A preliminary study of *Gelidium capense* in culture

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Subject: Phycology

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Abstract

Some preliminary investigations of the effects of temperature, light intensity, salinity and to a lesser extent, water movement, were performed under controlled laboratory culture conditions on the subtidal red alga *Gelidium capense,* with regards to its potential for aquaculture for its use in the agar and paper-making industries. Agar was also extracted from a wild population and its concentration measured. Four temperature conditions (10°C, 15°C, 20°C and 25°C) and three salinities (35ppt, 18ppt and 9ppt) were tested as well as four irradiances (120-140 µmol photons $m^{-2}s^{-1}$, 80-100 µmol photons $m^{-2}s^{-1}$, 60-70 µmol photons $m^{-2}s^{-1}$ and 30-50 µmol photons $m^{-2}s^{-1}$). Water movement was achieved either through aeration or using a flask shaker. A combination of full salinity (35ppt) and 15°C temperature with a light intensity of 80-100 µmol photons $m^{-2}s^{-1}$ yielded the most optimal growth rates (5.07% day^{-1}) while poor growth rates (-1.61% day^{-1}) were observed for the 18ppt and 9ppt salinities and the lowest light intensities. Interestingly, *G. capense* did not thrive at 20°C or 25°C and instead discoloured rapidly, showed necrosis within a few days, which is in contrast to the findings of most studies focussed on other species of *Gelidium* under similar conditions. Slow growth rates were achieved at 10°C. The mean agar yield measured ten percent (9.66% ± 1.81) of dry algal weight which is less than a third of the agar yields of some other species of *Gelidium* and *Gracilaria.* This suggests that this species may not be as valuable as other *Gelidium* species in terms of its agar content. It does, however, have high rhizine content and this may lend it to be beneficial in the papermaking industry.

Introduction

Several genera of red algae have become economically important globally as a source of agar, carrageenan and agarose for their uses in electrophoresis, fuels and as gelling agents in microbiological work as well as in the cosmetics and food industries. There is increasing demand worldwide for industrial agars. *Gelidium, Gracilaria* and *Pterocladia* are the most commercially important agarophyte genera (Sousa-Pinto *et al,* 1999; Armisen, 1995), and *Gelidium* and *Pterocladia* are suggested as being the most economically viable genera producing the best quality agar. *Gelidium* is valuable for this polysaccharide and there has also been recent interest for its use in paper-making (Seo *et al,* 2010). The shape, growth rate and amount of endofibres, or rhizines, of *Gelidium*

species may be used as criteria for considering their potential use in the pulp and papermaking industries (Seo *et al,* 2010). Physically, *Gelidium* species contain large amounts of mucilaginous agar which is easily extracted, leaving behind small amounts of solid material, the endofibres, which may then be bleached to make red algae pulp and processed into high quality paper (Seo *et al,* 2010).

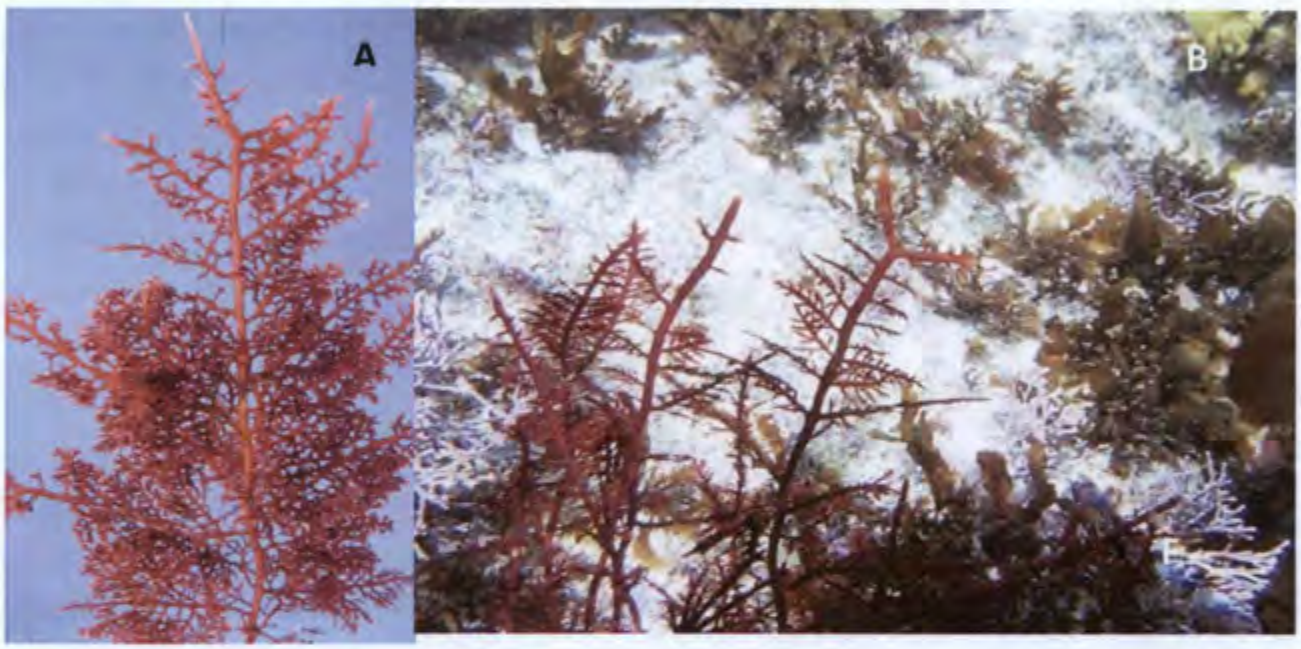


Fig. 1: Habit and morphology of *G. capense.* A: Morphology of *G. capense.* B: Habit of *G. capense,* taken in De Hoop Nature Reserve. Photos: R. J. Anderson, Seaweed Research Unit, MCM.

Gelidium has been harvested from natural populations for many years and therefore the availability and quality of its agar has varied between locations and over time and therefore there have been inconsistencies in global agar quality and availability (Macler & West, 1987). Interest in the cultivation of seaweeds over the last few decades has led to increased understanding of the biology and ecology of seaweeds and their resources (Avila & Seguel, 1993). While initial steps have been taken in developing a cultivation methodology for *Gelidium* (e.g. Rico, 1991; Carter & Anderson, 1986; Melo *et al,* 1991) and many studies have focussed on culture experiments (Macler & West, 1987; Ramiro *et al,* 1996; Robaina *et al,* 1990; Sousa-Pinto *et al,* 1999) a successful technology has not yet been developed. *Gelidium* is currently collected or harvested as opposed to cultivated. One of the main problems with the cultivation of *Gelidium* is its low growth rate and it is thus suggested that only high yield strains—potentially achieved through selection or genetic engineering—will be economically viable (Friedlander, 2008). The aquaculture of macroalgae carries large costs and the lack of expertise and knowledge about the

growth rates and agar yields of many *Gelidium* species has hampered the expansion of profitable aquaculture ventures (Sousa-Pinto *et al,* 1999).

Gelidium capense is a South African endemic robust, bushy red alga up to 40cm tall with several erect axes arising from a compact stoloniferous holdfast (Fig. 1) (Stegenga *et al,* 1997). This species in dark brownish red in colour with abundant rhizines in younger branches in the central medulla, and in older axes the rhizines are concentrated in a subcortical layer (Stegenga *et al,* 1997). Collections have been made from Melkbosstrand on the west coast of the Western Cape and along the south-east coast as far as Kenton on Sea in the Eastern Cape, situated between Port Elizabeth and East London (Stegenga *et al,* 1997).

The coast of southern Africa is physically and climatically varied and is therefore suggested to have the potential to support a range of commercial seaweeds (Anderson *et al,* 2003). In South Africa, *G. pristoides* is harvested along the Eastern Cape Province coastline and proves to be the most economical as it fetches a high price and produces high agar yields (Anderson *et al,* 2003). It is also strictly intertidal in its distribution, making it easier for collection than *G. capense* which occurs subtidally (Anderson *et al,* 2003). Global *Gelidium* production increased from the 1980s due to increased demand for the resource in Japan (Ko, 2010) and by 1991 Spain, Morocco and Portugal represented almost 50% of the global *Gelidium* (mostly *G. sesquipedale)* harvest (McHugh, 1991).

Gelidium species are often associated with high wave action (Santelices, 1991). Knowledge of the growth capabilities and environmental factors such as light intensity, temperature, water turbulence, salinity as well as life cycle strategies are all highly relevant information essential for the evaluation of potential algae for aquaculture cultivation and all have effects on the potential growth rates. These factors also have varying effects on the yield and quality of the agar. The recent interest in the use of red algae in the papermaking industry has also brought attention to the endofibre content of these algae (Seo *et al,* 2010).

The aims of this study are to produce several growth experiments investigating some of the effects of temperature, salinity and light intensity and to a lesser extent water turbulence on the growth rate *G. capense* in culture. There are no published studies on the culture of *G. capense.* Agar was also extracted from this species and the yield measured. This study offers a preliminary view of the potential for aquaculture of this species.

more sentences to above paragraphs

Materials and Methods

Collection site

Gelidium capense samples were collected from Kalk Bay (34°07'40"S, 18°26'54"E) on the east coast of the Cape Peninsula, South Africa. The average sea surface temperatures on the east coast of the peninsula show variation in winter and summer conditions ranging from 12.5°C to 19°C, respectively (McQuaid & Branch, 1984). The site consists of a rocky shore with *G. capense* occurring subtidally with high turbulence from breaking waves. Collections were made on four occasions in July, August and October. This encompasses winter and spring months. Healthy looking plants were chosen from several populations amongst the rocks to obtain an average representation of the plants within the different populations.

Laboratory culture

The Provasali enriched seawater medium, or PES solution (Anderson, 2005), was used in this study and constitutes the following: 2.8g $NaNO_3$, 0.4g Na_2 glycerophosphate, 0.004g Thiamine dichloride, 4g Tris buffer, 0.8ml vitamin B12 (1mg.10ml^{-1}), 0.8ml Biotin (1mg.20ml^{-1}), 200ml Fe EDTA solution, 200ml PII solution, all made up to a total volume of 1000ml. The Fe EDTA and PII solutions constitute the following, respectively: 0.07g Fe $(NH_4)_2(SO_4)_2.6H_2O$, 0.6g Na_2 EDTA, 1000ml distilled H_2O, and 1.14g H_3BO_3, 0.049g $FeCl_3.6H_2O$, 0.164g $MnSO_4.H_2O$, 0.022g $ZnSO_4.7H_2O$, 1000g Na_2 EDTA, 0.005g $CoSO_4.7H_2O$ and 1000ml distilled H_2O. Full PES was used and comprised 20ml stock solution per litre of seawater.

After collection the samples were rinsed twice with fresh water and visible animal and foreign plant material was removed. Several growth experiments were performed whereby some of the effects of temperature, salinity, light intensity and water turbulence were investigated. The daylength was kept constant at 16: 8 h light: dark, LD, in a walk-in controlled temperature growth room. Filtered seawater (2.2µm) was obtained from the Marine and Coastal Management (MCM) Research Aquarium (Beach Road, Sea Point). Four irradiances were used in this study, namely, 120-140 µmol photons $m^{-2}s^{-1}$, 80-100 µmol photons $m^{-2}s^{-1}$, 60-70 µmol photons $m^{-2}s^{-1}$ and 30-40 µmol photons $m^{-2}s^{-1}$ which, in the context if this study, will constitute high, medium-high, medium and low light intensities, respectively. Salinity experiments comprised 2.2µm filtered seawater diluted with distilled water added accordingly prior to nutrient enrichment. Salinities measured include 35ppt (high), 18ppt (medium) and 9ppt (low).

Growth measurements were taken every three to five days as increments in fresh weight in grams and the experiments lasted between one and a half to two weeks each over a three month period. All samples were randomised by moving the flasks, culture dishes or plastic bags three times a week to account for differing conditions at the microscale along laboratory benches. Fresh medium was provided weekly. Three temperature and three pH readings were taken weekly over a month for all four temperature conditions to

record mean measurements (CyberScan pH metre, 300/310, Eutech Instruments). In the salinity experiments, salinity readings were taken three times a week to monitor the salinity with adjustments being made accordingly (Portable refractometer, FG201, salinity 0-100ppt). The different irradiances were obtained by covering the light source with varying degrees of shadecloth. Irradiance was measured using a light meter (Skye Instruments Ltd, SKE 500).

Several different methods of culture were tested in this study. The aim was to test differing culturing methods focussing on the effects of different variables with the available facilities and apparatus. Due to logistical and time constraints, all the combinations of variables could not be measured in every instance. The growth studies involved several different approaches as follows:

Algae in bags with aeration at two temperature conditions and three irradiances

Firstly, five plastics bags (length: 54cm, width: 28cm) each constituting five replicates with approximately 10g of algal material in bunches of different lengths and sizes, excluding the holdfasts, were hung with air filtration provided by bubbling air (Electromagnetic air compressor, BOYU, ACQ-003) in 10°C (±2°C) and 15°C (±2°C) constant temperature rooms. The bags each contained 2l of 2.2µm filtered seawater and full PES. Light intensity was set at 120-140 µmol photons $m^{-2}s$, which constitutes the maximum for this study. A further two experiments were set up in triplicate under 15°C with the same parameters as before, except for the light intensity which was altered to 60-70 µmol photons $m^{-2}s^{-1}$ and 30-40 µmol photons $m^{-2}s^{-1}$ using different amounts of shadecloth to provide shading, and constitute the minima for this study. Growth measurements were recorded as the increments in fresh weight (g) of the samples using a scale (Ohaus, Scout Pro), before which they were first spun in a salad spinner for 60 rotations for consistency.

Further growth experiments made use of apical algal tips approximately 1cm in length (≈0.015g) in 100ml of 2.2µm filtered seawater and full PES. Growth measurements were recorded as increments in fresh weight (g) using a fine scale (Mettler AE100). For consistency, the algal tips were first dried for ten seconds on paper towel before being weighed. All the experiments using apical tips comprised three replicates of each condition, and are as follows:

Algal tips in still culture at four temperature conditions

Algal tips were placed in still 100ml culture dishes under 15°C and 10°C in the constant temperature rooms at medium-high irradiance. Still culture dishes were also set up in water baths (Lab Waterbath, LW Scientific, DSB-500E) under 20°C (±2°C) and 25°C (±2°C). Irradiance was set at 80-100 µmol photons $m^{-2}s^{-1}$.

Algal tips in agitated culture at two temperature conditions and three irradiances

Tips were also placed in 100ml flasks on a flask shaker (Stuart Scientific, SF1) set at 200 oscillations per minute, in both the 10°C and 15°C constant temperature rooms. In the 15°C room, three light intensities were measured, namely, 80-100 µmol photons $m^{-2}s^{-1}$, 60-70 µmol photons $m^{-2}s^{-1}$ and 30-40 µmol photons $m^{-2}s^{-1}$, again using different levels of shadecloth. In the 10°C room, 80-100 µmol photons $m^{-2}s^{-1}$ and 30-40 µmol photons $m^{-2}s^{-1}$ irradiances were measured. No data were available for 60-70 µmol photons $m^{-2}s^{-1}$ light intensity.

Algal tips in agitated culture under three salinities and two temperature conditions

Growth under three salinities was tested under both 10°C and 15°C using 100ml flasks on a flask shaker, including 35ppt, 18ppt and 9ppt salinities, under 80-100 µmol photons $m^{-2}s^{-1}$ irradiance.

Algal tips in aerated culture at two temperature conditions

Lastly, apical tips were also placed in aerated 250ml flasks in the 20°C (±2°C) and 25°C (±2°C) water baths. Irradiance was set at 80-100 µmol photons $m^{-2}s^{-1}$. The larger 250ml flasks were used as opposed to the 100ml flasks as a result of the turbulence created by the bubbling water.

The above experiments are summarized in Table 1 and Table 2, below.

Table 1. Table showing experiments with light intensities, temperatures & media used. In these experiments salinity was kept constant at 35ppt. Letters stand for: A=aerated tips, S=still culture tips, G=agitated tips, B=aerated bags.

	30-40 µmol photons $m^{-2}s^{-1}$	60-70 µmol photons $m^{-2}s^{-1}$	80-100 µmol photons $m^{-2}s^{-1}$	120-140 µmol photons $m^{-2}s^{-1}$
10°C	G	-	S G	B
15°C	G B	G B	S G	B
20°C	-	-	A S	-
25°C	-	-	A S	-

Table 2. Table showing experiments with salinities, temperatures and media used. In these experiments, light intensity was kept constant at 80-100 µmol photons $m^{-2}s$. Letters stand for: A=aerated tips, S=still culture tips, G=agitated tips, B=aerated bags.

	9ppt	18ppt	35ppt
10°C	G	G	S G
15°C	G	G	S G
20°C	-	-	A S
25°C	-	-	A S

Agar extraction

Agar was extracted from a population sample of *G. capense* collected from the field from several populations growing within a few metres of one another in October. Approximately 110g of fresh material was dried overnight at 60°C in an oven. The dry material was then passed through a fine mesh mill. The resulting milled material measured approximately 30g. Agar was then extracted with six replicates using a modified version of the method of Craigie & Leigh (1978) (Carter & Anderson, 1986). Approximately five grams of the dried material was added to a flask containing 250ml of distilled water. The flask was then heated to between 85°C and 95°C for an hour, with continuous stirring using a heated stirrer (FMH instruments). The resulting mixture was then passed through fine filter paper and the remaining dry material which did not pass through the paper was then subjected to the same procedure again. The two resulting mixtures were then combined and the leftover dry material discarded. The mixture was frozen overnight at -20°C, and then thawed. The thawed mixture was passed through filter paper and dried in the oven overnight at 60°C after which it was weighed.

Statistical analyses

The relative growth rates (RGR) were calculated as follows:

$$100 \ln\left(\frac{wt}{wo}\right)/t$$

Where *wo* is the weight at the start and *wt* is the weight at time t, and t represents the time interval in days (Rueness & Tananger, 1984).

The growth rates of *G. capensis* under the different culture experiments were analysed using STATISTICA v.9 (2009, Statsoft Inc., OK, USA). Prior to the analyses, all the data were tested for normality by examination of Komolgorov-Smirnov and Lilliefors tests for normality by normal probability plots and normality histograms,

and transformed where necessary. All the data were not found to be normally distributed and therefore the non-parametric Kruskal-Wallis ANOVA by ranks for multiple comparisons of independent groups was used to test for significance of relationships. Significance was tested at the five percent level.

Examination of rhizines

Cross-section and longitudinal sections of *G. capense* were made using a microtome. Photographs were then taken under the microscope and the internal structure was examined.

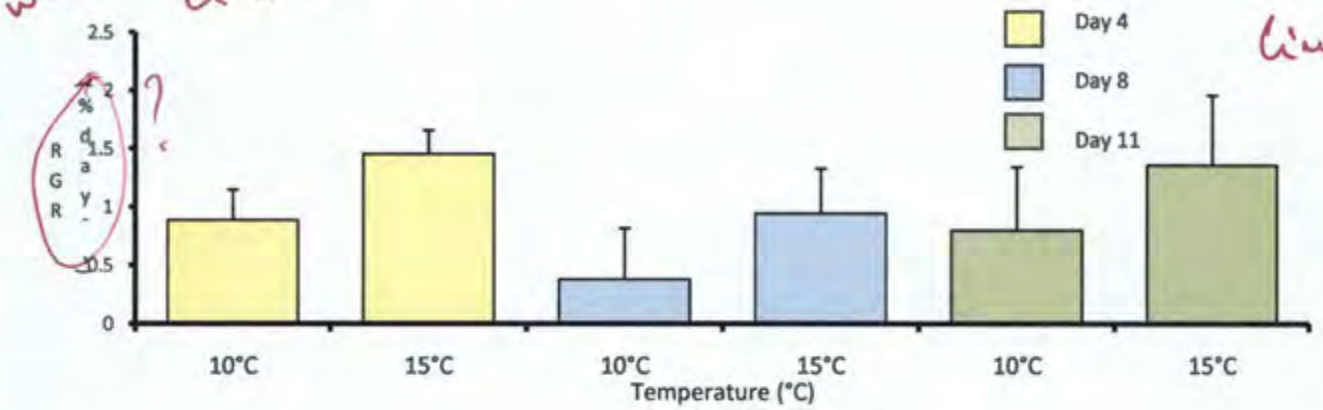
Results

Laboratory culture

The temperature of the medium under 10°C was recorded as averaging 10.9°C and in the 15°C constant temperature room the temperature averaged 14.4°C. The 20°C and 25°C water baths averaged temperatures of 20.2°C and 25.6°C, respectively. The mean pH was 8.2. While the mean temperature readings reflect readings close to the desired temperature, it must be noted that there were temperatures recorded that showed variation of around ±2°C of the desired temperature.

Algae in bags with aeration at two temperature conditions

The various growth experiments involving differences in temperature produced the following results. The plastic bags containing 10g of algal material under 10°C and 15°C (Fig. 2) at 120-140 µmol photons $m^{-2}s^{-1}$ irradiance were found to yield growth results that were significantly different from one another ($p < 0.05$) with mean RGRs of 0.69% ± 0.27 (mean ± standard deviation) and 1.25% ± 0.2, respectively. After two weeks of culture the algae had become moderately green in appearance, especially towards the tips. The growth experiment using the algal bags at 15°C under 60-70 µmol photons $m^{-2}s^{-1}$ irradiance and 30-40 µmol photons $m^{-2}s^{-1}$ irradiance (Fig. 3) were not found to be significantly different from each other ($p > 0.05$). Conversely, there were statistically significant differences in growth rate between the 120-140 µmol photons $m^{-2}s^{-1}$ irradiance and 30-40 µmol photons $m^{-2}s^{-1}$ irradiance irradiances at 15°C ($p < 0.05$).



Fig, 2. RGR (% day^{-1}) of algal bags under 10°C and 15°C constant temperature at 120-140 µmol photons m^{-2}s^{-1} irradiance. Bars represent three weigh-ins over eleven days, and the RGR value at each weigh-in represents the amount of growth since the previous weigh-in. Error bars represent standard deviations.

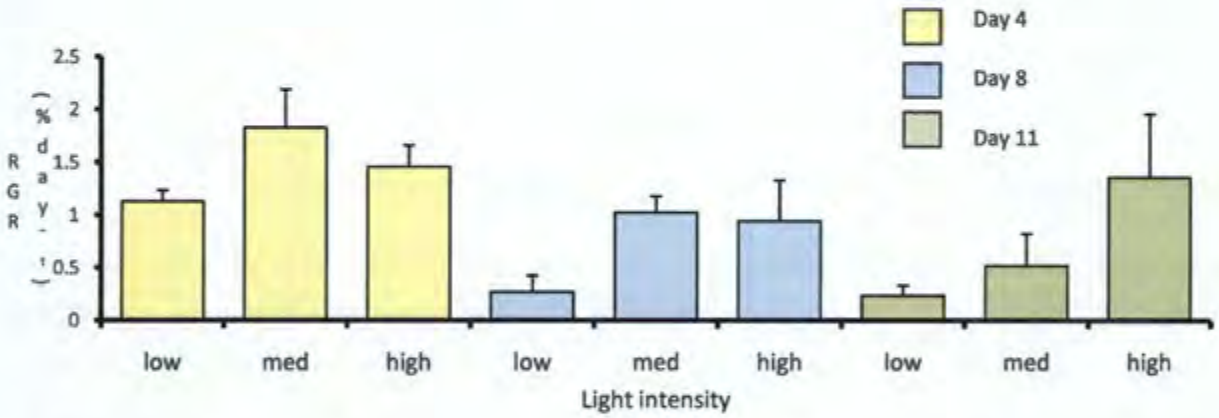


Fig. 3. RGR (% day^{-1}) of algal bags at high (120-140 µmol photons m^{-2}s^{-1}), medium (60-70 µmol photons m^{-2}s^{-1}) and low irradiances (30-40 µmol photons m^{-2}s^{-1}), under 15°C constant temperature. Bars represent three weigh-ins over eleven days, and the RGR value at each weigh-in represents the amount of growth since the previous weigh-in. Error bars represent standard deviations

Algal tips in still culture at four temperature conditions

The algal tips in still culture dishes under all four temperature conditions and 80-100 µmol photons m^{-2}s^{-1} light intensity produced the following results (fig. 4): the growth rate of the 15°C samples were found to be significantly different from the growth rates of both the 10°C and 25°C samples ($p < 0.05$) while the 20°C

sample was not found to differ significantly from the other conditions ($p > 0.05$). At 20°C (0.56% ± 0.84) and 25°C (-0.14% ± 0.63) the algal tips became bleached and the growth rate deteriorated and necrosis was observed, whereas the samples at 10°C (0.09% ± 0.89) and especially 15°C (1.3% ± 0.56) appeared healthier for longer.

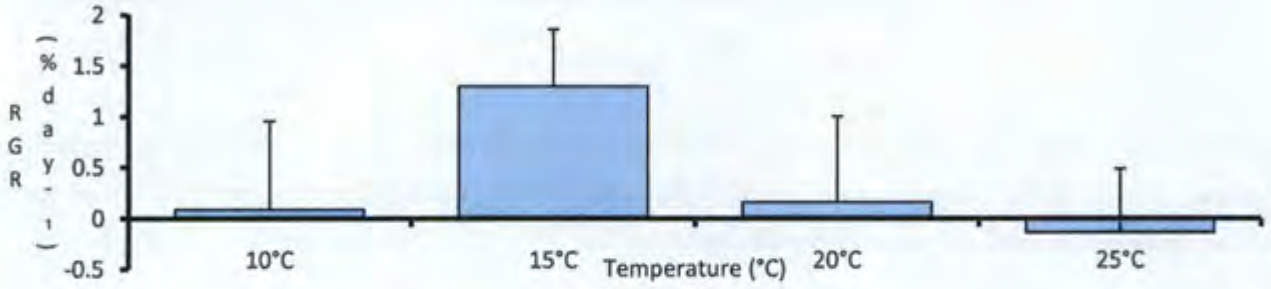


Fig. 4. Mean overall RGR (% day^{-1}) of algal tips in still culture dishes at four temperature conditions. Irradiance was set at medium-high (80-100 µmol photons $m^{-2}s^{-1}$). Bars represent mean RGR under each condition. Error bars represent standard deviations.

Algal tips in agitated culture at two temperature conditions and differing light intensities

The Kruskal-Wallis multiple comparisons values suggest that, for the growth experiment using algal tips on the shaker under 15°C (Fig. 5), there are significant differences between the growth rates of the 80-100 µmol photons $m^{-2}s^{-1}$ irradiance and 30-40 µmol photons $m^{-2}s^{-1}$ irradiances ($p < 0.05$), but no significant difference was found between the 60-70 µmol photons $m^{-2}s^{-1}$ irradiance and 30-40 µmol photons $m^{-2}s^{-1}$ irradiance or the 60-70 µmol photons $m^{-2}s^{-1}$ irradiance and 80-100 µmol photons $m^{-2}s^{-1}$ light intensities. All samples at 15°C appeared to stay healthier for longer and the first to deteriorate were the samples at 30-40 µmol photons $m^{-2}s^{-1}$ irradiance and 60-70 µmol photons $m^{-2}s^{-1}$ irradiance light intensities. The mean RGRs under 80-100 µmol photons $m^{-2}s^{-1}$ irradiance, 60-70 µmol photons $m^{-2}s^{-1}$ irradiance and 30-40 µmol photons $m^{-2}s^{-1}$ irradiance light intensities were 2.29% ± 0.74, 1.46% ± 1.01 and 1.29% ± 0.48, respectively. With regards to the samples under 10°C (figure x), a significant difference was found between the 80-100 µmol photons $m^{-2}s^{-1}$ irradiance and 30-40 µmol photons $m^{-2}s^{-1}$ light intensities ($p < 0.05$), with mean RGR values of 1.89% ± 0.55 and -0.69% ± 0.91 (no data for 60-70 µmol photons $m^{-2}s^{-1}$ irradiance was available for the 10°C samples). Furthermore, in a combined analysis of both temperature conditions, significant differences were found between the 80-100 µmol photons $m^{-2}s^{-1}$ irradiance (15°C) samples and the 30-40 µmol photons $m^{-2}s^{-1}$

irradiance (10°C) samples. The 10°C samples under 30-40 µmol photons $m^{-2}s^{-1}$ light intensity deteriorated fairly quickly as can be seen from the poor RGR.

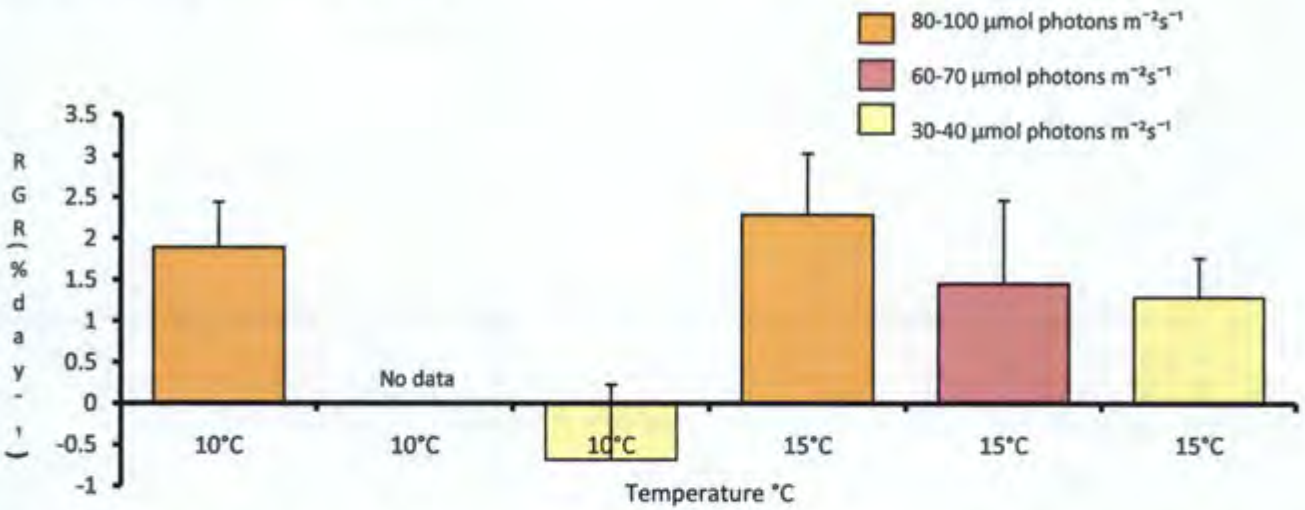


Fig. 5. Mean RGR overall (% day^{-1}) of algal tips under differing light intensities and two temperature conditions. Bars represent the mean values under 80-100 µmol photons $m^{-2}s^{-1}$, 60-70 µmol photons $m^{-2}s^{-1}$ and 30-40 µmol photons $m^{-2}s^{-1}$. No data is available for 60-70 µmol photons $m^{-2}s^{-1}$ intensity at 10°C. Error bars represent standard deviations.

Algal tips in agitated culture under differing salinities and two temperature conditions

The three salinity experiments under both 10°C and 15°C (Fig. 6) yielded the following results. The Kruskal-Wallis multiple comparison values suggest that, with regards to the samples under 10°C, significant differences were found between 35ppt (2.9% ± 0.5) and 18ppt (-0.4% ±0.9) and 35ppt and 9ppt (-0.7% ± 0.8) salinities ($p < 0.05$) while no significant difference was found between the 18ppt and 9ppt salinities ($p > 0.05$). With regards to the samples under 15°C, a significant difference was only found between the 35ppt (3.63% ± 1.3) and 9ppt (-0.26% ± 0.58) salinities ($p < 0.05$). In a combined analysis of both the 15°C and 10°C samples, significant differences were found between the 35ppt salinity (10°C) and 9ppt salinity (15°C) ($p < 0.05$) as well as the 18ppt and 9ppt salinities (10°C) against the high salinity (15°C). In both temperature scenarios, the 18ppt and 9ppt salinity samples deteriorated rapidly and appeared bleached and/or green in colour. The samples with the highest salinity in both scenarios appeared healthy throughout the experiment.

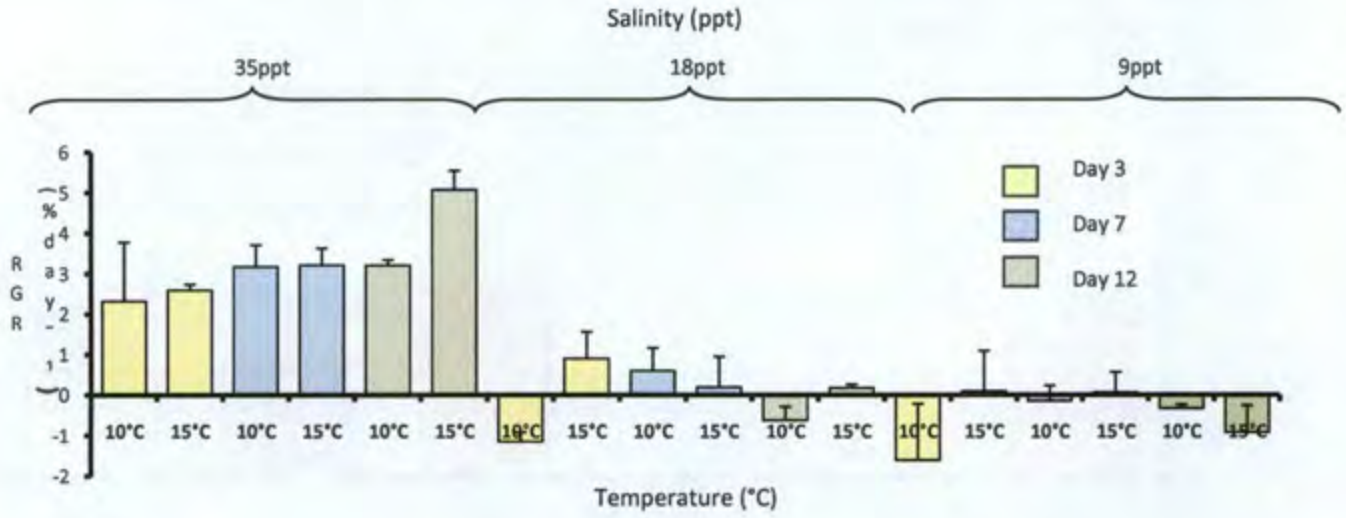


Fig. 6. RGR (% day^{-1}) of algal tips at three differing salinities (35ppt, 18ppt and 9ppt) and medium-high (80-100 µmol photons m^{-2}s^{-1}) irradiance, under both 10°C and 15°C constant temperatures. Bars represent three weigh-ins over twelve days, and the RGR value at each weigh-in represents the amount of growth since the previous weigh-in. Error bars represent standard deviations.

Algal tips in aerated culture at two temperature conditions

The growth experiment using the algal tips in the aerated flasks at 20°C and 25°C (Fig. 7) at 80-100 µmol photons m^{-2}s^{-1} irradiance were not found to be significantly different ($p > 0.05$). Interestingly, the mean RGR of the 25°C (-0.0003% ± 0.26) samples is greater than that of the 20°C samples (-0.21% ± 0.36) which is different to what was found in the still culture experiments at these temperatures (Fig. 5). In both cases, the algal tips became bleached and the growth rate deteriorated after a few days.

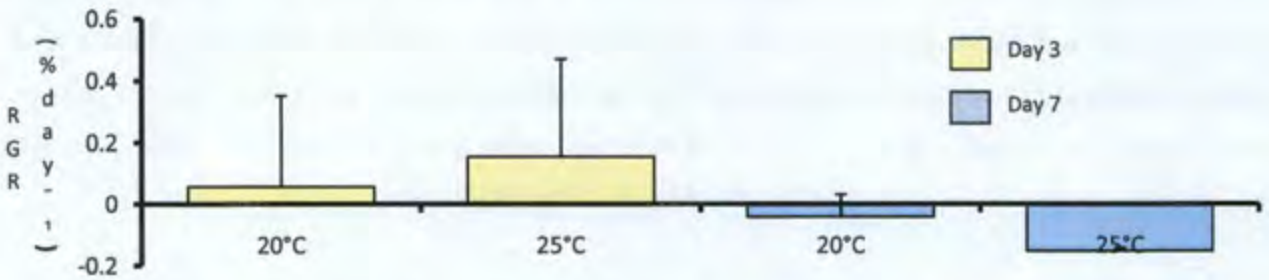


Fig. 7. RGR (% day^{-1}) of algal tips in aerated flasks under 20°C and 25°C temperature conditions. Irradiance was set at medium-high (80-100 µmol photons m^{-2}s^{-1}). Bars represent two weigh-ins over seven days, after which necrosis was observed. Error bars represent standard deviations.

A further test was applied to investigate whether there was a significant difference in growth rates between the four temperature conditions in still culture dishes versus those on a shaker in flasks and those in the water baths (Fig. 8). A significant difference was found between the 10°C still culture and the 10°C flasks on the shaker ($p < 0.05$), with the RGR of the sample on the shaker being significantly greater than that of the still culture sample. No significant difference was found between the other corresponding temperatures.

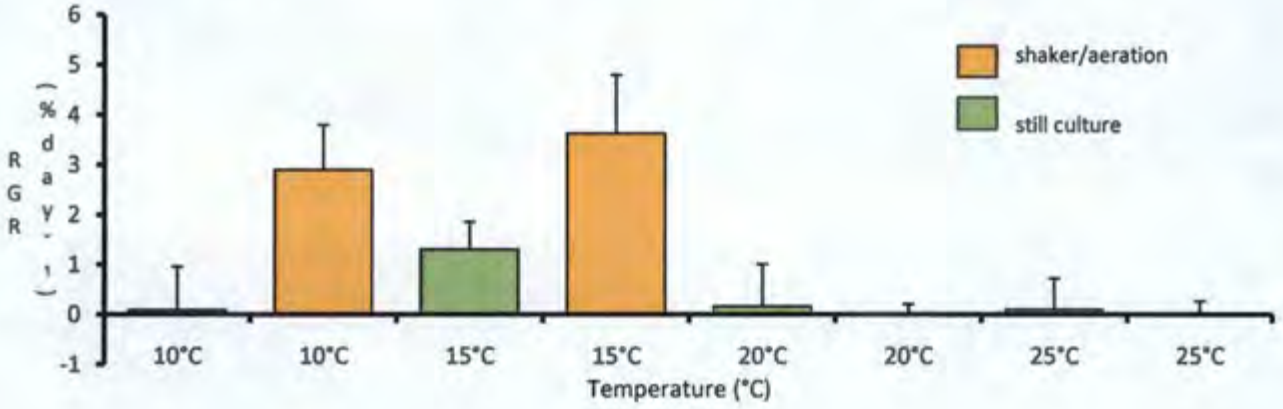


Fig. 8. Mean RGR (% day^{-1}) of algal tips under four temperature conditions with a comparison between still culture and cultures on a shaker/aerated flask. The irradiance for all conditions was set at medium-high (80-100 µmol photons m^{-2}s^{-1}) light intensity. Bars represent two temperature conditions under two water turbulence conditions. Error bars represent standard deviations.

Overview of effects of temperature, salinity and light intensity on growth

In general, the algal tips did not thrive at 20°C or 25°C and started to bleach and discolour after a few days. At 10°C and especially 15°C, the algal tips as well as the algal samples in bags tended to appear healthier for longer. Salinity had a major effect on the growth of this species with poor and negative growth rates being achieved at 18ppt and 9ppt salinities. Although water turbulence is a difficult parameter to measure in the context of this study, the experiments using still culture dishes under 10°C and 15°C showed poor growth rates compared with those experiments with water movement, although only the 10°C conditions were statistically significant. Overall, the greatest RGR (5.07% day^{-1}) was achieved using the algal tips in a flask on a shaker at 15°C with 35ppt salinity and 80-100 µmol photons m^{-2}s^{-1} irradiance, while the poorest RGR (-1.61% day^{-1}) was achieved at the lowest salinity value, 9ppt, and 80-100 µmol photons m^{-2}s^{-1} irradiance at 10°C.

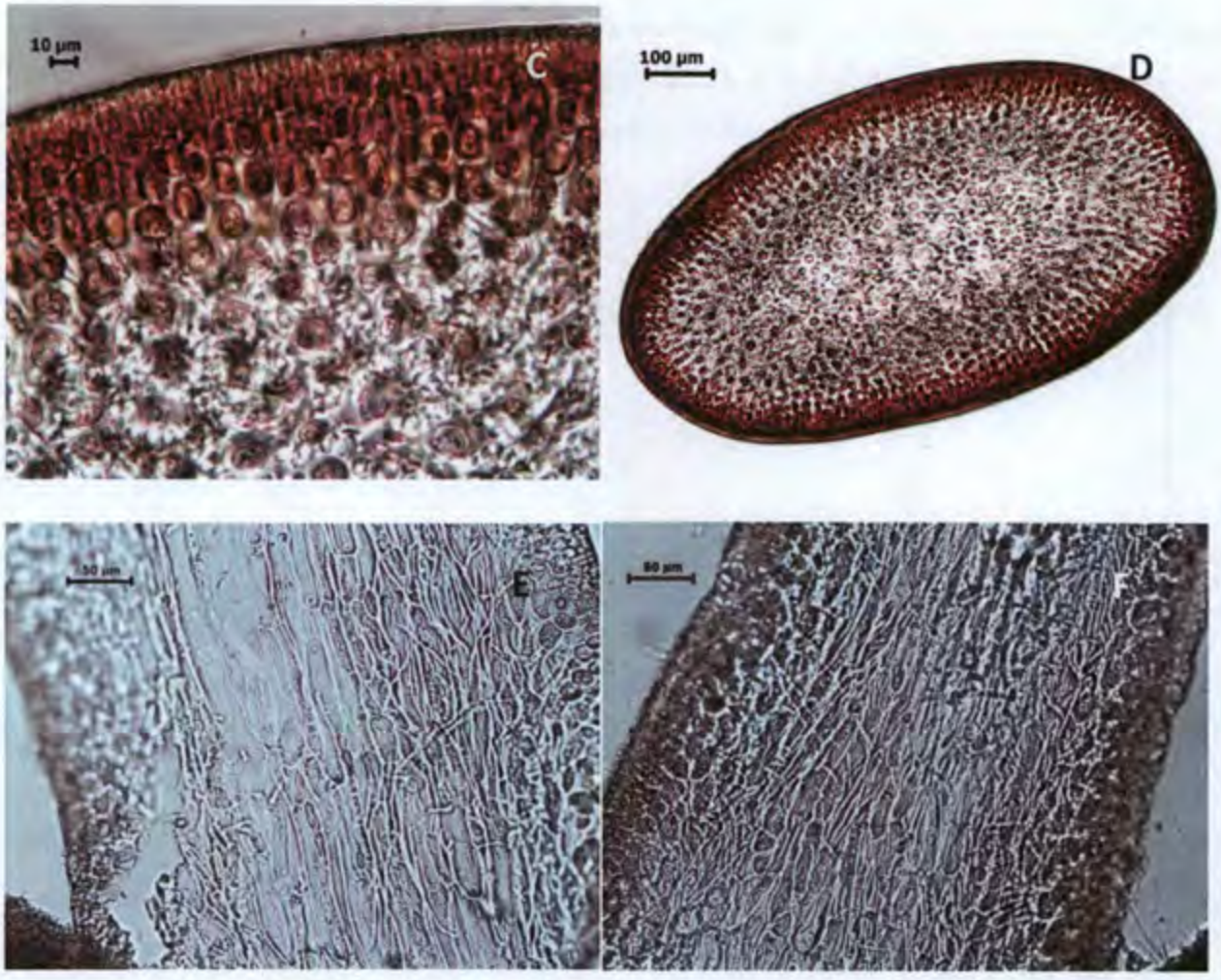


Fig. 9. Internal structure of *G. capense*. C & D: Cross-section of stem of *G. capense* showing medullary cells and endofibres. E & F: Longitudinal section of *G. capense* showing fibrous internal structure. Cross section photos: R. J. Anderson

Agar extraction

The agar yields from the six replicates are expressed as dry weight (g). The mean agar yield is 0.475g ± 0.089 per 4.92g of milled dry material which corresponds to 9.66% ± 1.81 of total mass. These results suggest that the amount of agar extracted from dry material is approximately ten percent of the weight of the dry material.

Internal Structure

The internal structure of *G. capense* appears highly fibrous. Cross-section and longitudinal section reveal high rhizine content (Fig. 9).

Discussion

The objective of this study was to determine the effects of light intensity, salinity and temperature, and to a lesser extent water movement, on the culture of *G. capense* in the laboratory, as well as to test its ability to be grown under laboratory culture conditions. Another aim was to measure the agar contents of this species and examine the rhizine content. Growth rates were measured and compared between culture experiments under differing conditions while agar content was measured from the wild population at the collection site.

Studies have found that other *Gelidium* species experience high growth rates at high irradiances ≥100 µmol photons $m^{-2}s^{-1}$ and show a positive response to irradiance range, while at lower irradiances growth rates are inhibited although plants remain healthy (Sousa-Pinto *et al,* 1999; Macler & West, 1987). In this study, the highest irradiance tested (≥120-140 µmol photons $m^{-2}s^{-1}$) was not found to produce an increased growth rate, while very low irradiances (30-40 µmol photons $m^{-2}s^{-1}$, 60-70 µmol photons $m^{-2}s^{-1}$) did produce slower growth rates. The optimum growth rate was achieved at 80-100 µmol photons $m^{-2}s^{-1}$ irradiance. Macler & West (1987) found growth rates to be maximal over the range of 150-250 µE $m^{-2}s^{-1}$ in their study of *G. coulteri* in culture, using whole vegetative plants. Sousa-Pinto *et al* (1999), in their study of the culture of a small intertidal *Gelidium* species *G. pulchellum,* achieved a highest growth rate of 10% day^{-1} with irradiances of between 130-240 µmol photons $m^{-2}s^{-1}$ using 2-3cm suspended algal pieces. The reason for the greater growth rate at the lower irradiance (80-100 µmol photons $m^{-2}s^{-1}$) in this study may be related to the amount of material in the medium. The highest RGR was achieved with a 1cm (or approximately 0.015g) algal tip in 100ml of medium at 15°C and 80-100 µmol photons $m^{-2}s^{-1}$ irradiance with the algal tip constituting about 0.00026% of the total volume of the medium, while the samples under 130 µmol photons $m^{-2}s^{-1}$ comprised 10g of algal material in two litres of medium at 15°C, with the algae constituting 0.0049% of the total volume. Growth limitations with regards to nutrients, for instance, may therefore have been reached faster in the samples in the bags. Another likely reason may be that this species occurs subtidally and may therefore not be able to tolerate the higher irradiances. In culture at 120-140 µmol photons $m^{-2}s^{-1}$, samples discoloured towards the tips after approximately two weeks in culture. This can be compared with samples at 80-100 µmol photons $m^{-2}s^{-1}$ which still appeared healthy after two weeks in culture.

Previous studies have also found that *Gelidium* and *Gracilaria* species have achieved maximum growth rates under 20°C-27°C (Choi *et al,* 2006; Rueness & Tananger, 1984; Macler & West, 1987; Boulus *et al,* 2007) which was not the case in this study. Rueness & Tananger (1984) found that both *Gelidium* sp. and *Gracilaria verrucosa* under culture grew best at 24°C, although this was the highest temperature tested, and naturally occur in sub-optimal temperature conditions in Norway. Similarly, Macler & West (1987) in a culture study of *G.*

coulteri, which occurs naturally in water temperatures ranging from 8-17°C, found that optimum growth was achieved in a temperature range of 20-27°C while inhibition and death were seen at temperatures greater than 30°C. Choi *et al* (2006), in their culture study of two intertidal *Gracilaria* species occurring in average annual sea temperatures of 7.11-23.73°C, found that both discoloured after a few days under 35°C and achieved optimal growth rates at around 25°C. However, the tolerance of the upper intertidal species to higher temperatures was found to be greater than the lower intertidal zone species. While the southern distribution of *G. capense* experiences temperatures of between 12.5°C to 19°C in winter and summer months (McQuaid & Branch, 1984), the northern most distribution of this species experiences mean maximum temperatures that do not exceed 22°C (Bolton, 1985).

The fact that *G. capense* is strictly temperate in its distribution may explain its poor tolerance to higher temperatures. There have been several studies that have found certain *Gelidium* species to thrive at lower temperatures and lower light intensities (Fredriksen & Rueness, 1989; Salinas & Valdés, 1993; Oliger & Santelices, 1981). Salinas & Valdés (1993), in their study of *G. sesquipedale* in the adult tetrasporophyte phase from Spain, found that necrosis was not observed at 16°C and at 18°C it did not cripple the eight week experiment and produced the best results, while temperature conditions of 20°C and 22°C resulted in the death of the plants by the fifth week. Oliger & Santelices (1981), in an ecological study of three Chilean species of *Gelidium,* found growth rates among adult plants to be maximal at 15°C for *G. rex* and *G. ligulatum,* while 20°C was optimal for *G. chilense.*

Rueness & Tananger (1984) found that *Gelidium* sp. and *G. verrucosa* grew best at 30ppt salinity, the highest salinity that was tested, and poor growth was achieved at 10ppt and 5ppt. They also found that the *Gelidium* sp. appeared to be more growth limited by salinity than the *Gracilaria.* Choi *et al* (2006) also found that the *Gracilaria* species in their study died in culture within seven days at 5ppt salinity. The salinity experiment results obtained in this study are not anomalous as other studies have found salinity to be a major limiting factor (Rueness & Tananger, 1984; Choi *et al,* 2006).

A positive correlation has been found between the relative abundance of *Gelidium robustum* and upwelling index (Hernandez-Guerrero *et al,* 2000) and growth has also been found to be directly proportional to water movement in semi-controlled cultivation conditions with treatments with the greatest water movement achieving growth rates of 3.6% day^{-1} (Pacheco-Ruiz & Zertuche-Gonzalez, 1995). Although the effect of water movement was not fully investigated in this study, Santelices (1991) suggests that water movement compensates for nutrient limitations and increased water movement along with the addition of nutrients may prevent bleaching by high light and high temperatures.

The maximum RGR observed in this study was 5.07% day^{-1}. Sousa-Pinto *et al* (1999) in their culture study of *G. pulchellum* achieved a maximum growth rate of 10% day^{-1} while Choi *et al* (2006) obtained highest RGRs of 4.95% day^{-1} and 4.47% day^{-1} for *Gracilaria verrucosa* and *G. chorda,* respectively. The maximum growth rate achieved by Rueness & Tananger (1984) of *Gelidium* sp. in culture was ≈7% day^{-1} and the highest RGR achieved by Macler & West (1987) was also ≈7% day^{-1}, although this achieved at a temperature that was not sustainable. The RGR in this study is therefore within the same range as that achieved in other culture studies with other species. Further culture investigations may improve the growth rates achieved.

Environmental and physiological as well as species-specific factors affect the yield and properties of agar. The agar characteristics of some other *Gelidium* species from previous experiments have proved to be of a far higher yield than that of *G. capense* in this study, ranging from around 30% to 48% of dry algal weight. Carter & Anderson (1986), in their study examining the growth and agar characteristics of the intertidal *Gelidium* species *G. pristoides* in South Africa, recorded agar contents ranging seasonally from 30% to 48% of dry weight. Similarly, Maclear & West (1984) measured agar yields of between 32% and 35% for *G. coulteri.* The agar content of *G. pulchellum* (Sousa-Pinto *et al,* 1999) was found to be around 38.6%. The highest agar content of *G. canariensis* in the wild was recorded as 32.6% with a mean value of 23.1% (Freile-Pelegrín, 1995). These values differ quite substantially from the agar contents measured in this study, which gave a value of 10.36% of dry algal weight. I would suggest that this species is probably not a viable option for agar production. However, the agar was extracted from one wild population within a several metre radius. Further culture experiments may find that agar yields can be improved through increasing growth rates, or with increased branching in the distal region. Carter & Anderson (1986) found agar concentrations to be between 8% and 15% higher in the distal than in the proximal plant halves which corresponded to season, with higher values recorded during summer months. Agar yields have also been found to increase with increased irradiance, and those plants grown at higher irradiances also tend to be more highly branched (Macler & West, 1987).

As it is suggested (Seo *et al,* 2010) that the shape, growth rates and amount of endofibres of the members of the Gelidiaceae family are potential criteria for analysing their potential uses in the pulp and papermaking industries, further investigation of the fibre content of *G. capense* could be investigated. Stegenga *et al* (1997), as mentioned earlier, suggest that *G. capense* has abundant endofibres in both younger branches and older axes. Through the observation of longitudinal and cross-sectional samples (Fig. 1) this species does appear to be highly fibrous internally. The fact that poor agar extractions were obtained may be directly related to the abundance of rhizines in this species.

Conclusion

This preliminary study suggests that *G. capense* may not be the most viable option as the agar yield was low compared with some other *Gelidium* species. However, the algal material for the extraction came from one wild population and further investigation into the yield from several different populations, including those occurring higher up on the coast in warmer waters, may produce different results. Seasonality may also play a role in the agar yield. The optimum temperature and irradiance for this species was lower than that found in many other studies, but also consistent with a few studies that found similar results. Increased water movement may improve growth rates and perhaps this aspect could be investigated further in future. A more detailed examination of the endofibre content may also be interesting, especially if future investigations can improve agar yield.

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