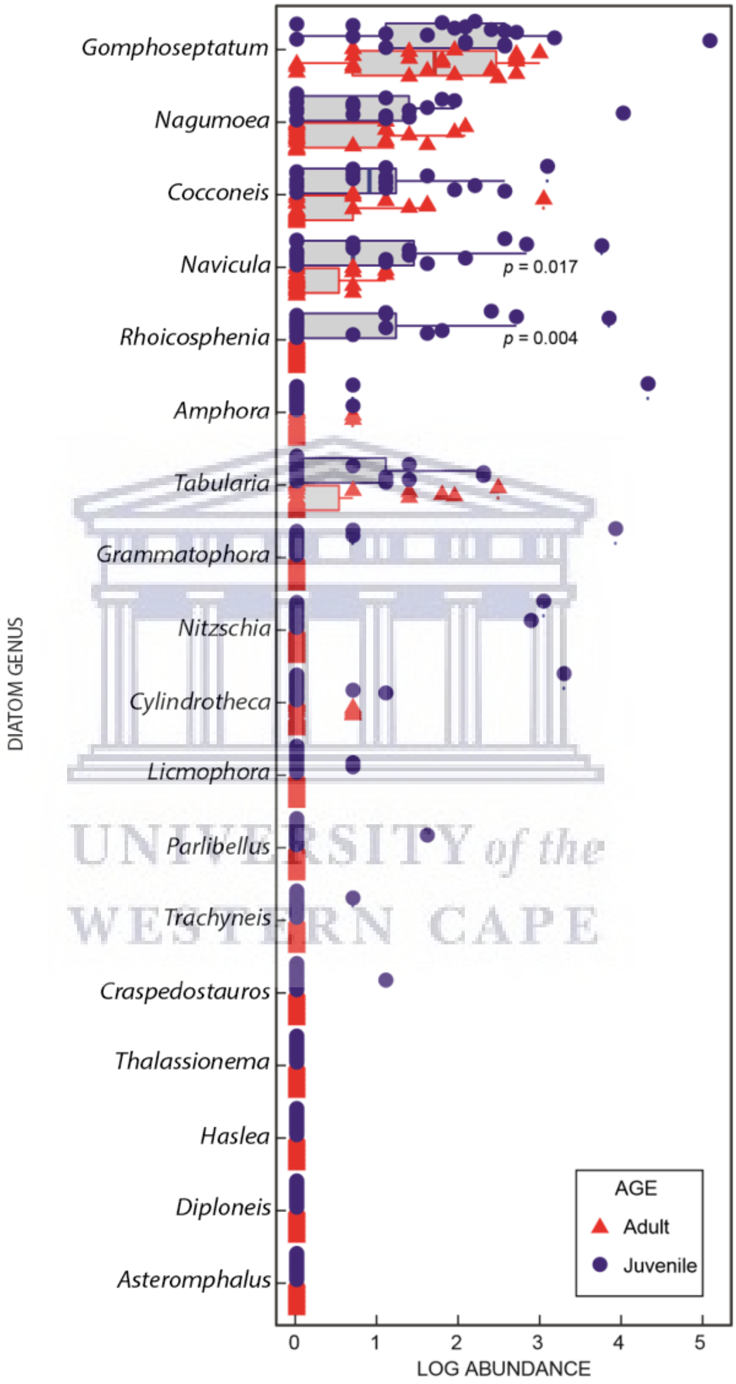

Figure 4: Univariate generalised linear models fitted individually to each diatom taxon found on adult and juvenile thalli of the kelps collected in False Bay, South Africa; small dots beneath some data points denote outliers

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3.3. Conclusion

In this Chapter, we tested the influence of the host species and age on the community composition and abundances of epiphytic diatoms on *Ecklonia maxima* and *Laminaria pallida*, two common kelps growing on the west coast of South Africa. Primary blades of adult and juvenile thalli of both kelp species were sampled in winter, 24 July 2017, and analysed using SEM. The results showed that both host kelp species harboured relatively low diatom densities. Juvenile specimens hosted more abundant and diverse diatom assemblages than adult thalli, with species belonging to *Navicula* and *Rhoicosphenia* genera contributing significantly to the observed dissimilarity. The low number of diatom taxa observed here coud be partly justified by the homogenous surface and uniform morphology of the examined kelps. Additionally, the low abundance values of epiphytic diatoms recorded on the specimens could be the result of efficient kelp defence strategies, *e.g.* secretion of chemically active metabolites and/or sloughing of the outer layer of the thallus. The non-metric multidimensional scaling ordination graphs showed overlapping and scattered sample points. Two-way PERMANOVA partitioning indicated significant both main effects, *e.g.* host species and age, as well as their interaction were significant. But, due the high residual variance, the overall ability of kelp species, their age, and their interaction to explain the variation in diatom community structure was limited. Future work will examine the biotic influences of the host species, age, and thallus part on the associated epiphytic diatom assemblages growing South African kelps.

Chapter 4

Does the substratum matter? An assessment of the influence of the host kelp species, age, and thallus part on epiphytic diatoms – a case study from South Africa

4.1. Preamble

The results of the analyses conducted on the primary blades of the sampled kelp specimens showed limited evidence to support that the host kelp species and age affect their associated epiphytic diatoms even though the main effects were both significant. And considering the size of the investigated kelp specimens, with juvenile thalli (<1.5 m) and adult ones (>3 m), I found it important to also analyse the other remaining thallus parts, *e.g.* holdfast, lower stipe, upper stipe, lower blade and the upper blade. Then, I used the whole dataset of diatom abundances on the six sampled thallus parts to assess the influence of the host species, age and thallus part on the epiphytic diatom communities, using the same advanced multivariate statistical analyses as in the previous Chapter. In this Chapter, I used a dataset of diatom abundances from 288 (48 x 6) subsamples of kelp specimens. I assume that different thallus parts harbour different epiphytic diatom communities because previous studies such as Russel (1983a), Blindow (1987) and Costa *et al.* (2014) reported different patterns of epiphytes growth on different parts of the macroalgae. These authors justified the differences in epiphytic communities on different thallus parts as a result of biotic as well as abiotic factors. The work conducted in this Chapter was aimed to test (1) if there is any differences in epiphytic diatom communities on different thallus parts of South African kelps, *E. maxima* and *L. pallida*, and (2) whether epiphytic diatom assemblages on different thallus parts were influenced by the host macroalgae species, age and/or thallus part.

Introduction

Kelp forests thrive along most of the rocky coastlines in temperate to polar waters across the globe (Krumhansl *et al.* 2016). These dynamic biogenic habitats host a large biodiversity of flora and fauna, forming the base of complex marine food webs and providing numerous valuable ecosystem services to humans, *e.g.* food provision, supply of raw materials for industry, atmospheric gas balance and climate regulation, disturbance prevention (flood and storm protection), nutrient cycling, *etc*. (Krumhansl *et al.* 2016, Arnold *et al.* 2016). Kelp thalli constitute suitable substrata for a wide range of epiphytic organisms, further contributing to the productivity of coastal environments worldwide (Leclerc *et al.* 2016). Among the epiphytic microalgae associated with kelps, diatoms, which are thought to supply at least 40% of the oceanic primary production, are often the most diverse and abundant group (Moncreiff *et al.* 1992; Falkowski *et al.* 1998; Mann 1999; Siqueiros-Beltrones *et al.* 2002, 2016; Uribe *et al.* 2015; Mayombo *et al.* 2019).

Epiphytic diatoms interact with their macroalgal hosts in different ways. These host-epiphyte interactions constitute the biotic factors that may enhance or deter epiphyte growth (Medlin and Juggins 2018). Some host macroalgal thalli were reported to be neutral substrata to their associated epiphytes, while others were found to deeply affect biofilm development on their surface (Gough and Gough 1981, Blindow 1987). The release of inorganic nutrients through the surface of some macroalgae has been reported (Burkholder *et al.* 1990). These nutrients are used by the associated epiphytic microalgal assemblages, particularly in oligotrophic waters (Burkholder *et al.* 1990). Snoeijs (1994) reported that the release of nutrients encouraged the settlement and proliferation of the diatom community on dying *Cladophora* Kützing in autumn in the Baltic Sea. Nutrients released through the surface of the macroalgae favoured also the development of a mutualistic relationship between the host thallus and epiphytic microalgal communities along the western Antarctic Peninsula (Amsler *et al.* 2019). The latter use the released nutrients for their primary production while the former is protected from grazing by the layer of epiphytes, because grazers would prefer to feed on epiphytic microalgae over the macroalgal tissues (Amsler *et al.* 2019).

Surface colonisation of macroalgal substratum is generally seen as deleterious for the host thallus. Epiphytes decreased the growth rate of the intertidal red alga *Rhodomela larix* (Turner) C. Agardh, thus increasing the probability of axis breakage and decreasing the host reproductive output (D'Antonio 1985). The host macralgal thallus and its associated epiphytic microalgal assemblages may compete for nutrient and sunlight (Hudon and Bourget 1983; Gosselain *et al.* 2005). Macroalgal hosts use a wide range of strategies to adequately respond to the biofilm development on their surfaces. Secretion of secondary metabolites enhancing or deterring the biofilm formation was reported in various species of macroalgae. Chemical defences against epiphytes are common and relatively well-known in marine macroalgae. Kubanek *et al.* (2003) isolated lobophorolide, a compound responsible for targeted antifungal activities in the small thalloid brown alga *Lobophora variegata* (J. V. Lamouroux) Womersley ex E. C. Oliveira. Amsler *et al.* (2005) studied the potential chemical defence of Antarctic macroalgae against diatom fouling. The authors found extracts of two species collected from McMurdo Sound to be toxic to diatoms at/or below concentrations originally present in the macroalgal thalli (the ‘‘natural concentration’’).

Mechanical defence strategies, such as the shedding of the outer layer of the thallus (epithallus), have been reported in a number of macroalgae (Moss 1982; Helio *et al.* 2001; Yamamoto *et al.* 2013; Halat *et al.* 2015). Epithallus shedding as a host macroalgae mechanical defence strategy against epiphytes in general might operate selectively in favour of some epiphytic species that have evolved the means to elude its effects (Russel and Veldkamp 1984). This seemed to be more active in young, fast-growing tissues than in older ones, promoting gradients in epiphytes distribution on different part of the host macroalgal thalli (Russell and Veldkamp 1984).

Several studies have reported that host macroalgal thalli with highly homogeneous surfaces and uniform morphology were found to harbour less-diverse and sometimes low numbers of epiphytic diatoms due to competition among epiphytes with overlapping niches (Majewska *et al.* 2013a, 2013b, 2016; Costa *et al.* 2016). Previously, we found that based on the evidence of our SEM observations, surface-cell sloughing occurs in both *Eckonia maxima* and *Laminaria pallada*, and this process seems to be an efficient antifouling mechanism (Mayombo *et al.* 2019). We speculated that this mechanical defence strategy controls the development of diatom communities on kelps surface and obscures the patterns in diatom community composition between the investigated host species and age groups (Mayombo *et al.* 2019).

According to Jennings and Steinberg (1997), water-soluble phlorotannin compounds exuded by the sublittoral kelp *Ecklonia radiata* (C. Agardh) J. Agardh were found to have negligible effect on epiphyte fouling, although previously thought to play a role in chemical defence. This study reported that some other factors such as the relative age of different thallus parts of the host plant (*e.g.* length of the time of exposure of the macroalgal host substratum in the water column), height of exposure in the water column, and tissue texture and rugosity have influenced epiphytes colonisation on the host macroalgae surface (Jennings and Steinberg 1997). High levels of polyphenols, as defence against grazers, were recorded in the thin outer meristoderm tissues of the holdfasts, stipes and blades of *E. maxima* and *L. pallida* (Tugwell and Branch 1989). Antagonistic effects of these polyphenolic substances on associated epiphytes growth were documented (McLachlan and Craigie 1964; Cundell *et al.* 1977; Rönnberg *et al.* 1986).

Moreover, Russell (1983a) observed some patterns of distal increase in epiphytes frequency on cultured excised blade discs of the brown macroalga *Laminaria digitata*. These patterns of distal increase in epiphytes densities proved indistinguishable despite seasonal differences in blade growth (Russell 1983b). The distribution and abundance of some epiphytic diatom taxa differed among host macrophyte species, while other taxa were more abundant on certain parts of the host (Blindow 1987). Even when epiphyton were compared between macrophytes growing close to each other, the age of every single host substratum affected the epiphyton community (Gough and Woelkerling 1976; Blindow 1987). Cundell *et al.* (1977) observed that the macrozonal distribution of epiphytic microorganisms on the brown alga *Ascophyllum nodosum* (Linnaeus) Le Jolis collected from a rocky shore on the New England coast could be explained in terms of the age of the algal surface, differences in light intensity, and the differential secretion of tannin by various parts of the host macroalga.

Considering the size of South African kelps, with their blade, stipe and holdfast standing at different depths in the water column and receiving different amount of light intensity, the attachment and development of epiphytic diatom assemblages would differ from a thallus part to another. The present study was aimed to examine separately different thallus parts of each kelp species to assess possible differences in associated diatom community composition and abundances along the host thalli based on host species, age and thallus part.

4.2. Materials and methods

4.2.1. Study site

See Chapter 2, section 2.3.1, page 24.

4.2.2. Material collection

See Chapter 2, section 2.3.2, page 25.

4.2.3. Sample processing

Each kelp part was split into three portions (subsamples) of approximately 1 cm^2. Every subsample was dehydrated by immersion in ethanol solutions at increasing concentrations. This included a 30-min immersion in a 30% ethanol solution, a 45-min immersion in a 50% ethanol solution, a 1-h immersion in a 60%, 70%, 80%, 90%, and 95% ethanol solution, and at least 24-h immersion in absolute ethanol. Subsequently, the specimens were treated with a HCP-2 critical point dryer (Hitachi, Japan), before being mounted on 13-mm aluminum stubs with adhesive carbon tape, and sputter-coated with gold/palladium (Au/Pd) using a Q150T ES (Quorum Technologies Ltd, UK) sputter coater. Diatom observation and counting were carried out at high magnification using an Auriga field emission SEM and a LEO 1450 SEM (Zeiss, Germany).

4.2.4. Microscopical observations

A total of 288 specimens (3 × subsamples, 4 × replicates, 6 × thallus parts, 2 × ages, 2 × species) were prepared and analysed under SEM to estimate diatom densities. Diatom cells, still attached to the dehydrated kelp thalli, were counted over a surface area of at least 1 mm^2 on each of the specimens. For taxonomic diatom identification, two or three sections of about 2 cm^2 of the analysed thalli were digested with boiling concentrated nitric and sulphuric acids at a ratio of 2:1. The resulting solutions were repetitively centrifuged and rinsed with distilled water until an approximately neutral pH was reached. The cleaned diatom material was used to prepare both permanent slides for LM observations and specimen stubs for SEM. The slides are stored at the Department of Biodiversity and Conservation Biology, UWC. All diatom frustules were identified to at least genus level, following the available literature (Giffen 1971, 1973, 1976; Medlin and Round 1986; Al-Handal and Wulff 2008a, 2008b; Navarro and Lobban 2009; Witkowski *et al.* 2011; Lobban *et al.* 2012; Al-Handal *et al.* 2016, Siqueiros-Beltrones *et al.* 2016; Park *et al.* 2018).

4.2.5. Statistical analysis

Statistical and graphical analyses of diatom abundance data where performed using the open-source statistical software R version 3.5.3 (R development Core Team 2019). The **vegan** package version 2.5.5 (Oksanen *et al.* 2019) and the **mvabund** package version 3.13.1 (Wang *et al.* 2012, 2018) were used for multivariate analysis of diatom assemblages. Multivariate statistical analysis refers to the mathematical tools aimed to reduce variation in community (of diatoms in this instance) composition and taxon densities into visible patterns that can inform the biotic or abiotic parameters that may structure them (Medlin and Juggins 2018). Generic diatom abundance data were used rather than species abundance data to detect the major structural patterns and avoid possible misidentification. The latter is unavoidable in studies analysing diatom flora from very poorly documented regions and habitats, such as the South African kelp forests. Generic diatom abundance data were used to calculate richness, Shannon diversity and evenness, Simpson diversity and evenness and rarefied richness indices.

Diatom abundance data were transformed by applying either proportion normalisation or logarithmic standardisation. Proportion normalisation entailed dividing the count for each taxon in a sample (number of cells mm^{-2}) by the total number of all the taxa counts in that sample or total cells mm^{-2} (also known as Total Sum Normalisation). This form of normalisation was used to calculate and plot the relative abundance of different epiphytic diatom genera on each analysed kelp specimen. Logarithmic standardisation, used for multivariate statistical analyses, applied the function $\log_2 x + 1$, for $x > 0$, where x is the number of cells per 1mm^2. Both standardisation methods reduced the weight of taxa present at a greater abundance relative to less abundant ones in the samples (Anderson *et al.* 2006).

Before performing permutational multivariate analysis of variance (PERMANOVA) using the **vegan** function '`adonis2()`', the vegan function '`betadisper()`' was used to check the multivariate homogeneity of groups dispersion. Subsequently, the function '`permutest()`' was run to implement a permutation-based test of multivariate homogeneity of group dispersions (variances) for the results of a call to '`betadisper()`'. Unconstrained ordination plots were produced by principal coordinates analysis (PCoA) to visualise the multivariate homogeneity of group dispersion. Non-metric multidimensional scaling (nMDS) was also conducted using the vegan's '`metaMDS()`' function and the Bray-Curtis similarity index with 999 permutations in order to plot how samples were related based on distance matrices. Lastly, the '`manyglm()`' function of the **mvabund** package version 3.13.1 (Warton *et al.* 2012; Beneth and Kalbe 2016) was applied to develop multiple univariate Generalised Linear Models (GLMs) to determine which diatom taxa were responsible for differences between diatom assemblages on different parts of the host kelps.

4.3. Results

4.3.1. Diatom abundances

Average absolute diatom abundances recorded on *Ecklonia maxima* ranged from 4 (SD 2) cells mm^{-2} (lower blade) to 404 (SD 647) cells mm^{-2} (upper blade) on adult specimens, and from 43 (SD 66) cells mm^{-2} (primary blade) to 248 (SD 395) cells mm^{-2} (lower blade) on juvenile specimens (Table 1). Similarly, on *Laminaria pallida*, the average diatom density ranged from 7 (SD 5) cells mm^{-2} (primary blade) to 120 (SD 130) cells mm^{-2} (lower stipe) on adults and from 38 (SD 77) cells mm^{-2} (primary blade) to 187 (SD 161) cells mm^{-2} (holdfast) on juvenile specimens (Table 1). On twelve subsamples, including thallus pieces of seven adult (one primary blade, three lower blade and three upper stipe specimens) and four juvenile (primary blade) specimens of *L. pallida* and one adult specimen of *E. maxima* (primary blade), diatoms were not found. Those subsamples were excluded from further analyses. In general, the highest diatom abundances were recorded on the upper blade (197 cells mm^{-2}; SD 376) and lower stipe (126 cells mm^{-2}; SD 217) and the lowest on the primary blade (27 cells mm^{-2}; SD 52) and upper stipe (52 cells mm^{-2}; SD 138). Diatom abundance were higher on juvenile (114 cells mm^{-2}; SD 218) than on adult specimens (80 cells mm^{-2}; SD 221) and on *E. maxima* (122 cells mm^{-2}; SD 285) than on *L. pallida* (72 cells mm^{-2}; SD 117; Table 2, Figs 4.1–4.4).

Table 4.1: Mean diatom abundance [cells mm^{-2}] and standard deviation (SD) calculated for different thallus parts of adult and juvenile specimens of *Ecklonia maxima* and *Laminaria pallida*.

Host species	Host age	Thallus part	Mean diatom abundance	SD
Ecklonia maxima	Adult	Holdfast	39	51
		Lower stipe	139	105
		Upper stipe	19	14
		Primary blade	21	15
		Lower blade	4	2
		Upper blade	404	647
Ecklonia maxima	Juvenile	Holdfast	104	116
		Lower stipe	198	400
		Upper Stipe	139	258
		Primary blade	43	66
		Lower blade	248	395
		Upper Blade	120	263
Laminaria pallida	Adult	Holdfast	73	116
		Lower stipe	120	130
		Upper stipe	9	15
		Primary blade	7	5
		Lower blade	24	69
		Upper blade	110	118
Laminaria pallida	Juvenile	Holdfast	187	161
		Lower stipe	49	32
		Upper stipe	41	54
		Primary blade	38	77
		Lower blade	54	110
		Upper blade	155	201

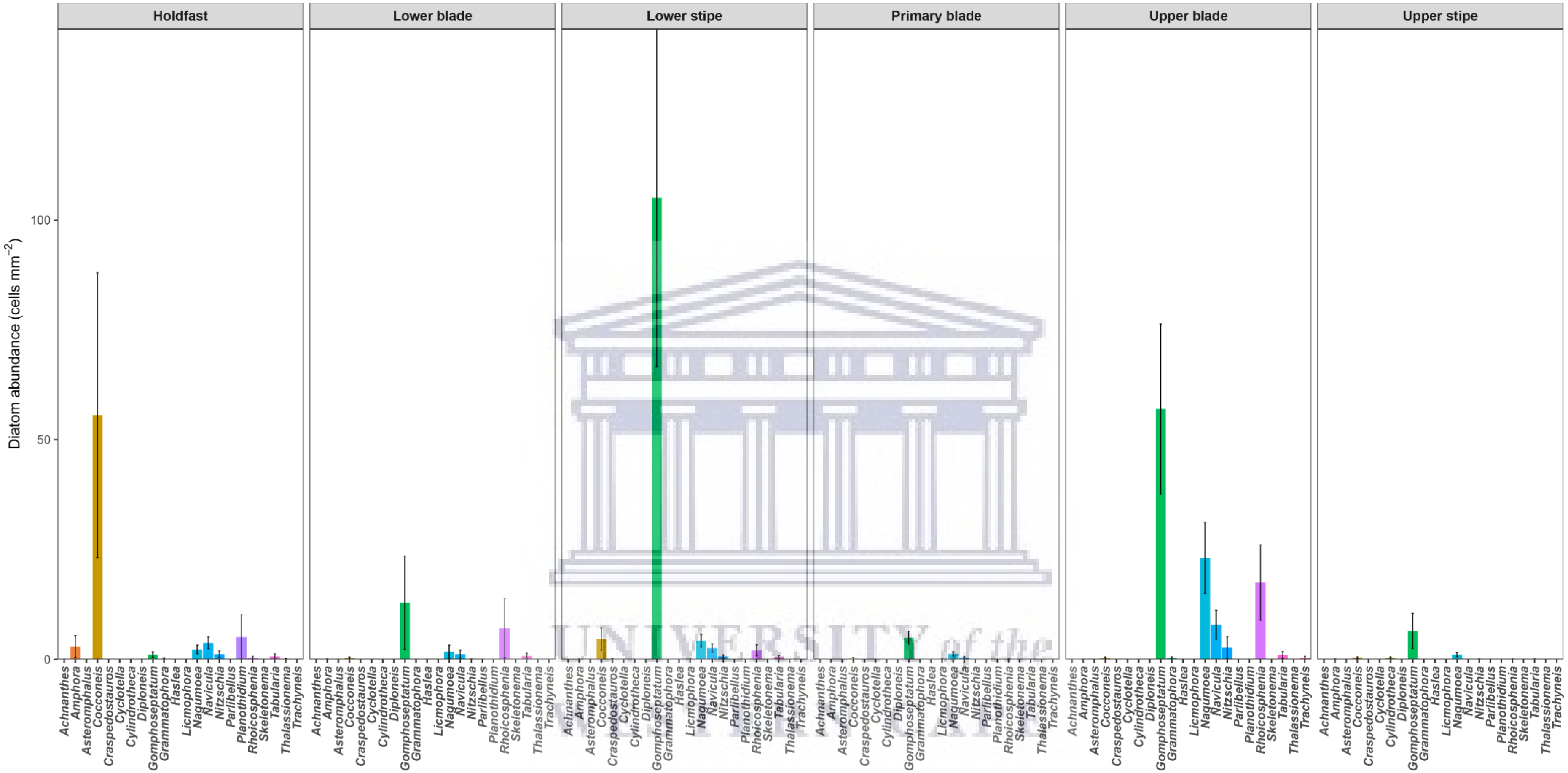

Figure 4.1: Mean diatom abundances (± Standard Error: SE) on different thallus parts of adult *Laminaria pallida*

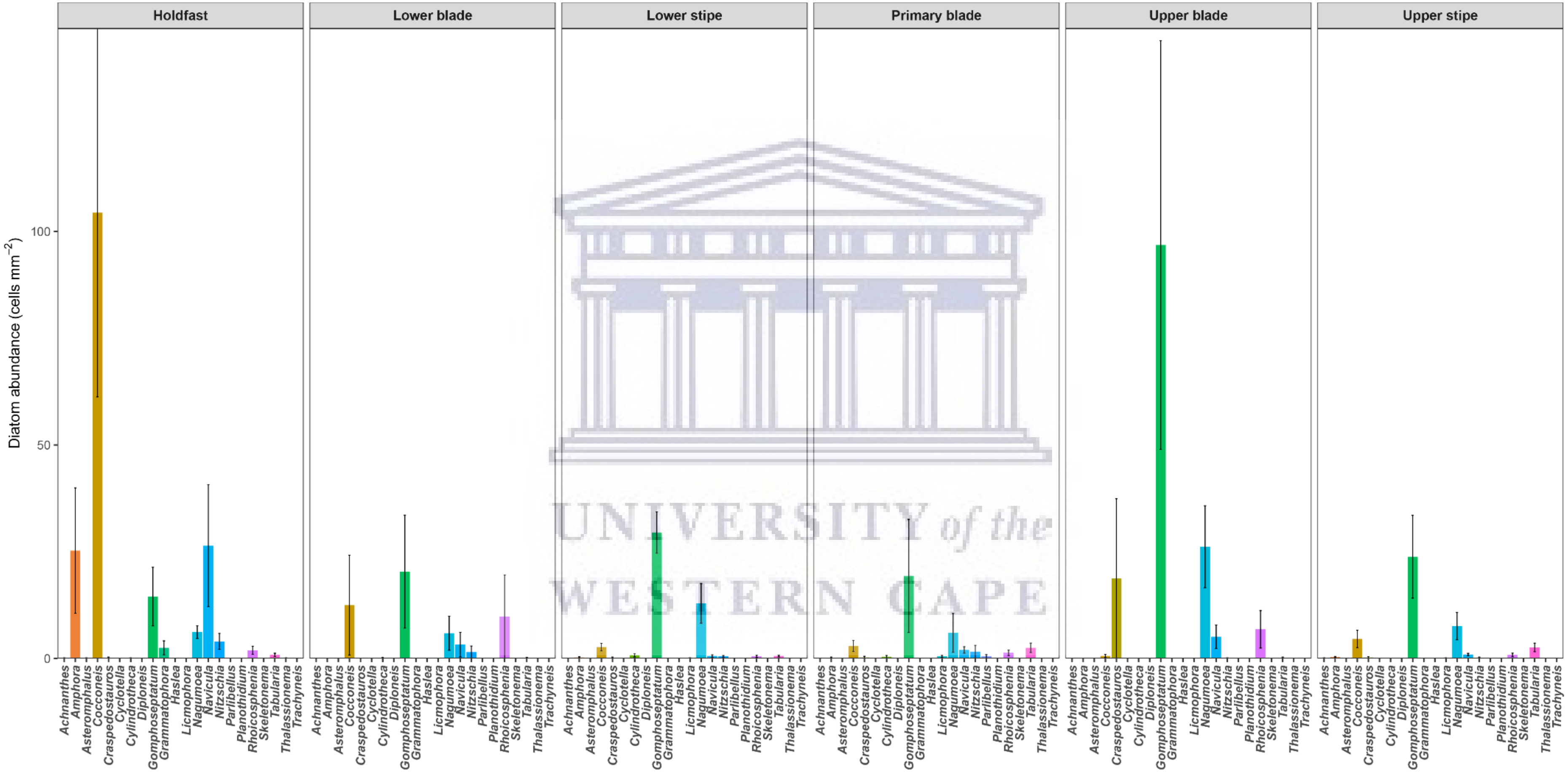

Figure 4.2: Mean diatom abundances (±SE) on different thallus parts of juvenile *Laminaria pallida*

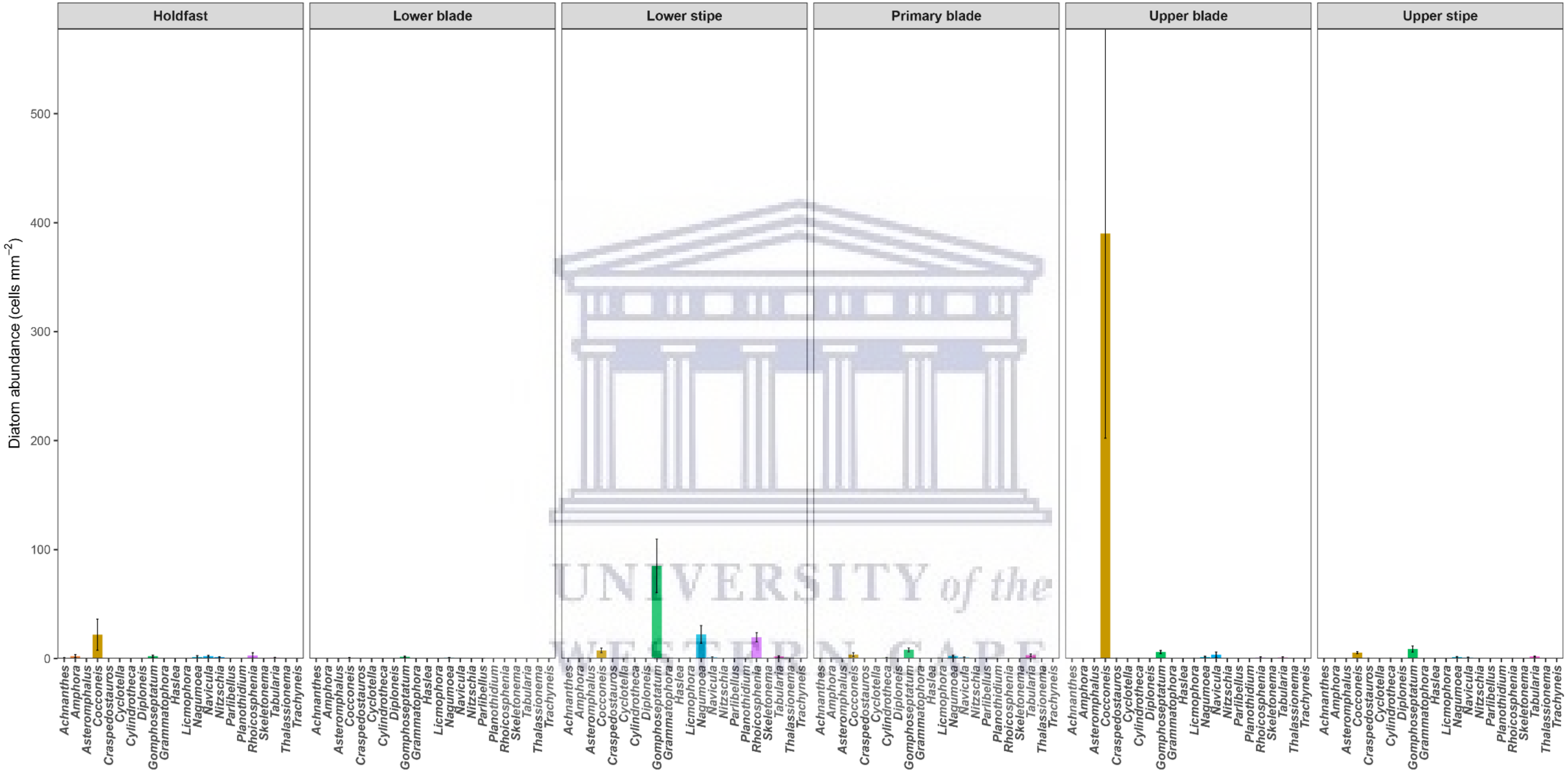

Figure 4.3: Mean diatom abundances (±SE) on different thallus parts of adult *Ecklonia maxima*

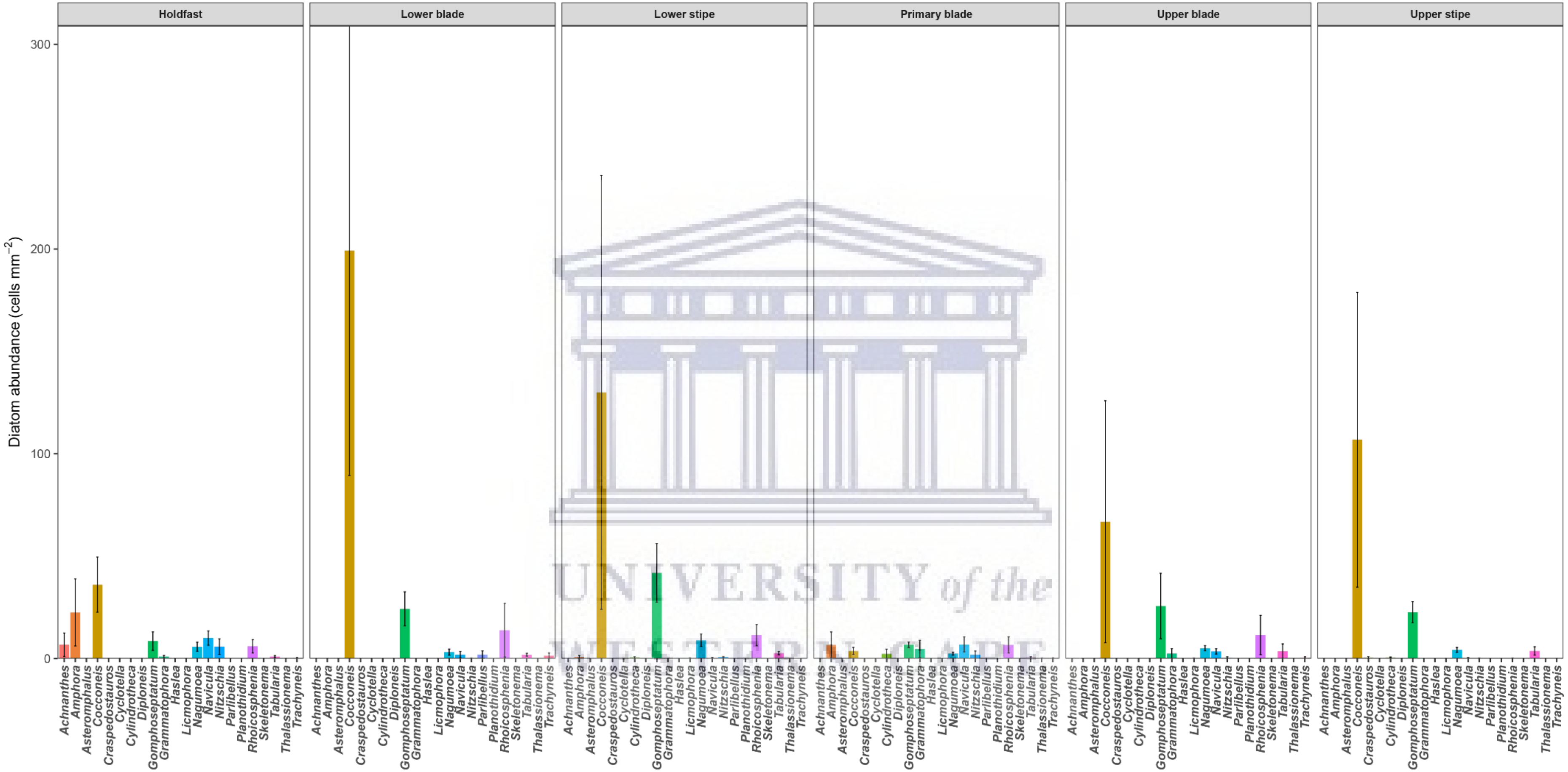

Figure 4.4: Mean diatom abundances (±SE) on different thallus parts of juvenile *Eckolnia maxima*

Table 2. Average diatom abundance [cells mm^{-2}] and standard deviation (SD) calculated for all samples of *Ecklonia maxima*, *Laminaria pallida*, juvenile and adult specimens, and each of the six thallus parts.

Substratum	Mean abundance	SD
Holdfast	100	127
Lower stipe	126	217
Upper stipe	52	138
Primary blade	27	52
Lower blade	82	224
Upper blade	197	376
Adult	80	221
Juvenile	114	218
Ecklonia maxima	122	285
Laminaria pallida	72	117

4.3.2. Diatom community composition

A total of 22 diatom genera were observed attached on the surfaces of examined kelp specimens under SEM. Diatom genera belonging to *Gomphoseptatum* Medlin, *Nagumoea* Witkowski and Kociolek, *Cocconeis* Ehrenberg and *Navicula* Bory genera were the most frequently encountered, occurring respectively in 242, 187, 180 and 130 subsamples out of 288, such as being present in 84%, 65%, 62.5% and 45% of the analysed subsamples respectively (Figs 4.5–4.8). Among these, *Cocconeis* and *Gomphoseptatum* were the most abundant, contributing 50% and 27% of the total diatom cells counted collectively across all subsamples, respectively (Figs 4.5–4.8). The two genera were sometimes observed growing in densely packed clusters, covering a significant portion of the analysed kelp surface. Eight genera, including the above mentioned *Cocconeis*, *Gomphoseptatum*, *Nagumoea*, and *Navicula* as well as *Amphora* Ehrenberg ex. Kützing, *Craspedostauros* E.J. Cox, *Grammatophora* Ehrenberg, and *Rhoicosphenia* Grunow, contributed 100% of the total diatom number in at least one subsample (Figs 4.5–4.8). On average, *Cocconeis* dominated in holdfast samples of *L. pallida*, holdfast and upper blade samples of adult *E. maxima*, and almost all thallus parts (except for primary blade) of juvenile *E. maxima*, whereas *Gomphoseptatum* dominated on the remining thallus parts. Typically planktonic forms, such as *Cyclotella* (F.T. Kützing) A. de Brébisson*, Cylindrotheca* Rabenhorst and *Skeletonema* Greville, were rarely observed.

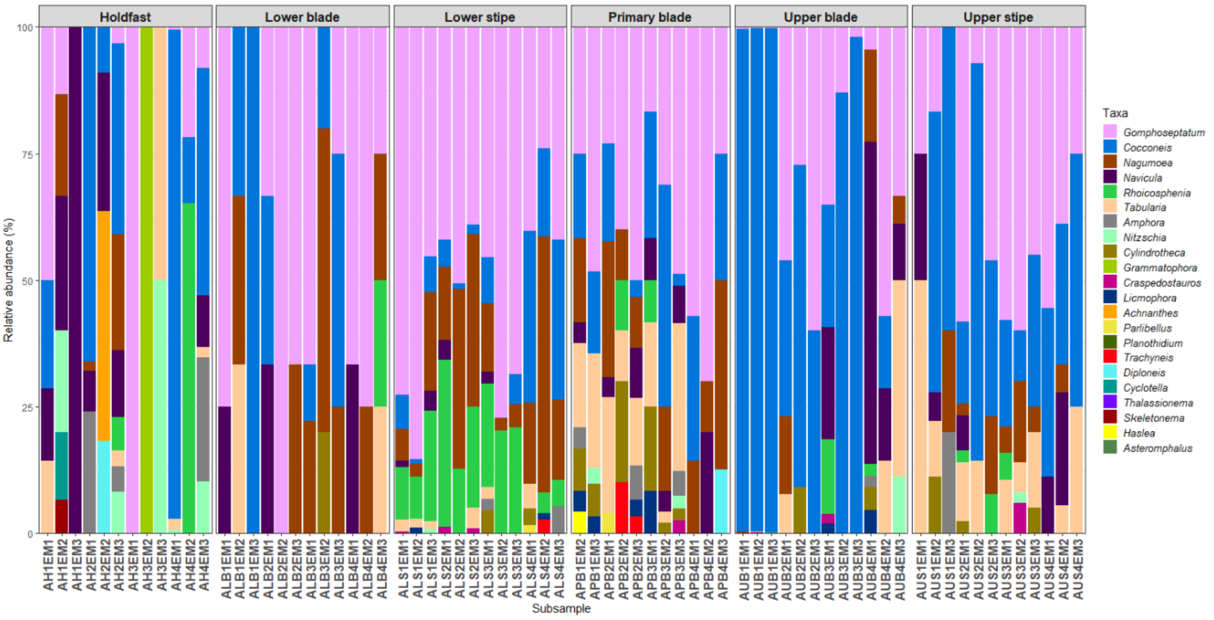

Figure 4.5: Relative abundance of diatom genera on different parts of adult specimens of *Ecklonia maxima*.

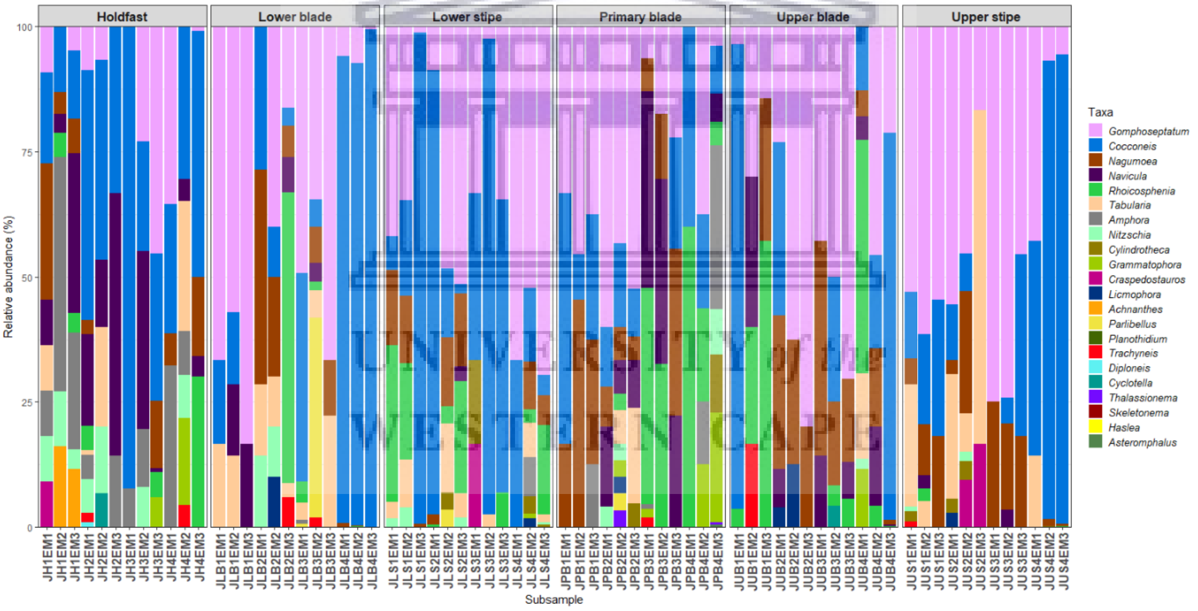

Figure 4.6: Relative abundance of diatom genera on different parts of juvenile specimens *Ecklonia maxima*.

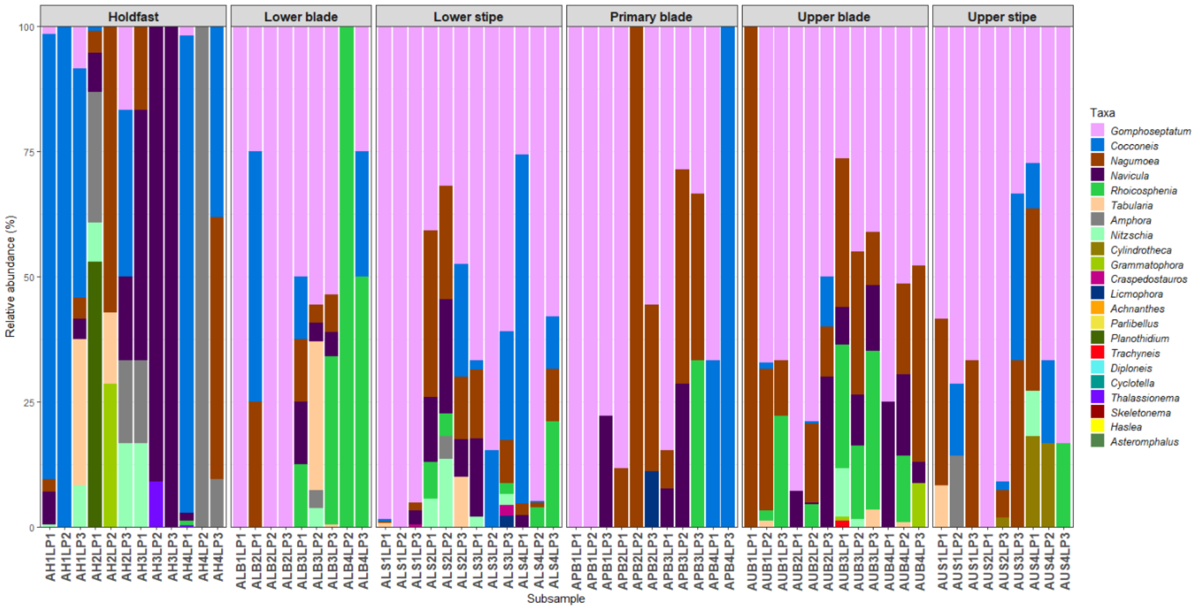

Figure 4.7: Relative abundance of diatom genera on different parts of adult specimens of *Laminaria pallida*.

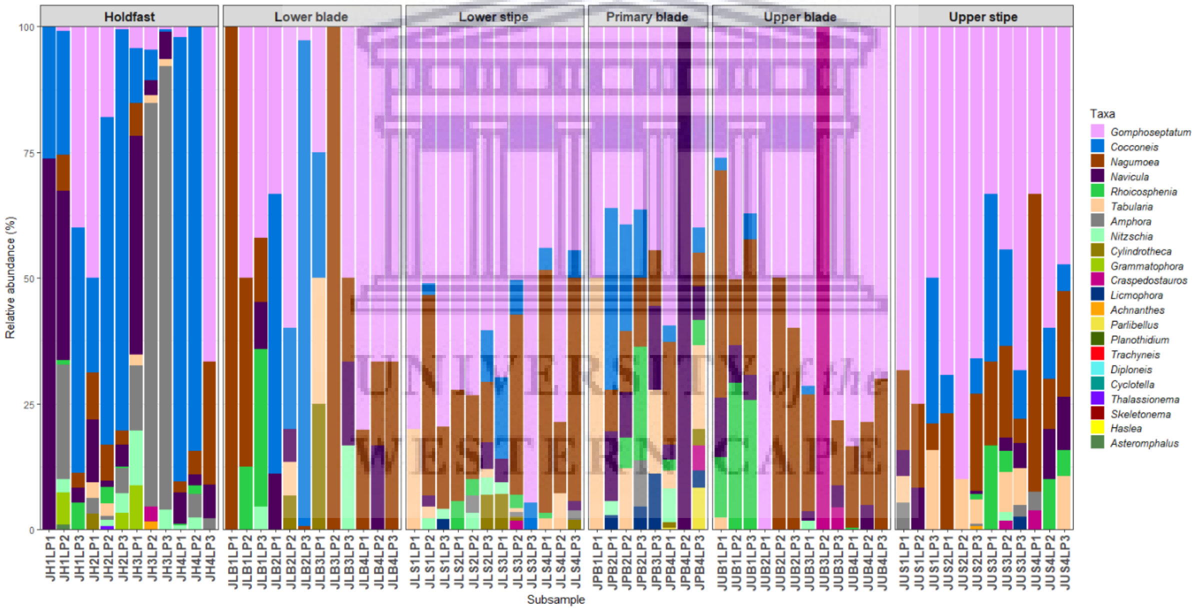

Figure 4.8: Relative abundance of diatom genera on different parts of juvenile specimens *Laminaria pallida.*

Alpha diversity indices computed on untransformed diatom community abundance data revealed that generic richness values ranged from 1 to 11 (Fig. 4.9a) and Shannon diversity index (base e) from 0 to 1.979 (Fig. 4.9b). Simpson diversity index ranged from 1 to 6.368 (Fig. 4.5c), Shannon evenness (Hill's ratio) from 0.256 to 1 (Fig. 4.9d), Simpson evenness (Hill's ratio) from 0.193 to 1 (Fig. 4.9e), and rarefied richness ranged from 1 to 11 (Fig. 4.9f).

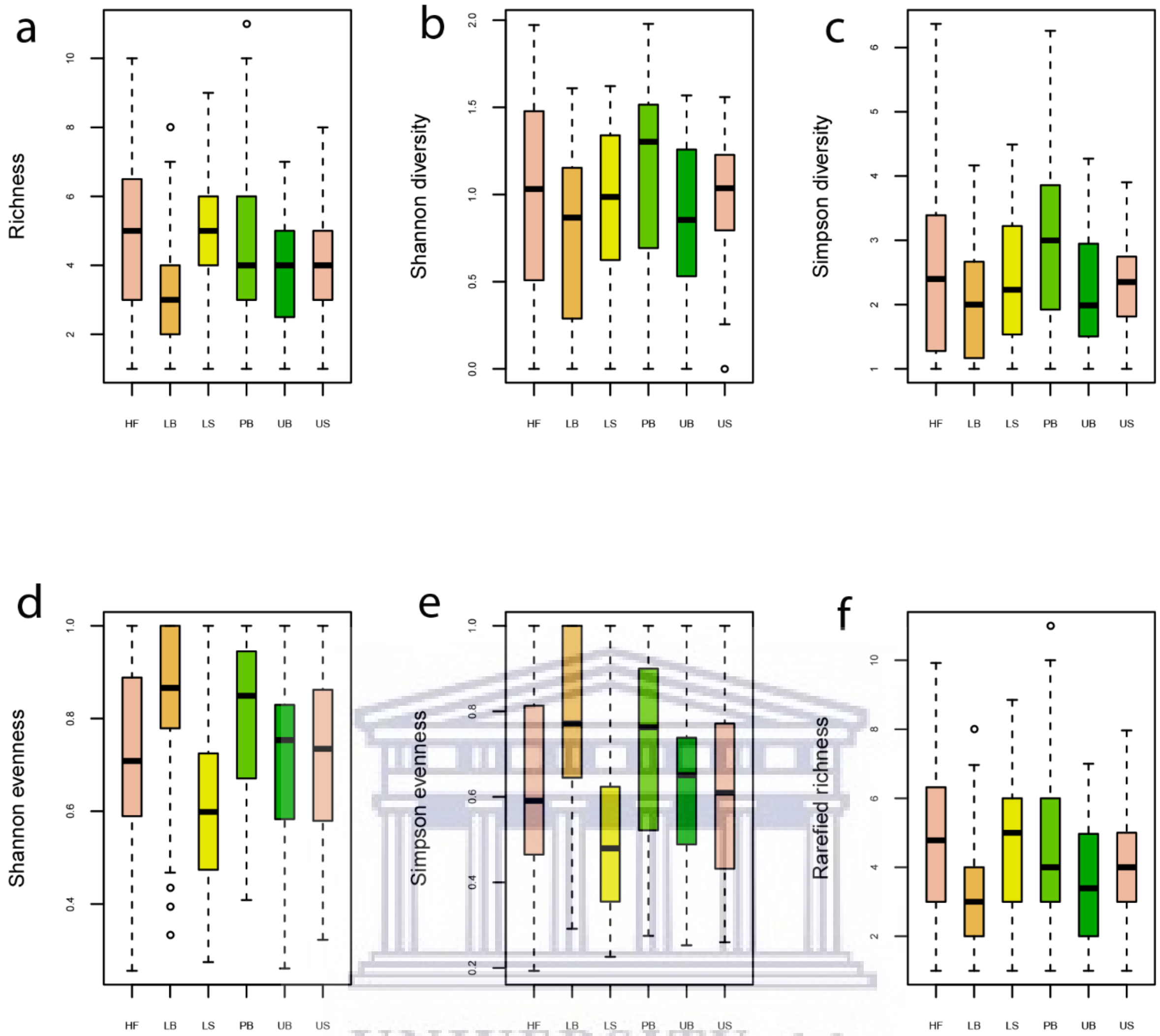

Figure 4.9: Boxplots visualising differences in diatom generic diversity indices along kelp thalli. HF = Holdfast, LB = Lower blade, LS = Lower stipe, PB = Primary blade, UB = Upper blade, US = Upper stipe.

The estimated coefficients of linear models revealed that generic diatom richness was significantly lower on lower (Estimate = -1.749, $p < 0.0001$) and upper blade (Estimate = -1.125, $p = 0.005$; Fig. 4.9a) as well as *L. pallida* (Estimate = -0.633, $p = 0.008$) and was significantly higher on juvenile specimens (Estimate = 0.936, $p < 0.0001$). Similarly, Shannon diversity index (base e) was significantly lower on lower blade (Estimate = -0.227, $p = 0.029$; Fig. 4.9b) as well as *L. pallida* (Estimate = -0.222, $p = 0.0001$) and was significantly higher on juvenile kelp thalli (Estimate = 0.157, $p = 0.007$). Other significant differences included: i) Simpson diversity index lower on lower blade (Estimate = -0.488, $p = 0.0233$) (Fig. 4.9c) and *L. pallida* (Estimate = -0.516, $p < 0.0001$); ii) Shannon evenness lower on lower stipe (Estimate = -0.106, p = 0.0069) and juvenile specimens (Estimate = -0.07, $p = 0.0027$) and higher on lower blade (Estimate = 0.117, $p = 0.0035$) and primary blade (Estimate = 0.108, $p = 0.0079$; Fig. 4.9d); iii) Simpson evenness lower on lower stipe (Estimate = -0.116, $p = 0.0079$) and *L. pallida* (Estimate = -0.09, $p = 0.0001$) and higher on lower blade (Estimate =

0.137, $p = 0.0021$) and primary blade (Estimate = 0.11, $p = 0.0152$; Fig. 4.9e); iv) rarefied richness lower on lower (Estimate = -1.662, $p < 0.0001$) and upper blade (Estimate = -1.166, $p = 0.0034$) as well as *L. pallida* (Estimate = -0.617, $p = 0.0086$) and higher on juvenile specimens (Estimate = 0.908, $p = 0.0001$; Fig. 4.9f).

SEM analyses revealed that some samples contained low abundances of epiphytic diatoms (Fig. 4.10, panels 1–3 and Figure 4.11, panels 1–9), while others were densely populated (Figure 4.10, panels 4–12). Most diatoms associated with South African kelps were pennate diatoms. However, some planktonic forms, such as *Cyclotella* (F. T. Kützing) A. de Brébisson*, Cylindrotheca* Rabenhorst and *Skeletonema* Greville, were also found on the analysed specimens (Figs 4.5–4.8). Bacteria were also visible on some critical point dried subsamples under SEM.

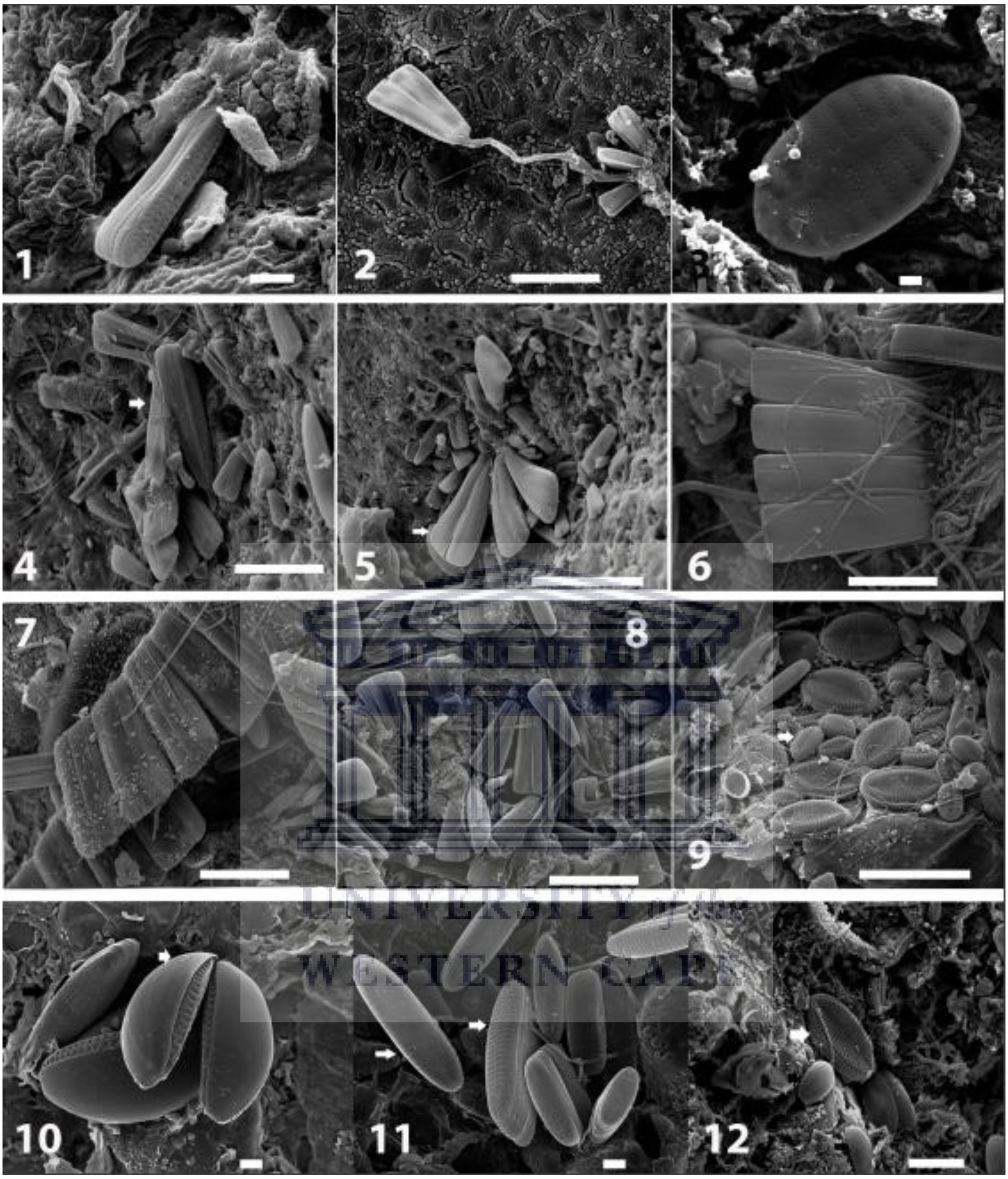

Figure 4.10: SEM micrographs showing diatom assemblages on South African kelps. 1. & 2. *Gomphoseptatum pseudoseptatum*; 3. *Cocconeis* cf*. costatum*; 4. & 8. Colonies dominated by *Gomphoseptatum pseudoseptatum*; 5. *Rhoicosphenia abbreviata* (arrow) and other diatoms covering the surface of a cavity; 6. *Tabularia* sp.; 7. *Grammatophora* cf. *marina*; 9. *Cocconeis scutellum* (white arrow) and *Navicula* sp. (black arrows); 10. *Amphora* cf. *helenensis* (white arrow) and *Navicula* sp. (black arrow); 11. *Rhoicopshenia genuflexa* (white arrows) and *Navicula* sp. (back arrow). 12. *Cocconeis* cf. *scutellum* (white arrow) and *Amphora* cf. *helenensis* (black arrows). Scale bars: 1 = 4µm; 2, 4 & 8 = 10µm; 3, 10 & 11 = 2µm; 5 = 20µm; 6, 7 & 9 = 15µm, 12 = 7µm.

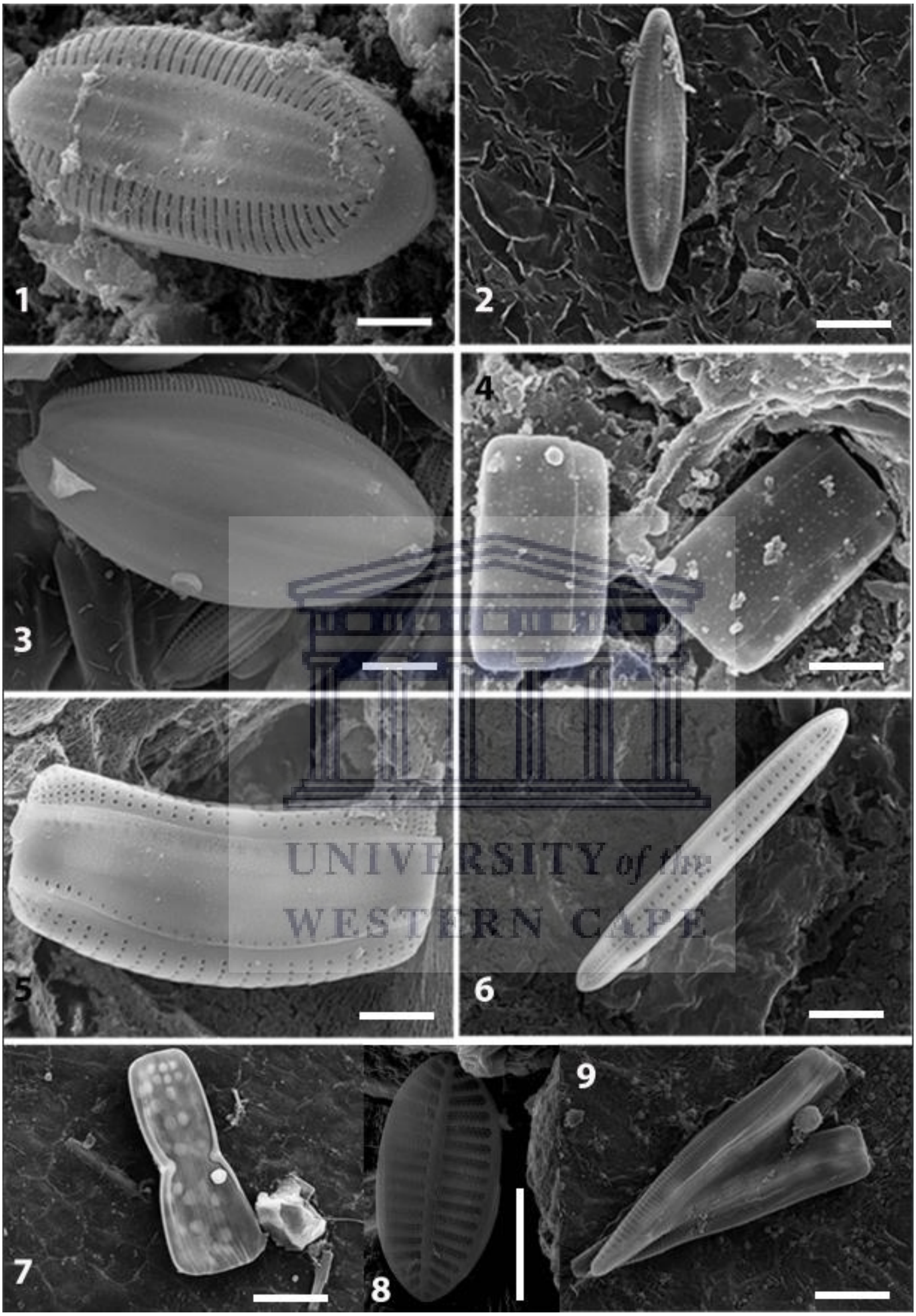

Figure 4.11: SEM micrographs showing diatom assemblages on South African kelps. 1. *Diploneis* sp. 2. *Navicula* sp. 3. *Amphora* sp. 4. *Nagumoea* sp. 5. *Rhoicosphenia genuflexa*, 6. *Gomphoseptatum pseudoseptatum*. 7. *Craspedostauros* sp. 8. *Cocconeis* cf. *costatum*. 9. *Licmophora* cf. *abbreviata*. Scale bars: 1–6 & 9 = 2µm, 7 = 10 µm, 8 = 4 µm.

4.3.3. Host species, age and thallus part effects on the diatom communities

The multivariate homogeneity of group dispersion of diatom assemblages based on host species factor showed no significant differences (df = 1, SS = 0.031, *F* = 1.397, *p* = 0.229) (Figure 4.8 (a)). Differences in the multivariate homogeneity of group dispersion were significant for the examined kelp age (df = 1, SS = 0.193, *F* = 9.283, *p* = 0.002) and parts (df = 5, SS = 0.771, *F* = 7.339, *p* = 0.001) effects (Fig. 4.12 (b) and (c) respectively).

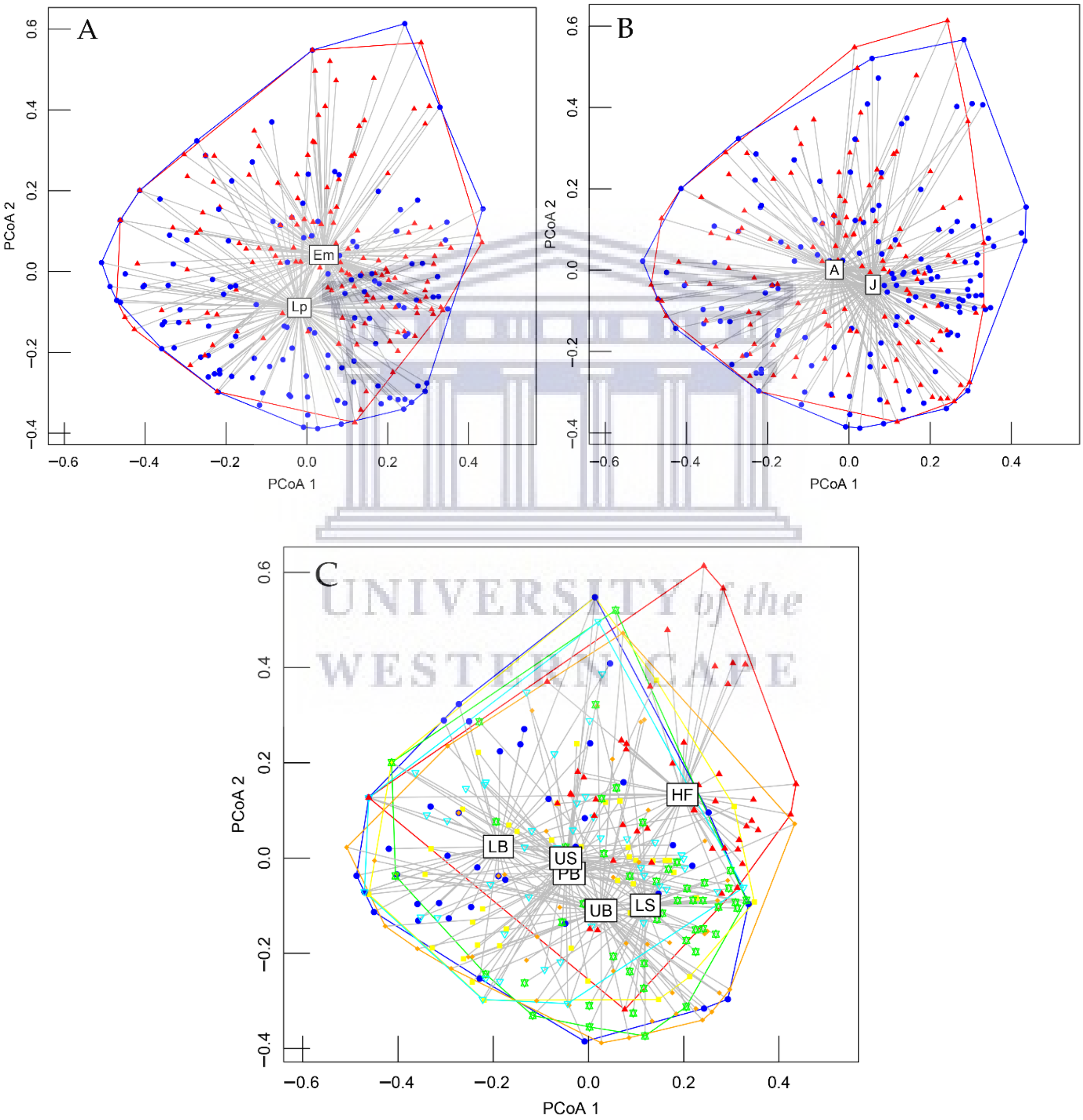

Figure 4.12: PCoA plots visualising the multivariate homogeneity of group dispersion based on the epiphytic diatom abundances data grouped by (a) host kelp species: *Ecklonia maxima* (Em, red) and *Laminaria pallida* (Lp, blue); (b) age: A = Adult (red), J = Juvenile (blue); and (c) thallus part: HF = Holdfast (red), LS = Lower stipe (green), US = Upper stipe (cyan), PB = Primary blade (yellow), LB = Lower blade (blue), UB = Upper blade (orange).

The nMDS analysis revealed a strong correlation between the observed dissimilarity and the ordination distance ($R^2 = 0.96$), but the rather high stress value (~0.20) indicated a significant distortion of the 2-dimensional arrangement of the sample points (Fig. 4.13). Thus, attempting to show the effects of different factors in the nMDS ordination plots is just uninterpretable because of the high residual variation.

PERMANOVA partitioning revealed that the epiphytic diatom community composition and abundances on South African kelps were significantly influenced, to some extent, by thallus part ($df = 5$, $SS = 8.752$, $R^2 = 0.147$, $F = 10.261$, $p = 0.001$), host species ($df = 1$, $SS = 1.701$, $R^2 = 0.029$, $F = 9.972$, $p = 0.001$) and age ($df = 1$, $SS = 0.730$, $R^2 = 0.012$, $F = 4.278$, $p = 0.001$). Some small-scale differences in diatom community structures could also result from statistically significant interactions between host species and thallus part ($df = 5$, $SS = 1.377$, $R^2 = 0.023$, $F = 1.615$, $p = 0.013$), as well as between age and thallus part ($df = 5$, $SS = 2.034$, $R^2 = 0.034$, $F = 2.384$, $p = 0.001$). Even though there was no evidence of interaction between host species and age ($df = 1$, $SS = 0.196$, $R^2 = 0.003$, $F = 1.146$, $p = 0.335$), the three-way interaction between the three tested factors of host species, age and thallus part was significant ($df = 5$, $SS = 1.838$, $R^2 = 0.031$, $F = 2.155$, $p = 0.002$). However, the high residual variance ($df = 252$, $SS = 42.989$, $R^2 = 0.721$) suggests that other unexplained factors contribute the largest components of variation to the overall model (Table 4.3).

Table 4.3: Results of three-way crossed PERMANOVA of the effects of kelp species, kelp age and thallus part on the composition and abundance of epiphytic diatoms growing on *E. maxima* and *L. pallida*.

Factor	*df*	*SS*	R^2	*F*	*p**
Species	1	1.701	0.029	9.972	**0.001**
Age	1	0.730	0.012	4.278	**0.001**
Part	5	8.752	0.147	10.261	**0.001**
Species × Age	1	0.196	0.003	1.146	0.335
Species × Part	5	1.377	0.023	1.615	**0.013**
Age × Part	5	2.034	0.034	2.384	**0.001**
Species × Age × Part	5	1.838	0.031	2.155	**0.002**
Residual	252	42.989	0.721		
Total	275	59.617	1.000		

* Significant ($p < 0.05$) main effects and interactions are highlighted in bold. *df*: degrees of freedom; *SS*: sum of squares; *F*: *F*-statistic.

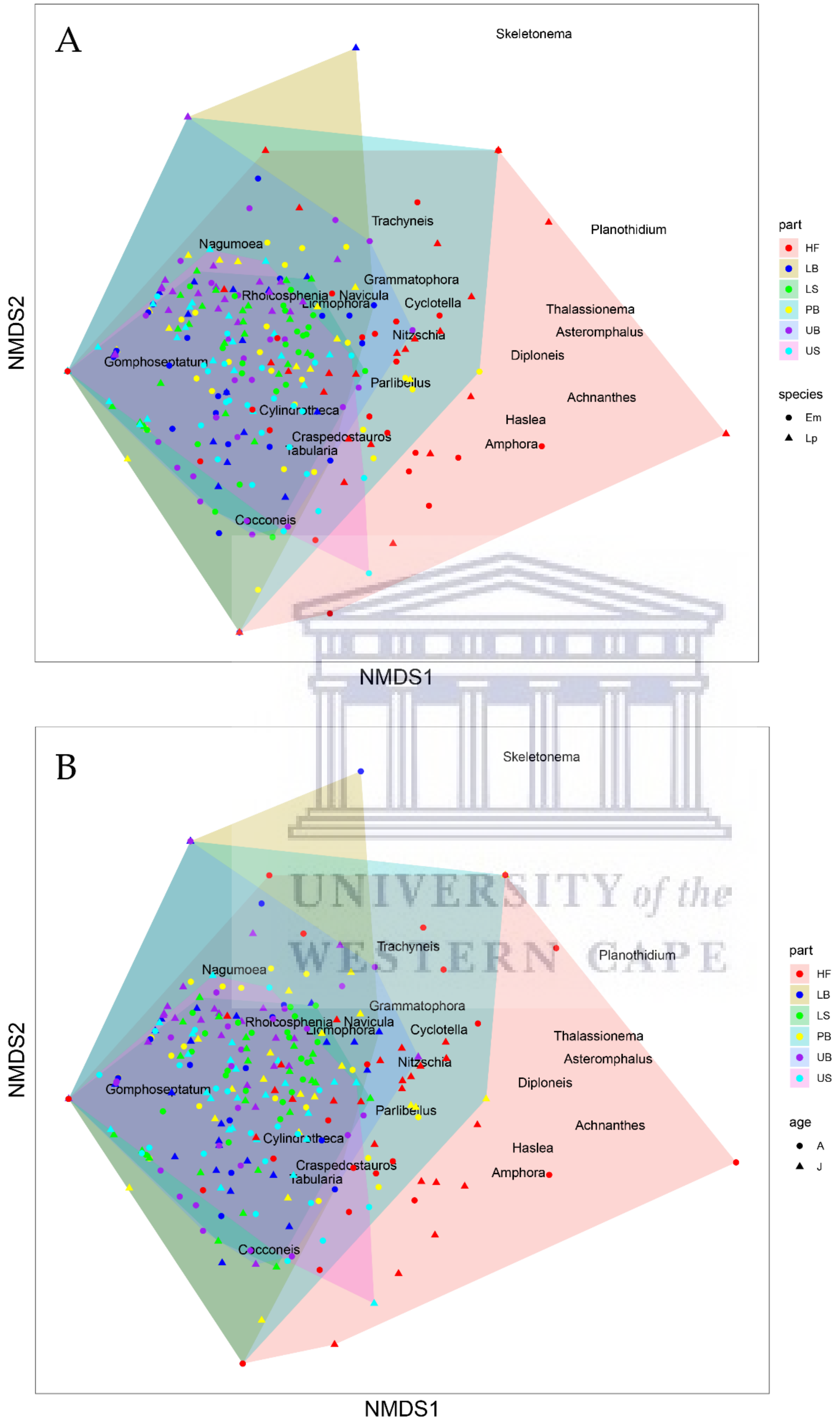

Figure 4.13: nMDS graphs of the changes in epiphytic diatom community compositions and abundances along South African kelp thalli. (2D Stress = ~0.20), (a) host species and parts, (b) host age and parts. HF = holdfast, LB = lower blabe, LS = lower stipe, PB = Primary blade, UB = Upper blade, US = upper stipe; Em = *Ecklonia maxima*, Lp = *Laminaria pallida*; A = Adult, J = Juvenile.

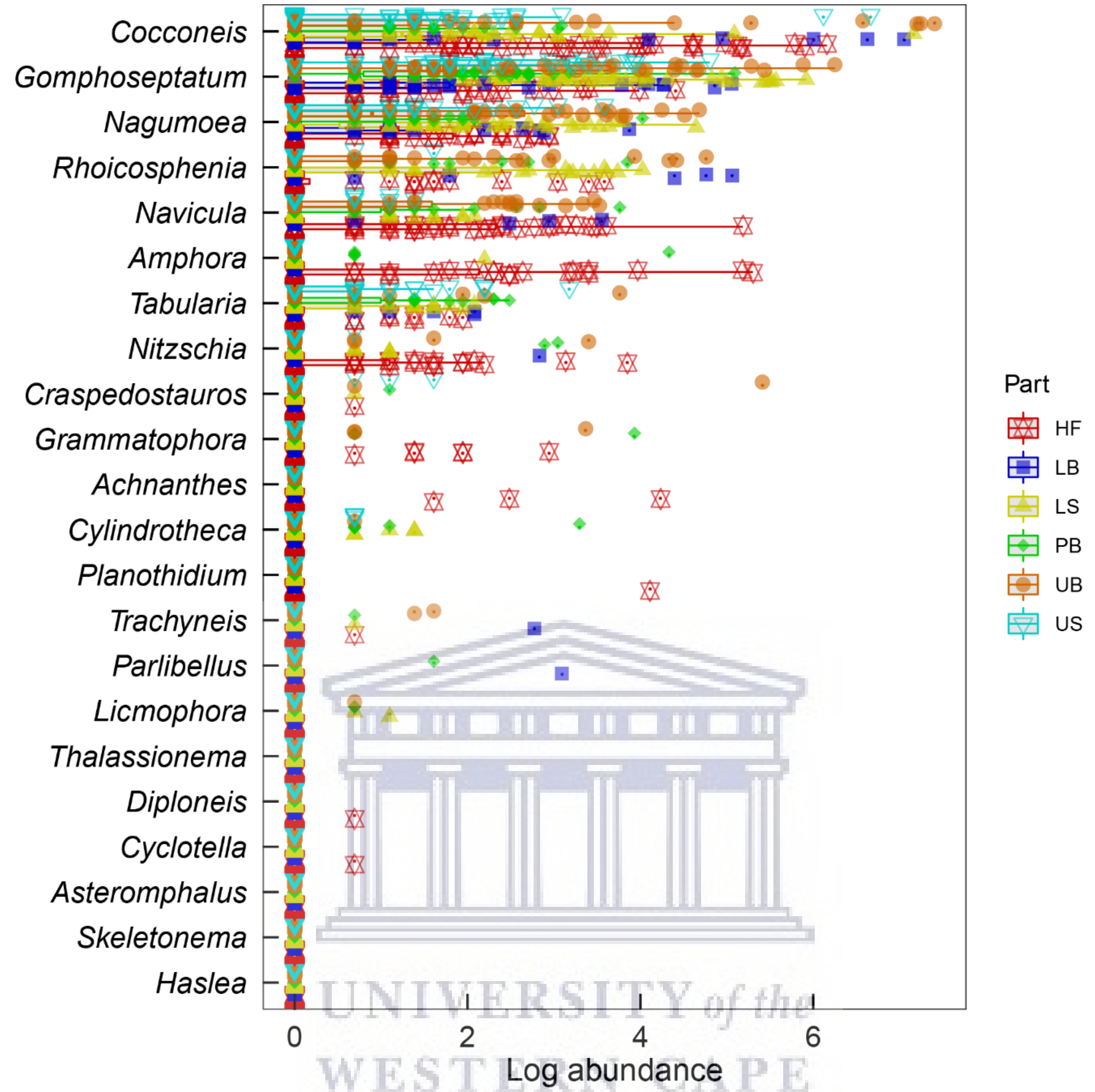

Figure 4.14: Univariate GLMs fitted individually to each diatom genus based on different parts of South African kelp thalli collected in False Bay, South Africa; Small dots beneath some data points denote outliers. HF = Holdfast, LB = Lower blade, LS = Lower stipe, PB = Primary blade, UB = Upper blade, US = Upper stipe.

The ‘`manyglm()`’ function showed also that diatom taxa belonging to *Cocconeis*, *Gomphoseptatum*, *Nagumoea* genera were the most abundant on all the thallus parts of the examined kelp specimens. The ‘manyglm’ model confirmed that the influence of all three tested factors, i.e. the host species (Dev = 88.9, p = 0.002), the host age (Dev = 69.0, p = 0.005) and the thallus part (Dev = 546.5, p = 0.001) on epiphytic diatom communities was significant. Similarly, the effects of the two-way interactions between the host species and thallus part (Dev = 189.7, p = 0.001) and the host age and thallus part (Dev = 165.9, p = 0.001), as well as the three-way interaction between the host species, age and thallus part (Dev = 151.1, p = 0.001) were also significant. Furthermore, univariate ‘genus by genus’ results of the ‘manyglm’ model revealed that the abundance of 11 diatom genera, including *Ampohora* (Dev = 74.9, *p* = 0.001), *Cocconeis* (Dev = 59.3, *p* = 0.001), *Craspedostauros* (Dev = 18.8, *p* = 0.004), *Cylindrotheca* (Dev = 26.8, *p* = 0.002), *Gomphoseptatum* (Dev = 92.2, *p* = 0.001), *Grammatophora* (Dev = 19.6, *p* = 0.026), *Licmophora* (Dev = 21.7, *p* = 0.011), *Nagumoea* (Dev = 49.9, *p* = 0.001), *Navicula* (Dev = 45.6, *p* = 0.001), *Nitzschia* (Dev = 25.5, *p* = 0.017) and *Rhoicosphenia* (Dev = 31.1, *p* = 0.001), were significantly influenced by the thallus part effect, as shown in the ‘mvabund’ plot (Fig. 4.14). Additionally, the abundance of *Cocconeis* (Dev = 23.5, *p* = 0.010) and *Amphora* (Dev = 13.9, *p* = 0.047) were also significantly affected by host species and age effects, respectively. There was no significant influence of the two-way interaction host species and host age, thus no diatom genus was significantly influenced in this case. However, the abundance of *Cocconeis* (Dev = 55.5, *p* = 0.001) and *Nagumoea* (Dev = 25.4, *p* = 0.011) was significantly influenced by the interaction between host species and thallus part. Similarly, the interaction host age and thallus part significantly affected the abundance of *Cocconeis* (Dev = 33.7, *p* = 0.001), *Gomphoseptatum* (Dev = 28.9, *p* = 0.003) and *Navicula* (Dev = 23.9, *p* = 0.008). Finally, the abundance of *Cocconeis* (Dev = 20.9, *p* = 0.037), *Gomphoseptatum* (Dev = 19.9, *p* = 0.041) and *Tabularia* (Dev = 35.9, *p* = 0.001) were significantly influenced by the three-way interaction of host species, age and thallus part effects. The plot of the residual vs. fits which allows to check the quadratic mean–variance assumption of negative binomial regression (with diatom assemblages on different thallus parts coded in different colours); showed little pattern suggesting that the assumption is plausible (Fig. 4.15).

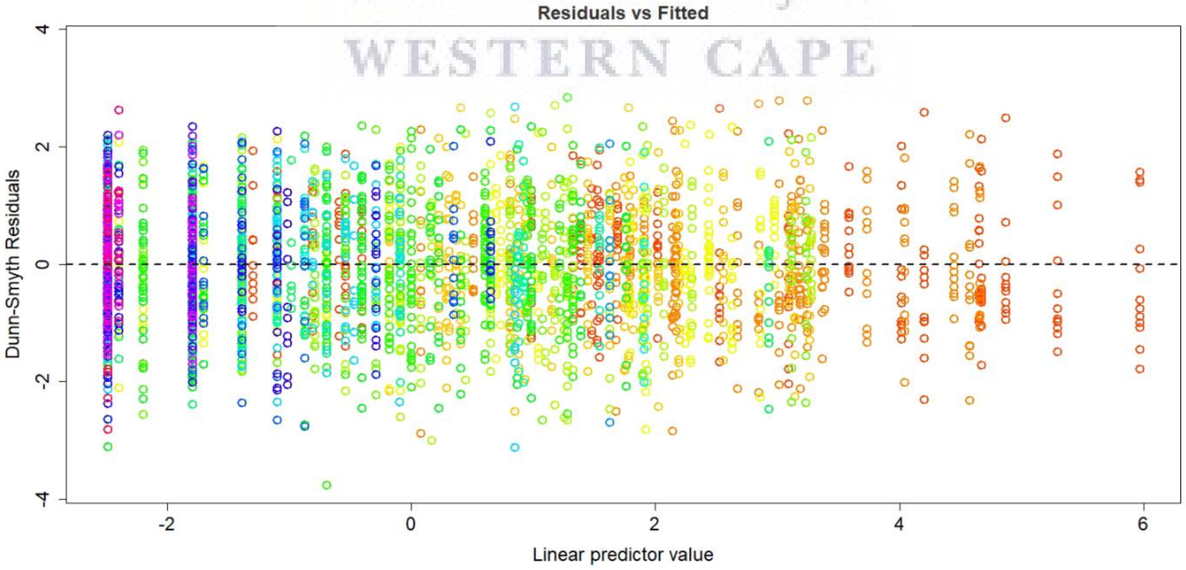

Figure 4.15: Residual vs. fits plot to check the quadratic mean–variance assumption of negative binomial regression (with diatom assemblages on different thallus parts coded in different colours); little pattern suggests the assumption is plausible).

4.4. Discussion

4.4.1. Diatom community composition and densities

Information on epiphytic diatom community composition and densities on host macroalgae, particularly kelps, are very fragmentary in many regions around the world. In this Chapter I tested the effect of the host kelp species, age and thallus part on associated epiphytic diatom assemblages. The density of the epiphytic diatom colonisation per unit area of the host surface was not uniform across the kelp thallus part. The average epiphytic diatom densities recorded in this study, ranging from 4 (SD 2) to 404 (SD 647) cells mm^{-2} on *E. maxima* and from 9 (SD 15) to 187 (SD 161) cells mm^{-2} on *L. pallida*, suggest that diatom abundances on these two macroalgae species of very similar morphology are at the lower end of those recorded on different macroalgal hosts from other regions around the world (Tanaka *et al.* 1984; Snoeijs 1994, 1995; Totti *et al.* 2009; Siqueiros-Beltrones *et al.* 2002, 2014a, 2016; Majewska *et al.* 2013a, 2013b, 2015, 2016; Costa *et al.* 2014, 2016). However, in many of the quantitative studies reporting epiphytic diatom densities on host macroalgae different metrics, such as cells g^{-1} wet or fresh weight of seaweed (Malkin *et al.* 2009; Costal *et al.* 2014, 2016; Moncer *et al.* 2017), chlorophyll a biomass (Nelson 1997; Pinckney and Micheli 1998), biomass µg C cm^{-2} (Accoroni *et al.* 2016), biovolume, i.e. total cell volume mg^{-1} dry weight sample (Snoeijs 1994, 1995), were used, which makes direct comparison challenging if not impossible.

The few studies available in the literature that have reported epiphytic diatom abundances in cells mm^{-2} counted up to over 10 000 diatom cells mm^{-2} of the host macrothallus. Siqueiros-Beltrones *et al.* (2002) estimated between 3000 and 4000 frustules of *Cocconeis* cf. *britannica* per mm^{2} on the kelp *Macrocystis pyrifera* (Linnaeus) C. Agardh blades from Baja California Peninsula, Mexico. Totti *et al.* (2009) counted between 7 (SD 5) and 7524 (SD 3491) diatom cells mm^{-2} when analysing ten species of brown, red and green macroalgae from the west coast of Iceland. The lowest and the highest diatom abundances values were recorded on the brown macroalgae *Laminaria saccharina* (Linnaeus) J. V. Lamouroux and *Fucus vesiculosus* Linnaeus respectively. Majewska *et al.* (2013a, 2016) counted from 21 (SD 13) to more than 8000 diatom cells mm^{-2} on three red algae species from the Ross Sea Antarctica. Majewska *et al.* (2015) recorded mean total diatom abundances ranging from 2 951 (SD 78) to 10 919 (SD 2 260) cells mm^{-2} on *Plocaminium cartilagineum* (Linnaeus) P.S. Dixon (Rhodophyta) from Admiral Bay, King George Island, still in Antartica.

The low epiphytic diatom densities recorded in this study suggests that kelps are not preferred substrata for epiphytes colonisation. Both the architecture of the macroalgal thallus and the surface characteristics may have a role in affecting the abundance and taxonomic composition of the epiphytic diatom communities (Totti *et al.* 2009). Wuchter *et al.* (2008) working in the North Sea, reported that diatoms were almost absent from the smooth and slimy surface of *Laminaria digitata* (Hudson) J.V. Lamouroux, which offers little protection against grazing and abrasion. Low epiphytic diatom abundances were observed on host macroalgae with poorly branched and flat smooth surface, *e.g. Alaria esculenta* (Linnaeus) Greville, *Laminaria hyperborea* (Gunnerus) Foslie and *L. saccharina* (Linnaeus) J.V. Lamouroux from Iceland (Totti *et al.* 2009).

Furthermore, SEM observations confirmed that both kelp species employed surface-cell sloughing which appears to be an efficient antifouling strategy (Mayombo *et al.* 2019). This mechanical defence against surface colonisation may be one of the major biotic factors controlling the density of epiphytic diatoms on South African kelp specimens. Low-magnification observations of epiphyte load on the brown algal thallus *Ascophyllum nodosum* (Linnaeus) Le Jolis using SEM allowed to distinguish highly fouled host substratum with continuous layers of epiphytes that cover the epidermal cells completely and in which diatoms were particularly conspicuous, to uncovered areas where the apical walls of epidermal cells were clearly visible (Halat *et al.* 2015). Skin shedding a method for controlling epiphytes was also reported in the brown alga *Halidrys siliquosa* (Linnaeus) Lyngbye (Moss 1982).

Studies conducted on various host macroalgae from the three major classes (red, brown and green) revealed that not all host substrata were equally used (Al-Handal and Wulff 2008a; Medlin *et al.* 1985; Snoeijs 1994; Totti *et al.* 2009; Majewska *et al.* 2016; Medlin and Juggins 2018). Epiphytic diatoms showed host preferences, *e.g.* rhodophyte hosts (*Pantoneura plocamioides* Kylin, *Delesseria* lancifolia J. Agardh and *Georgiella confluens* (Reinsch) Kylin, were more epiphytised than phaeophytes, and no diatoms were recorded on chlorophytes in the sublittoral zone of Potter Cove, in King George Island, Antarctica (Al-Handal and Wulff 2008). Majewska *et al.* (2016) reported that *Plocamium cartigilaneum* (Linnaeus) P.S.Dixon was the preferred host substratum among three studied Rhodophytes from Antarctica. Diatom community was related to the host macroalgal shape, with erect species being dominant on flat thalli (Totti *et al.* 2009). Medlin and Juggins (2018) reported a dominance of adnate species on *Gelidium* J. V. Lamouroux, which was the most flattened host seaweed in their study.

The high dominance of epiphytic diatom taxa belonging to *Cocconeis* and *Gomphoseptatum* genera, making up respectively 50% and 27% of the total frustule counts in this study, may be explained by the flat and smooth morphology of the examined kelp specimens. *Cocconeis* genus showed the strongest host preference in the Baltic Sea (Snoeijs 1994).

They were absent on the brown alga *Pilayella littoralis* (Linnaeus) Kjellman, which was too narrow to allow the cells to adhere to its surface, whereas they occur abundantly on *Ceramium gobii* Waern, which had larger areas of a flattened thallus (Snoeijs 1994). Majewska *et al.* (2016) observed the dominance of two *Cocconeis* species, *C. antiqua* Tempère & Brun on *Iriadae cordata* (Turner) Bory de Saint-Vincent and *C. fasciolata* (Ehrenberg) N.E. Brown on *Phyllophora antarctica* A. Gepp & E. S. Gepp. They justified the dominance of *Cocconeis* species on these two macroalgae of very similar morphology by their homogenous surface (micro-topography), which might appear to favour competition among epiphytic organisms with overlapping niches (Majewska *et al.* 2016). High abundance of taxa belonging to *Cocconeis* genus was also noted on the blades of the kelp *Macrocystis pyrifera* (Linnaeus) C. Agardh from Baja Carlifonia, Mexico (Siqueiros-Beltrones *et al.* 2002).

4.4.2. Effects of host kelp species, age and thallus part on associated epiphytic diatom communities.

The present Chapter investigated the effects of three factors, *e.g.* host kelp species, age and thallus part, on associated epiphytic diatom community composition and densities. PERMANOVA revealed significant influences of all these three tested main effects. In the previous Chapter of this Master's thesis the effects of host kelp species and age on associated epiphytic diatom community composition and densities were reported (Mayombo *et al.* 2019). Significant influences of the host kelp species and age were revealed by PERMANOVA. Both more abundant and diverse diatom communities were recorded on juvenile thalli, whereas the species of macroalgal hosts seemed to be of less importance (Mayombo *et al.* 2019).

The influence of kelp thalli on their associated epiphytic diatom community composition and densities could be due to variations in a number of biological and chemical factors. The low diatom diversity and abundances observed on South African kelps could be explained by their smooth and flat shape and lack of branchlets to induce epiphyte settlement. The effect of host thallus shape on the colonisation sequence of epiphytes was reported in Medlin *et al.* (1985). Medlin and Juggins (2018) documented that the diatom community composition and abundances on host macroalgal thalli were significantly different and contributed up to 8% of the community variation. The authors found *Gelidium* J. V. Lamouroux to harbour the most distinctive diatom community, and they justified this as a result of the absence of projections which break up the fluid dynamics around the host (Medlin and Juggins, 2018). The diameter of the host macroalga thallus influenced the type of diatoms growing on it (Snoeijs 1994). Chung and Lee (2008) found that the species composition of epiphytic diatoms was closely related to host morphology and lifespan of the leaf tissues on three *Zostera* Linnaeus species distributed on the southern coast of Korea.

Significant differences were also observed in the structure of the epiphytic diatom community, in terms of richness, growth forms, and cell abundance, among three seaweeds species (*Bachelotia antillarium* (Grunow) Gerloff, *Caulerpa verticillata* J. Agardh, *Haloplegma duperreyi* Montagne) from north-eastern Brazil, according to the distance-based PERMANOVA ($p < 0.05$) (Costa *et al.* 2016). The community structures of diatom assemblages varied between three *Zostera* species (*Zostera marina* Linnaeus, *Zostera japonica* Ascherson & Graebner and *Zostera caespitosa* Miki) distributed along the southern coast of Korea (Chung and Lee 2008). Majewska *et al.* (2013b) observed a pronounced difference between the epiphytic diatom communities on three rhodophyte species (*Iridaea cordata* (Turner) Bory, *Phyllophora antarctica* Gepp & Gepp, and *Plocamium cartilagineum* (L.) Dixon) from Terra Nova Bay, Ross Sea, Antarctica. The authors noted that most of the dissimilarities occurred between diatom taxa of the same growth form. However, still in Ross Sea, Antarctica, the differences between epiphytic diatom communities associated with different macroalgal species were reflected better in species composition than in growth form structure (Majewska *et al.* 2016).

A number of studies reported no significant effect of the host macroalga on associated epiphytic diatom assemblages. In terms of growth form, no significant differences between diatom communities associated with different macroalgal species were observed in Antarctica (Majewska *et al.* 2013a). The authors found the epiphytic diatom communities growing on macroalgal blades to be often very uniform and homogenous. Algal epiphyte flora on *Ulva* sp on the Patagonian coast, Argentina, were not determined by the host species (Gauna *et al.* 2016). Sterrenburg *et al.* (1995) found no differences in epiphytic diatom floras on young and older leaves of two host species from South Sulawesi (Indonesia). Gordon *et al.* (2008) reported no significant differences in epiphytic diatom community composition on two host thalli from Saint Lucia Estuary, South Africa. Epiphytic microalgal assemblages were not related to a particular host species in a study of the temporal dynamics of algal epiphyton on *Leathesia marina* (Lyngbye) Decaisne and *Colpomenia sinuosa* (Mertens ex Roth) Derbès & Solier macrothalli (Phaeophycaea) in the Patagonian Atlantic Coast of Argentina (Poza *et al.* 2018).

Several other explanations could also be put forward to justify the significant effects of the three tested factors, *e.g.* host species, age, and thallus part. Firstly, macroalgae may control diatom biofilm development on their surfaces through chemical defence strategies. The secretion of chemically active metabolites which act as inhibitory compounds, particularly in growing regions of the thallus have been reported in many species of macroalgae (Kubanek *et al.* 2003; Wikström and Pavia 2004; Lam *et al.* 2008; Wang *et al.* 2017a, 2017b). These chemical compounds are mobilised in reaction to stress or herbivore attack (Pavia and Toth

2000; Amsler 2001), thus inducible on specific parts of the thallus as observed in other genera of macroalgae (Sotka *et al.* 2003). Although the influence of secondary metabolites produced by kelps on epiphytic microalgae is not yet well understood, diatom distribution, being strongly linked to grazing activity of herbivores, will be indirectly affected by anti-grazing compounds excreted by the host macroalga as well as the taxonomic composition of the local grazer communities. Therefore, adnate diatom taxa (e.g. *Cocconeis*), which are more susceptible to grazing by non-selective herbivores but can attach to and develop on smooth homogenous surfaces, may gain competitive advantage in poorly grazed thallus parts, whereas tissue-boring forms (*Gomphoseptatum*, *Rhoicosphenia*) may prefer heavily grazed, damaged areas that provide them access to the underlying more porous tissue layer.

Secondly, mechanical defence strategies such as shedding of the outer layer of the thallus or epithallus was also reported as an efficient means for macroalgal thalli to control epiphyte loads on their surfaces (Lam *et al.* 2008; Yamamoto *et al.* 2013; Halat *et al.* 2015). SEM observations confirmed that surface-cell sloughing occurs in both kelp species examined and appears to be a particularly efficient antifouling mechanism (Mayombo *et al.* 2019). However, in some instances, diatom frustules were visible under the sloughing surface layer of the kelp. As cross-sections of the thallus were not analysed, it remains unknown whether tissue-boring forms such as *Gomphoseptatum pseudoseptatum* (Giffen) Witkowski, Lange-Bertalot & Metzeltin and *Rhoicosphenia genuflexa* (Kützing) Medlin observed during this study are adapted to fully endophytic lifestyle. Furthermore, it is not clear whether these diatoms caused or significantly contributed to the further degradation of certains part of the thallus as observed under SEM.

Klotchkova *et al.* (2014) showed that tissue damage in two species of the red algae *Neoabbottiella* Perestenko was related to the presence of a diatom identified as *Pseudogomphonema* sp. that developed both in the medullar layer and cortex of the host macroalgae as well as on its surface. It was shown that both endo- and epiphytic forms were normally pigmented and most likely photosynthetically active despite the very low light intensities that could be transmitted into the deeper layers of the thallus. Thus, the authors proposed a mixotrophic mode of nutrition for the investigated diatom taxon (Klotchkova *et al.* 2014). I speculate that tissue-boring taxa observed in the current study that share the “gomphonemoid” shape and growth form with the endobiotic *Pseudogomphonema* sp. may utilise the same mechanisms to colonise the kelp hosts. It is possible that clavate elongated shape of the diatom frustules represents functional morphology that facilitates spacing of the host cells and thus colonisation of the deeper tissue layers. As previously observed by Klotchkova et al. (2014), the tissue-boring diatoms, despite causing visible tissue damage and often inducing the so-called warping of the thallus, do not seem to change the host physiology or affect its growth rates, and their ecological role is not well understood.

Thirdly, among the epiphytic diatoms, there is a variety of attachment morphologies that are species-specific (Medlin *et al.* 1985), *e.g.* flat embedded taxa like *Cocconeis* sp. need a wider surface area for their attachment. However, they resist strong wave actions in exposed areas. According to Tanaka (1986), the micro-distribution and dispersion patterns of various attached diatoms showed close relations with their motility, adhesion and ability to form colonies; *e.g.* active motile and solitary species (solitary cell scattering distribution), tightly adhering with the secreted mucus and colony forming species (small clump scattering distribution), and non-active motile species (large clump).

Host part was the most influential biotic factor as revealed by PERMANOVA partitioning. It contributed about 14.7% of the observed dissimilarities, followed by host species (2.9%), and then host age (1.2%) of the variations. The PCoA as well as the nMDS ordination plots showed the holdfast group (HF) of samples (points) to distinguish itself from the other thallus part. The nMDS plot showed also the HF group to have the largest variance (*e.g.* differences between sample point and the group centroid), followed by the upper blade group (UB). Although diatom densities recorded in this study were not uniform on all the thallus parts, mean generic richness values were higher on lower parts (holdfast and lower stipe) than on upper portions the thallus, *e.g.* frond. The lowest mean diatom generic richness value was noted on the lower blade. We speculate that this is due to the hydrodynamic actions of the water because the frond is more exposed to waves than the holdfast, which may dislodge some loosely attached diatom taxa. Siqueiros-Beltrones *et al.* (2016) recorded lower than usual values of diversity (H'), coupled with an extremely high dominance of *Pteroncola inane* (Giffen) Round. They compared the observed structure of the diatom assemblage to those from extreme environments (Siqueiros-Beltrones *et al.* 2016). Costa *et al.* (2014), observed the greatest abundance of epiphytic diatoms on basal parts of *Galaxaura rugosa* (J. Ellis & Solander) J. V. Lamouroux regardless of the collection sites in the Fernando de Noronha archipelago, Brazil. They attributed this vertical zonation pattern to the hydrodynamic features of the study area.

The high residual variance reported in this study suggests that other unexplained factors, not investigated in the present study, contributed the largest part of variations in kelp associated diatom assemblages. However, since all kelp samples were collected on the same day from the same sampling spot, abiotic factors related to seasonality and hydrological conditions of the site should be excluded as a possible explanation for the observed patterns even though abiotic or environmental factors were reported to also shape the community composition and abundances of epiphytic diatoms. Epiphytic diatom flora along the Swedish west coast were influenced by temperature, salinity, light, tide, geographical position and water currents (Edsbagge 1966). Diatom species composition, cell abundance and vertical stratification were dependent on depth and ultimately light intensity in Canadian great lakes (Hudon and Bourget 1983). Snoeijs (1994) reported season and salinity as the main drivers of epiphytic diatom community compositions in the Baltic and Bothnian Seas. Epiphytic diatom assemblages growing on *Thalassia testudinum* Banks ex König within the Florida Bay and Florida Keys were affected by salinity, nutrient availability and to some extend by water column temperature (Franckovich *et al.* 2006). Sutherland (2008) observed a decrease in diatom species diversity with increase in depth below the sea ice at Cape Evans, Antarctica.

4.5. Conclusion

Diatom communities associated with *Ecklonia maxima* and *Laminaria pallida* were significantly affected by all three biotic factors tested, i.e. the thallus part, host species, and host age. The relatively high residual variance indicated that other factors contributed to the diatom community structuring and distribution. I suggest grazing as well as chemical (active compound production) and mechanical (cell sloughing) defence strategies against herbivores and biofouling employed by the kelps as important processes, directly and indirectly, affecting the epiphytic diatoms, whose influence may depend on the kelp species and age and the thallus part further complicating the observed patterns of diatom colonisation.

The macroalgal surface was dominated by strongly adhering adnate and erect diatoms showing a highly uneven distribution across the host thalli. Naturally occurring cavities and damaged tissue were diatom abundance hot-spots and were colonised by potentially endophytic tissue-boring diatom forms that, however, did not seem to affect the overall fitness of the host. Further studies are required to shed more light on the physiology and ecological role of kelp-associated diatoms and their influence on the host macroalgae. Future works should relate kelp associated diatom assemblages to environmental variables such as temperature, light intensity, turbidity and waves in order to assess the influence of these abiotic factors on kelp associated diatoms.

Chapter 5

Conclusion

This Master’s thesis focused on the ecology and biodiversity of very poorly studied epiphytic diatoms associated with two common and commercially valuable South African kelp species, *Ecklonia maxima* (Osbeck) Papenfuss and *Laminaria pallida* Greville. It involved deep assessment of the structural and compositional changes in epiphyton among and within host kelp species (*E. maxima* and *L. pallida*), as well as among and within age (adult and juvenile) and thallus part (holdfast, lower stipe, upper stipe, primary blade, lower blade and upper blade). This is the first report providing an overview and baseline description of marine epiphytic diatom assemblageas in South African kelp forests in order to better understand the ecology within these highly productive coastal ecosystems.

The first floristic report of the epiphytic diatom assemblages associated with two kelp species is presented in the second Chapter of this thesis. Marine diatoms are very poorly investigated in African coastal waters. There is no updated marine benthic diatom foristic list for the region. Thus, the objective set in the second Chapter of this thesis was to identify all the diatom taxa occurring on kelp samples collected in False Bay and to determine their propotional abundances. Overall 48 taxa of epiphytic diatoms belonging to 28 genera were identified. Most of these taxa were rarely observed during the diatom counts, and some genera, *e.g. Actinoptychus* Ehrenberg, *Campyloneis* Grunow, *Delphineis* G.W.Andrews, *Halamphora* (Cleve) Mereschkowsky, *Mastogloia* Thwaites ex W.Smith, *Pleurosigma* W.Smith, were only observed during the additional observations of the cleaned material. The most abundant and frequently occurring diatom taxa included *Gomphoseptatum pseudoseptatum*, *Cocconeis costata*, *Cocconeis scutellum*, *Nagumoea* sp., *Rhoicosphenia genuflexa*, *Rhoicosphenia flexa* and *Navicula* spp. These baseline floristic data may serve to investigate different biotic and abiotic factors driving the community composition and structures of kelp associated diatom assemblages on the west coast of South Africa.

The influence of the host species and age on the community composition and abundances of epiphytic diatoms on primary blades of adult and juvenile thalli of both kelp species was assessed in the third Chapter. Diatom abundances on the host macroalgae surface was estimated by counting the density of frustules per unit area (mm^2) of the host substratum using a SEM. The results showed that both host kelp species harboured relatively low diatom densities. Juvenile specimens hosted more abundant and diverse diatom assemblages than adult thalli, with species belonging to *Navicula* and *Rhoicosphenia* genera contributing significantly to the observed dissimilarities in community composition. The low number of diatom taxa observed here could be due to the homogenous surface and uniform morphology of the examined kelps. Additionally, the low abundance values of epiphytic diatoms recorded on the host specimens could be the result of efficient kelp defence strategies, *e.g.* secretion of chemically active metabolites and/or sloughing of the outer layer of the thallus.

An assessment of the host species, age, and thallus part influence on kelp-associated diatoms was conducted in the fourth Chapter. The results presented in this chapter indicated that all investigated biotic factors, i.e., the host species, age, and different part of the thallus, significantly affected both the abundances and taxonomic composition of the kelp-associated diatom communities. Among these, the influences of the kelp thallus part and the host age were the most and least pronounced, respectively. The high residual variance (72%) indicated that other factors likely contributed to the diatom community structuring. However, since all kelp samples were collected on the same day from the same sampling spot, abiotic factors related to seasonality and hydrological conditions of the site should be excluded as a possible explanation for the observed patterns. Based on the SEM analysis of the kelp surface, we infer that both grazing and the kelp response to grazer-caused damage and biofouling by epiphytes, as well as specific life strategies employed by kelp-associated diatoms, may be responsible for a large portion of the unexplained variation.

Diatoms are known for their excellent bio-indication properties and have long been used in biomonitoring studies worldwide (Witon and Witkowski 2006; Walsh and Wepener 2009; Desianti *et al* 2017). In the future, kelp-associated diatoms may serve as indicators of the multifactorial changes occurring in the most vulnerable coastal ecosystems of Southern Africa. However, currently, little is known about either the kelp-associated diatom community composition or the interplay between biotic and abiotic factors affecting the epiphytic assemblages. This knowledge is required before accurate diatom-based indices of the marine habitat health can be developed. Therefore, future work should relate diatom community compositions and abundances on kelps, as well as other macroalgae (red, green and brown) collected from different sampling sites and seasons along the entire distributional range of South African kelp forest, to assess any qualitative and quantitative changes in community composition and structure due to variations in host morphology, sites and seasons. The effects of environmental conditions (*e.g.* temperature, light intensity, turbidity and wave intensity) prevailing at sampling sites should also be investigated.

References

It should be noted that this section contains all the references cited in Chapters 1, 2, 4 and 5 of this thesis. This means that some sources cited in Chapter 3 may not be found here. This has been done intentionally to maintain the distinction between the published work in this thesis (Chapter 3) and the other chapters not yet published.

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Plates

Most of the light micrographs were taken at 1000× magnification.

If otherwise the specific magnification appears next to the taxon name.

Plate 1

Fig. 1. C*occoneis costata var costata*

Fig. 2. *Cocconeis californica*

Fig. 3. *Cocconeis scutellum*

Fig. 4. *Pleurosigma marinum*

Fig. 5. *Navicula* cf. *ramosissima*

Fig. 6. *Pinnularia cfr rectangulata* (600×)

Fig. 7. *Pleurosigma intermedium*. (600×)

Fig. 8. *Diploneis crabro*

Fig. 9. *Achnanthes* sp. (cf. *A. brevipes* var. *intermedia)*

Fig. 10. *Cocconeis dirupta*

Scale bars = 10 μm


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Plate 2

Fig. 11. *Tabularia investiens*

Figs. 12, 18. *Navicula cfr johanrossii*

Fig. 13. *Rhoicosphenia flexa*

Figs. 14–15. *Rhoicosphenia genuflexa*

Figs. 16, 21. *Diploneis littoralis*

Fig. 17. *Diploneis papula*

Fig. 19. *Navicula ramosissima*

Fig. 20. *Pseudogomphonema kamtschaticum*

Figs. 22–23. *Cocconeis pseudomarginata.*

Scale bars = 10 µm.

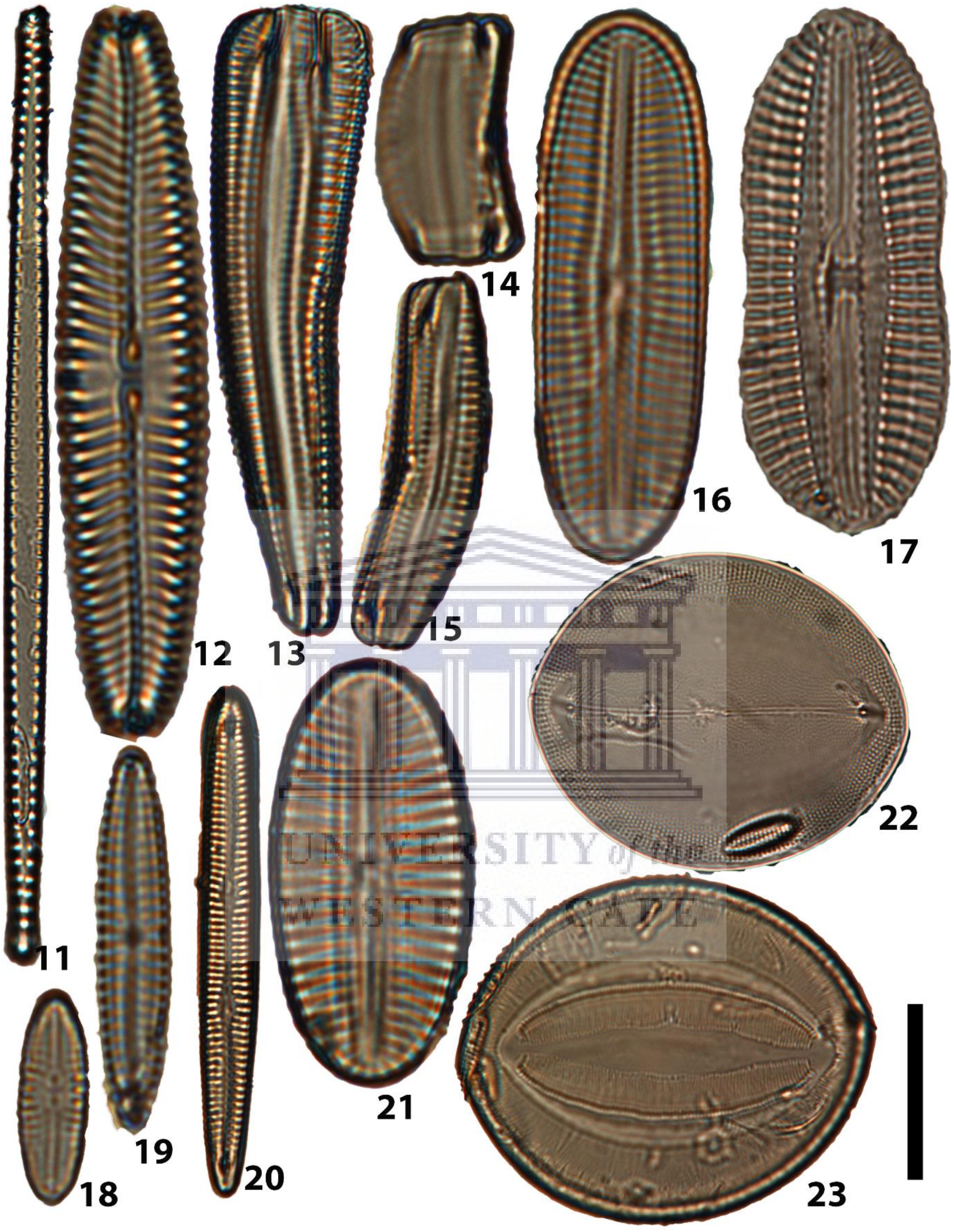
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Plate 3

Figs. 24, 25, 30. *Trachyneis aspera*

Fig. 26. *Grammatophora angulosa*

Fig. 27. *Grammatophora oceanica*

Fig. 28. *Grammatophora marina*

Fig. 29. *Fragilariopsis* sp.

Figs. 31–32. *Grammatophora angulosa* var. *islandica*

Fig. 33. *Cocconeis* sp.

Fig. 34. *Licmophora* sp.

Scale bars = 10 µm.

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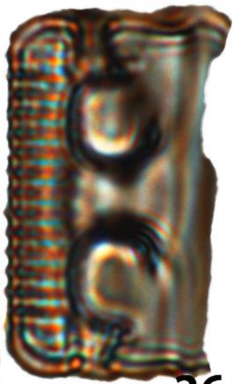

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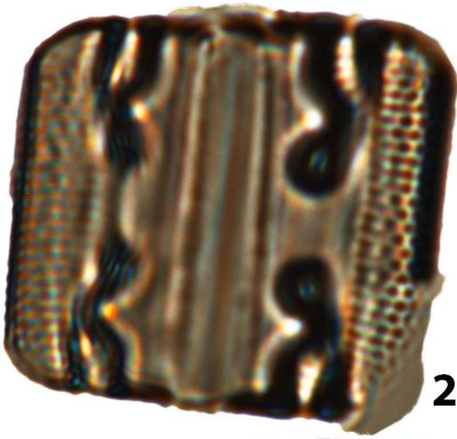

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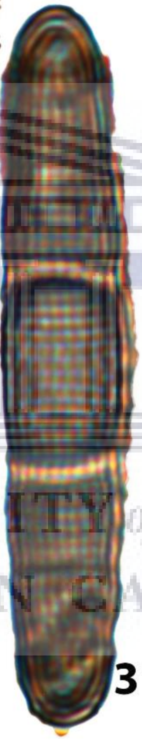

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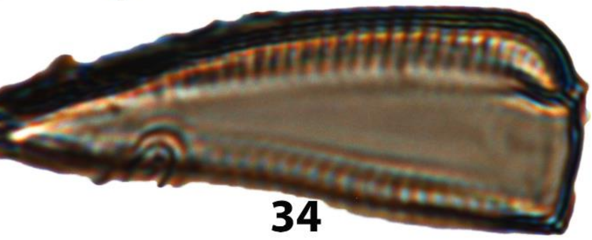

34

Plate 4

Fig. 35. *Amphora* cfr *proteoides*

Figs. 36, 39. *Amphora helenensis*

Fig. 37. *Amphora bigibba*

Fig. 40. *Halamphora luciae*

Fig. 38. *Asteromphalus* sp.

Fig. 41. *Actinoptychus* cfr *aster*

Fig. 42. *Trigonium* cfr *arcticum*

Fig. 43. *Delphineis* sp.

Fig. 44. *Campyloneis grevillea* var. *grevillei.*

Scale bars = 10 µm.

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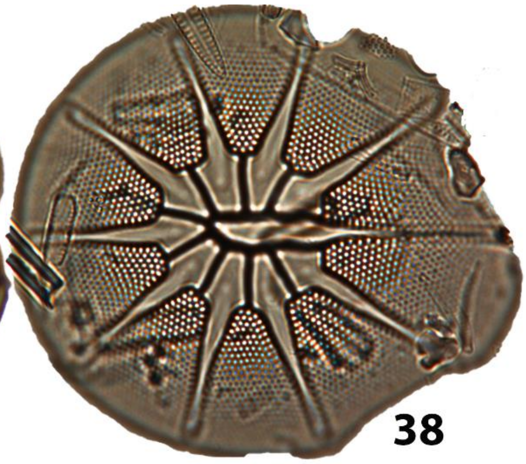

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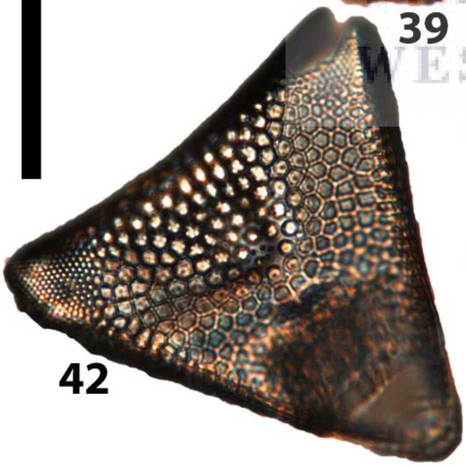

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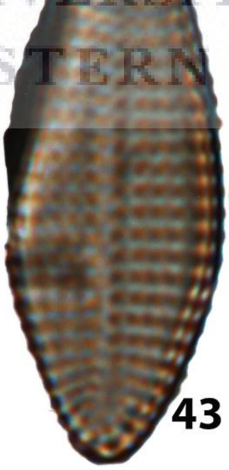

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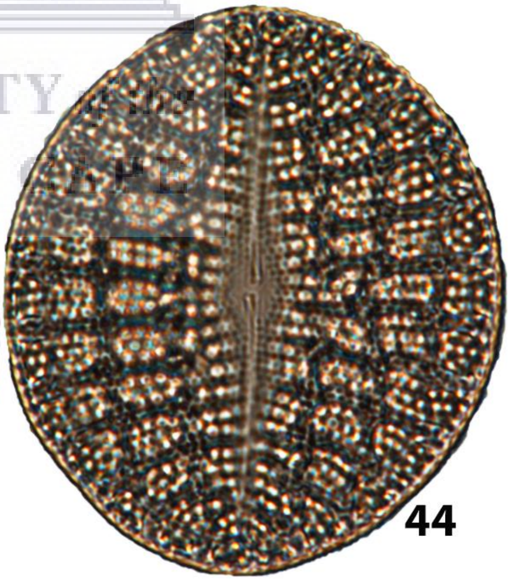

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Plate 5

Figs. 45, 46. *Craspedostauros* sp. (600×)

Fig. 47. *Mastogloia manokwariensis*

Fig. 48. *Planothidium* sp.

Fig. 49. *Nitzschia* sp1.

Fig. 50. *Nitzschia* sp2.

Fig. 51. *Nitzschia* sp3.

Fig. 52. *Hantschia marina*

Figs. 53-56. *Gomphoseptatum pseudoseptatum*

Fig. 57. *Cocconeis costata var. hexagona*

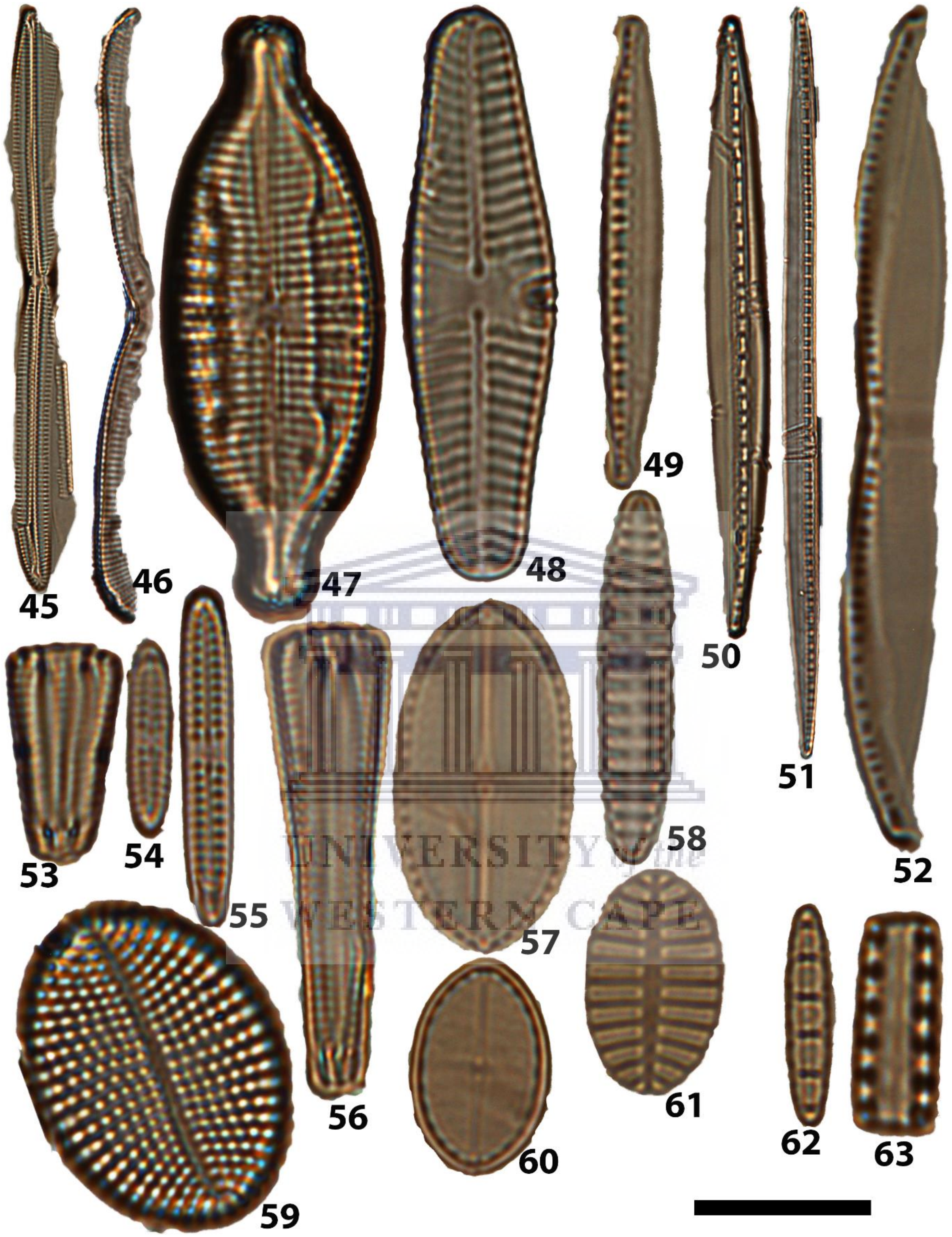
Fig. 58. *Opephora* cf. *pacifica*

Fig. 59. *Cocconeis* cfr *scutellum*

Fig. 60. *Cocconeis placentula*

Fig. 61. *Cocconeis costata var. costata*

Figs. 62–63. *Nagumoea* sp.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