VARIATION IN MORPHOLOGY AND ANATOMY OF *GRACILARIA VERRUCOSA*: IMPLICATIONS FOR SELECTION OF PHENOTYPES FOR CULTIVATION

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ABSTRACT

Cultivation of the economically important seaweed, *Gracilaria verrucosa*, on the west coast of Southern Africa, has received little attention despite the economic implications. To ensure maximum productivity from cultivation, it is essential that appropriate strains be selected. It is believed that the productivity of plants can be predicted from their surface:volume ratio. Therefore, the most productive strains can be selected on this basis. A study was undertaken to determine the extent of phenotypic variation between populations of *G. verrucosa* from St. Helena, and Saldanha Bay (west coast of Southern Africa) in order to establish whether phenotypes exist for cultivation. Anatomical and morphological features of plants at different depths were measured to give an indication of overall phenological variation. It was found that plants at St. Helena Bay were phenotypically distinct from those at Saldanha Bay. The St. Helena plants had a higher surface:volume ratio, and consequently were predicted to be suitable for productive cultivation. Phenotypic variation between cultivated plants at Saldanha Bay and Luderitz was also assessed. There was evidence that plants cultivated on rafts may also become phenotypically distinct. Although there has been doubt about whether plants at Luderitz are members of the species *Gracilaria verrucosa*, preliminary findings in this study suggest this is likely to be true. The phenotypic response to salinity and depth was also assessed. It was found that salinity plays a role in stunting plant growth, and thus also affects the phenotype of individuals in this species. The greatest phenotypic response to depth was found to be greatest in St. Helena plants, as these plants have to adapt their growth form to cope with murky water conditions.

INTRODUCTION

The red alga, *Gracilaria verrucosa*, is a member of one of the most economically important families of seaweeds- the Gracilar~~e~~iaceae (McLachlan and Bird 1986). In addition to being extensively used for agar production, *Gracilaria* has also been used for human consumption (Abbott 1988, as cited by Molloy 1992), energy production (Hanisak 1987, as cited by Molloy 1992) and feed for abalone (Santileces and Doty 1989).

The natural stocks of *Gracilaria* in South Africa and Namibia are among the world's most viable for commercial exploitation. Currently, the seaweed industry at Luderitz and Saldanha Bay depends on the beach cast of *Gracilaria* (Figure 1).

Figure 1: Harvesting of beach cast at Saldanha Bay

However, this beach cast is not constant through the year or from year to year (Molloy 1990). As a result, the only means to expand the industry is through cultivation (Molloy 1990). A consequence of cultivation is that strains with the highest growth rates and biomass production can be selected. Furthermore, as this species has extensive vegetative propagation, a selected plant can be maintained without genetic changes over a long period of time (Hanisak *et al.* 1988).

Such strain selection has been demonstrated by Hanisak *et al.* (1988) based on the functional-form model by Littler and Littler (1980). These authors determined morphological, physiological and ecological differences between strains within *Gracilaria tikvahiae*, to select the most appropriate strain for mariculture.

According to the model, the productivity of a species, or strain, can be predicted from their thallus form. A thallus-form with a high surface area:volume ratio is predicted to have rapid nutrient uptake, higher growth rates, and therefore more rapid production (Hanisak *et al.* 1990). However, continuous screening of strains is essential in this genus, because contrary to Hanisak *et al.* (1988), genetic changes in selected plants may occur over time. Recently, Santelices and Varela (1993) have found that clones of the same plant of *Gracilaria chilensis* may develop into different phenotypes under the same conditions.

To date, two systems of *Gracilaria* cultivation have been employed. The first is the floating cultivation system, where fragments of the plants are attached to ropes on rafts or barges (Figure 2).

Figure 2: Floating raft cultivation system at Saldanha bay

The second is the bottom cultivation system, where ropes are tied around polyethylene tubing and fixed to the substratum (Figure 3).

Figure 3: Bottom cultivation system at Saldanha Bay

Tufts of *Gracilaria* may also be pegged into the sediment with forks. In both cases, plants propagate vegetatively. Floating raft cultivation systems have been established at Saldanha Bay, and both floating and bottom cultivation systems have been established at Luderitz. However, to date, no work has been done on strain selection for cultivation purposes. Despite this, there is an indication that the raft grown algae may differ phenotypically from that of natural populations.

According to the function-form model, such phenotypic variation would affect productivity. Therefore, it would be of value to experimentally determine any phenotypic variation of raft samples compared to that of natural populations to assess the effect on productivity. To date, little attention has been paid to differences in the initial and eventual morphologies of transplants (Buschmann *et al.* 1992).

Phenotypic variation may also be high within and between natural plant populations. Thus, it is likely that distinct phenotypes exist in natural populations as well. If so, these could also be reviewed for possible strain selection.

In the past, this phenotypic variability and lack of tangible features (Bird *et al.* 1982) in the genus, makes *G. verrucosa* difficult to study, and there is much controversy concerning the identification of this species. In particular, it is extremely difficult to distinguish the genus, *Gracilariopsis*, from *G. verrucosa*-type seaweeds.

Such confusion is particularly the case along the Southern African Coast. Here, although one of the main distinguishing features between these two genera is the presence (in *Gracilariopsis*) or absence (in *Gracilaria)* of nutritive filaments in the cystocarp (Fredericq and Hommersand 1989), fertile material is rarely seen, and in Luderitz, no fertile material has ever been reported (Molloy 1990). Thus, although plants from this region are currently considered to be *G. verrucosa*, other methods, eg. genetics (Bird and Rice 1990), should be employed for confirmation of the identity of these plants. Furthermore, distinct phenotypes may be identified by evaluating the population variation between and within sites. These phenotypes could be taxonomically described, and thereby facilitate the identification of this species.

In light of this taxonomic confusion and the need for strain selection, a study is warranted to determine the extent of phenotypic variation in *G. verrucosa* within and between natural populations and cultivated plants. An assessment of the nature of the factors affecting variation would also be beneficial. Such a study has recently been undertaken. Various morphological and anatomical features of plants from Saldanha Bay, St. Helena Bay and Luderitz were assessed and compared in an attempt to address the following issues: the extent of phenotypic variation of *Gracilaria* between natural and cultivated plants; the existence of possible phenotypes within and between natural and cultivated plants; whether plants from Luderitz are members of the species, *Gracilaria verrucosa*; the effect of depth and salinity on *Gracilaria* and finally, the implications for selecting the most productive phenotypes for cultivation.

MATERIALS AND METHODS

Study sites

The Luderitz Bay system is essentially divided into Luderitz Bay, Luderitz Lagoon and Shearwater Bay (Figure 4).

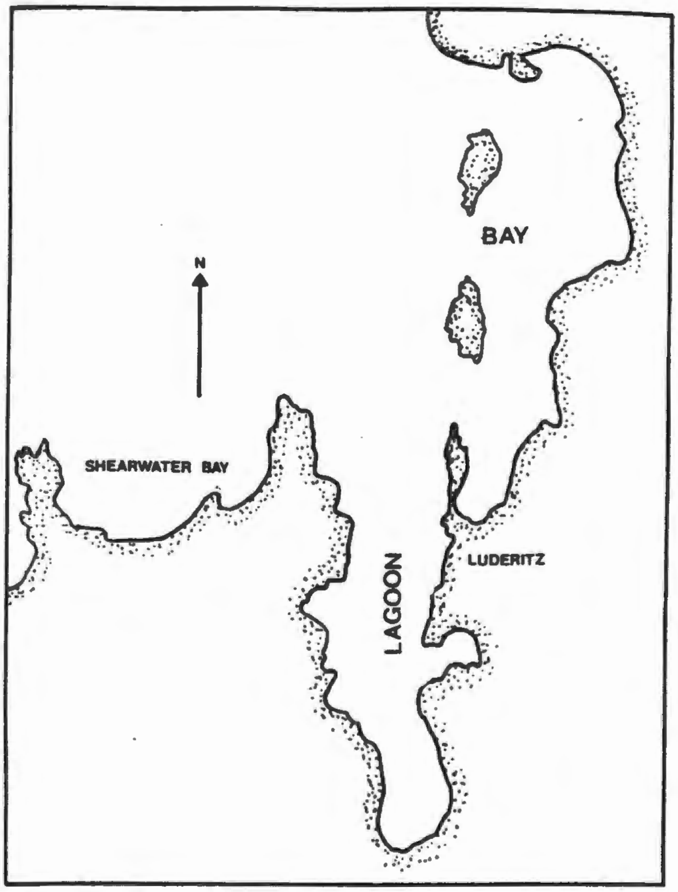


Figure 4: The Luderitz Lagoon and Bay system on the western coast of Southern Africa

From Molloy (1992)

Samples from this study were collected from the Luderitz Lagoon (26°39'S), and only cultivation material was collected. This material was approximately a month old when it was collected in March. Four samples were collected from floating raft systems (sites A, B, C and E) at approximately 0.5m below the water surface. Two other samples were collected from bottom raft systems at approximately 0.2m (site F) and 0.6m (site D) below Chart Datum.

Saldanha Bay, on the West Coast of Southern Africa, is the northern part of a larger embayment known as the Saldanha Bay/Langebaan complex (Figure 5).

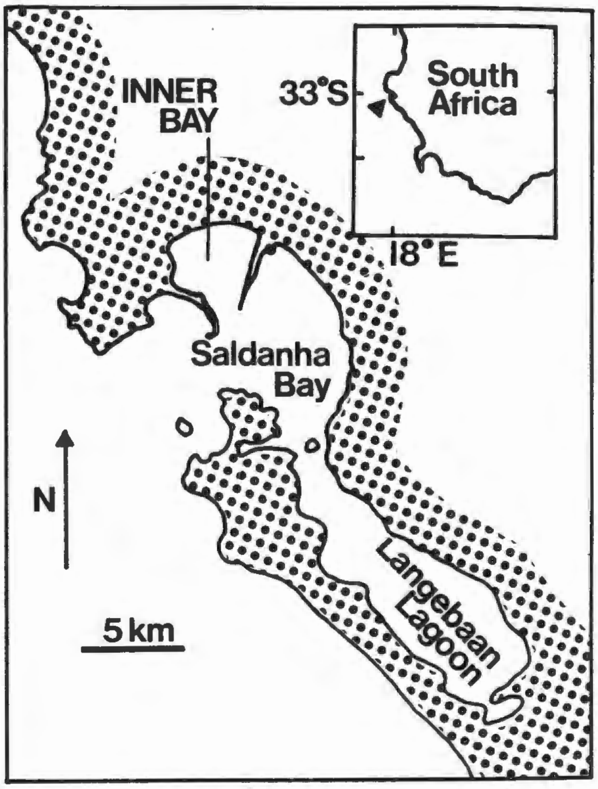


Figure 5: The Saldanha Bay/Langebaan Lagoon embayment on the western coast of South Africa

From Anderson *et al.* (1993)

It opens to the sea via a wide mouth. The western shores of the Bay are rocky and relatively steep to between 5 and 10m depth. The northern and eastern shores are sandy and slope gradually into the sea. Samples were collected from Inner Bay (Figure 5) in March at a shallow depth of 2.5, at intermediate depths of 4m and 6m, and a deep depth of 8m. Floating raft samples (approximately 0.5m below the water surface) were also collected. Analyses of these cultivated plants was comparable to that of Luderitz plants as the material was also only a month old.

The area sampled at St. Helena Bay (Figure 6) (latitude 32° 45'50" and longitude 18° 08'

36"; Figure 7) is essentially shallow with a sandy substratum.

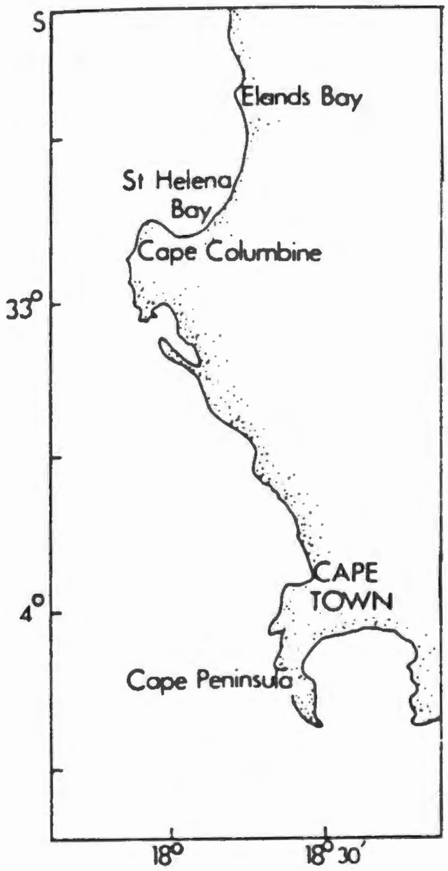


Figure 6: General location of St. Helena Bay on the western coast of Southern Africa

From Pitcher (1988)

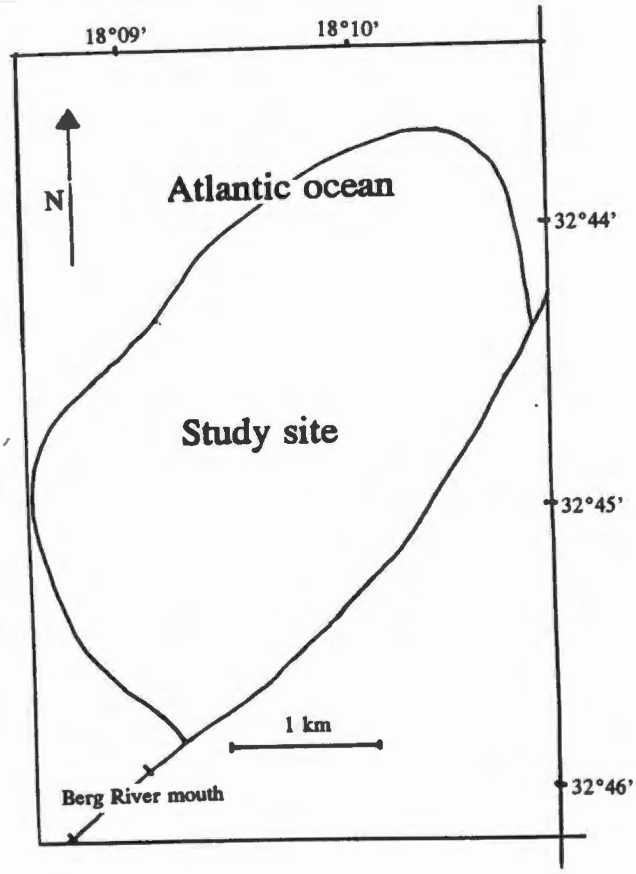


Figure 7: Study site at St. Helena Bay

Sedimentation from the Berg River mouth (Figure 7) is responsible for the often murky waters at this site. Samples were collected in May from depths of 3, 6 and 8m. As there are no current cultivation experiments at St. Helena Bay, no cultivation material could be collected. The Berg River Mouth, at 4m was also sampled so that the phenotypic response to salinity effects could be assessed.

Collection procedures

Random sampling at the various depths was achieved by throwing a $1m^2$ core sampler off a boat. All material in the core sampler was collected. Material from floating cultivation systems was randomly collected off raft ropes, and material from bottom cultivation systems was randomly collected off ropes tied around polyethylene tubing. Samples were brought back to the lab and preserved in 5% formalin in seawater. As prolonged exposure to light is known to result in bleaching of the plants, all samples were stored in the dark until analysis was commenced. Luderitz samples were freighted to Cape Town under these appropriate conditions.

Assessment of variation

In an attempt to establish variation within and between sites, both anatomical and morphological variation was assessed. Because *G. verrucosa* tends to grow as a tangled mass on the surface of the substratum (Figure 8), plants are often broken in the collection process.

Figure 8: Typical tangled growth mass of *Gracilaria verrucosa* on the substratum

Since *G. verrucosa* lacks a distinct holdfast, it was difficult to distinguish whole plants from broken plants or even branches of plants. However, since any portion of *G. verrucosa* has the potential to grow vegetatively into a new plant, it was assumed that broken portions could be treated as individual plants.

Morphological variation

Despite the great phenotypic variability in this species, *G. verrucosa* has a relatively simple morphology. Essentially it has a main axis which gives rise to short (0-1cm), medium (>1-10cm) and long branches (>10cm). In this study, eight morphological features were measured: 1. the **total length** of the plant; taken as a measure of the main axis length 2. the length of the **primary branch**; measured as the first long branch from the bottom of the plant 3. the length of the **secondary branch**; taken as the longest branch on the primary branch 4. the **base length**; taken as the length from the bottom of the plant to the first primary branch 5. the **tip length**; taken as the length from the last long or medium branch, to the top of the plant 6. the total number of short branches on the main axis 7. the total number of medium branches on the main axis 8. the total number of long branches on the main axis. Accuracy of measurements was achieved by marking 5cm intervals on the main axes with string before measuring. The number of plants that were measured depended on the number of intact plants in each sample. This ranged from 3-21 plants.

Averages were obtained for the above parameters, and data analysis was performed using the spreadsheet and statistical functioning programme, Quattro Pro. Zar (1984) was consulted for statistical analyses.

Anatomical variation

Using a Wetzlar Freeze-microtome, 5µm cross sections of the main axis, the tip and the primary branch were cut. Only one plant was sectioned for each depth and raft site, and measurements from five sections were averaged per plant part. The following parameters were measured: total diameter of cross section; cortical width; medulla width; cuticle width; number of cortical cells and number of medulla cells. The main axis, tip and primary branch of a *Gracilariopsis* plant was also sectioned to establish any obvious anatomical differences between this species, and *Gracilaria verrucosa*. This specimen was obtained from Luderitz at a depth of 0.5m. The presence of fertile material in this plant allowed for positive identification, based on the presence of nutritive filaments (as discussed above). Data analysis was done using Quattro Pro and Zar (1984) was consulted for statistical analyses.

RESULTS

Phenological comparison of naturally growing *G. verrucosa* from Saldanha Bay and St. Helena Bay

more ?

The branching pattern was fairly constant in all plants. In general, plants had the most short branches, followed by medium and then long branches (Figure 9). However, the degree of this branching pattern changed between plants from Saldanha and St. Helena Bay. Plants at St. Helena Bay had the most short and medium branches at all depths, and the most longer branches at mid and deeper depths (Figure 9).

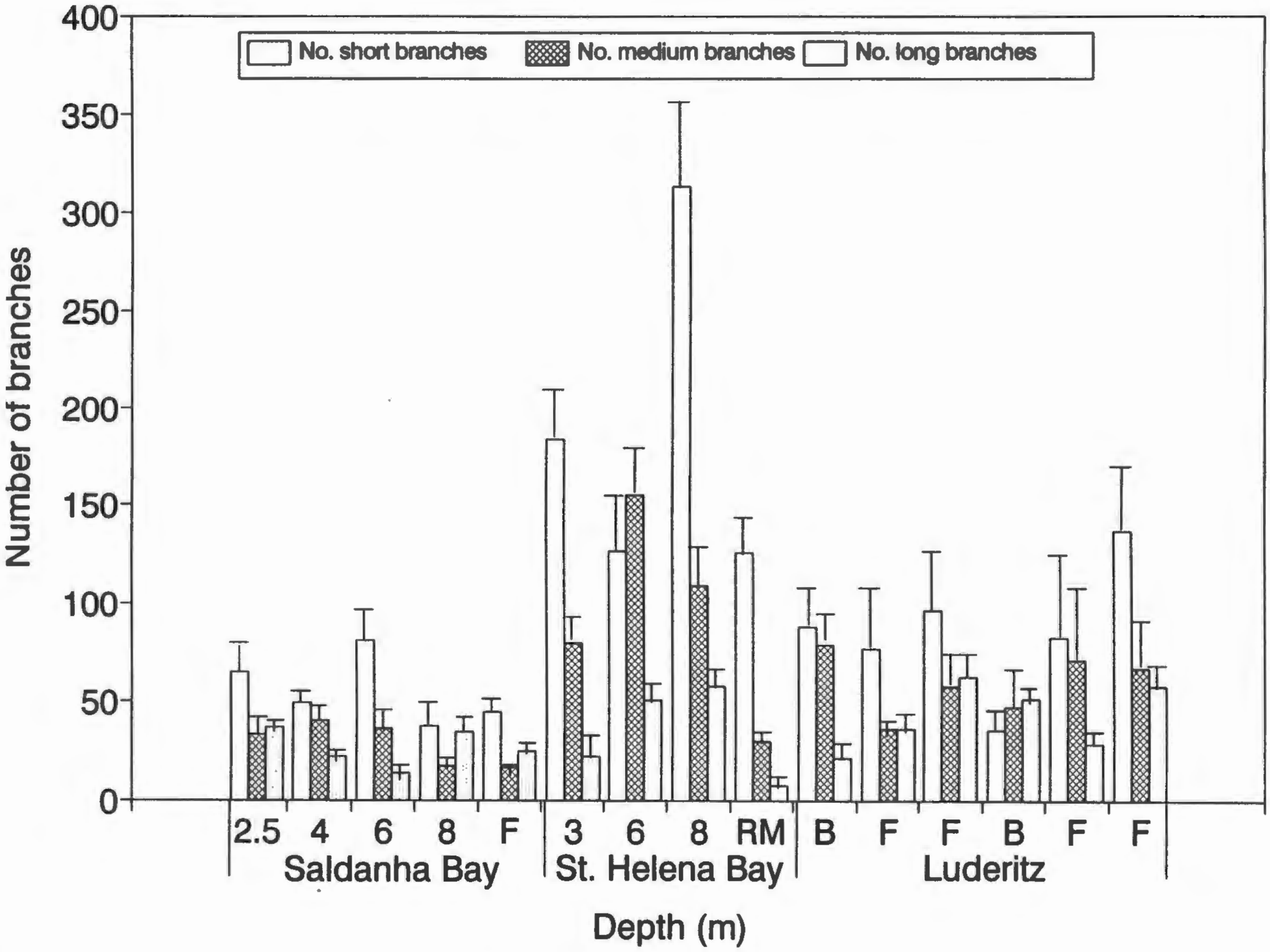


Figure 9: Number of short, medium and long branches of plants at Saldanha Bay, St. Helena Bay and Luderitz at various depths. Saldanha Bay: 2.5m, n=7; 4m, n=20; 6m, n=13; 8m, n=7; F (Raft 0.5m), n=24. St. Helena Bay: 3m, n=5; 6m, n=5; 8m, n=5; RM (River Mouth), n=20. Luderitz: B (Site F) 0.2m, n=5; F (Site B) 0.5m, n=5; F (Site C) 0.5m, n=6; B (Site D) 0.6m, n=5; F (Site E) n=5, 0.5m; F (Site A) 0.5m, n=5

The distinct phenotypic differences between the two sites is also clear when other morphological attributes are compared. For example, the plants from Saldanha Bay had much longer secondary branches than those at St. Helena Bay (Figure 10).

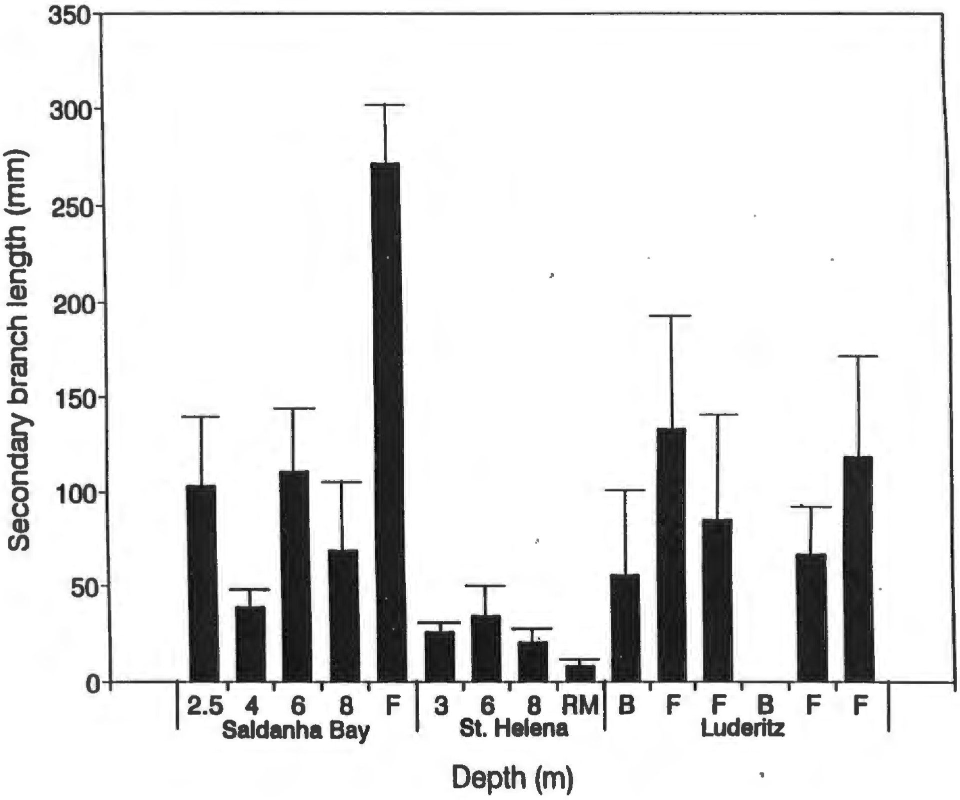


Figure 10: Secondary branch length of plants at Saldanha Bay, St. Helena Bay and Luderitz at various depths. Saldanha Bay: 2.5m, n=7; 4m, n=20; 6m, n=13; 8m, n=7; F (Raft 0.5m), n=24. St. Helena Bay: 3m, n=5; 6m, n=5; 8m, n=5; RM (River Mouth), n=20. Luderitz: B (Site F) 0.2m, n=5; F (Site B) 0.5m, n=5; F (Site C) 0.5m, n=6; B (Site D) 0.6m, no data; F (Site E) n=5, 0.5m; F (Site A) 0.5m, n=5

The primary branches of plants at comparable depths from St. Helena, were shorter than those of Saldanha Bay plants (Figure 11).

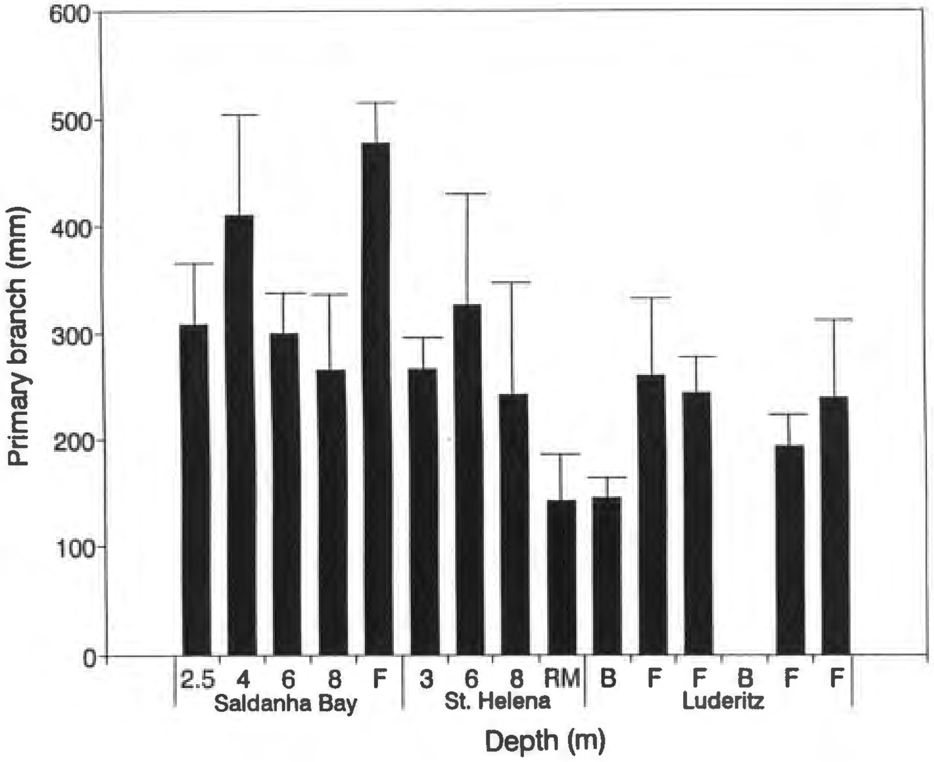


Figure 11: Primary branch length of plants at Saldanha Bay, St. Helena Bay and Luderitz at various depths. Saldanha Bay: 2.5m, n=7; 4m, n=20; 6m, n=13; 8m, n=7; F (Raft 0.5m), n=24. St. Helena Bay: 3m, n=5; 6m, n=5; 8m, n=5; RM (River Mouth), n=20. Luderitz: B (Site F) 0.2m, n=5; F (Site B) 0.5m, n=5; F (Site C) 0.5m, n=6; B (Site D) 0.6m, no data; F (Site E) n=5, 0.5m; F (Site A) 0.5m, n=5

The tips of Saldanha plants were also longer than those from St. Helena Bay (Figure 12). However, at both sites, tip lengths were greatest at mid depths.

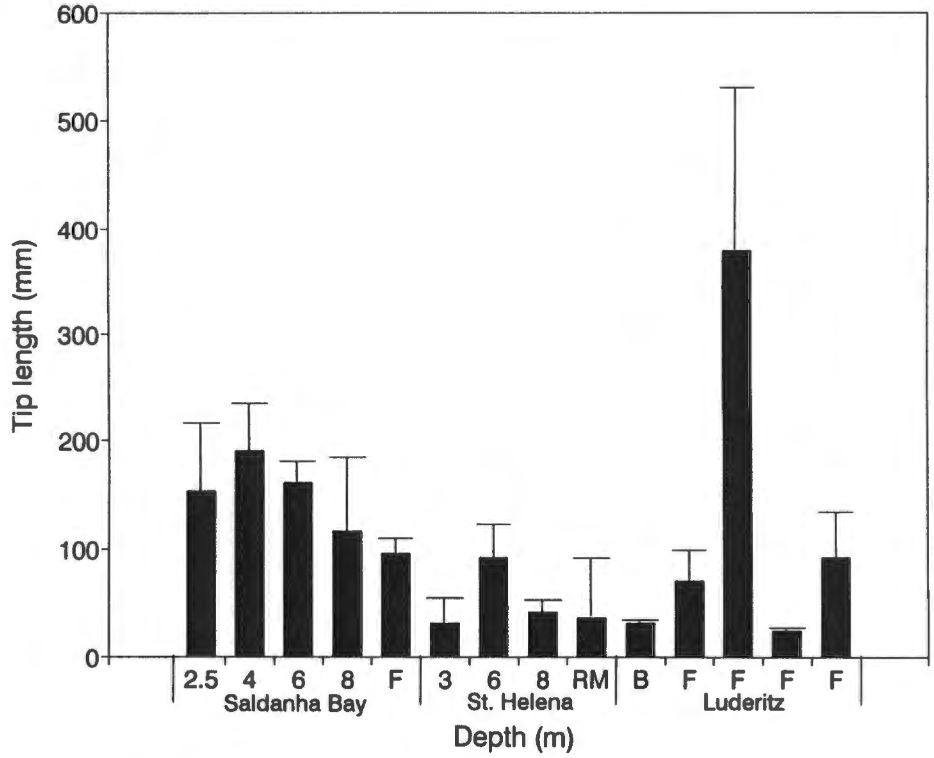


Figure 12: Tip length of plants at Saldanha Bay, St. Helena Bay and Luderitz at various depths. Saldanha Bay: 2.5m, n=7; 4m, n=20; 6m, n=13; 8m, n=7; F (Raft 0.5m), n=24. St. Helena Bay: 3m, n=5; 6m, n=5; 8m, n=5; RM (River Mouth), n=20. Luderitz: B (Site F) 0.2m, n=5; F (Site B) 0.5m, n=5; F (Site C) 0.5m, n=6; F (Site E) n=5, 0.5m; F (Site A) 0.5m, n=5. No data for site D

Plants from St. Helena bay were much longer than those from Saldanha Bay (Figure 13).

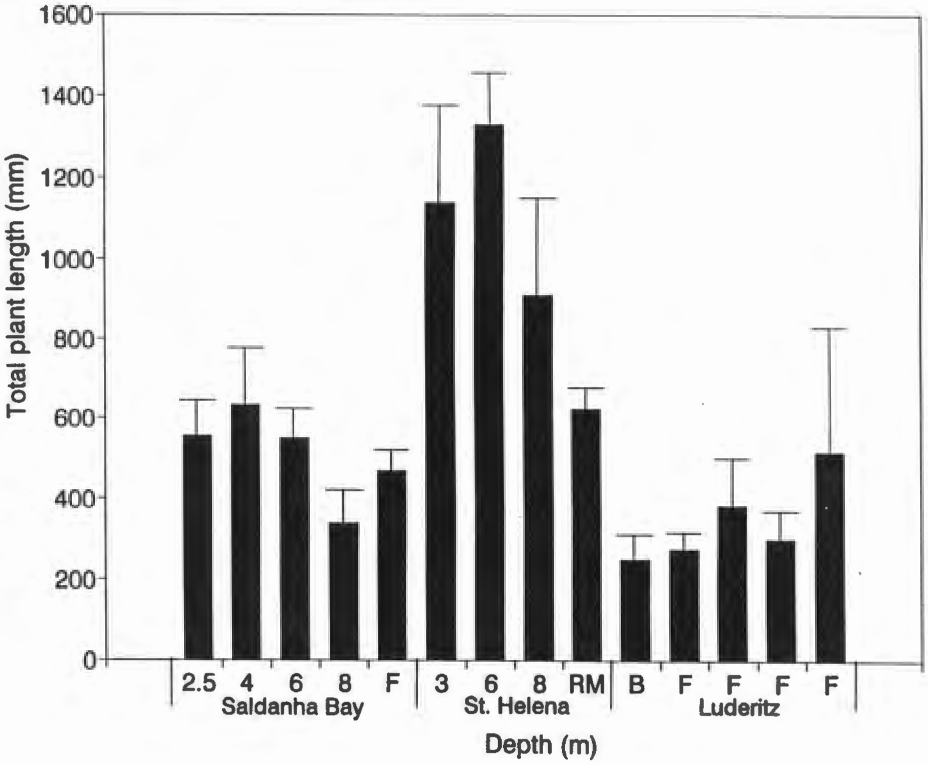


Figure 13: Total plant length of plants at Saldanha Bay, St. Helena Bay and Luderitz at various depths. Saldanha Bay: 2.5m, n=7; 4m, n=20; 6m, n=13; 8m, n=7; F (Raft 0.5m), n=24. St. Helena Bay: 3m, n=5; 6m, n=5; 8m, n=5; RM (River Mouth), n=20. Luderitz: B (Site F) 0.2m, n=5; F (Site B) 0.5m, n=5; F (Site C) 0.5m, n=6; F (Site E) n=5, 0.5m; F (Site A) 0.5m, n=5. No data for site D

Plant weight was clearly not related to plant length, because the long plants from St. Helena Bay did not weigh more than shorter plants from Luderitz and Saldanha Bay (Figure 14).

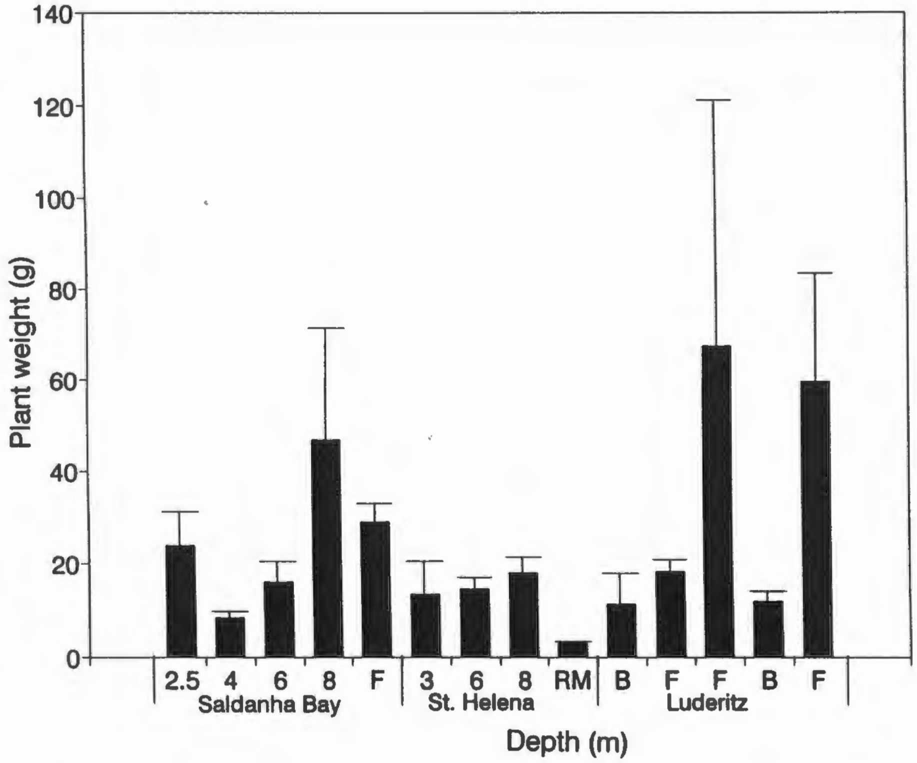


Figure 14: Weight of plants at Saldanha Bay, St. Helena Bay and Luderitz at various depths. Saldanha Bay: 2.5m, n=7; 4m, n=20; 6m, n=13; 8m, n=7; F (Raft 0.5m), n=24. St. Helena Bay: 3m, n=5; 6m, n=5; 8m, n=5; RM (River Mouth), n=20. Luderitz: B (Site F) 0.2m, n=5; F (Site B) 0.5m, n=5; F (Site C) 0.5m, n=6; B (Site D) 0.6m, n=5; F (Site E) n=5, 0.5m; F (Site A) 0.5m, n=5

In fact, deep plants from Saldanha Bay, as well as plants from sites 3 and 5 at Luderitz, weighed more than St. Helena plants (Figure 14).

There are also certain anatomical differences that distinguish St. Helena plants from those of Saldanha Bay. Firstly, plants from St. Helena Bay had smaller cortex diameters (Figure 15), medulla diameters (Figure 16) and total diameters (Figure 17) of their tips and primary branches.

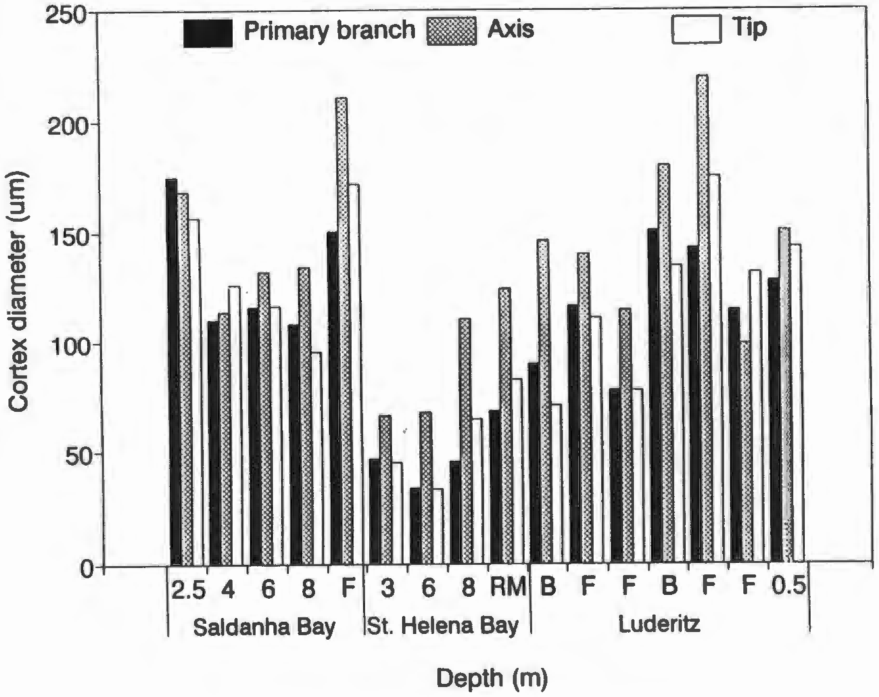


Figure 15: Cortex diameter of the primary branch, axis and tip of plants from Saldanha Bay, St. Helena Bay and Luderitz at various depths. n=1 for all plant; RM (River Mouth). Luderitz: B=Site F; F=Site B; F=Site C; B=Site D; F=Site E; F=Site A; 0.5=*Gracilariopsis*

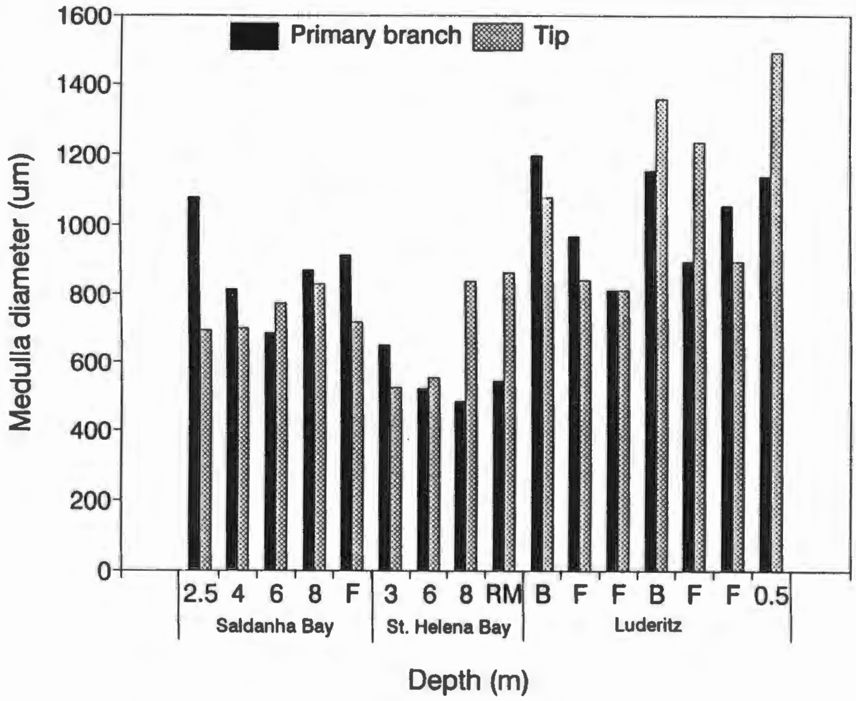


Figure 16: Medulla diameter of the primary branch and tip of plants from Saldanha Bay, St. Helena Bay and Luderitz at various depths. n=1 for all plants; RM (River Mouth). Luderitz: B=Site F; F=Site B; F=Site C; B=Site D; F=Site E; F=Site A; 0.5=*Gracilariopsis*

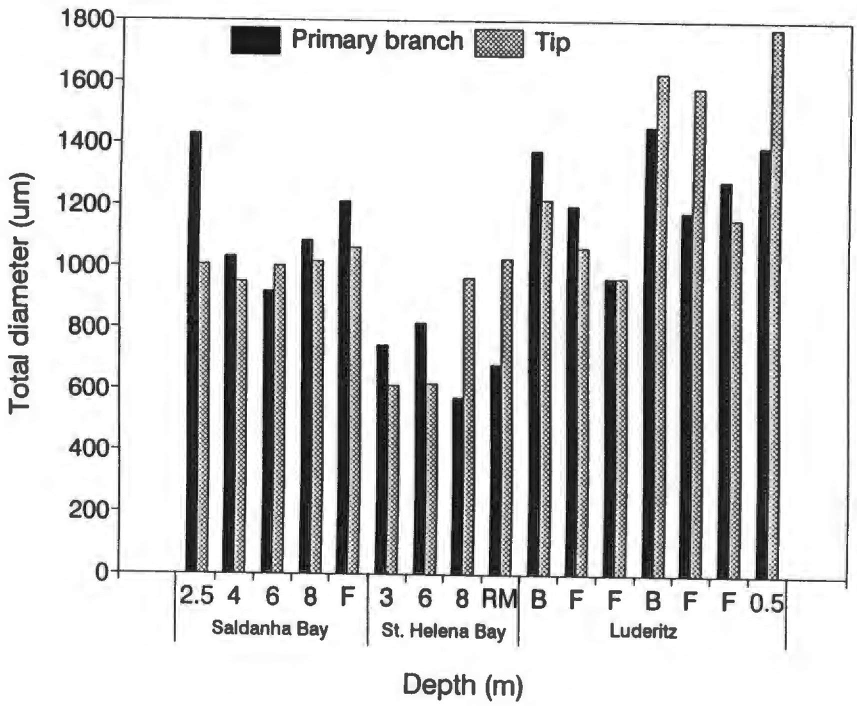


Figure 17: Total diameter of the primary branch and tip of plants from Saldanha Bay, St. Helena Bay and Luderitz at various depths. n=1 for all plants; RM (River Mouth). Luderitz: B=Site F; F=Site B; F=Site C; B=Site D; F=Site E; F=Site A; 0.5=*Gracilariopsis*

However, while the cortex diameters of their axes was also small (Figure 15), plants from St. Helena Bay had large medulla diameters of their axes (Figure 18); larger than the medulla diameters of the primary branches and tips.

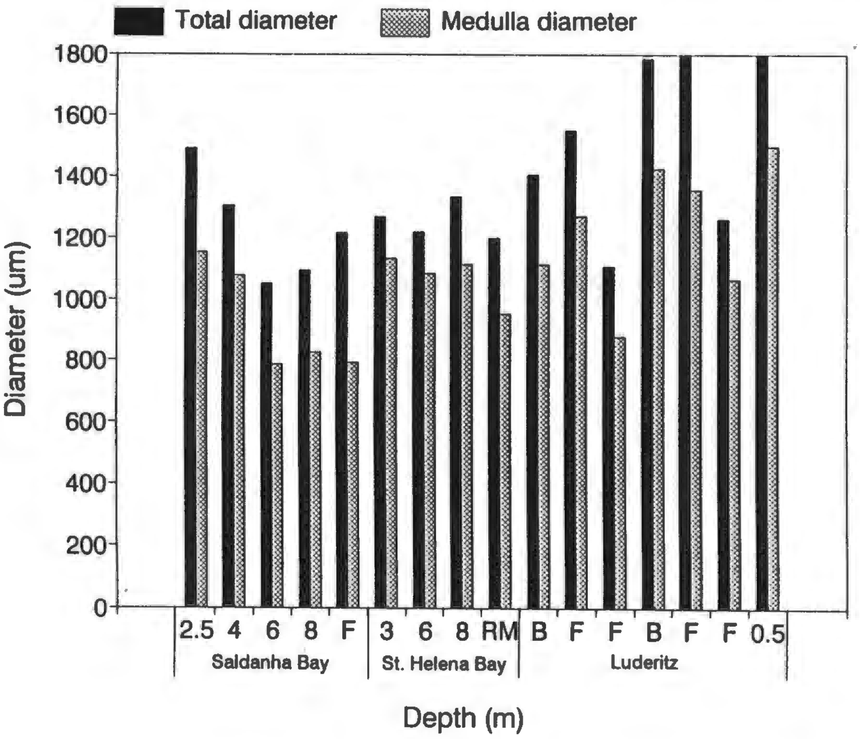


Figure 18: Total diameter and medulla diameter of the axes of plants from Saldanha Bay, St. Helena Bay and Luderitz at various depths. n=1 for all plants; RM (River Mouth). Luderitz: B=Site F; F=Site B; F=Site C; B=Site D; F=Site E; F=Site A; 0.5=*Gracilariopsis*

Thus, the axes of these plants had a large medulla:cortex ratio (Table 1), with a total diameter similar to that of the axes of other Saldanha Bay plants (Figure 18)

Table 1: The Medulla:Cortex ratios of *G. verrucosa* plants from different from Saldanha Bay, St. Helena Bay and Luderitz. The Medulla:Cortex ratio for *Gracilariopsis* is included for comparison. Ratios were obtained by averaging the ratios from all depths at each site

Medulla:Cortex Ratio

	Primary branch	Axis	Tip
Saldanha Bay	3	3.5	3.1
Saldanha Raft	3	3	3
St. Helena	5.1	7.8	6.3
River Mouth	4.0	3.8	5.2
Luderitz	4.6	4.8	4.2
Gracilariop.	4.46	5.2	5

A correlation analysis revealed that there was a close relationship between the medulla and cortex diameters in the tips (correlation coefficient=0.73, n=15; $p<0.05$) and primary branches (correlation coefficient=0.92, n=15; $p<0.01$). This stats? was significantly similar for all sites. Thus, while the cortex and medulla diameters were much smaller in the St. Helena plants, the medulla:cortex ratio in their tips and primary branches was similar to that in plants from other sites (Table 1). The medulla:cortex ratios of the axes were higher in plants from St. Helena Bay (Table 1). Since there were no significant differences between the number of cortical and medulla cells between plants at all parts of the plants, it is clear that the diameter size was a function of the relative sizes of, rather than the number of cells.

In plants from Saldanha Bay, and St. Helena Bay, the axes had largest total diameters (Figure 18). The primary branches were generally the next thickest branches in Saldanha Bay plants and in plants from shallow and mid depths at St. Helena (Figure 17). However, the tips were thicker than the primary branches at deep sites at St. Helena.

Analysis of other anatomical features reveals that plants from Saldanha Bay generally had larger axis cuticles, and to a lesser extent, primary branch and tip cuticles than plants from St. Helena Bay (Figure 19).

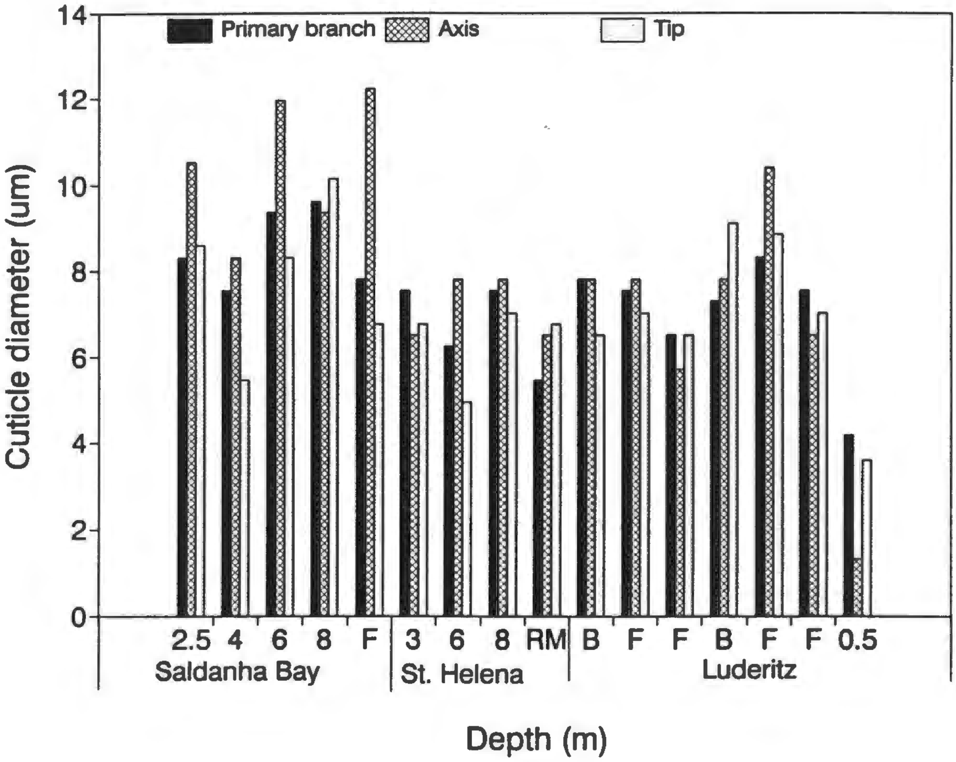


Figure 19: Cuticle diameter of the primary branch, axis and tip of plants from Saldanha Bay, St. Helena Bay and Luderitz at various depths. n=1 for all plants; RM (River Mouth). Luderitz: B=Site F; F=Site B; F=Site C; B=Site D; F=Site E; F=Site A; 0.5=*Gracilariopsis*

Phenotypic response of *G. verrucosa* to the effect of salinity

Plants from the river mouth were also phenotypically distinct with regard to certain morphological and anatomical features. Firstly, although they had the same branching pattern as other St. Helena plants, ie. more short than medium branches, and more medium than long branches, they were distinct in the degree of branching. They mainly had short branches, with very few medium and long branches (Figure 9).

These plants also had the shortest secondary and primary branches (Figures 10 and 11) compared to most other plants. In general, they seemed to have stunted growth, because they were also shorter and weighed less than other St. Helena Bay plants (Figures 13 and 14). Their tips lengths were only different to the tip lengths of other St. Helena plants at mid depths (Figure 12)

In terms of their anatomy, river mouth plants had larger cortex diameters in all parts of the plant compared to other St. Helena plants (Figure 1 5). The medulla (Figure 16) and thus, total diameters (Figure 17) of the tips of these plants were also much larger. However, the medulla (Figure 16) and total diameters (Figure 17) of the primary branches were similar to other St. Helena Bay plants.

Nevertheless, these plants still had similar medulla:cortex ratios of both the tips (correlation coefficient=7.3 0.73?, $n=15$; $p<0.05$) and primary branches (correlation coefficient=9.2 0.92?, >1 ? $n=15$;$p<0.01$) (Table 1) as other St. Helena Bay plants. However, their medulla:cortex ratios of their axes were not as high (Table 1), and they had smaller total axes diameters (Figure 18). For tips, primary branches and axes, the number of medulla and cortical cells were similar in all other plants. Thus, as in other plants, the medulla:cortex ratio was a function of the relative sizes, rather than the number of these cells. The cuticle diameter was not different from other St. Helena Bay plants (Figure 19).

Effect of depth on the growth form of *Gracilaria verrucosa*

There were significant correlations of certain morphological and anatomical characteristics with depth in St. Helena plants. Firstly, they were found to have the most short branches (correlation coefficient=-0.5, n=15; $p<0.05$) at a deeper depth. Long branches were also more numerous at a deeper depth (correlation coefficient=0.7, n=15; $p<0.002$), and the most number of medium branches occurred at a mid depth (correlation coefficient=0.53, n=15; $p<0.05$) (Figure 9).

The longest plants (correlation coefficient=0.53, n=15; $p<0.05$) (Figure 12), longest secondary branches (correlation coefficient=-0.57, n=15; p,0.01) (Figure 10), longest primary branches (Figure 11), and longest tips (Figure 13) were also found at a mid depth. The total axis was thinnest at mid depths (correlation coefficient=0.5, n=15; $p<0.05$) (Figure 10), and the cuticle of the tip and primary branch were also thinnest at mid depths (Figure 19). Plants had generally thicker tips at deeper depths (correlation coefficient=0.8, n=15; $p<0.001$) (Figure 9), and plant weight also increased with depth (correlation coefficient=0.5, n=15; $p<0.05$) (Figure 14).

Trends with depth were not as distinct in Saldanha Bay plants, and a correlation was only found for the number of medium branches versus depth, where this was maximal at 6m (correlation coefficient=-0.23, n=47; p=0.1).

Phenotypic differences between Gracilaria verrucosa from the natural populations and those from cultivation

In general, no distinct differences could be found in the morphology and the anatomy of bottom versus raft cultivated plants. The branching pattern of naturally growing Saldanha and St. Helena plants does not seem to be as distinct in Luderitz and cultivated Saldanha plants (Figure 1). All plants have the most short branches. However, the branching pattern of the medium and long branches is variable between sites.

Luderitz plants seem distinct from other Saldanha cultivation and natural population plants in their degree of branching (Figure 9). They had more short, medium and long branches than Saldanha plants, but fewer branches than plants at St. Helena Bay.

Other evidence of dissimilar morphology between the Saldanha and Luderitz cultivation material, and natural population plants, is found in that Saldanha Bay raft samples had much longer primary and secondary branches (Figures 11 and 10) than any plants. With respect to the secondary branch length, plants from Luderitz were similar to naturally growing plants from Saldanha. However, as with the Saldanha raft material, they had much longer secondary branches than those of St. Helena plants (Figure 10). They did not differ much from naturally growing Saldanha and St. Helena plants with respect to the length of primary branches (Figure 11).

Plants at Site C at Luderitz had phenomenally long tips (Figure 4). However, the tip lengths of the other plants at Luderitz as well as the cultivation plants at Saldanha Bay had shorter tips than the naturally growing Saldanha Bay plants. On the other hand, they were generally similar to the St. Helena plants with respect to tip lengths.

Plants were also generally smaller than cultivation plants of Saldanha Bay (Figure ?), and in general, cultivation material was shorter than plants of natural populations (Figure 13). When considering the weight of cultivation material, it is obvious that this varies within sites, and between cultivation material from Luderitz and Saldanha Bay (Figure 14). Sites C and A at Luderitz had the heaviest plants.

No differences were found between the cuticle diameter, and the number of cortical or medulla cells of branches, tips and axes of cultivation material and natural populations. However, Luderitz material tended to have larger tip, primary branch and axial diameters than that of natural populations (Figure 18). Thus, in general, they were the thickest plants, and even their medulla:cortex ratios of the various plant parts were slightly higher than that of other plants (Table 1).

Although a big cortex diameter was characteristic of the tips and primary branches of Saldanha Bay cultivation plants (Figure 15), their medulla diameters in the tips and primary branches were not as big as those of Luderitz (Figure 16). Thus, although they had large tip and primary branch diameters (Figure 17), these were of similar size to other Saldanha Bay plants. As a result, they had a medulla:cortex ratio which was similar to other Saldanha plants, but lower than that of the Luderitz material.

Nevertheless, the cortex:medulla ratio of the primary branches (correlation coefficient=0.92) and the tip (correlation coefficient=0.73) of all cultivation material was still significantly similar to that of other plants. And, cultivation plants from Luderitz and Saldanha Bay also had similar medulla:cortex ratios in their axes compared to other Saldanha Bay plants. Thus, as in other plants, the medulla:cortex ratio is a function of the relative sizes of the medulla and cortical cells, rather than the number of cells.

The cuticle diameters of cultivation plants were similar to that in natural populations (Figure 19), although Saldanha raft plants tended to have larger cuticle diameters of their main axes.

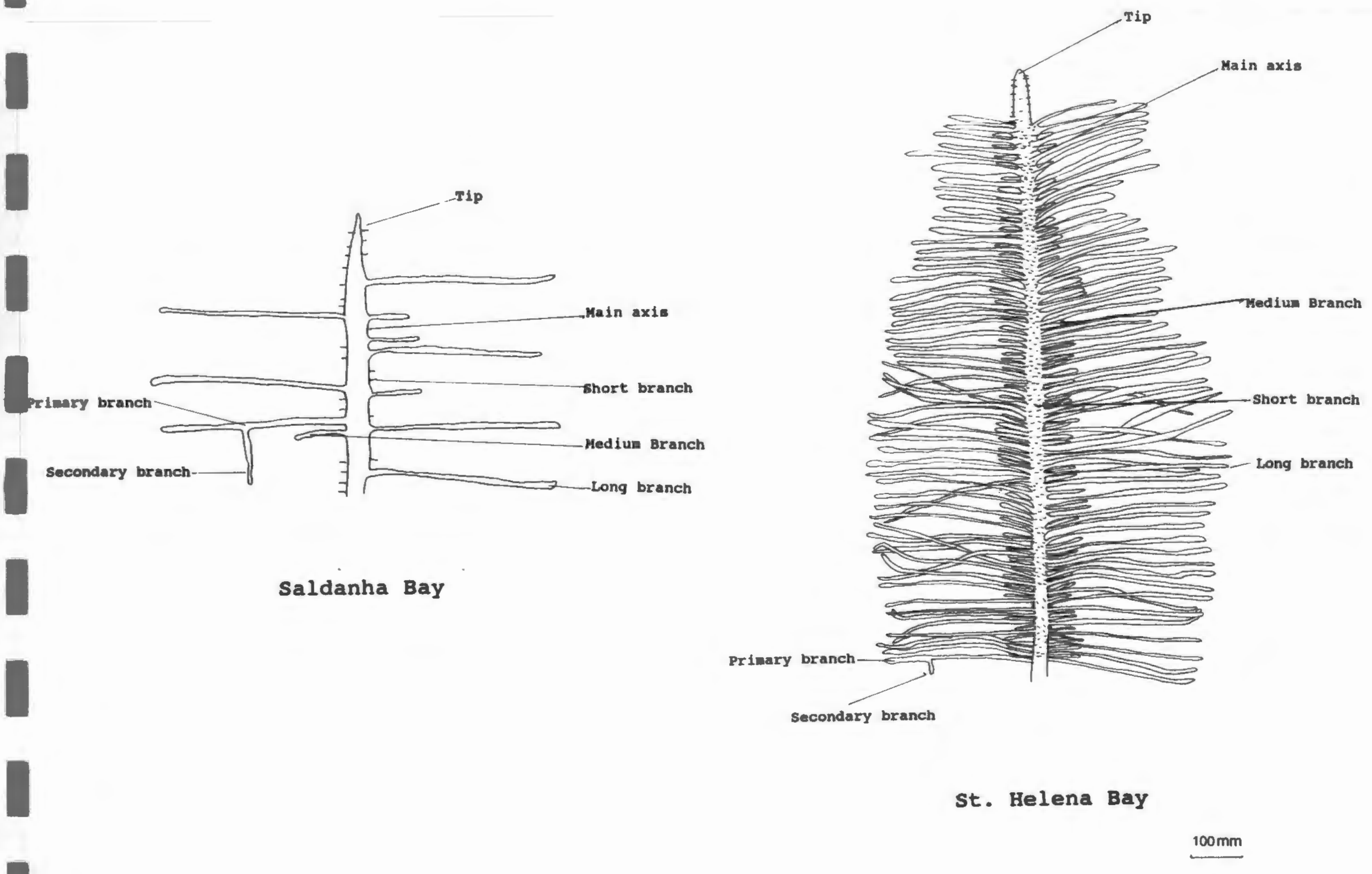
Anatomical differences between *Gracilariopsis* and *Gracilaria verrucosa*

Gracilariopsis differed from all other plants in some anatomical respects, but was similar in others. It had the largest tip (Figure 17) and main axis (Figure 18) diameters. Furthermore, the primary branches were also quite thick in comparison to other plants (Figure 17). The axes, tips and branches of these plants also had noticeably thinner cuticles (Figure 19). However, the medulla:cortex ratio of the primary branches (correlation coefficient=0.92) and tips (correlation coefficient=0.73) is similar to that of St. Helena and Saldanha Bay plants. Also, the ratio in the main axis, appeared similar to that of Saldanha Bay plants. Thus, as in other plants, the medulla:cortex ratio is a function of the relative sizes of the medulla and cortical cells, rather than the number of cells.

DISCUSSION

Extent of phenotypic variation and the existence of possible phenotypes in naturally growing *Gracilaria*

From the results, it is clear that there is extensive phenotypic variation in the morphology and anatomy of plants from Saldanha Bay and St. Helena Bay. The number, length of branches, degree of branching, plant length, tip length, and the thickness of plant parts all varied. Furthermore, these differences are characteristic of all plants at a site, regardless of depth. It is therefore suggested that two different phenotypes do indeed exist at Saldanha Bay and St. Helena Bay. From the results, the gross morphology of typical plants representing these distinct phenotypes have been drawn (Figure 20).



Saldanha Bay

St. Helena Bay

Figure 20: Phenotypic differences in the gross morphology of *Gracilaria* in Saldanha Bay, and St. Helena Bay

It is well known that intraspecific variations in the morphological and functional characteristics of benthic algae may be a reflection of differences in the abiotic and biotic factors in a habitat (Norton *et al.* 1981; Lobban *et al.* 1985). In particular, light, nutrients, temperature and wave action are the most important factors affecting the growth of seaweeds (Lobban *et al.* 1985). An analysis of temperature and nutrient conditions reveals that these conditions differ between St. Helena and Saldanha Bay. The sea surface temperature at Saldanha Bay is almost 2° higher than that at St. Helena Bay (Table 2). However, *Gracilaria* has been shown to grow well at 15°C, and poorly at lower temperatures (Engledow and Bolton 1992).

Table 2: **Sea surface temperatures at Saldanha Bay, St. Helena Bay and Luderitz**

	Sea Surface Temperature (°C)	References
Saldanha Bay	15.66	Shannon and Stander (1977)
Luderitz	13.4	Molloy (1992)
St. Helena Bay	14	Pitcher (1989)

Thus, it is unlikely that temperature is playing a big role in promoting such prolific growth in St. Helena Bay plants. An analysis of nutrients, reveals that surface nitrate levels are over 30 times higher in St. Helena Bay compared to Saldanha Bay (Table 3).

Table 3: Sea surface nitrate levels at Saldanha Bay, St. Helena Bay and Luderitz. Nitrate levels as a function of depth at Saldanha Bay are also included

Nitrate level (μmol.l^{-1})

	0m	5m	10m	References
Saldanha Bay	0.4	0.52	0.8	Shannon and Stander (1977)
Luderitz	10			Molloy (1992)
St. Helena Bay	10-15			Pitcher (1989)

This is to be expected since St. Helena Bay is a major upwelling zone (Pitcher G.C. 1989), and, as a consequence plants are continuously exposed to nutrients. Saldanha Bay on the other hand, is only exposed to seasonal upwelling, and thus nutrient cycling is limited. Thus high nitrate levels are believed to improve the growth of *Gracilaria* at St.Helena Bay. Furthermore, phosphate levels at St. Helena Bay are over 10 times higher than phosphate levels in Saldanha Bay (Table 4). Thus it is likely that the higher phosphate levels at St. Helena Bay are also responsible for the improved growth of plants. The fact that they have the most number of branches is also an indication that they are increasing their surface area to maximising nutrient uptake.

Table 4: Sea surface phosphate levels at Saldanha Bay, St. Helena Bay and Luderitz. Phosphate levels as a function of depth at Saldanha Bay are also included

Phosphate levels (μmol.l^{-1})

	0m	5m	10m	References
Saldanha Bay	0.14	0.14	0.15	Shannon and Stander (1977)
St. Helena Bay	1.5			Pitcher (1989)
Luderitz	2			Chapman and Shannon (1985)

Although epiphytes (mussels, bryozoans, smaller epiphytic red algae) were present in *Gracilaria* samples from St. Helena Bay, the degree of epiphytism was greater in Saldanha Bay.

Epiphytism has often been noted in the past to have substantial effects on the survival and productivity of *Gracilaria* (Bravo *et al.* 1992). Thus, it is possible that epiphytes may also have affected the morphology and growth of Saldanha plants. Of interest is that the bigger surface-volume ratio of St. Helena plants did not enhance epiphytism. However, this finding is consistent with that of Buschmann *et al.* (1992) who also found that *Gracilaria* plants with a higher surface-volume ratio did not enhance epiphytism.

Implications for strain selection.

According to the predictions made by Littler and Littler, (1980), Gracilaria with a higher surface-volume ratio have higher production capacities (Hanisak *et al.* 1988, 1990). Thus, following this argument, it would be expected that St. Helena plants, with a higher-surface to volume ratio would have higher biomass production rates. Furthermore, Buschmann *et al.* (1992) found that plants, with a high surface-volume ratio, like those from St. Helena Bay, had a significantly higher gel strength. Also, plants with a heavier central axis (presumably a function of thickness) had higher agar yields. The fact that plants from St. Helena have a higher surface-volume ratio, and thicker axes as well, they may well produce high agar yields of high gel strength.

These authors also give evidence that some *Gracilaria* plants have a certain degree of resistance to epiphytism (Buschmann *et al.* 1992). The fact that the higher-surface to volume ratio of St. Helena plants did not promote more epiphytism in St. Helena plants suggests that these plants may also have this resistance to epiphytes. Thus it is suggested that plants from St. Helena Bay are good candidates for strain selection. However, further testing of agar strength and yields, and resistance to epiphytes is required.

Phenotypic variation and the existence of possible phenotypes between naturally growing and cultivated *Gracilaria*

No distinct differences were found between bottom and raft cultivation systems, and there was no indication of a distinct phenotype in Luderitz. Plants within Luderitz sites were phenotypically different from each other eg. some weighed more than others. Moreover, they were phenotypically different to cultivated plants, of similar age, from Saldanha Bay. They tended to have shorter primary and secondary branches, and with the exception of site C, they also have shorter tip lengths.

Since optimal growth of *Gracilaria* has been found for temperatures over and above 15°C, (Engledow and Bolton 1992), a lack of growth in these plant parts may be a result of the colder temperatures at Luderitz (2.26°C lower) (Table 2). However, it has to be questioned whether Saldanha plants can be considered to have "better growth" than Luderitz plants. After all plants are of a similar length in both cultivation sites, and even though branches are longer in Saldanha plants, Luderitz plants have many more branches. Furthermore, plants from Luderitz also generally had thicker primary branches, tips and axes. Thus, it is difficult to establish which plants have better growth, even though nitrate and phosphate values may be more favourable for growth in Luderitz (Tables 2 and 3).

It is difficult to offer an explanation for the increased length of plant tips at site C in Luderitz as no additional information about the nature of conditions at the site are available. It may be possible that plants were not stored appropriately. This is based on the fact that there was evidence of many broken branches in the samples from this site. Thus, it could very well be that the tip seemed longer because of missing branches near the tip.

Even though Saldanha raft plants had certain phenotypic differences to natural population plants, it is suggested that differences in these parameters (namely a lack of a distinct branching pattern; longer secondary and primary branches), were not enough to identify them as distinct phenotypes. Furthermore, they were similar to Saldanha and or St. Helena plants in most other respects.

Luderitz plants also lacked the distinct branching pattern characteristic of natural population plants. However, where these plants seemed distinct from Saldanha plants (eg. shorter tip lengths) they were similar to St. Helena plants (similar tip lengths). Furthermore, where these plants seemed distinct from St. Helena plants (eg. longer secondary branches) they were similar to Saldanha plants (similar lengthed secondary branches). With respect to the length of their primary branches, they were similar to plants at both sites. Thus, as in Saldanha raft material, the phenological differences in these plants are not substantial enough to classify these plants as a distinct phenotype.

However, it should be noted that because raft material was only a month old (possibly the reason for shorter plants), phenotypic differences may not have been distinct at this stage, and may only have been obvious at a later stage. Buschmann *et al.* (1992) found that a great deal of phenotypic variation in cultivation material occurred over time. In this study, plants at Luderitz had thicker axes, tips and branches, and it is suggested that with time, phenotypic differences may become more distinct. Furthermore, the effect of bottom versus floating cultivation systems might have been established had the plants not been collected at such a young age.

Buschmann *et al.* (1992) found that cultivation conditions induced thicker plants and increased branching. Furthermore, this was found to improve the quality and quantity of agar. It has already been established that Luderitz plants are quite thick, and that this may become more pronounced with time. Thus, the potential exists for these plants to develop into growth forms similar to those in the study by Buschmann *et al.* (1992). As a consequence, these plants may also produce large amounts of this high quality agar.

Effect of depth on the growth form of *Gracilaria*

The effect of depth on plant growth form was most pronounced in St. Helena plants. The coastal waters of St. Helena Bay are often murky. Plants have to adapt to decreased light levels, especially at mid and deep depths. Thus, these plants have more short, medium and long branches at these depths to maximise their surface area for photosynthesis.

However, the decrease in plant length, length of branches and length of tips at 8m, suggests that light levels are generally too low at this depth to sustain adequate plant growth. On the otherhand, light levels at 6m seem sufficient to maintain adequate growth. Thus, longer branches, tips and longer plants were found at 6m. Axes diameters, and tip and primary branch cuticles were thinnest at 6m. It is suggested that this is a mechanism to reduce the distance that light has to travel before it reaches photosynthetic cells at this depth. The fact that the plants have a greater mass at 8m may be a consequence of these plants accumulating storage reserves in light limiting conditions.

Saldanha plants had increased numbers of medium branches at mid depths. This may be a strategy to increase their surface area for maximum uptake of higher nutrient levels at deeper depths. (Nitrate and phosphate levels are found to increase with depth at Saldanha Bay; Tables 3 and 4)

Effect of salinity on the growth form of *Gracilaria*

Although *Gracilaria* is known to be a euryhaline species (Engledow and Bolton 1992), decreased salinity was found to stunt the growth of *Gracilaria* Branches were shorter and fewer, axes were thinner, and plants weighed less. This has important implications for the productivity of these plants. Based on the predictions by Hanisak *et al.* (1988, 1990) using the functional form model, it is clear that these phenotypes would have little use in strain selection

Possibility of a different species at Luderitz

Based on the limited evidence of phenotypic differences between plants at Luderitz, plants at Luderitz cannot conclusively be considered to be different entities. Where they were different to Saldanha plants, they were found to be similar to St. Helena plants, and *vice versa.*

Furthermore they were found to be similar to plants at both Saldanha and St.Helena Bay in other respects. In particular, the medulla:cortex ratios of the primary branches and tips were found to be statistically similar to that in other plants. The axes had similar ratios as well, and no differences were found in the cuticle diameter, and the number of cortical or medulla cells of branches, tips and axes.

Furthermore, a comparison form with *Gracilariopsis* shows that there is a distinct differences in *Gracilariopsis* that is not found in Luderitz plants. Namely, *Gracilariopsis* plants had the thinnest cuticles in all plant parts, whereas cuticle thickness did not differ to a great extent between other plants.

CONCLUSION

Although this is a complex study, and has invoved the comparison of a number of factors, I would like to draw conclusions on the following main points. Firstly, this study showed that there was a high degree of phenotypic variation between natural populations of *Gracilaria* growing in Saldanha Bay and St. Helena Bay. Plants showed marked variation in both their anatomy and morphology. It was suggested that plants from Saldanha and St. Helena Bay are different phenotypes of *Gracilaria verrucosa*. Furthermore, it was proposed that the phenotype from St. Helena Bay should be selected for cultivation purposes. But [illegible]

Cultivated plants also varied phenotypically from natural population plants, as well as from each other. However, the extent of variation was not believed to be significant enough for distinct phenotypes to be recognised. It was suggested that cultivated plants examined in this study were too young for any meaningful variation to have occurred. Variation was also not distinct enough to have considered plants at Luderitz to be a different species.

Depth was also found to affect the growth form of St. Helena plants. Plants had adapted their growth forms to cope with the murky conditions in the Bay. Reduced salinity from the fresh water outlet of the Berg River, resulted in stunted growth and a generally poorly developed phenotype.

A study such as this, not only provides insight into the physiology, taxonomy and ecology of *Gracilaria verrucosa*, but has further implications for its cultivation. While the economic potential of this species for the Southern African region has been realised, this industry has not yet been adequately developed. The harvesting of beach cast has severe limitations. Cultivation of this species would overcome these, and yet this has not been done on a commercial basis. The current study was undertaken in attempt to address this problem. However, it is suggested that such a study be expanded to more fully assess possible phenotypes and/or strains for cultivation.

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