Effects of Commercial Seaweed Concentrate (Kelpak) on Growth of *Gracilaria gracilis* (Stackhouse) Steentoft (Rhodophyta, Gigartinales) in Laboratory Culture.

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ABSTRACT

The growth of *Gracilaria gracilis* (Stackhouse) Steentoft, Irvine *et* Farnham was examined by studying the effect of various concentrations of commercial seaweed liquid fertilizer (Kelpak®) on growth of apical segments, in laboratory culture, with a view to the potential in mariculture. Over the 14-days in seawater only, *Gracilaria gracilis* apical segments obtained maximal growth at 0.1% (1:1000) seaweed concentrate dilution with an increase in growth of 11% in length, 41% in weight and 13% in specific growth rate compared to the control. However the apical segments of *Gracilaria gracilis* growing in seawater culture with 1% (1:100) seaweed concentrate dilution had a significant inhibition in growth. In nutrient-enriched seawater medium, *Gracilaria gracilis* obtained maximal growth in 0.1% (1:1000) and 0.2% (1:500) seaweed concentrate dilution. Relative to the control, apical segments in 0.1% (1:1000) seaweed concentrate dilution in ES culture obtained an increase in growth of 63% in length, 120% in weight and 51% in the specific growth rate. Similarly in apical segments growing in 0.2% (1:500) seaweed concentrate dilution obtained a 41% increase in length, 32% increase in weight and 65% increase in specific growth rate, compared to the control. *Gracilaria gracilis* apical segments growing in 1% (1:100) seaweed concentrate dilution in ES culture had a significant inhibition in growth. Apical segments of *Gracilaria gracilis* did not survive at very high concentrations of Kelpak (1:1 and 1:5 seaweed concentrate dilutions) in both seawater and ES culture. Overall apical tips growing in ES culture had a higher growth than seawater culture for all the treatments. Hence, Kelpak ® may have commercial potential in the seaweed mariculture industry.

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INTRODUCTION

In many parts of the world, the gracilaroid red algae (*Gracilaria*, *Gracilariopsis*) are currently the most important algae for the production of agar, and thus the basis of a multimillion-dollar phycocolloid industry (Oliveira *et al.*, 2000). Agar is the main thickening agent used in Japanese cooking and is common additive to prepared foods in the western world. Agar is also the familiar medium on which microorganisms are cultured (agar plates).

In recent years, the large and increasing international demand for *Gracilaria* as a raw material for agar production has necessitated a shift away from utilization of limited natural stocks towards cultivation of *Gracilaria* on a large scale (Oliveira *et al.*, 2000). Consequently research has begun to focus on algal mariculture as a viable alternative, supplying not only the demand for agarophytes such as *Gracilaria*, but also for algae cultivated specifically for food and fodder, such as *Porphyra*, *Laminaria* and *Eucheuma* (Oliveira and Alveal, 1990). Successful commercial *Gracilaria* farming ventures are currently established in many countries of the world including Chile, Venezuela, Namibia, Taiwan and China, and a wide variety of cultivation methods have been employed (Dawes, 1995). These include intensive cultivation in tanks, cultivation in ponds and cultivation in the sea. In recent years, cultivation in the open sea has gained

much popularity as it allows for extensive production of *Gracilaria* without the necessity for constant monitoring and manipulation. It can be divided into two main techniques; bottom cultivation, where algae are planted directly onto the sea floor, and suspended cultivation, in which plants are suspended above the sea bottom on ropes or lines (Oliveira *et al.* 2000). In South Africa, cultivation of macroalgae, particularly *Gracilaria gracilis* (Stackhouse) Steentoft, Irvine *et* Farnham, has gradually been shifting from the conceptual to practical methodology. Suspended cultivation on floated rafts is presently being investigated on a small scale with a view to future expansion (Anderson *et al.* 1996).

Characteristics of *Gracilaria* species that make them desirable for cultivation are fast growth rate, good agar yield and quality and relative ease of growth (Buschmann *et al.*, 1995; Smit & Bolton, 1999). The most important attribute is that almost all cultivated species produce solely through fragmentation, leading to a high regenerative capacity. This along with the fact that *Gracilaria* species are morphologically plastic has initiated many studies focusing on selection of strains with good gel characteristics and fast growth rates (Smit & Bolton, 1999). Several studies have been done in southern Africa since World War II. Most of these have dealt with the ecology of *Gracilaria* (Anderson *et al.*, 1993; Molloy & Bolton, 1995). Several other studies have looked at ecophysiological aspects of *Gracilaria* cultivation or dealt with commercial aspects of its utilization (Engledow & Bolton, 1992; Anderson *et al.*, 1996; Smit & Bolton, 1999). Interest in seaweed mariculture, has necessitated research into factors inherent to *Gracilaria* itself, which affect growth and regrowth performance (Smit & Bolton, 1999). Santelices and

Varela (1995) termed these factors 'organismic determinants'. Since productivity of *Gracilaria* is tightly coupled to vegetative regeneration, it is important to understand the processes leading to the development of new tissue and to apply this knowledge to seaweed mariculture operations in order to enhance production.

Increases in the yield of the seaweed have been accomplished by a variety of methods, including supplementing natural stocks by planting methods, and tank or fishpond cultivation (Engledow & Bolton, 1992). When any attempt is made to farm natural stocks or begin mariculture of a seaweed, it is obviously a benefit to know the environmental tolerances of the organism to be used (Engledow & Bolton, 1992) as well as the main constraints to cultivation, namely nutrient supply (Oliveira *et al* 2000).

According to Hanisak (1990), nitrogen is the nutrient most frequently reported to limit the growth of seaweeds in natural ecosystems. Macroalgae have physiological mechanisms to acquire, utilize, and store various forms of nitrogen in environments that have tremendous spatial and temporal variations in the concentration of this nutrient. The successful cultivation of seaweeds requires the knowledge of nitrogen relationships of seaweeds (Hanisak, 1990). *Gracilaria* has the capacity to take up and store nitrogen in excess of immediate requirements, and use it to sustain growth during subsequent periods of nutrient deficiency (Smit *et al*., 1997; Navarro-Angulo & Robledo, 1999). Storage can be in the form of inorganic nitrogen and/or metabolites such as proteins and pigments (Navarro-Angulo & Robledo, 1999). This capacity has been utilized in cultivation of seaweeds to minimize the growth of epiphytes and provide the physiological basis for

pulse feeding. Nutrient supply is an important operating parameter in the management of seaweed cultivation systems. The development of a separate management strategy is required for each species under cultivation since their physiological requirements differ (Navarro-Angulo & Robledo, 1999).

Maximal yields are possible only when nutrient conditions do not limit growth. At first, it would seem that continuous nutrient enrichments would maximize growth (Hanisak, 1990). However constant high nutrient availability is usually not natural for most seaweeds and is unnecessary for their cultivation. Given their high nitrogen uptake rates, the continuous enrichment of high concentrations of nitrogen quickly saturates the nitrogen requirement for growth; furthermore fertilization is not only wasteful and uneconomical, but also provides nutrients for opportunistic epiphytes, which often are considered to be the most serious treat to maintaining seaweed cultures (Hanisak, 1990). Thus the optimal management of nutrients in a seaweed cultivation system includes the application of enough fertilizer to sustain maximum yields, but without the substantial excesses that would contribute to epiphyte problems and/or unfavourable economics (Hanisak, 1987).

The use of marine algae as manures and fertilizers for crops dates back to the Ancient Greeks. As early as the twelfth century, algae from the phylum Phaeophyta (brown seaweeds) were used as manure on the coastal lands of Europe (Crouch, 1990). Much more recently, the use of various seaweed extracts and concentrates as a foliar spray on agricultural plants is increasing in popularity, even though, the literature on seaweed

extracts is unbalanced and contradictory. Well-documented studies (Crouch et al. 1990, Featonby-Smith and van Staden, 1983, 1987), mostly from universities and research institutes, report that seaweed extracts improve growth and yield of plants, as well as prevent pests and improve quality of the product. Other well-documented reports indicate that seaweed extracts often have no effect at all (Verkleij, 1992). Besides these studies, there are leaflets and reports from producers of seaweed extract publicizing the benefits of their products. The value of this information is hard to assess since often a statistically valid experimental design is lacking. However, the significance of scientific evaluation should not be overestimated considering the experience of many farmers, who use seaweed extracts to their satisfaction. The commercial seaweed extracts are mostly applied as a spray to foliage, although sometimes they are applied as a flush into the soil. Liquid extracts are diluted in a volume by a factor 1:200 to 1:600 and are applied at a rate of diluted product of 500-1000 l ha^{-1}, corresponding to a rate of undiluted product of 1-50 l ha^{-1} and to a rate of seaweed dry matter of 0.08 to 4.0 kg ha^{-1} (Verkleij, 1992).

The algae are regarded as a less advanced group of organisms than higher plants, although they show great diversity in morphology and in many of their biochemical systems. Although the morphology is simpler than that of higher plants, many of the processes of development are similar. The differentiation of complex structures can occur and photoperiodism is commonly observed. Alterations in growth in response to light quantity, quality, and mineral availability have been reported. Hence, today, commercial liquid algae fertilizers are being used in hatch tanks to grow *Gracilaria* in Hawaii (in

traditional Hawaiian fishponds on the fringing reefs of Molokai) (Glenn *et al.* 1996), although there is no scientific evidence to show Kelpak is beneficial for mariculture.

Growth is an orientated process. In algae polarities in cells and thalli are established from the start and are maintained throughout development. Presumably apico-basal polarity and apical dominance in seaweeds are caused by growth regulators (Lobban and Harrison, 1994) also known as plant growth hormones. Plant hormones, specialized chemical substances produced by plants, are the main internal factors controlling growth and development. The auxins, cytokinins, gibberellins, abscissic acid, and ethylene have all been identified in plant tissues. Biosynthesis mutants have in every case established that these endogenously synthesised substances play a functional role, but the precise function is still a matter of debate and argument (Lobban and Harrison, 1994). Plant hormones are produced in one part of a plant and transported to others, where they are effective in very small amounts. Depending on the target tissue, a given hormone may have different effects. One current view of their function is that they regulate the rate at which individual parts of the plant grow, integrate growth of those parts to form the whole organism, and control reproduction (Bradley, 1991). Plant hormones also allow mature plants to respond to changes in their environment. In 1958 Bentley (cited in Evans and Trewavas, 1991) postulated that algae might also have similar biochemical systems to the 'more advanced' higher plants. Since then there has been accumulating evidence that some of the hormones that operate in higher plants could have a similar role in the algae.

Much of this speculation arose due to observations made when bioassays, performed to determine the presence of plant hormone-like substances, resulted in a positive responses from seaweed material. Many studies have looked at the effect of applying higher-plant regulators, such as auxins and gibberellins, to seaweeds (Lobban and Harrison, 1994). Many other studies have attempted to extract and characterize seaweed compounds with growth-regulatory effects. Auxins, abscissic acid (ABA), cytokinins and gibberellins have been identified in seaweeds by using various techniques, including high performance liquid chromatography (HPLC). However no technique has been able to show that the compound is active as a growth substance, nor could they allow recognition of a growth substance that did not fall into one of the classic substance groups (Lobban and Harrison, 1994).

Evans and Trewavas (1991) pointed out that microorganisms synthesize representatives of all the five plant growth substance groups as secondary metabolites. Consequently there is a possibility that microorganisms could provide the precursors for growth regulator biosynthesis by associated algae. Therefore according to Bradley (1991) if bacteria are a source of plant growth substances, one can speculate that changes in environmental conditions might affect their growth, which in turn might change the plant hormone supplied to the seaweed on which they live. Growth substances have also been identified in seawater, although their origin has not been traced (Evans and Trewavas 1991). Nevertheless, plant growth substances have been identified in commercial seaweed liquid fertilizer although the response is unknown; the seaweed concentrate has been scientifically reported to promote increase in yields of many agricultural plants

(Crouch, 1990, Featonby-Smith and van Staden, 1983, 1987 and Finnie and van Staden, 1985).

The principal aim of this report is to investigate the growth response in laboratory culture of *Gracilaria gracilis* to the application of the commercial liquid algae fertilizer, Kelpak, with the view of applying this knowledge to *Gracilaria* mariculture operations.

Taxonomy of Gracilaria

Gracilaria (Rhodophyta) has presented a problem to taxonomists over the years in that the occurrence of similar morphology among different taxa, and extreme phenotypic variability in certain identities, combine and impede species recognition. Despite much effort in the past decade to resolve the taxonomy of algae in the Gracilariaceae, the family remains problematic. Generic concepts have become questionable, as their diagnostic reproductive features have been reported as mixed in single species (Govender 2001).

Fredericq and Hommersand (1989) have reinstated the genus *Gracilariopsis*, which in the past has been misidentified as *Gracilaria verrucosa*. The reason for the general uncertainty is partially due to *Gracilaria* species being notably difficult in their taxonomy owing to poorly understood species limits, large amounts of variation in morphological features selected for taxonomy, large numbers of taxa mostly previously studied in narrow geographic ranges and finally misapplication of species names due to the lack of reference to type specimens, although now it has learn to be conspecific.

The member of the Gracilariaceae most commercially utilized in southern Africa was originally known in past years as *Gracilaria confervoides* (L.) Greville (Anderson *et al.* 1989), then subsequently as *Gracilaria verrucosa* (Hudson) Papenf. and more recently as *Gracilaria gracilis* (Stackhouse) Steentoft, Irvine *et* Farnham (Steentoft *et al.* 1995). The South African *Gracilaria* from Saldanha Bay has been shown to be co specific with the European *Gracilaria gracilis* by molecular methods.

General Characteristics of Gracilaria

The Gracilariaceae in general have a pantropical distribution with the majority of species concentrated in the warmer waters of the northern hemisphere and with no more than a few species established in more temperate waters (Oliveira and Plastino, 1994, Oliveira *et al.* 2000). Plants of the genus *Gracilaria* consist of over 100 described species and are widely distributed trough the world. *Gracilaria gracilis* has been recorded from various locations around the world such as Chile, China, Taiwan, Namibia, and South Africa. In southern Africa *Gracilaria gracilis* extends from Lüderitz, Namibia to Port Elizabeth and possibly Port Alfred, South Africa (Smit, 1998). Major accumulations of *Gracilaria* frequently occur free-floating, attached to substrates, or anchored to the seafloor by sand (Santelices and Doty, 1989). They grow attached or free-living from the eulittoral to sublittoral zone, usually shallower than 10m. The gracilaroids in general (cylindrical branched species of *Gracilaria* and *Gracilariopsis*) rarely grow in areas of extreme wave action but can be found in partly turbulent waters where the thalli may be attached to the

tubes of annelid worms, or to the byssal fibres of mussels, entangled with other algae, attached to small shells, pebbles or small stones (Santelices and Doty, 1989).

Gracilaria gracilis normally occurs in subtidal regions of the South African coast. *Gracilaria* elsewhere often attached to a solid surface by a holdfast, which is a crustose clump of 5-6 fused sporelings from which many erect thalli grow. However South African material does not posses holdfasts. Oliveira *et al.* (2000) described members of the *Gracilariaceae* as macroscopic algae from about 0.1 to about 5 m long, in various shades of red and brown-yellowish. Green forms and other colours are found that appear to be the result of sample genetic controls (Santelices and Doty, 1989). The algae are perennial and can regenerate readily from the holdfast as well as from small thallus pieces. The solid thalli of pseudoparenchymatous, filamentous construction can be branched, cylindrical to flattened or even leafy (Oliveira *et al.* 2000). However in South Africa, the commercial species are less than 30 cm in length, cylindrical and highly branched. Simons (1977) describes *Gracilaria* from Saldanha Bay, South Africa as consisting of 'ramifying, stringy streamers and looks like branching, reddish-brown, bootlaces'. The description also applies to many other members of the genus.

Life Cycle of Gracilaria

Typically *Gracilaria* has a life history that follows the basic pattern of most red algae, the three-phase 'Polysiphonia-type', with an alteration of morphologically inseparable yet genetically distinct generations, with sexes separated in the gametophyte phase (Kain and Destombe, 1995) (Figure A). South African *Gracilaria* are seldom fertile and

gametophytes have rarely been observed in the field. In field populations fertile female thalli are recognized without the aid of a microscope, as the cystocarps, which bear the carpospores, are hemispherical lumps readily visible to the naked eye. None of the plants used in these experiments were fertile, and it is thus not possible to know which life history phase or phases were present.

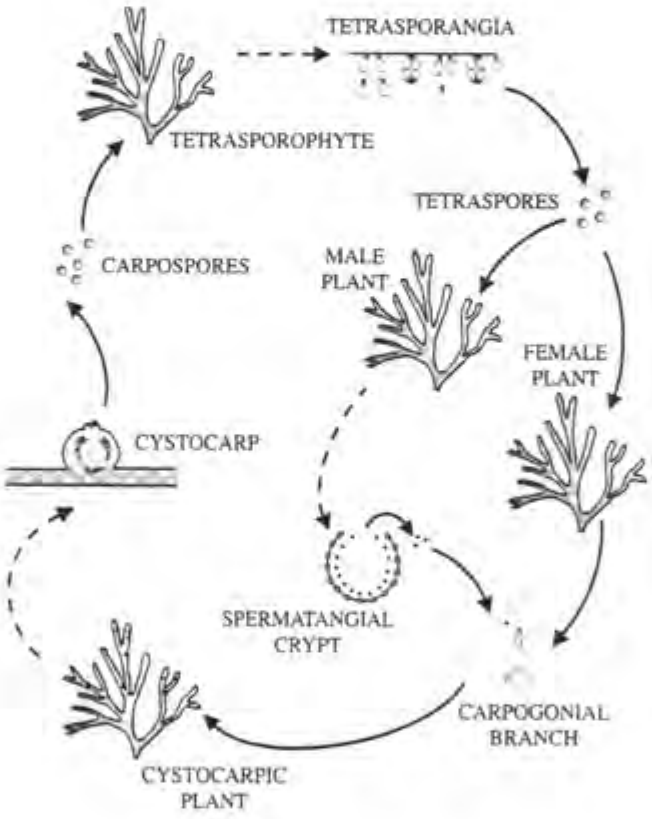

Figure A. Typical life history of *Gracilaria* (Rhodophyta, Gracilariales). (From Oliveira *et al.* 2000).

MATERIALS AND METHODS

Specimens of *Gracilaria gracilis* were collected from Saldanha Bay ($33^0$01'S, $17^0$59'E), on the west coast of South Africa, about 100 km north of Cape Town. The experimental material was collected in July and August 2001.

Plant Material

Healthy plants collected from Saldanha Bay were placed in a laboratory-holding tank with re-circulated seawater at the University of Cape Town, until required, usually within a week of collection. Before the experiment, thallus fragments were rinsed in 2 µm-filtered seawater and any visible epiphytes were removed manually. The plant material towards the top was cut and placed in a glass dish filled with seawater. The glass dish was place on a dissecting microscope; with a sheet of graph paper underneath and 15-mm unbranched apical segments were cut using a sterilized blade and fine forceps. Each experiment consisted of three replicate 200-ml crystallizing dishes, each containing five 15-mm apical segments of *Gracilaria gracilis*, obtained randomly from 8-10 individual plants. The set up of the dishes in this experiment was similar to that described by Engledow and Bolton (1992).

Seaweed Concentrate

Kelpak was the commercially available seaweed concentrate used in this experiment. Kelpak is manufactured by Kelp Products (Pty) Ltd., Simons Town, Republic of South Africa. The seaweed concentrate is manufactured from the stipes of the brown alga *Ecklonia maxima* (Osbeck) Papenfuss, using a cell burst process. This process involves the use of pressure on fresh material to compress the cells in the absence of air or water followed by a sudden release resulting in the rupture of the cell walls and release of the contents. That is, the seaweed is progressively reduced in particle size, and the particles pass under extremely high pressure into a low-pressure chamber where they disintegrate, resulting in the liquid extract. The process excludes the use of heat, chemicals or dehydration that could affect some of the organic components of the concentrate (Verkleij, 1992).

Culture Medium

Two different culture media were used in the experiment. Two hundred millilitres of culture medium was added to each 200-ml crystallizing dish containing five 15-mm apical segments of *Gracilaria gracilis*, for each culture medium. One experiment used seawater as its basis. Thereafter different concentrations of Kelpak were added. The other experiment used Provasoli Enriched Seawater medium. The enriched seawater (ES) medium used was prepared according to a standard recipe (Starr and Zeikos, 1987) (Appendix A). One-third strength standard Provasoli's Enriched Seawater was made up by adding 6 ml ES concentrate to a litre (1L) of seawater. This is the concentration used for many red algae in laboratory culture and has been used for tips of *Gracilaria gracilis*

by Engledow and Bolton, 1992. The resulting media were sterilized by autoclaving at 120^{0}C for sixty minutes. After sterilization and cooling, different concentrations of Kelpak were added to the seawater and enriched seawater media.

Experimental design

Seven experimental conditions were set up for seawater medium and enriched seawater medium. The Kelpak : medium concentration dilutions were control (no Kelpak), 1:1, 1:5, 1:100; 1:500, 1:1000, 1:5000. A small amount of GeO_2 (germanium dioxide) (2.5 gl^{-1} of culture solution) was added to both culture media to inhibit the growth of diatoms (Markham and Hagmeier, 1982). The culture medium in both experiments was changed every 3 days. The small amount of material per culture dish prevented problems with pH changes. The experiments were carried out in a 15^{0}C temperature controlled room. The irradiance of between 75 and 80$\mu E.m^{-2}.s^{-1}$ was provided by means of cool white fluorescent tubes and the photoperiod was 16 hours (Light): 8 hours (Dark). To obtain a uniform environment for all the dishes, the dishes were rotated on a daily basis to ensure that they were all exposed to similar light conditions.

Data Analysis

Fragment lengths were measured to the nearest millimetre and the total number of branches counted after seven and fourteen days. At the end of the 14-day experiment, the fresh mass of each fragment was determined as well as the specific growth rate (S.G.R.) using the following formula:

$$SGR\ (\%\ day^{-1}) = (100 \ln N_t/N_o)/t$$

Where t is the time in days N_o is the initial length (mm) and N_t is the length at time t (mm) (as used by Engledow and Bolton 1992).

Statistical Analysis

The effect of the various Kelpak treatments on the growth rate of *Gracilaria gracilis*, were analysed using ANOVA, the single factorial analysis of variance (p=0.05) statistical package in STATISTICA. The least significance difference (LSD) test or planned comparison test was conducted at the 95% level, to distinguish significantly different results following the ANOVA test.

RESULTS

Gracilaria gracilis apical segments growing in seawater culture medium as well as in ES culture medium with 1:1 and 1:5 seaweed concentrate dilution, survived the first two days of the experiment. They appeared fully intact during the first two days of the experiment but by the third day they appeared colourless. Therefore no measurements were recorded as these treatments were discontinued. For apical segments growing in seawater culture medium and in ES culture medium with the various other seaweed concentrate dilutions, the measurements are presented below.

The results presented in Figure 1A shows that apical segments in 1:1000 seaweed concentration dilution remained unchanged compared to the control, however significantly longest compared to the other treatments. The apical segments in 1:500 and 1:100 seaweed concentrate dilution were significantly the shortest compared to the control. After 14 days in seawater culture medium (Figure 1B), the *Gracilaria gracilis* apical tips were significantly longer in the 1:1000 seaweed concentrate dilution (47.27 mm) compared to the control (42.53 mm). The 1:100 seaweed concentrate dilution had significantly the shortest apical segments of *Gracilaria gracilis* (24.93 mm) compared to the control and all the other treatments.

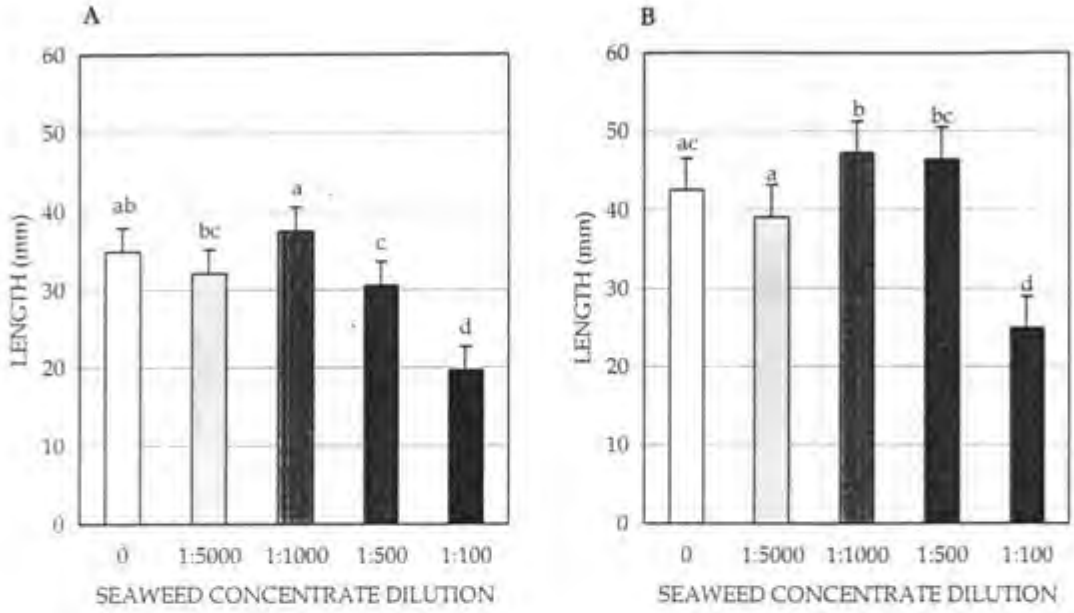

Figure 1. The effect of various seaweed concentrate dilutions on the average length (mm) of main axis of apical tips of *Gracilaria gracilis*. **A.** After 7 days in seawater culture medium. **B.** After 14 days in seawater culture medium. Different letters indicate significant differences at 5 percent level (one-way ANOVA and LSD post-hoc test). Vertical lines represent ± one Standard error, p=0.05.

Table 1 shows the effect of different seaweed concentrate dilutions on the total number of branches of *Gracilaria gracilis* per dish in seawater medium. After 14 days there was no change in the total number of branches per dish in *Gracilaria gracilis* growing in the different treatments compared to the control.

Table 1. The effect of various seaweed concentrate dilutions on the total number of branches of *Gracilaria gracilis* apical segments per dish after 14 days in seawater medium. Different letters indicate significant differences at 5 percent level (one-way ANOVA and LSD post-hoc test). Vertical lines represent ± one Standard Error, p = 0.05.

Treatment	Total Number of Branches
Control	3 a
1:5000	6 a
1:1000	3 a
1:500	2a
1:100	2 a

The average weight (mg) of apical segments at different seaweed concentrate dilutions after 14 days culture in seawater medium is shown in Figure 2. *Gracilaria* apical segments growing at 1:1000 and 1:500 seaweed dilution concentrate had significantly more mass (23.3 mg and 21.88mg respectively) compared to the control (16.5 mg). Segments growing in 1:100 showed a significant inhibition of 10.3 mg in relation to the control and the other treatments.

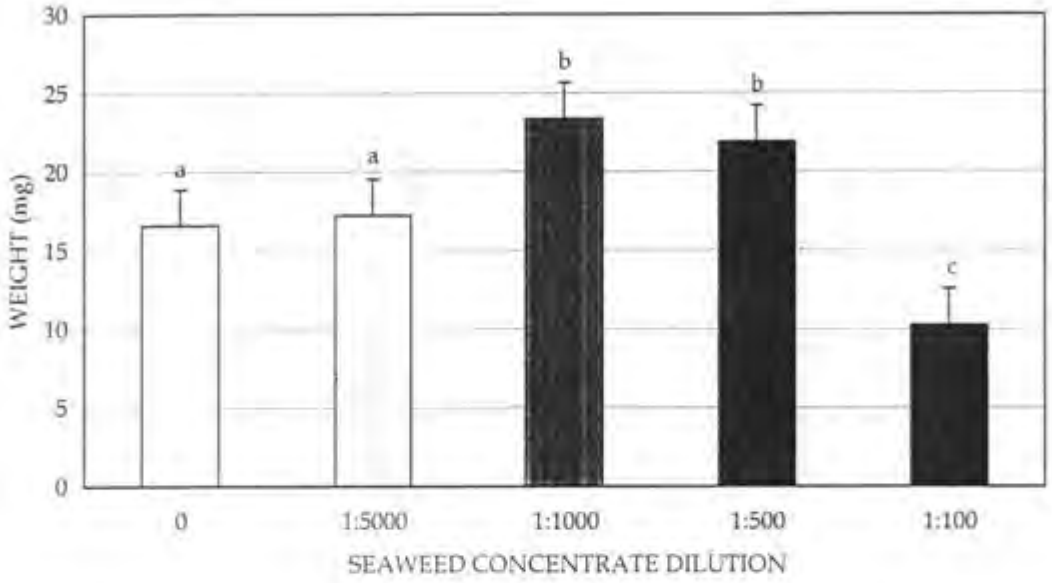

Figure 2. Fresh mass (mg) of *Gracilaria gracilis* apical segments after 14 days in seawater medium. Apical segments were treated with seaweed concentrate at various dilutions. Different letters indicate significant differences at 5 percent level (one-way ANOVA and LSD post-hoc test). Vertical lines represent ± one Standard Error, p = 0.05.

The effect of different dilutions of seaweed concentrate on growth of *Gracilaria gracilis* after 14 days in seawater medium is as shown in Figure 3. Two weeks after the treatment, the 1:100 seaweed concentrate dilution showed a significant major inhibition in specific growth rate (S.G.R) of 3.74 % day^{-1} in relation to the control (7.12% day^{-1}) and the other treatments. The cultures of *Gracilaria gracilis* treated with 1:1000 seaweed concentrate dilution showed significant maximum specific growth rate of 8.05 % day^{-1} compared to the control and treatments 1:5000 and 1:100 seaweed concentrate dilution.

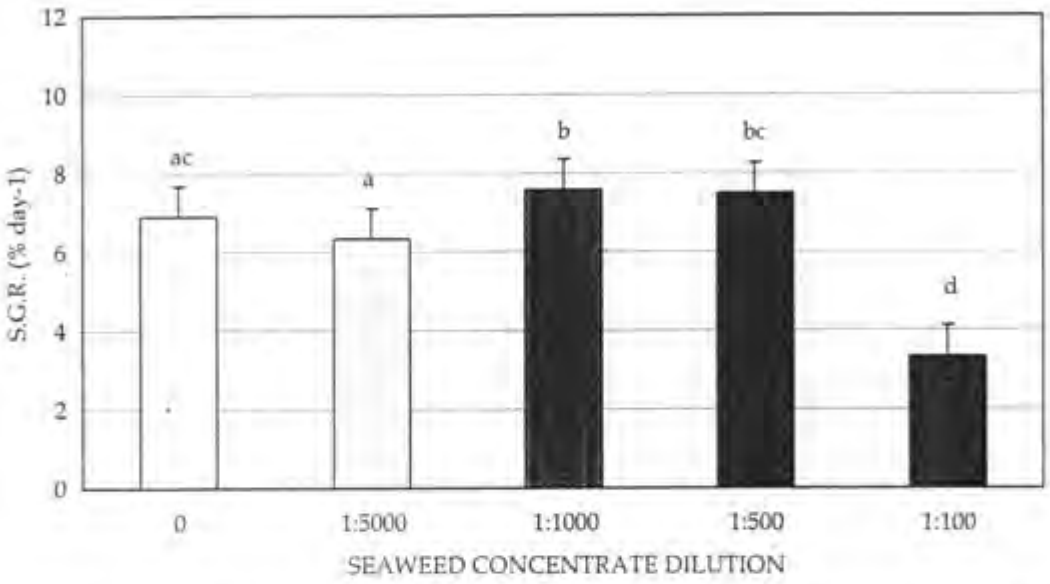

Figure 3. The effect of various seaweed concentrate dilutions on the specific growth rate (S.G.R.) on length of the main axis and branches of apical tips of *Gracilaria gracilis* after 14 days in seawater medium. Different letters indicate significant differences at 5 percent level (one-way ANOVA and LSD post-hoc test). Vertical lines represent ± one Standard error, p=0.05.

In Figure 4A the main axis of the apical segments of *Gracilaria gracilis* in ES medium after 7 and 14 days were significantly longer at 1:1000 seaweed concentrate dilution (47.73 mm) when compared with the control (31.53 mm) and other treatments. *Gracilaria gracilis* in 1:100 seaweed concentration dilution (17.13 mm) after 7 days had significantly shorter apical segments when compared to the control and other treatments. After 14 days in culture (Figure 4B), apical segments of *Gracilaria gracilis* were significantly longest in 1:1000 seaweed concentrate dilution (61.27 mm), followed by 1:500 seaweed concentrate dilution (53.00 mm) compared with the control (37.67 mm) and the other treatments. When compared with the control the apical segments in 1:100 seaweed concentrate dilution had the significantly shortest apical segments (20.73 mm).

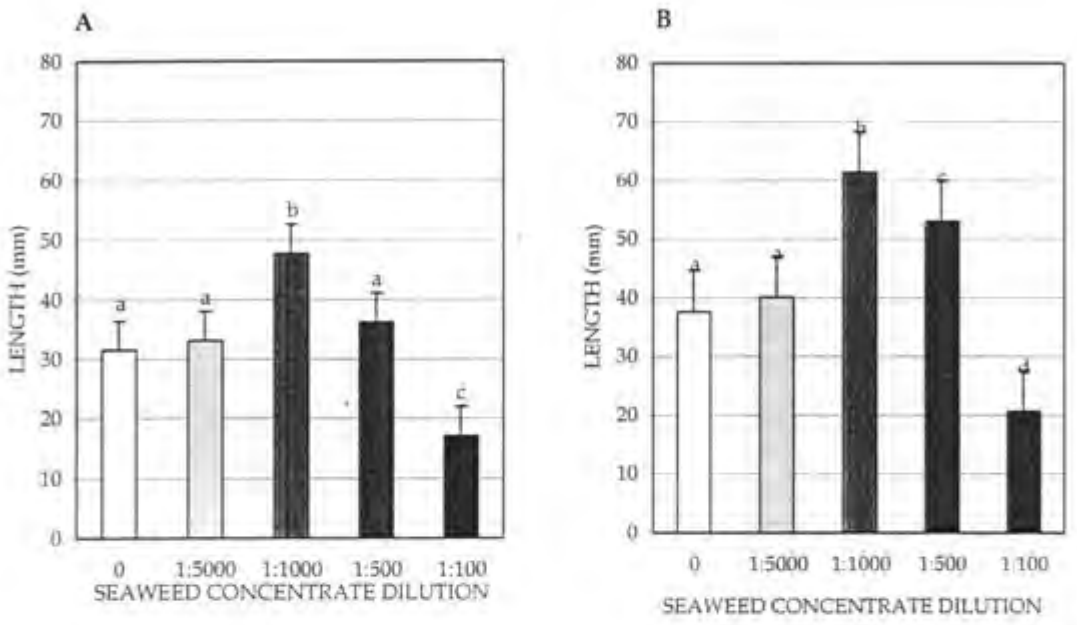

Figure 4. The effect of various seaweed concentrate dilutions on the average length (mm) of main axis of apical tips of *Gracilaria gracilis*. **A.** After 7 days in ES culture medium. **B.** After 14 days in ES culture medium. Different letters indicate significant differences at 5 percent level (one-way ANOVA and LSD post-hoc test). Vertical lines represent ± one Standard error, p=0.05.

The effect of different seaweed concentrate dilutions on the total number of branches of *Gracilaria gracilis* per dish in ES medium is shown in Table 2. There was no change in the total number of branches per dish in apical segments growing at different seaweed concentrate dilutions compared to the control. However, apical segments growing in 1:5000 had significantly more branches per dish compared to apical segments growing at 1:100.

Table 2. The effect of various seaweed concentrate dilutions on the total number of branches of *Gracilaria gracilis* apical segments per dish after 14 days in ES medium. Different letters indicate significant differences at 5 percent level (one-way ANOVA and LSD post-hoc test). Vertical lines represent ± one Standard Error, p = 0.05.

Treatment	Total Number of Branches
Control	7 ab
1:5000	10 a
1:1000	7 ab
1:500	5 ab
1:100	3 b

Gracilaria gracilis apical segments (Figure 5) treated with 1:1000 and 1:500 seaweed concentrate dilution in ES medium, harvested after 14 days had significantly more mass (36.58 mg and 21.88 mg respectively) compared to the control (16.56 mg). The culture with seaweed dilution concentrate 1:100 showed a significant inhibition in mass of 10.28 mg in relation to the control and other treatments.

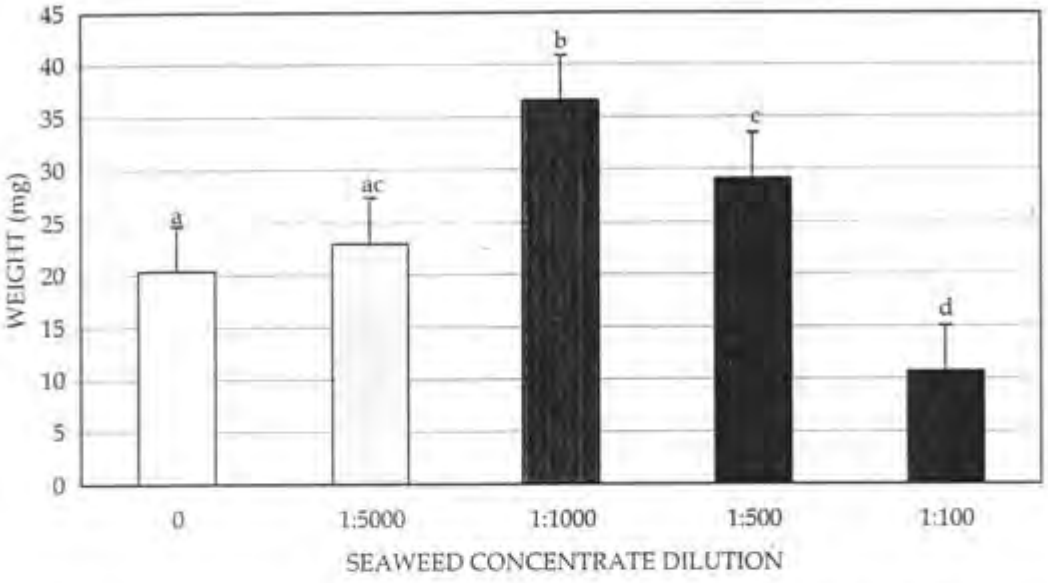

Figure 5. Fresh mass (mg) of *Gracilaria gracilis* apical segments after 14 days in ES medium. Apical segments were treated with seaweed concentrate at various dilutions. Different letters indicate significant differences at 5 percent level (one-way ANOVA and LSD post-hoc test). Vertical lines represent ± one Standard Error, p = 0.05.

The specific growth rate of *Gracilaria gracilis* growing in different seaweed concentrate dilutions for 14 days in ES medium is represented in Figure 6. The apical segments growing at 1:100 seaweed concentrate dilution significantly had the lowest specific growth rate of 2.09 % day^{-1} when compared to the control (6.09 % day^{-1}) and the other three treatments. *Gracilaria gracilis* showed significantly increase in the specific growth rate at 1:1000 and 1:500 seaweed concentrate dilution of 9.22 % day^{-1} and 8.24 % day^{-1}, respectively, compared to the control .

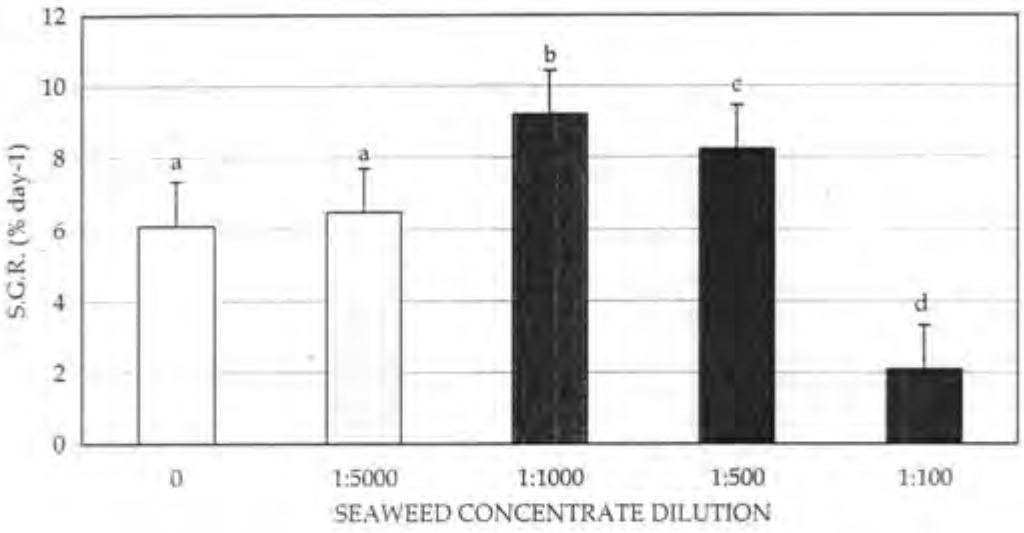

Figure 6. The effect of various seaweed concentrate dilutions on the specific growth rate (S.G.R.) on length main axis and branches of apical tips of *Gracilaria gracilis* after 14 days in ES medium. Different letters indicate significant differences at 5 percent level (one-way ANOVA and LSD post-hoc test). Vertical lines represent ± one Standard error, p=0.05.

DISCUSSION

For a commercial seaweed farm to be economically viable cultivation must be continuous and the system easily and cheaply operated (Smit *et al.* 1997). According to Smit *et al.* (1997), the most cost effective form of seaweed tank cultivation is possibly using mariculture effluent water, where running seawater is readily available and no fertilization is needed. It is possible however, that the ambient nutrient levels in the effluent water are too low to sustain the high seaweed densities required for biomass production, in which case external nutrient sources are needed. In this case the amount of fertilizer to be added to provide maximal growth has to be determined. To our knowledge no one has ever tried to ascertain the effect of seaweed concentrate on seaweed growth in culture, as done here. However, the beneficial effect of seaweed concentrate on the growth and yield of field crops has been documented for the last 30 years. In biological agriculture and horticulture diluted extracts of seaweed are applied to promote growth, prevent pests and diseases and improve the quality of the products, hence Kelpak is not a fertilizer, it is additional to normal nutrients requirments

Maximal yields are possible only when nutrient conditions do not limit growth. Hanisack (1990) has suggested that nitrogen is the nutrient most frequently reported to limit the growth of seaweeds. Past studies have found that levels of pigment proteins are often closely correlated with the N content (Lapointe and Ryther 1979). As pigment protein such as phycoerythrin in red algae are largely responsible for determining the colour of seaweeds, changes in the concentrations of these pigments according to N availability

cause lightening or darkening of seaweed colour. Hence there is a relationship between the colour and the healthiness of the seaweed. Throughout the experiment, with the exception from the highest seaweed concentration dilution (1:1 and 1:5), *Gracilaria gracilis* appeared dark red indicating that the seaweed was very healthy. In contrast *Gracilaria gracilis* growing at 1:1 and 1:5 seaweed concentrate dilution had a yellow colour after the second day of experiment, becoming colourless by the third day of the experiment, *Gracilaria* did not died at such strong seaweed concentrate dilutions from starvation, but quite possibly due to alteration of the salinity. This observation was identical on *Gracilaria* apical segments growing in the seawater medium and ES medium.

Finnie and van Staden (1985) have demonstrated that the water dilution ratio of *Ecklonia maxima* kelp concentrate is an important factor controlling its efficacy. In land plants low dilution ratios (1:100 seaweed concentrate:water) were found to have an inhibitory effect upon root growth, whereas higher dilution ratios (1:400, 1:500 and 1:600) were stimulatory. Such seaweed concentrate dilution effects upon plant root growth could be attributed to growth inhibitors in the concentrate that, upon increasing dilution, becomes less effective than the growth promoting substances. Furthermore, Temple *et al.* (1989) suggested that optimal dilution ratios of the concentrate to land plants might be dependent on the particular environmental conditions to which plants are subjected. The concentrations of seaweed concentrate (Kelpak) used in this study fall within the range of concentrations commonly used in land plant studies. After 14 days, both the seawater and ES medium culture with various Kelpak dilutions were effective in altering *Gracilaria*

gracilis growth in length, weight and specific growth rate, however differences existed in the efficacy of the two media under various Kelpak concentrations.

Treatment of *Gracilaria gracilis* apical segments with 1:1000 seaweed concentrate dilutions after fourteen days in seawater medium and ES medium obtained the maximum growth compared to the control. The results demonstrated that apical segments growing in seawater medium with 1:1000 seaweed concentrate dilution had a significant increase of 11% in length, 41% in weight and 13% in the specific growth rate relative to control. Similarly, apical segments growing in ES medium with 1:1000 seaweed concentrate dilution had a significant increase of 63% in length, 120% in weight and 51% in the specific growth rate compared to control. These results contradict those of Beckett and van Staden (1990), which showed that the growth of wheat did not change with very low concentrations (1:1000) of Kelpak compared to control. However, *Gracilaria gracilis* growing at 1:5000 seaweed concentrate in both seawater and ES media remained unchanged compared to the control, hence consistent with results obtained by Beckett and van Staden (1990).

Gracilaria gracilis apical segments growing in 1:500 seaweed concentrate dilution in both seawater and ES medium also had the second highest increase in growth compared to the control. There was an increase in length, weight and specific growth rate on apical segments compare to the control. This is consistent with the report that seaweed concentrate (Kelpak) at dilution 1:500 applied regularly, improved the total biomass of *Beta vulgaris* and *Phaseolus vulgaris* (Crouch, 1990). In a study conducted by Nelson

and van Staden (1984), they found that root growth was stimulated in greenhouse studies with cucumber plants sprayed weekly with 1:500 seaweed concentrate, leading to 56% increase in the total plant biomass. In that study, the seaweed treatment tended to increase the P content in the leaves and to decrease the N content. This led to the suggestion that the seaweed treatment had induced the uptake of 'unavailable' nutrients by cucumber roots or had improved the efficiency of utilization of 'available' nutrients. Similarly, the shoot and root dry mass of wheat, in a study conducted by Nelson and van Staden (1986), increased the application of 1:500 seaweed concentrate. Interestingly, maximum yield was obtained at submaximal rates of seaweed concentration, indicating that the seaweed did not have a direct effect on growth but acted as a stimulant. The same was observed in this experiment with *Gracilaria gracilis*.

A pronounced inhibitory effect on *Gracilaria gracilis* length after one week in seawater and ES medium with 1:100 seaweed concentrate dilution treatment was observed. After two weeks in 1:100 seaweed concentrate dilution, the inhibitory effect prevailed, although there was a small increase in length of *Gracilaria gracilis* from week one to week two. This could be due to the fact that the seaweed concentrate dilution was too strong, hence causing a change in salinity of the media. Another possibility could be due to the fact that Kelpak inhibits light to penetrate into the seaweed, although it shouldn't kill it. According to Engledow and Bolton (1992), the maximum growth of *Gracilaria* is obtained at 80 μmol m^{-2} s^{-1}, followed by a considerable decrease in growth at 40 $\mu molm^{-2}s^{-1}$. Thus there must be enough light penetrating for *Gracilaria* to grow. However, these results are consistent with Finnie and van Staden (1985), which reported

that tomato roots were inhibited with the application of 1:100 seaweed concentrate dilution.

In their study on *Gracilaria chilensis*. Santelices and Varela (1995) suggested that intercalary growth is more important than apical growth in contributing to elongation since thallus length increment was found to be positively related to thallus length up to 20 cm, while S.G.R was inversely related to length. According to M. Steentoft (Smit *et al.* 1997), *Gracilaria gracilis* growth occurs throughout the thallus and not particularly near the apex. Smit and Bolton (1999), suggested that growth was significant over the entire thallus, however it was found that the apical region contributes more to the overall elongation than does the proximal part of the thallus. Overall there has been an increase in the growth of *Gracilaria gracilis* apical segments when exposed to specific concentrations of the commercial seaweed liquid fertilizer (Kelpak). The highest growth rates can be obtained from the concentration of 0.1 % (1:1000) and 0.2% (1:500). The weakest concentration of seaweed concentrate of 0.02 % (1:5000) does not change the growth of *Gracilaria gracilis* apical segments. The stronger the concentration the most likely it is to inhibit growth, as for the case of *Gracilaria gracilis* growing at 1% concentration (1:100). An even stronger concentration of 20% (1:5) and 100% (1:1) seaweed concentrate causes the death of the *Gracilaria gracilis* apical segments. The results obtained for the apical segments of *Gracilaria gracilis* in ES solution medium are constantly higher then the apical segments growing in seawater medium. A possible explanation could be due to the fact that in seawater there may be a shortage of specific

nutrient, essential for the growth of *Gracilaria*. Since ES solution is enriched seawater, it has the ability to provide all the nutrients required for the seaweed to grow.

The results of this investigation confirms previous findings on the effect of seaweed concentrate applications on the growth of certain field crops (Featonby-Smith and Van Staden, 1983). The reasons for the growth increase achieved with the use of seaweed concentrate are not completely understood. It is however, thought that the hormonal content, particularly cytokinin content of the seaweed, plays an important role (Featonby-Smith and Van Staden, 1983). In one study, the application of a water-soluble algal extract to groundnut plants produced a significant increase in growth and yield of one the cultivar tested but not the other (Ketring and Schubert 1981). This increase was attributed to the cytokinin content of the extract, a suggestion that was later repeated by Featonby-Smith and van Staden (1987). Perhaps, the only direct evidence of cytokinin promotion of plant growth is the study by Blunden and Wildgoose (1977), who showed aqueous seaweed extract of known cytokinin activity significantly increased the yield of potatoes. Close correlations were also found to exist between the results obtained from the use of synthetic cytokinin, kinetin and seaweed extract of equivalent cytokinin activity. A more recent investigation (Finnie and Van Staden 1985) showed that excised tomato roots exposed to low concentrations of seaweed concentrate (Kelpak) mimicked the effect of low levels of cytokinin. The stimulatory effect of the seaweed was lost if the material was ashed, indicating that the regulatory substance is associated with the organic rather than with the inorganic fraction. Reports have been made (Yokoya *et al.* 1999; Kaczyna and Megnet, 1993) that auxins and cytokinins can regulate growth in red algae. Hence one

could be led to speculate that the results obtained from this study could be due to the fact that the cytokinins-like promoted the growth of *Gracilaria gracilis* apical segments. This study is however preliminary, the data are inadequate to draw any final conclusions or to the mechanism of action of the seaweed concentrate. Nevertheless Kelpak ® may have commercial potential in the seaweed mariculture industry.

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APPENDIX A: *SOLUTION*

ES-enriched seawater medium (Provasoli, 1963)

Fe-solution

Dissolve 351 mg of $Fe(NH_4)_2(SO_4)_2.6H_2O$ and 300 mg of Na_2EDTA in 500 ml of glass distilled water. Autoclave.

PII metal solution

Na_2EDTA	100 mg
H_3BO_3	114 mg
$FeCl_3.6H_2O$	4.9 mg
$MnSO_4.7H_2O$	16.4 mg
$ZnSO_4.7H_2O$	2.2 mg
$CoSO_4.7H_2O$	0.48 mg

Dissolve all materials in 100 ml glass-distilled water. Adjust the pH 7.8 and autoclave. Store at 10°C.

ES-enrichment solution

$NaNO_3$	350 mg
Na_2glycerophosphate.$5H_2O$	50 mg
Fe-solution	25 ml
PII metal solution	25 ml
Vitamin B_{12}	10 g
Thiamine	0.5 mg
Biotin	5 g
Tris buffer (Sigma)	500 mg

Dissolve all materials (except Fe-solution and {PII metals) in 50 ml glass distilled water. Autoclave. Add 25 ml Fe-solution and 25 ml PII metals solution and store at 10°C. Dissolve 6 ml ES-enriched solution in 1000 ml autoclaved, filtered seawater.

APPENDIX B: RAW DATA

SEA WATER SOLUTION

SEAWEED CONCENTRATE DILUTION	AFTER 7 DAYS					AFTER 15 DAYS				
	Replicate Number	Length of main axis (mm)	Length including Branches (mm)	Number of Branches	Specific Growth Rate (%day-1)	Length of main axis (mm)	Length including Branches (mm)	Number of Branches	Specific Growth Rate (%day-1)	Weight (mg)
0	1	35	35	0	5.649	46	46	0	7.471	14.5
0	1	41	46	1	6.703	40	40	0	6.539	14.6
0	1	29	29	0	4.395	50	56	1	8.026	14.2
0	1	40	40	0	6.539	35	35	0	5.649	19.5
0	1	34	34	0	5.455	42	42	0	6.864	17.2
0	2	33	33	0	5.256	51	58	3	8.159	16.5
0	2	40	40	0	6.539	39	43	1	6.370	16.6
0	2	28	28	0	4.161	45	45	0	7.324	19.6
0	2	37	39	1	6.019	43	44	1	7.021	18.7
0	2	33	33	0	5.256	44	44	0	7.174	13.8
0	3	39	39	0	6.370	41	42	1	6.703	12
0	3	36	38	2	5.836	36	36	0	5.836	24.6
0	3	37	38	1	6.019	49	49	0	7.892	21.5
0	3	31	31	0	4.840	42	46	2	6.864	11.3
0	3	30	30	0	4.621	35	35	0	5.649	13.8
1:5000	1	36	42	2	5.836	42	76	4	6.864	13.7
1:5000	1	36	36	0	5.836	34	36	1	5.455	13.9
1:5000	1	35	50	4	5.649	37	44	2	6.019	26.4
1:5000	1	25	25	0	3.406	41	51	2	6.703	45
1:5000	1	33	34	1	5.256	46	47	1	7.471	18.6
1:5000	2	37	37	0	6.019	32	32	0	5.051	16.1
1:5000	2	25	25	0	3.406	42	42	0	6.864	12.7
1:5000	2	33	33	0	5.256	36	36	0	5.836	14.1
1:5000	2	34	34	0	5.455	37	37	0	6.019	13.2
1:5000	2	36	37	1	5.836	40	44	0	6.539	15.4
1:5000	3	45	45	0	7.324	46	52	1	7.471	11.5
1:5000	3	26	26	0	3.667	36	39	1	5.836	12.5
1:5000	3	24	24	0	3.133	34	37	1	5.455	12.8
1:5000	3	33	33	0	5.256	50	61	4	8.026	9.6
1:5000	3	23	23	0	2.850	34	34	0	5.455	22.5

SEA WATER SOLUTION

SEAWEED CONCENTRATE DILUTION	AFTER 7 DAYS					AFTER 15 DAYS				
	Replicate Number	Length of main axis (mm)	Length including Branches (mm)	Number of Branches	Specific Growth Rate (%day-1)	Length of main axis (mm)	Length including Branches (mm)	Number of Branches	Specific Growth Rate (%day-1)	Weight (mg)
1:1000	1	35	35	0	5.649	46	46	0	7.471	28
1:1000	1	36	36	0	5.836	41	41	0	6.703	19.5
1:1000	1	35	35	0	5.649	47	47	0	7.614	21.3
1:1000	1	35	35	0	5.649	55	59	1	8.662	14.6
1:1000	1	45	45	0	7.324	45	45	0	7.324	23.2
1:1000	2	50	50	0	8.026	46	46	0	7.471	30.1
1:1000	2	36	36	0	5.836	65	65	0	9.776	17.4
1:1000	2	30	30	0	4.621	38	38	0	6.197	32.9
1:1000	2	38	49	2	6.197	45	66	4	7.324	25.4
1:1000	2	30	30	0	4.621	39	39	0	6.370	12.9
1:1000	3	41	43	1	6.703	53	58	1	8.415	21.3
1:1000	3	40	42	1	6.539	57	57	0	8.900	28
1:1000	3	42	42	0	6.864	47	53	1	7.614	24.6
1:1000	3	31	41	1	4.840	43	64	3	7.021	27
1:1000	3	39	39	0	6.370	42	42	0	6.864	24
1:500	1	32	34	1	5.051	45	52	1	7.324	22.1
1:500	1	27	28	1	3.919	44	49	1	7.174	24.8
1:500	1	21	23	1	2.243	51	57	1	8.159	20
1:500	1	37	39	1	6.019	37	44	1	6.019	27.9
1:500	1	31	31	0	4.840	49	49	0	7.892	26.2
1:500	2	33	33	0	5.256	53	53	0	8.415	23.5
1:500	2	35	35	0	5.649	41	41	0	6.703	14.9
1:500	2	25	25	0	3.406	43	43	0	7.021	19.9
1:500	2	25	25	0	3.406	42	46	1	6.864	23.9
1:500	2	27	27	0	3.919	56	56	0	8.782	14.2
1:500	3	33	33	0	5.256	51	51	0	8.159	17.3
1:500	3	32	32	0	5.051	48	48	0	7.754	22.9
1:500	3	36	36	0	5.836	45	45	0	7.324	21.4
1:500	3	34	34	0	5.455	50	50	0	8.026	24.8
1:500	3	30	33	1	4.621	41	53	2	6.703	24.4

SEA WATER SOLUTION

SEAWEED CONCENTRATE DILUTION	AFTER 7 DAYS					AFTER 15 DAYS				
	Replicate Number	Length of main axis (mm)	Length including Branches (mm)	Number of Branches	Specific Growth Rate (%day-1)	Length of main axis (mm)	Length including Branches (mm)	Number of Branches	Specific Growth Rate (%day-1)	Weight (mg)
1:100	1	19	19	0	1.576	30	34	1	4.621	12
1:100	1	19	20.9	1	1.576	27	33	1	3.919	14.7
1:100	1	23	24	1	2.850	25	27	1	3.406	9.4
1:100	1	20	24	2	1.918	24	24	0	3.133	10.5
1:100	1	20	22	1	1.918	25	38	3	3.406	10.3
1:100	2	22	22	0	2.553	24	24	0	3.133	11.6
1:100	2	21	21	0	2.243	30	30	0	4.621	9.9
1:100	2	24	24	0	3.133	19	19	0	1.576	7.9
1:100	2	16	16	0	0.430	22	22	0	2.553	9.1
1:100	2	17	17	0	0.834	26	28	1	3.667	8.2
1:100	3	20	20	0	1.918	26	26	0	3.667	10.2
1:100	3	19	19	0	1.576	28	28	0	4.161	10.8
1:100	3	17	17	0	0.834	22	22	0	2.553	10.5
1:100	3	17	17	0	0.834	21	21	0	2.243	12.2
1:100	3	21	21	0	2.243	25	25	0	3.406	6.9

ENRICHED SEAWATER SOLUTION

SEAWEED CONCENTRATE DILUTION	AFTER 7 DAYS					AFTER 15 DAYS				
	Replicate Number	Length of main axis (mm)	Length including Branches (mm)	Number of Branches	Specific Growth Rate (%day-1)	Length of main axis (mm)	Length including Branches (mm)	Number of Branches	Specific Growth Rate (%day-1)	Weight (mg)
0	1	35	35	0	5.649	38	42	2	6.197	27.3
0	1	35	43	4	5.649	39	42	1	6.370	19.7
0	1	35	36	1	5.649	40	40	0	6.539	28.2
0	1	35	35	0	5.649	36	36	0	5.836	15
0	1	35	35	0	5.649	47	77	5	7.614	36.5
0	2	24	24	0	3.133	39	52	1	6.370	25
0	2	34	34	0	5.455	34	35	1	5.455	12.5
0	2	32	38	1	5.051	31	31	0	4.840	17.5
0	2	23	23	0	2.850	30	30	0	4.621	10.8
0	2	20	20	0	1.918	41	44	2	6.703	19.4
0	3	30	33	1	4.621	36	56	2	5.836	10.8
0	3	37	41	1	6.019	35	43	1	5.649	23.4
0	3	40	42	1	6.539	43	53	1	7.021	18.5
0	3	33	39	2	5.256	32	32	0	5.051	19.4
0	3	25	25	0	3.406	44	51	5	7.174	21.6
1:5000	1	36	46	2	5.836	41	48	2	6.703	15.8
1:5000	1	35	40	1	5.649	36	49	2	5.836	22.7
1:5000	1	33	36	1	5.256	38	47	1	6.197	23.2
1:5000	1	34	34	0	5.455	30	32	1	4.621	12.3
1:5000	1	23	23	0	2.850	37	47	1	6.019	24
1:5000	2	33	35	1	5.256	35	35	0	5.649	19.5
1:5000	2	29	29	0	4.395	41	49	2	6.703	19.3
1:5000	2	35	35	0	5.649	38	51	3	6.197	18.3
1:5000	2	24	24	0	3.133	41	45	1	6.703	12.9
1:5000	2	27	28	1	3.919	39	48	2	6.370	12.5
1:5000	3	35	64	2	5.649	46	79	7	7.471	25.7
1:5000	3	31	34	1	4.840	50	54	2	8.026	31.9
1:5000	3	37	43	2	6.019	49	62	2	7.892	37.9
1:5000	3	40	53	4	6.539	35	39	1	5.649	36.6
1:5000	3	45	45	0	7.324	45	67.8	2	7.324	32.5

ENRICHED SEAWATER SOLUTION

SEAWEED CONCENTRATE DILUTION	AFTER 7 DAYS					AFTER 15 DAYS				
	Replicate Number	Length of main axis (mm)	Length including Branches (mm)	Number of Branches	Specific Growth Rate (%day-1)	Length of main axis (mm)	Length including Branches (mm)	Number of Branches	Specific Growth Rate (%day-1)	Weight (mg)
1:1000	1	50	50	0	8.026	70	70	0	10.270	37
1:1000	1	55	55	0	8.662	59	61	1	9.130	22.3
1:1000	1	39	39	0	6.370	45	45	0	7.324	20.8
1:1000	1	25	25	0	3.406	31	31	0	4.840	34.6
1:1000	1	49	49	0	7.892	60	60	0	9.242	29
1:1000	2	60	70	2	9.242	75	94	2	10.730	61.4
1:1000	2	72	84	2	10.457	63	63	0	9.567	45.2
1:1000	2	43	43	0	7.021	80	104	2	11.160	46
1:1000	2	45	60	4	7.324	66	109	6	9.877	55
1:1000	2	50	78	2	8.026	67	123	2	9.978	33
1:1000	3	42	42	0	6.864	64	71	1	9.672	38.5
1:1000	3	50	50	0	8.026	52	52	0	8.288	29
1:1000	3	41	41	0	6.703	48	58	1	7.754	30.3
1:1000	3	52	52	0	8.288	65	65	0	9.776	22.3
1:1000	3	43	43	0	7.021	74	117	4	10.640	44.3
1:500	1	47	47	0	7.614	55	55	0	8.662	22.6
1:500	1	44	47	1	7.174	68	80	1	10.076	37.8
1:500	1	29	30	1	4.395	70	70	0	10.270	40.3
1:500	1	34	34	0	5.455	50	50	0	8.026	30.2
1:500	1	35	35	0	5.649	48	56	0	7.754	17.9
1:500	2	50	52	1	8.026	65	71	1	9.776	46.7
1:500	2	45	45.2	1	7.324	47	62	1	7.614	31.8
1:500	2	36	58	6	5.836	77	84	2	10.905	42.8
1:500	2	40	44	3	6.539	46	126	6	7.471	36.8
1:500	2	45	51	1	7.324	62	71	1	9.461	26.8
1:500	3	25	25	0	3.406	36	36	0	5.836	21.5
1:500	3	26	26	0	3.667	37	37	0	6.019	22.9
1:500	3	35	35	0	5.649	42	42	0	6.864	17.6
1:500	3	25	25	0	3.406	51	51	0	8.159	21.9
1:500	3	27	27	0	3.919	41	41	0	6.703	19

ENRICHED SEAWATER SOLUTION

SEAWEED CONCENTRATE DILUTION	AFTER 7 DAYS					AFTER 15 DAYS				
	Replicate Number	Length of main axis (mm)	Length including Branches (mm)	Number of Branches	Specific Growth Rate (%day-1)	Length of main axis (mm)	Length including Branches (mm)	Number of Branches	Specific Growth Rate (%day-1)	Weight (mg)
1:100	1	16	16	0	0.430	22	22	0	2.553	12.4
1:100	1	16	16	0	0.430	28	37	3	4.161	8.5
1:100	1	16	16	0	0.430	18	18	0	1.215	7.5
1:100	1	23	23.4	2	2.850	22	22	0	2.553	12.2
1:100	1	18	18	0	1.215	20	22	1	1.918	10.2
1:100	2	16	16	0	0.430	21	24	2	2.243	12.8
1:100	2	21	24	1	2.243	17	17	0	0.834	11.1
1:100	2	16	16	0	0.430	17	20	1	0.834	9.5
1:100	2	16	16	0	0.430	20	20	0	1.918	9.8
1:100	2	16	16	0	0.430	26	33	1	3.667	8.4
1:100	3	16	16	0	0.430	20	20	0	1.918	16.4
1:100	3	17	17	0	0.834	17	19	1	0.834	7.1
1:100	3	18	18	0	1.215	20	20	0	1.918	12.9
1:100	3	17	17	0	0.834	21	21	0	2.243	16.2
1:100	3	15	15	0	0.000	22	26	1	2.553	6.2