THE EFFECTS OF TEMPERATURE AND LIGHT ON THREE SOUTH AFRICAN *ULVA* SPECIES, AND THEIR POTENTIAL IN INTEGRATED AQUACULTURE

BY

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Contents

Abstract

Three *Ulva* species namely; *U. lactuca, U. rigida* and *U. capensis* were cultivated in the laboratory over a range of temperatures and irradiances. The aim was to assess the ability of these algae to tolerate various light and temperature and ultimately select one that can be recommended for cultivation by in integrated systems by the abalone industry. Such an *Ulva* species must ideally be able to withstand a range of temperature and irradiances, and one that often sporulates would be disadvantageous. The collected algae were cut into discs (1 cm in diameter) which were then cultivated in the enriched seawater media for seven days, crystallizing dishes at the desired experimental conditions. The range of temperature used was 10, 15, 20, 25 and 30°C. The range of irradiances used was 60, 120,180 and 240 $\mu E.m^{-2}.s^{-1}$. Through out the experiment, sporulation was a problem, resulting in the loss of a lot data in the form of discarded discs. Multivariate Analysis of Anova was done to determine the effect of the imposed factors and found that temperature significantly affected the mean growth of the *Ulva*. Overall *U. lactuca* was the most recommendable for integrated aquaculture because it sporulated least and it produced the maximum mean growths.

Introduction

Ulva species, commonly known as "sea lettuce" have been widely used as biofilters for marine fishpond effluents (Cohen & Neori, 1991; Neori *et al.*, 1991; Neori *et al.*, 1996). The alga is very efficient in removing nitrogenous compounds from wastewater, and the effluents in turn support the growth of *Ulva* (Cohen & Neori, 1991; Neori *et al.*, 1991; Jimenez Del Rio *et al.*, 1996; Neori *et al.*, 1996; Goldberg *et al.*, 1998). Indeed Goldberg *et al.* recorded a removal of 95% of the ammonia contained in the effluent in which *Ulva rigida* was grown. The resulting protein rich seaweed biomass may in turn be used as food for aquaculture fisheries such as, the fish, *Sparus aurata* L. (Neori *et al.* 1996) or abalone, *Haliotis midae* (Simpson and Cook, 1998; Steyn, 2000).

Long before the current methods of integrated systems evolved, other methods of nutrient removal from aquaculture ponds such as microbial oxidation, the use of seaweeds and bivalves in a two step removal process (Jimenez Del Rio, *et al.*, 1996) or flushing (Vandermeulen and Gordin 1990) have been in use. Of these three methods, the microbial oxidation by means of the sludge technique requires long retention times. A combination of seaweeds and bivalves in two separate systems requires shorter retention times but it is very complicated and unstable (Jimenez Del Rio, *et al.*, 1996). The flushing method on the other hand is very impractical because of the expenses involved, especially in cases where enclosed ponds or culture tanks are used (Vandermeulen and Gordin, 1990). The most viable method would be one that can absorb dissolved nitrogen from within the system at minimal costs while

allowing biofiltration of higher water volumes in shorter time, and this can be achieved by seaweeds, which in turn may also be of other benefit to the venture.

Various species of the genus *Ulva* have been successfully cultured both in the laboratory and outdoors, and grown in either artificial seawater or wastewater, showing a high capacity to take up nutrients (Neori *et al.*, 1990; De Busk *et al.*, 1986; Parker, 1981). *Ulva* species that have been studied in detail include *Ulva rigida* C. Agardh (Jimenez. *et al.*, 1996; Steyn, 2000; Altamirano *et al.*, 2000), *U. lactuca* L. (Parker, 1981; Friedlander *et al.*, 1996), *U. pertusa* Kjellman (Floreto *et al.*, 1996), *U. fasciata* Delile (Lapointe and Tenore 1981; Steyn, 2000) and *Ulva curvata* (Kutz) de Toni (Duke *et al.*, 1989).

Factors that primarily control the growth of seaweeds are light and temperature, and these in turn have strong influence on nutrient uptake and growth (Duke *et al.*, 1989; Lüning, 1990). According to Duke *et al.* (1989) growth and nutrient uptake are not regulated to the same degree by the same factor. This has been demonstrated in experiments whereby *Gracilaria tikvahiae, U. curvata,* and *Codium decorticatum* were all shown to accumulate nitrogen at temperatures limiting to growth and have lower nitrogen contents at nonlimiting temperatures (references in Duke *et al.*, 1989). Meanwhile Lapointe and Tenore (1981) have established that the yield of *U. fasciata* generally increased with increased light, irrespective of nitrogen loading.

Other factors that have an influence on growth and nutrient uptake are stocking density (Lapointe and Tenore, 1981; De Busk *et al.*, 1986; Vandermeulen and Gordin 1990; Israel *et al.*, 1995), aeration or water motion (Parker, 1981; De Busk *et al.*

1986), as well as DIC (dissolved inorganic carbon) and pH (Frost Christensen and Sand-Jensen 1990). The stocking density that according to Debusk *et al.* that produced highest yields was 0.8 kg. wet wt m^{-2}. High stocking densities result in carbon limited systems (Vandermeulen and Gordin 1990). Aeration enhances the growth of *Ulva* especially when the alga is grown in nutrient poor environments by breaking down diffusive boundary layers at the thalli surface which may impede nutrient and carbon uptake while exposing the thalli to light (Parker, 1981; Vandermeulen and Gordin 1990). *Ulva* has a high affinity for DIC whereas increased pH of about 10 is highly growth inhibitory.

Of all these factors however, the magnitude of this study could only investigate the effect of temperature and light on the growth of local species.

The biology of the genus *Ulva*

Ulva species are membranous green algae and are two cell layers thick. The alga attaches to substrata by means of a holdsfast composed of rhizoids produced by the extension of the proximal cells. Floating plants have also been observed, as is the case with *Ulva lactuca* from the Simons Town Harbour, which have also been cultured in this study. The arrangement of cells in surface view varies depending on what part of the blade is examined and this arrangement is also used to distinguish species. The cells contain a parietal chloroplast and one to a few pyrenoids, which varies in number from species to species although there is a high degree of overlap.

Reproduction

The life cycle is composed of two isomorphic generations, the diploid sporophyte and the haploid gametophyte. Haploid gametophytes are unisexual and produce gametes by mitosis, which return to the sporophytic phase after copulation with gametes of the opposite mating strain (Tanner, 1981). The diploid sporophyte (2n) produces spores (n) in sporangia through meiosis. The sporangia result from peripheral somatic cells becoming reproductive. Propagule release may result in total loss of biomass or the sloughing of the marginal portions of the thallus. During sporulation the periphery of the plant disintegrates to produce numerous propagules. The cause of sporulation is not yet well defined. A study by Niesenbaum (1988) found that propagule release normally occurs during the warmer months of the year, or when the alga is cultivated at high temperatures in laboratory experiments. Some studies (cited in, Steyn 2000) found the tidal cycle to be a major driving force behind sporulations, whereas Oza and Sreenivasa Rao (1977) expressed that the production of spores depends on the culture media used. A recent study by Stratmann *et al.*, (1996) on *U. mutabilis*, produced a new line of thinking as to what causes sporulation. They found that the blade cells of *U. mutabilis* produces regulatory factors, which they excrete into their cell walls and into the environment, and these factors are apparently vital for the maintenance of the vegetative state.

Another form of reproduction that is common members of the genus *Ulva* is parthenogenesis whereby gametes develop into parthenosporophytes, with a small percentage (1-2%) developing into gametophytes of the same mating type (Tanner,

1981 and references therein). Apart from reproduction by means of swarmers, *Ulva* may also propagate by means of vegetative fragmentation.

Sterile strains of *Ulva* have also been isolated in *Ulva pertusa* (Migita, 1985). This nonspore producing alga could make a very good cultivar to replace the spore producing strains, which lead to loss of biomass.

Notes on the taxonomy of *Ulva* species

Ulva is a widespread genus is mainly confined to the tidal zone of the sea. Their form of growth maybe by attachment onto substrata using the holdfast, or simply freefloating. The genus belongs to the division Chlorophyta and class Ulvophyceae. Wynne and Kraft (1981), Silva *et al.*, (1996) and Stegenga *et al.* (1997) classified the *Ulva* genus under the order: Ulvales, and family: Ulvaceae and the genus includes the following South African west coast species: *U. fasciata, U. lactuca, U. rhacodes, U. rigida and U. capensis*. These are but a few of the species found in southern Africa.

Not too long ago there was a controversy as to whether the group belongs to the order Ulotrichales or Ulvales. According to Tanner (1981), there is sufficient support for the distinction between the two orders and they thus proposed that the two orders be separated. Their main reasoning is based on the life histories and forms of reproduction, which are different in the said groups. Another, rather more recent change in local species of *Ulva* is the nomenclatural change resulting in the inclusion of *U. capensis* in *U. uncialis* by Silva *et al.*(1996). It is likely that *U uncialis* and *U capensis* are not conspecific (J.J. Bolton *pers. Comm*) and thus this change is not followed here.

The distribution of the genus is cosmopolitan, and of the local species some are much more widespread than others. *U. rhacodes* (Holmes) Papenfuss has only been recorded in northwest Spain in addition to the South African records (Stegenga *et al.*, 1997). *U. rigida* C. Agardh is a warm temperate species, but has also been recorded from the tropics (Joska 1992; Silva *et al.*, 1996; Stegenga *et al.* 1997). *U fasciata* Delile is widely distributed but it is said to be a pantropical species (references in Stegenga *et al.*, 1997). The records of *U. lactuca* L. showed that this species have a wide distribution but, as Stegenga *et al.* suggested, most of the records will probably not be correct and that is why many are marked *cf* an indication of a doubtful record. Bolton (*pers. comm.*) is also of the opinion that people tend to lump all the *Ulva* species that they could not identify into *U. lactuca*. There is no conclusive evidence that southern Africa *U. lactuca* is genetically identical to the original European *U. lactuca*.

Introduction to seaweed mariculture

Several seaweed species are cultivated worldwide because of their importance to man as sources of food, particularly in the oriental countries, or as gels and as chemicals used in everyday commodities. However due to the ever-increasing demand, natural populations of commercial algae are on the brink of collapse as a result of overharvesting and thus a resort to mariculture was necessary.

Recent developments in cultivation techniques have prompted the cultivation of the majority of commercially important seaweeds. As well as increasing yields, mariculture improves quality of the food products by exposing the algae to optimal conditions for the desired standard (Ohno and Critchley, 1993).

The main cultivated species today are *Laminaria japonica*, *Undaria pinnatifida* (Phaeophyta), *Porphyra tenera*, *Eucheuma* spp. *Gracilaria* spp. (Rhodophyta) *Monostroma nitidum* (Chlorophyta). Other species that have entered the commercial sector of recent are the greens, *Enteromorpha* spp. and *Caulerpa* spp. (Ohno and Critchley, 1993). The cultivation of *Ulva* species is still mainly confined to laboratory tanks and no major operations are in place as yet. Unlike other algae that are mainly cultivated to improve yields or quality, *Ulva* cultivation is aimed at biofiltration. In integrated systems, the setup becomes very beneficial whereby the resulting algal biomass is used as a supplement or as a primary feed for the cultivated herbivorous fish and mollusks.

In our context the cultivated organisms are Abalone (*Haliotis midae*), an industry that is still in its developing stages (Steyn, 2000). Naturally occurring populations of the abalone feed on *Ecklonia maxima*, on the west coast and mainly on *Plocamium corallorhiza* (Turn.) Harv., a red alga on the south coast. The sustainability of this have not been examined and no mariculture attempts have been tried thus far (Steyn, 2000). Attempts to feed the abalone on artificial feed proved to be unviable because it results in the discolouration of the shell and the meat (Steyn, 2000) and this may have a negative effect on the economics of the venture. A diet made up of mixed algae

produces abalone that are red to brown in colour, the colour most favoured by the consumers in the east (Simpson and Cook, 1998; Steyn, 2000).

Background of the study

This study is a complement of a study done earlier this year by Steyn who primarily compared two species of *Ulva, U.fasciata* and *U. rigida* to determine which of the two species currently cultivated for abalone feed by Marine Growers Ltd., Port Elizabeth, South Africa should be preferred. The two species were compared with respect to their morphological and physiological adaptations to varied chemical conditions in large-scale tank cultures. Our study aims to fill the gap left by Steyn's study, by looking at Temperature and light and their effect on the growth of the algae.

A total of three species namely; *U. lactuca*, *U. rigida* and *U. capensis*. were cultivated under a variety of light and temperature conditions in an attempt to determine the one that could best withstand range of conditions that commonly occur in land-based pond systems. It is envisaged that the results will be used as the basis for larger scale studies that in the end can be used to advise the mariculture industry on what species to pick for the best results in pond cultivation systems.

Materials and Method

The first part of the project involved the identification of the *Ulva* species that were to be used for the experiment and the specimens were collected from Dalebrook, Simonstown and Camps Bay. The species identified were *Ulva rigida* and *U. fasciata*.

from Dalebrook, *U. lactuca* form Simonstown and *U. capensis* from Camps Bay area (Joska 1992, Stegenga et al. 1997, Steyn, 2000). Specimens have been preserved in formalin in the Phycology Laboratory of the University of Cape Town.

Despite the fact that a total of four species were identified, our experimental set-up could only accommodate three at time. Due to the difficulty in separating *U. rigida* and *U. fasciata* the later had to be excluded from the experiment. Practically cutting *U. fasciata* into one cm wide discs would also be very difficult due to the plants stringy/ribbon-like morphology, and taking into account that one would not like to cut the discs from the outer one centimetre of the blade.

The enriched seawater (ES) media used was prepared according to the Provasoli protocol (see Chapman, 1979) (Appendix A). The media used was made up to a 1/3 strength by adding 6 ml ES to a litre (1L) seawater, this was necessary to control bacterial growth (Chapman, 1979). The seawater used in the experiment was filtered through Millipore prefilters using an electric suction pump. The resulting media was then autoclaved for an hour before the cultivation commenced.

Experimental design

A. The temperature experiment

Five experimental conditions were set up; 10, 15, 20, 25 and 30°C. Temperature experiments conducted at 10, 15 and 20°C were carried out in temperature-controlled rooms. For the 25 and 30°C temperature settings, water bath heaters dipped in baths were used. To obtain a uniform environment for all the dishes, water baths were used

in every temperature set-up, in addition to the dishes were rotated on a daily basis to make sure they all are exposed to the same outside factors. The irradiance of between 60 and 70 $\mu E.m^{-2}.s^{-1}$ was provided by means of cool white fluorescent tubes and the photoperiod was 16 L: 8 D.

B. Light experiment

The light experiment was conducted in a large water bath (120 x 40 x 66 cm) and the dishes were immersed to about 50 mm. Shading was provided by using nets to achieve irradiances of 60, 120, 180 and 240 $\mu Em^{-2}s^{-1}$. There was some variation within treatments but the effect thereof has been minimized by the daily rotation of the dishes, such that by the end of the cultivation period, all the dishes will have been exposed to the same intensities. Illumination was provided by fluorecent light bulbs.and the photoperiod was 16h (light): 8h(dark).

Field collection of the algae and preparation of the dishes

The algae were collected during low tide in plastic bags filled with seawater and a single collection lasted over two hours due to distances between the collection sites. Upon arrival in the laboratory the plants are cut into discs of one (1) centimetre each, using a cork borer. A total of 6 discs cut from different plants of a particular species were put in a crystallizing dish, altogether 9 crystallizing dishes three per species.

The discs were cut from below 1 cm of the periphery of the blade, while avoiding the basal region. The periphery of the blade would not be ideal to cut from because this is where sporulation takes place and this could have devastating consequences to have

all one's discs sporulating especially since the diameter of the disc is the measure of growth. The middle and the base of the blade is also not an ideal part to cut the disc from as this is the thickest part of the plant and as a result it would grow much slower than the thinner outer portions.

During the eight days of study (each experiment), the experiment was closely monitored on a daily basis, at which times the dishes the were rotated and the unhealthy discs (due to sporulation) were removed. The degree of sporulation was recorded, using a subjective scale whereby if only one disc sporulated or if only the periphery of the affected disc presents signs of sporulation then the situation would be described as minor. When more than one disc has completely sporulated then the condition is described as moderate. When all or 5/6 discs have sporulated or have turned pale then the situation is described as severe.

A method that was described by Steffensen (1976) was used to produce permanent state and sizes of the discs that would also cause little problem when measuring, compared to wet *Ulva* discs which are rather slippery and margin of error is higher. In this method the discs are laid on an unexposed photographic paper, which is then exposed by placing it under an enlarger, and developing the paper as usual using a developer, washer and a fixer. The process was carried-out in the dark room. The diameters of the discs was measured from these prints using a ruler. Both the normal and the sporulated discs were measured for increase in diameter.

Data analysis

The means, standard deviations and the mean standard error of the diameter measurements were calculated using the Descriptive statistics package in the STATISTICA programme and graphs produced there-from. The data from measurements of the diameters of the discs were analysed using the MANOVA/ANOVA (Multivariate/Analysis of Variance) statistical package in STATISTICA. Because some of the results were missing due to sporulation, the averages of the data sets, in which some of the data were missing, were used as replacements.

Results

One problem that was encountered in this culture work and has also been reported in many previous experiments is sporulation (Steffensen, 1976; Chapman, 1979; Parker, 1981, Steyn, 2000), and to a lesser extent bacterial growth resulting in a marked smell of ammonia in some culture vessels. Another problem that did not seem to affect the growth of the plants very much was the condensation, particularly in the 25 and 30°C experiments. This occurred as a result of covering the crystallizing dishes with glass lids, which apart from preventing contamination could also counter evaporation.

Ulva lactuca sporulates very fast when cultivated at high temperatures (Table 1) and thus no measurements were recorded at this temperatures of 25 and 30°C. At 25°C the algae however survived the first three days of exposure to this temperature. *Ulva capensis* was fully intact during the first three days of the experiment but on the fourth day signs of sporulation were beginning to show. The sporulation was however not restricted to higher temperatures, as was the case with *U. lactuca*.

Table 1. The sporulation data from the temperature experiment

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
U. lactuca 10°C							
U. lactuca 15°C							
U. lactuca 20°C							
U. lactuca 25°C				**	***		
U. lactuca 30°C	*	**	***				
U. rigida 10°C							
U. rigida 15°C							
U. rigida 20°C					*		
U. rigida 25°C							
U. rigida 30°C					***		
U. capensis 10°C						*	*
U. capensis 15°C				*	*	*	**
U. capensis 20°C				*	*	**	**
U. capensis 25°C				*		**	***
U. capensis 30°C				*	***		

* = minor sporulation

** = moderate sporulation

*** = severe sporulation (discontinuation)

During the light factor experiment it was found that all the studied species responded similarly to the different irradiances and sporulation only became apparent during the fourth day of the experiment. This is in exception of one case that was detected in one

disc of the *U. capensis* grown at 180μEm^{-2}s^{-1}. There is consistently a high prevalence of sporulation at the higher irradiances.

Table 2 The sporulation data from the Light experiment

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
U. lactuca @ 60μEm^{-2}s^{-1}							*
U. lactuca @ 120μEm^{-2}s^{-1}					*	*	*
U. lactuca @ 180μEm^{-2}s^{-1}				*	*	*	*
U. lactuca @ 240μEm^{-2}s^{-1}				*	*		*
U. rigida @ 60μEm^{-2}s^{-1}						**	*
U. rigida @ 120μEm^{-2}s^{-1}				*	*	*	*
U. rigida @ 180μEm^{-2}s^{-1}				*	**	**	*
U. rigida @ 240μEm^{-2}s^{-1}					**	**	*
U. capensis @ 60μEm^{-2}s^{-1}					**		*
U. capensis @ 120μEm^{-2}s^{-1}				*	*	*	*
U. capensis @ 180μEm^{-2}s^{-1}		*		*	*		*
U. capensis @ 240μEm^{-2}s^{-1}				*	*		**

* = minor sporulation

** = moderate sporulation

*** = severe sporulation (discontinuation)

Temperature results

Fig. 1 shows that *U. capensis* did not grow much adding on a mere 2 cm over seven days. There is very little difference in the mean growth between 10, 15, 20 and 25°C.

Although there is a suggestion of a drop towards high temperatures, this is weakened by the strong overlap in standard deviations and the mean errors.

All the discs of U. lactuca at 25 and 30°C died out (Fig.2), but overall best growth were recorded. In *U rigida* (Fig 2) there seem to be a problem at 15°C resulting in a very low mean growth. The maximum mean growth was recorded at 20°C. Growth at 10 and 20°C was average and poor growth was recorded at 30°C.

The statistical analysis of the temperature data

The MANOVA results presented in Table 3 and 4 below, both indicate that there is a significant difference ($P < 0.000001$) in the mean growths that are a direct result of the temperature factor. There is also a significant difference in the mean growth of different species ($P < 0000001$) as shown in Table 3. As shown in table 4, there is no significant difference between the mean growth of *U.rigida* and *U capensis* ($P = 0.333905$). The Tukey test, a test for multiple comparisons was used to determine which of the three species was more different (Zar, 1999) and it showed that the mean growth values of the three species were equally different.

Table 3 Table 4. A summary of the results of the MANOVA (temperature experiment, only including the data sets without missing data, 10, 15, and 20°C, all *U. lactuca* at 25 and 30°C died-out)

Sources of variation	Degrees of freedom	Mean square	Variation ratio
Temperature main effect	2*	47.5802*	20.7383*
Species main effect	2*	410.0355*	178.7177*
Interaction Temperature × species	4*	60.6619*	26.4401*

$P < 0.00001$, * = significant result.

Table 4. A summary of the results of the MANOVA (temperature experiment, *U. rigida* and *U. capensis* at 25 and 30°C)

Sources of variation	Degrees of freedom	Mean square	Variation ratio
Temperature main effect	1*	19.5313*	23.6778*
Species main effect	1	0.7813	0.9471
Interaction Temperature × species	1*	102.9613*	124.8201*

$P < 0.00001$, * = significant result.

The light results

Fig. 4. shows that the mean growth value of *U. capensis* does not change much apart from a slight hike at 60 $\mu Em^{-2}s^{-1}$and a little ditch at 240 $\mu Em^{-2}s^{-1}$. In Fig. 5. highest mean growth for *Ulva lactuca* is recorded at 120$\mu Em^{-2}s^{-1}$. The mean growth in *U. rigida* (Fig. 6) is fairly uniform, and the only shift is at an irradiance of 120 $\mu Em^{-2}s^{-1}$.

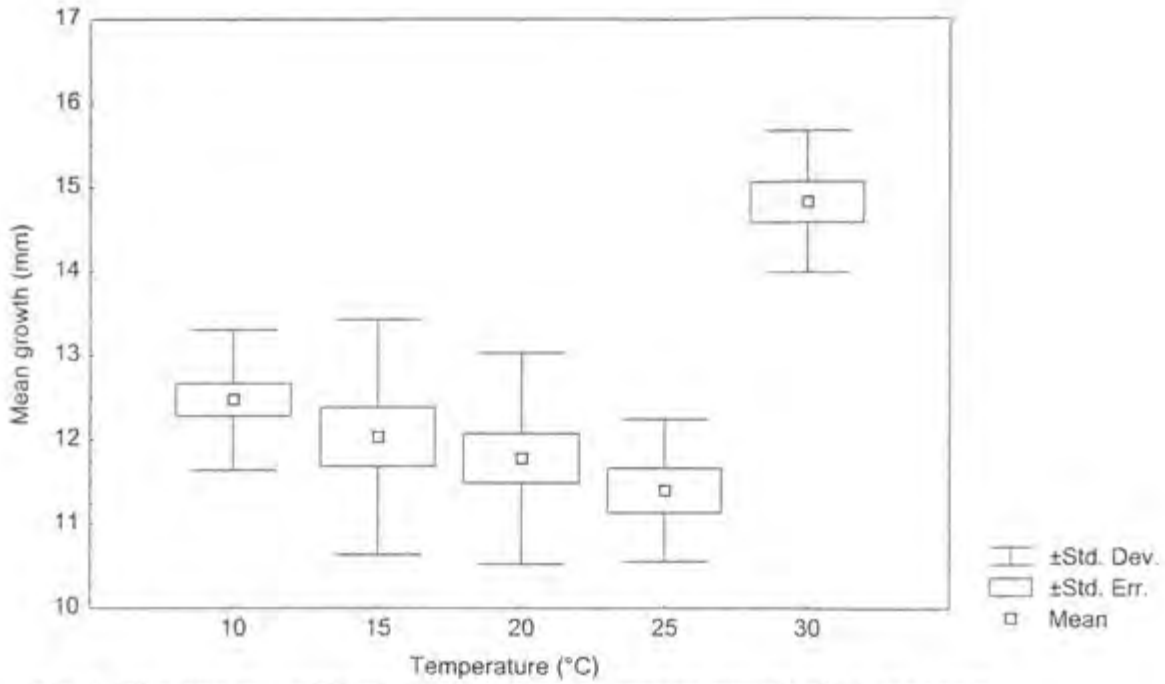

Fig. 1. The box and whisker plot showing the means growth of *U. capensis*

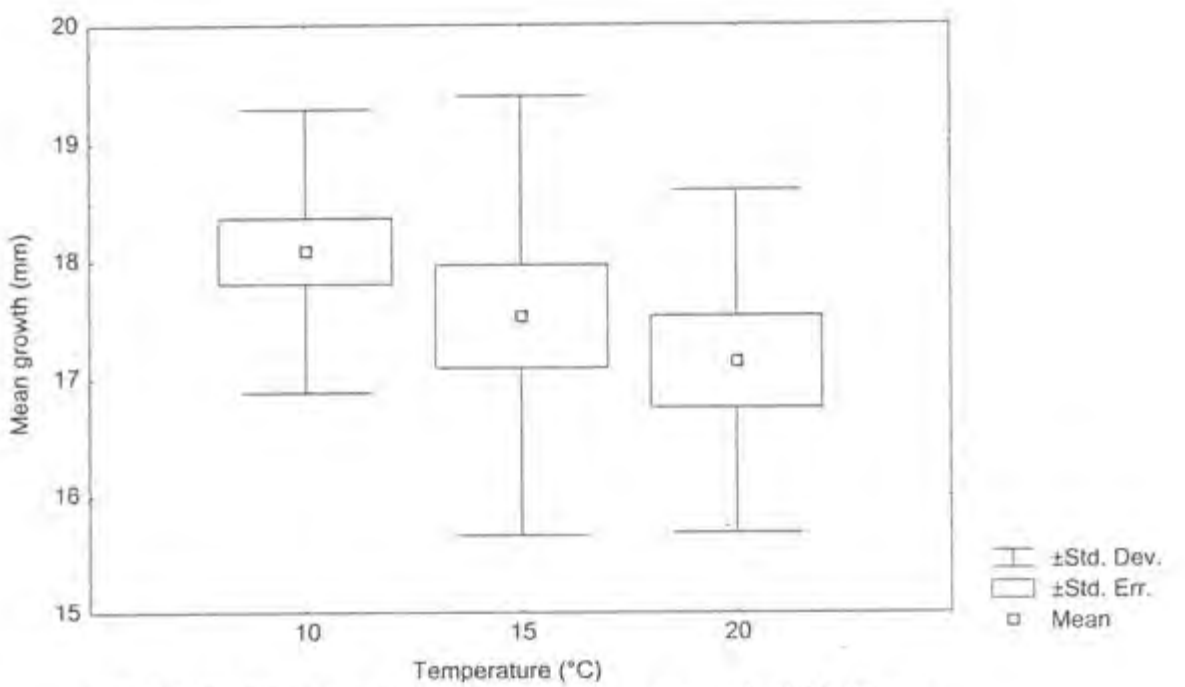

Fig. 2. The box and whisker plot of the mean growth of *U. lactuca*

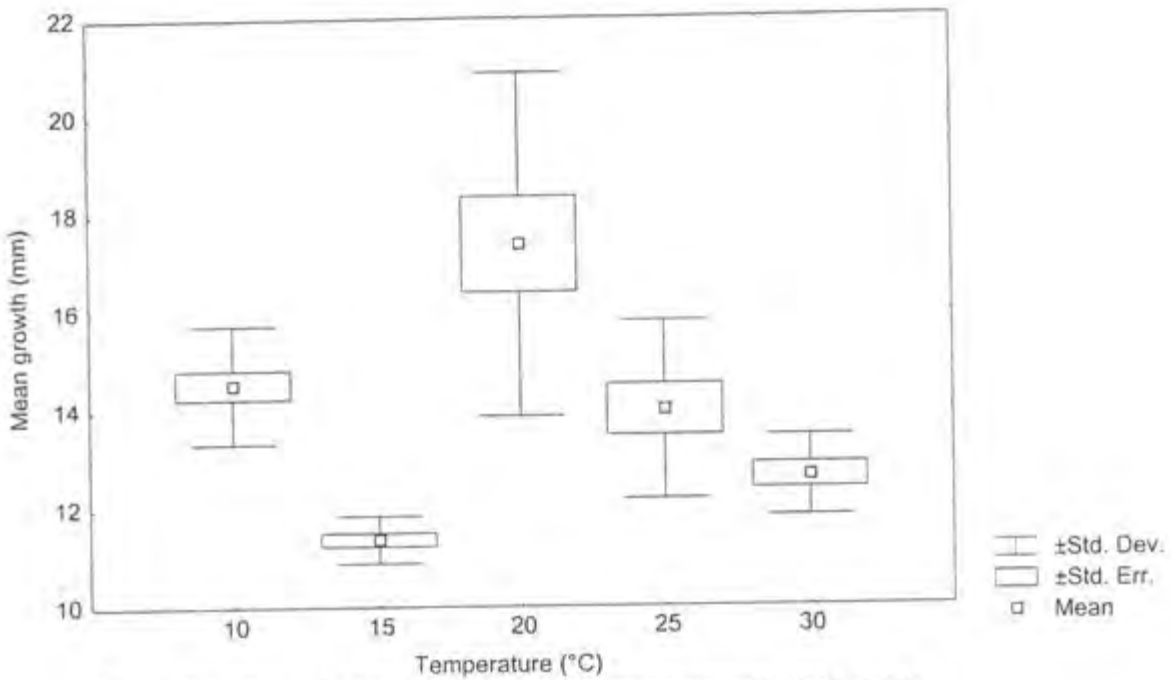

Fig. 3.The Box and Whisker plot of the mean growth of *U. rigida*

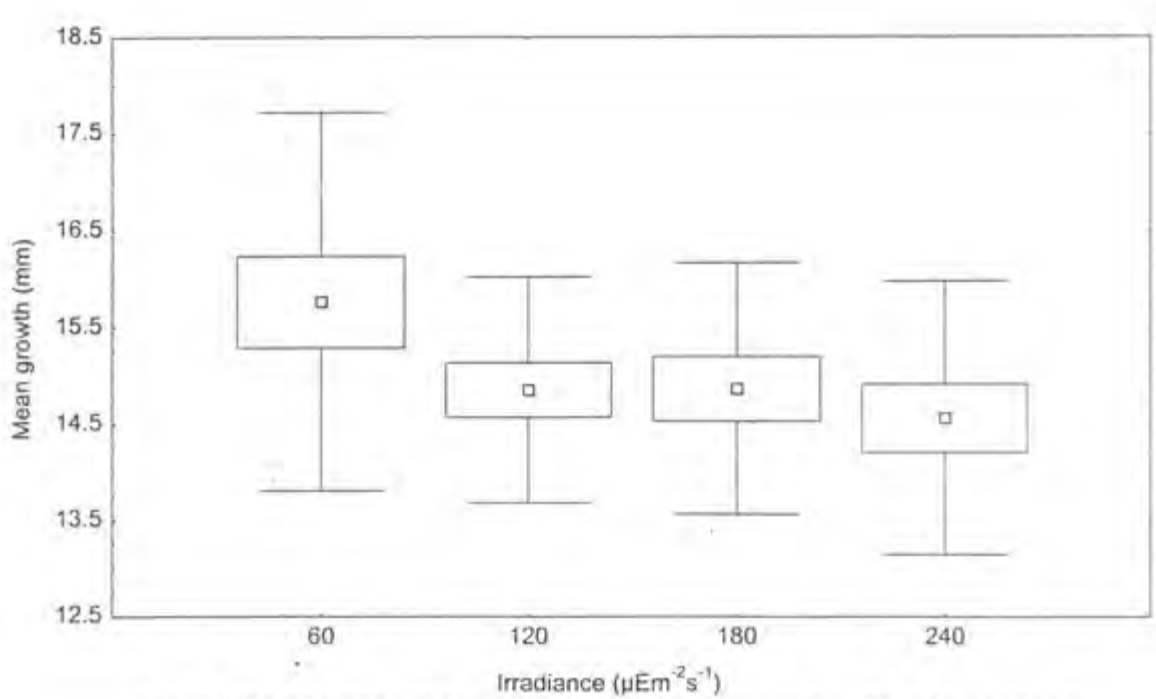

Fig. 4. The box and whisker plot showing the mean growth of *U. capensis*

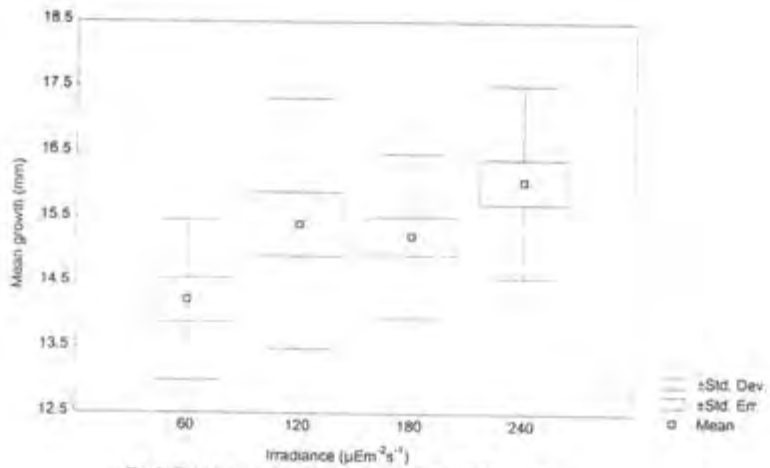

Fig. 5. The box and whisker of the mean growth of *U. lactuca*

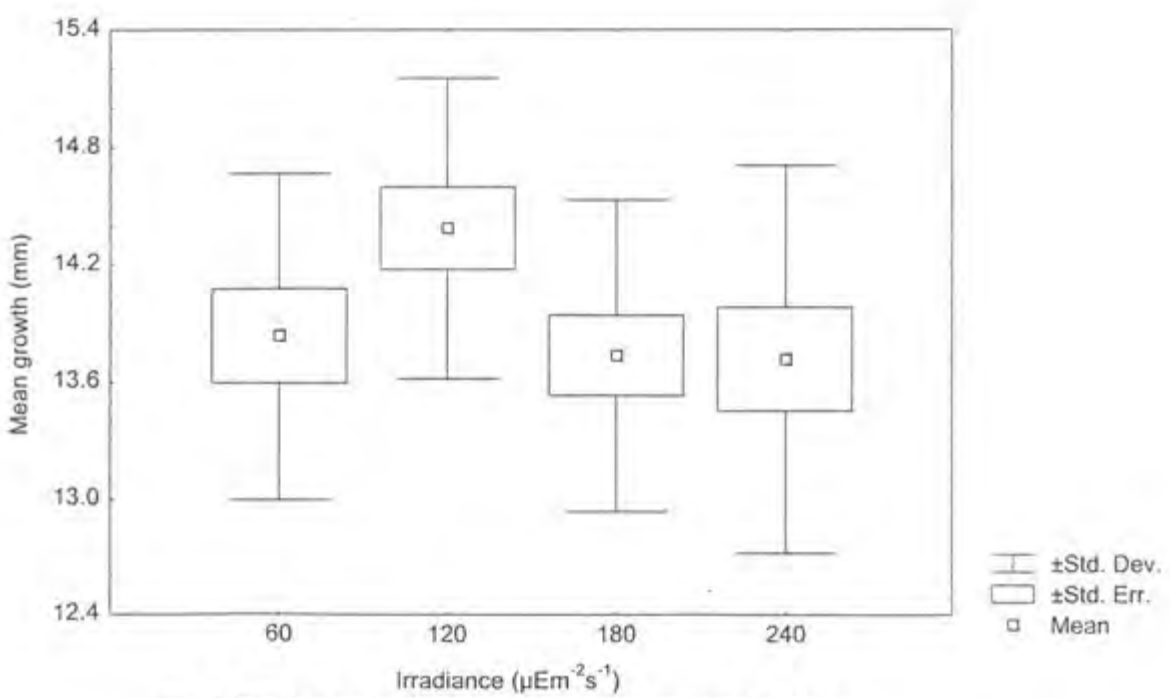

Fig. 6. The box and whisker plot of the mean growth of *U. rigida*

Table 5. A summary of the results of the MANOVA (Irradiance experiment)

Sources of variation	Degrees of freedom	Mean square	Variation ratio
Light main effect	3	0.91038	0.60467
Species main effect	2*	35.78447*	23.76784*
Interaction Irradiance × species	6*	8.06547*	5.35704*

$P < 0.00001$, * = significant result.

The statistical analysis of the irradiance data

According to Table 5. light does not have a significant effect on the mean growth of the algae ($P = 0.612675$). The species effect on the mean growth is significant ($P < 0.000000$). The effect of the interaction between the light and the species factors is highly significant ($P < 0.00001$).

Discussion

Sporulation

The prolem of disc sporulation have been addressed before by several authors such as Steffensen (1976), Parker (1981) and Steyn (2000). The sloughing of the discs leads to a pronounced loss of biomass and such that the growth measurements recorded in the final analysis are but really fractions of the real measurements. At this point let me put forward some observations that could explain the severity of sporulation or propagule release as Steyn refers to it. During the photographic undertakings, discs that had sporulated but still intact, were also measured to determine the increase in diameter,

but then most would be so damaged that no matter how one tries handle them they will tear. If they do not tear then one might still experience problems with lining them on the photographic paper as they become very thin. Some discs however, especially those from *U. rigida* are a hardy so that one can still align them on photographic paper even when they are completely pale. The implications for this is' a lot of missing data for for the softer species like *U. lactuca* even when sporulation in this discs were as severe.

The sporulation data showed that sporulation only started three after the start of the aquaculture experiment. But we also know that in nature or atleast in the South African context, it is not possible to get temperatures as high as 30°C that persist for longer than a 24 hours. Therefore, the information at hand does not really allow us to discriminate against any of the studied species but it can help us select the most hardy.

Temperature

The results above show that temperature is indeed a major factor in physiological processes and as such a variation in temperature has profound effects on the affected organisms.

In our case it was found that temperature affects the mean growth in *Ulva* spp. and each species is affected differently. But overall *U. lactuca* produced high mean growth values but *U. rigida* had a wider range of temperature tolerance. *U. capensis* was too fertile and this totally disqualifies it as a possible candidate for integrated aquaculture. But it must be noted that at the time during the field collections of the seaweed material many fertile U. capensis plants were observed compared to none in the other

two species. Therefore it could also have been the effect of the season that alga became fertile while in culture. This aspect needs more investigation.

Irradiance

Different algae may however have different requirements of light requirements, for instance those occupying deeper oceans will be expected to have lower light optima compared to those inhabiting the tidal regions. The Ulva genus, being a tidal group, would therefore be expected to have high light optima.

It has been shown above that irradiance between 60 –240 $\mu Em^{-2}s^{-1}$ does not have any significant effect on the mean growth of *Ulva*. However, I strongly believe that if the sporulation data could be quantified and tested similarly for the Irradiance effect the result was going to be significant because sporulation was consistently higher at elevated levels of irradiance.

The effect of irradiance on different species is marked and this was illustrated in Table 5 above. One would have expected that the sporulation results would be related to the mean growth values but this is not so, the reason being that many discs that had fully sporulated still retained their structure intact and as a result they were measured along with the healthy discs.

This study was fairly preliminary and therefore drawing conclusions from the results presented above would be a bit Premature. If I was however put in a position to pick one species from the three discussed above for an integrated aquacultue, I would most likely pick *Ulva lactuca*. *U. lactuca* sporulated least apart form that one instance at

25-30°C. The alga also produced the highest mean growth. *U. capensis* on the other hand had a lot of sporulation and recorded poor growth. *U rigida* recorded a lot of sporulations as well and the highest growth recorded was also much lower than that recorded for *Ulva capensis*.

Recommendations

Parker (1981) suggested that the longest study period of studies of this nature not be longer than two days citing problems of sporulation and nutrient starvation as the main reasons. From the problems experienced during the project recommend that Parker's suggestion be considered although a day or two beyond may still be safe. Nutrient starvation was not a problem as in some cultures discs were still healthy at the end of the experiment. Another aspect that could be considered would be the use of disc from a single parent plants to determine the effect of genetic differences on the outcome on the resulting ratios.

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Appendix 1

Preparing the Enriched Seawater (ES) medium

The Provasoli protocol was used in the setting up of the media and the constituents were as follows:

ES media	1L
Sodium Nitrate, $NaNO_3$	2.331g
Na_2 glycerophosphate	333.1mg
Fe solution	166.5ml
PII metals	166.5ml
Vitamin B_{12} (10 000X)	0.1ml
Thiamine (10 000X)	0.1ml
Biotin (1000X	1.0ml
Tris buffer	3.333g