

Can kelp extract (KELPAK[®]) be useful in seaweed mariculture?

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Abstract

The addition of low concentrations of commercial kelp extract (*Ecklonia maxima*: Kelpak[®]) in addition to fertiliser has proven to be beneficial in agriculture. It triggers rooting in field crops, increases yields and has other useful effects, such as parasite reduction. Its efficacy has been attributed to the fact that Kelpak[®] is produced by a cold process, and is a high auxin/low cytokinin product. The aim of this study was to investigate if seaweeds (which do not have a root system) grown in culture systems, would benefit from the addition of Kelpak[®] or a combination of Kelpak[®] and fertilizer. A preliminary laboratory experiment was carried out by growing excised 15 mm tips of the red alga *Gracilaria gracilis* in culture dishes containing Provasoli Enriched Seawater medium to which various concentrations of Kelpak[®] were added. *Gracilaria* tips in some of the Kelpak[®] treatments (1:2500; 1:1000; 1:500) grew significantly better than the control. Further experiments were carried out on a pilot commercial scale at Jacobsbaai Sea Products Ltd. on the South African west coast. *Ulva lactuca* was grown in effluent from fish (turbot) culture, with additions of 1:5000, 1:2500 and 1:500 concentrations of Kelpak[®] once a week. The intermediate Kelpak[®] concentration (1:2500) produced the highest growth of *Ulva* in the turbot water, while the highest Kelpak[®] concentration (1:500) inhibited *Ulva* growth. In another *Ulva* experiment, various combinations of aquaculture effluent water, commercial fertiliser and Kelpak[®] at 1:2500 were used. Best growth of *Ulva* was obtained in turbot water containing both fertiliser and Kelpak[®]. The results suggest that Kelpak[®] could be useful in commercial seaweed mariculture operations.

Introduction

The use of seaweed extracts as soil drenches and foliar sprays on agricultural plants is increasing, even though the literature on seaweed extracts is contradictory. Some studies suggest that seaweed extracts have no effect on plant growth (Verkleij, 1992). In contrast, documented studies on a commercial extract of the brown kelp *Ecklonia maxima* (Kelpak[®]: Featonby-Smith & van Staden, 1983, 1987; Crouch, 1990) have reported that these seaweed extracts improve the growth rates and yields of crops, as well as preventing pests and improving the overall quality of the product. Many of the physiological responses shown by crop plants treated with seaweed concentrates are thought to be

due to cytokinins and auxins, a number of which have been demonstrated to occur in Kelpak[®] (Stirk & van Staden, 1996, 2004; Crouch et al., 1992). The beneficial effects of this product have been attributed to the plant hormone content of the extract. Since seaweed concentrates are applied in small doses, the active compounds in these concentrates need to be effective at low concentrations. Many studies have looked at the effect of applying plant growth regulators (PGR), such as auxins and gibberellins, on seaweed growth (review in Lobban & Harrison, 1997; Yokoya et al., 1999, 2003).

Kelpak[®] is a commercially available seaweed extract and is marketed as a plant growth stimulator due to its hormonal content and not its nutrient content

(Featonby-Smith & van Staden, 1983, 1987). It is manufactured by Kelp Products (Pty) Ltd. in Simons Town, South Africa, from epiphyte-free fronds and stipes of the brown alga *Ecklonia maxima* (Osbeck) Papenfuss, using a cold cell-burst process (Verkleij, 1992; Stirk & van Staden, 1996, 2004; Stirk et al., 2004). This process excludes the use of heat, chemicals or dehydration that could affect some organic components of the concentrate (Verkleij, 1992).

The aim of this paper was to test the effects of Kelpak on growth of seaweeds in culture. This was initially done in controlled conditions in the laboratory, using excised tips of *Gracilaria*, which are easy to grow and measure. Subsequently, pilot commercial-scale experiments were carried out to test the effects of the kelp extract on the growth of *Ulva* in tank culture on a commercial abalone/fish farm. *Ulva* was chosen for this, as we have considerable experience in growing *Ulva* in these systems as potential feed for abalone. Nutrient content of the cultured *Ulva* was also measured, with regard to its use as abalone feed. Experiments on *Gracilaria* growth in these systems were not as successful as *Ulva*, particularly due to low temperatures on the farm, and these are not presented.

Björnsäter and Wheeler (1990) and De Busk et al. (1986) showed that additions of fertilizer to nutrient-depleted water significantly increased Specific Growth Rate (SGR) of *Ulva*. Species of *Ulva* have been successfully grown in effluent water (abalone, fish and human) and SGR's are significantly higher compared to *Ulva* sp. grown in seawater (Ryther et al., 1975; Vandermeulen & Gordin, 1990; Cohen & Neori, 1991; Neori et al., 1991; Neori, 1996; Jimenez del Rio et al., 1996; Shpigel et al., 1997; Goldberg et al., 1998). As some land plants grown with fertilizer and Kelpak[®], showed significant increases in SGR (Featonby-Smith & van Staden, 1983, 1987; Crouch, 1990), the authors wished to test this observation for seaweeds using an effluent aquaculture medium as the source of nutrients for the seaweeds.

Materials and methods

Four different experiments were run in order to investigate the effects of Kelpak[®] on cultivated algae. Kelpak[®] adds only a very small amount of nutrients as a proportion of the total nutrients applied to the seaweeds at these low concentrations: it has an N:P ratio in mg N/P per g DW of Kelpak[®] of 55.98: 49.15 (± 0.01 ; $n = 6$) (Robertson-Andersson, 2004). This was tested

in the first instance in the laboratory, using excised tips of *Gracilaria*, selected for ease of growth and measurement, followed by measurements of growth rates of *Ulva* in an existing experimental system on a commercial abalone farm. Growth of *Ulva* was tested in combinations of seawater, abalone and turbot effluent, with various concentrations of Kelpak[®], with and without additional fertilization. Nitrogen content of the seaweed thalli was measured as a physiological parameter of seaweed health.

Laboratory experiments

The material was collected one day prior to the start of the experiment. *Gracilaria gracilis* (Stackhouse) Steentoft, Irvine *et* Farnham was collected from Saldanha Bay on the South African west coast, and was washed with running fresh water and sterile seawater and brushed with a paint brush to eliminate contaminants. The darkest thallus fragments were selected and 15 mm unbranched apical segments cut.

One-third strength standard Provasoli Enriched Seawater medium (PES) was prepared according to a standard recipe (Starr & Zeikos, 1987). The Kelpak[®] treatments were; 1:100, 1:250, 1:500, 1:1000, 1:2500, and 1:5000 added to one third strength PES. The control consisted of one-third strength PES medium with no Kelpak[®] added. The culture medium was changed every 2 days. The experiments were carried out at 15 °C temperature with an irradiance 50–80 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ provided by cool white fluorescent tubes and a photoperiod of 16 h. (light) : 8 h. (dark). Culture vessels were 200 cm³ crystallizing dishes to which 200 mL of PES was added as well as five 15 mm apical segments of *Gracilaria gracilis*. There were four replicates for each treatment. The flasks were moved within the experimental setup on a daily basis to ensure a uniform environment for all flasks. The initial, and on completion of the experiment, final biomass (in fresh weight) was measured, from which the SGR could be calculated using the following formula (Evans, 1972) and calculated as: $\text{SGR} = [\ln(W_t/W_0)]/(t_t - t_0)$

Where W_0 and W_t are initial and final wet weights (wwt) in grams and t_0 and t_t are initial and final times in days respectively.

Number of branches per tip was measured for each treatment at the end of the experiment.

Pilot commercial scale experiments

The commercial scale experiments were run on the Jacobsbaai Sea Products (JSP) Aquaculture Farm

(west coast, South Africa), a land-based intensive mariculture operation of *ca.* 11 h. The farm predominantly cultivates abalone (*Haliotis midae*) and turbot (*Scophthalmus maximus*). The *Ulva* material used was *U. lactuca* Linnaeus, originally from Simon's Town Harbour near Cape Town, which had been grown in tanks on the farm for a year prior to the experiments. The experimental seaweeds were cultivated in 96 L white PVC (0.60 m × 0.40 m × 0.40 m) tanks, using an air curtain produced by a U-shaped pipe system. Abalone-effluent and turbot-effluent generated on the farm were the two culture media used in these experiments with unfiltered seawater as a control. The experiments ran over winter from May 2002 to August 2002. The average seawater temperature on the farm was 14.6 °C (min 6 °C, max 20 °C) (Robertson-Andersson, 2004).

The first experiment consisted of determining the correct Kelpak[®] concentration to use in conjunction with fertilizer to obtain optimal SGR. The second and third experiments were run to test the effect of fertilizer and Kelpak[®] concentration (as determined from the first experiment) in stand alone additions or combined in both effluent culture media. The water volume exchange rate was 20 times per day. The fertilizer used was a combination of Maxiphos[®] (a commercially available fertilizer) and ammonium sulphate, with a ratio of 10:1 (Duke et al., 1986). Fertilizer and/or Kelpak[®] was added once a week. After the addition of the fertilizer no water exchange occurred for 20 h., after which a normal water exchange resumed. The seaweeds were acclimatized to the new culture conditions for two weeks after which all material was harvested and then tanks restocked to original stocking density (2 kg m⁻²) using the harvested material. The experiment was then run for two weeks and the seaweeds collected for data analysis.

To determine the optimum concentration of Kelpak[®], a tank system was set up at JSP to run 12 tanks with seaweed on turbot effluent. A control system consisted of a seawater control (four tanks) and a turbot effluent control (three tanks). The remaining 9 tanks were divided into 3 series of three turbot effluent tanks, with the following Kelpak[®] dilutions (1:500; 1:2500; and 1:5000).

After completion of the first experiment the seaweeds were left for two weeks to acclimatize to normal farm conditions (background nutrient concentrations) after which the algal biomass was harvested and used to restock the tanks. SGR of *Ulva* determined which Kelpak[®] concentration would be used in the following

two effluent experiments. The Kelpak[®] concentration of 1:2500 showed best results and was used in both the turbot and abalone effluent media. The experiment was then rerun for two periods of two weeks in each effluent medium with the following treatments:

- 3 effluent tanks with fertilizer and a 1:2500 concentration of Kelpak[®]
- 3 effluent tanks with fertilizer only
- 3 effluent tanks with Kelpak[®] at 1:2500 concentration only
- 4 seawater and 3 effluent control tanks

A two week break between experiments was left to allow the algae to acclimatize to the new effluent media.

Nutrient analysis

During harvest, samples were taken for wet to dry weight ratio analysis, and tissue total nitrogen was determined using the micro-Kjeldahl technique (Solorzano, 1969).

Statistical analysis

The effect of different concentrations of Kelpak[®] treatments on the SGR were statistically analyzed using ANOVA, single factorial analysis of variance ($p = 0.05$) (STATISTICA V6.1), to test the null hypothesis that the means of the SGR of all tested Kelpak[®] concentrations were not significantly different. The least significance difference (LSD) test or planned comparison test was conducted at the 95% confidence level, to distinguish significantly different results. The data were not transformed. The pilot commercial scale experiments showed that there was a greater effect on the seaweed growth during the second run in all the experiments implying that the algae required two weeks to acclimatize to the conditions. Thus the statistics quoted in this paper are for the second run only.

Results

Laboratory experiment

All concentrations of Kelpak[®] tested, with the exception of the most concentrated (1:100), significantly increased *Gracilaria* tip growth (Figure 1). *G. gracilis* apical segments had significantly higher SGR after 15 days in PES medium with the 1:1000 Kelpak[®] dilution, followed by 1:500 and 1:2500 Kelpak[®] dilution

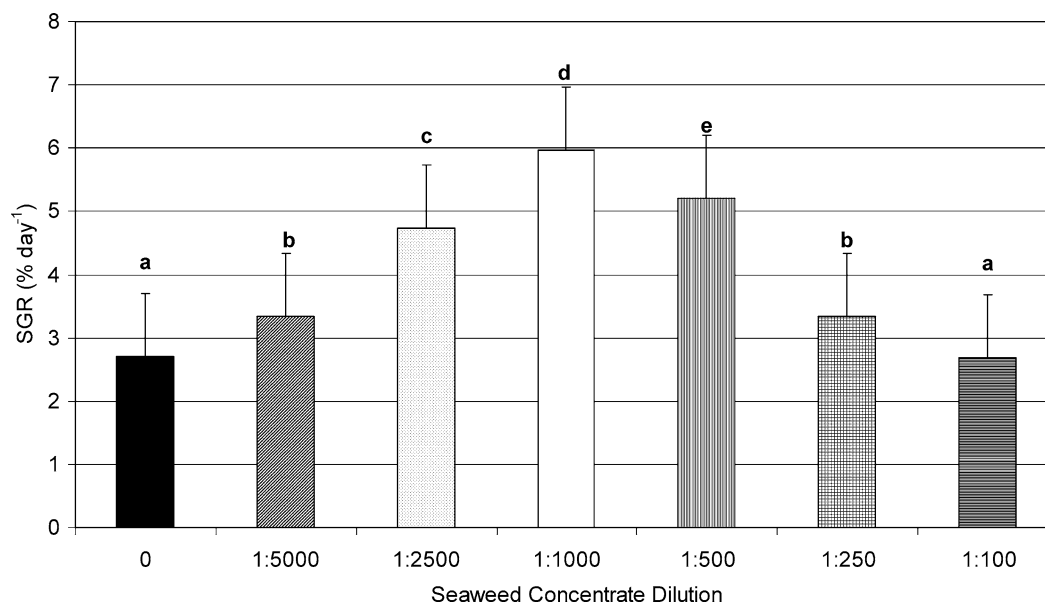


Figure 1. The effect of various Kelpak[®] dilutions on the SGR (% \cdot d⁻¹) of apical segments of *Gracilaria gracilis* after 15 days growth under laboratory conditions. Different letters indicate significant differences ($p < 0.05$: one-way ANOVA and LSD post-hoc test). Vertical lines represent \pm one Standard Error, $p = 0.05$.

compared to the control and other treatments. Apical segments growing at 1:250 and 1:5000 Kelpak[®] dilution had significantly higher SGR compared to the control and 1:100 Kelpak[®] dilution. There was no significant difference in the SGR of apical segments growing in 1:100 kelp concentrate dilution and control. Although the 1:5000 treatment showed slightly increased branching, there was no significant change in the number of branches of *G. gracilis* in the different Kelpak[®] concentrations compared to the control. Branching was, however, significantly reduced in the 1:100 treatment, compared to the 1:5000 treatment (Table 1).

Effect of various Kelpak[®] concentrations added to turbot effluent in pilot commercial scale experiment

The SGR of the *Ulva* grown using the 1:5000 and 1:2500 Kelpak[®] concentrations were not significantly different to the turbot control (Table 2). The SGR of the *Ulva* grown in both the seawater and the 1:500 Kelpak[®] concentration were significantly lower than all other treatments (LSD post-hoc test, $p < 0.01$ in all cases). Material from the seawater treatment had significantly lower tissue nitrogen levels (Table 2), compared to all other treatments (ANOVA, $df = 20$, $p < 0.01$; LSD post-hoc test, $p < 0.01$). The 1:2500 treatment

had slightly higher growth and nitrogen content than the other effluent treatments, but not significantly so.

Effect of Turbot effluent - Kelpak[®] - Fertilizer combination in a pilot commercial scale experiment

In all cases the addition of either fertilizer, or Kelpak[®] (1:2500) or a combination of both, significantly

Table 1. The effect of various Kelpak[®] dilutions on the total number of branches and the average branches of apical segments of *Gracilaria gracilis* per dish after 15 days in growth under laboratory conditions.

Treatment	Average no of branches
Control	1.4 \pm 1.6 ab
1:5000	1.9 \pm 1.6 a
1:1000	1.3 \pm 1.8 ab
1:500	0.9 \pm 1.7 ab
1:100	0.7 \pm 0.9 b

Different letters indicate significant differences ($p < 0.05$: one-way ANOVA and LSD post-hoc test).

Table 2. The effect of Kelpak[®] dilutions on SGR (% d⁻¹) and tissue nitrogen (mg N g⁻¹ DW) of *Ulva lactuca* cultivated in turbot effluent on a pilot commercial scale.

Treatment	SGR (% d ⁻¹)	Tissue N (mg N g ⁻¹ DW)
Turbot Effluent		
Turbot control	4.2 ± 1.2 b	58.9 ± 2.0 a
Turbot + Fertilizer	6.1 ± 0.8 a	59.8 ± 5.5 a
Turbot + Fertilizer + Kelpak [®]	6.6 ± 0.4 a	62.0 ± 6.5 a
Turbot + Kelpak [®]	5.1 ± 0.3 a	62.6 ± 4.8 a
Seawater control	3.5 ± 0.1 c	48.4 ± 6.1 b

All values are ± one Standard Error, $p = 0.05$. Different letters indicate significant differences ($p < 0.05$: one-way ANOVA and LSD post-hoc test).

Table 3. The effect of combinations of fertilizer and or Kelpak[®] concentrate (1:2500) on SGR (% d⁻¹) and tissue nitrogen (mg N g⁻¹ DW) of *Ulva lactuca* cultivated in turbot and abalone effluent on a pilot commercial scale.

Treatment	SGR (% d ⁻¹)	Tissue N (mg N g ⁻¹ DW)
Abalone effluent		
Abalone control	6.1 ± 1.2 a	60.6 ± 3.0 a
Abalone + Fertilizer	6.7 ± 1.0 a	63.7 ± 2.5 a
Abalone + Fertilizer + Kelpak [®]	7.2 ± 0.0 a	58.0 ± 4.5 a
Abalone + Kelpak [®]	6.3 ± 0.0 a	59.0 ± 2.3 a
Seawater control	4.9 ± 0.2 b	53.0 ± 6.5 b

All values are ± one Standard Error, $p = 0.05$. Different letters indicate significant differences ($p < 0.05$) (one-way ANOVA and LSD post-hoc test).

increased the SGR of the *Ulva* above the turbot control and the seawater control (Table 3). The combined Kelpak[®] – fertilizer – turbot effluent media was significantly higher than all other treatments except the turbot – fertilizer treatment (LSD post-hoc test, $p < 0.01$).

The tissue N of *Ulva* in the seawater control (Table 3) was significantly lower than all other treatments (ANOVA, $df = 20$, $p < 0.01$; LSD post-hoc test, $p < 0.01$).

Effect of Abalone effluent – Kelpak[®] – fertilizer combination in a commercial scale experiment

The results of the abalone effluent experiments were similar to those of the turbot effluent experiment (Table 3). Addition of Kelpak[®] (1:2500) and/or fertilizer increased the SGR of *Ulva* above that of the abalone effluent and seawater controls. The abalone effluent combined with Kelpak[®] (1:2500) and fertilizer

had a significantly higher SGR than all other treatments (ANOVA, $df = 20$; $p < 0.01$; LSD post-hoc test, $p < 0.01$) except the abalone – fertilizer treatment. In contrast to the turbot experiments, there was no significant difference in SGR between the abalone control and effluent plus Kelpak[®] treatments in the abalone effluent experiments.

There was a significant decrease in tissue nitrogen of *Ulva* cultivated in seawater compared to all other treatments (ANOVA, $df = 20$; $p < 0.01$; LSD post-hoc test, $p < 0.01$).

Discussion

As far as we are aware, this is the first study to examine the effect of commercial kelp concentrate on seaweed growth. This is surprising, as the beneficial effect of seaweed concentrate on the growth, yield and disease reduction in crop plants has been well documented over the past 30 years (Finnie & van Staden, 1985; Featonby-Smith & van Staden, 1983, 1987; Stirk & van Staden 1996, 2004; Stirk et al., 2004). Finnie and van Staden (1985) demonstrated that the concentration ratio of *Ecklonia maxima* kelp extract is an important factor in controlling its efficiency. In tomato plants, strong concentrations (1:100 seaweed extract: water) were found to have an inhibitory effect upon root growth, whereas weak concentrations (1:600) had a stimulatory effect. The Kelpak[®] concentrations used in this study are within the range commonly used in land plant studies.

Treatment with 1:5000 Kelpak[®] concentration did not significantly increase SGR of the seaweed, which is in agreement with Beckett and van Staden (1990), who showed that the growth of wheat was not stimulated by low concentrations (1:1000 retail product–5 times more dilute than commercial product) of Kelpak[®] compared to the controls. The 1:2500 Kelpak[®] concentration caused the highest SGR increase in the tank experiments on the aquaculture farm. This is slightly more concentrated than the Kelpak[®] treatment which produced maximum growth in *Gracilaria* tips in our laboratory experiment. This could be due to the fact that on the commercial farm a once off pulse addition was used, while in the laboratory the segments were constantly exposed to a more dilute concentration. The field experiment results are in agreement with studies in which Kelpak[®] used at a concentration of 1:2 500 and applied regularly, improved the total biomass of *Beta vulgaris* and *Phaseolus vulgaris* (Crouch, 1990)

and the root growth of cucumber plants (Nelson & van Staden, 1984).

A pronounced inhibitory effect was observed using a 1:500 concentration in both the laboratory and the aquaculture farm, for both *Gracilaria* and *Ulva*. This effect was also found in the study of Finnie and van Staden (1985) who reported inhibition of tomato roots at this concentration.

The experiments using abalone and turbot effluent water indicate that additions of both fertilizer and Kelpak[®] (1:2500 concentration) significantly increase SGR of *Ulva* above that of effluent or seawater controls alone. Moreover, a combination of Kelpak[®] (1:2500 concentration) with fertilizer significantly increased SGR of *Ulva* over all other treatments. This increase in SGR is similar to that of the kelp concentrate in agriculture, where it is used as a very diluted 'root drench' for a short period, in addition to traditional fertilisers. Therefore the positive effects on agricultural crops are not due to addition of major nutrients from the Kelpak[®]. This might be the case in the seaweed experiments in this study. The effects on agricultural crops have been reported to be caused by the concentrations of plant growth hormones (especially auxins and cytokinins) in Kelpak[®]. It is known from the literature that seaweeds contain plant growth substances (Bradley, 1991; Stirk et al., 2004), and that additions of plant growth hormones to media increases growth (callus production) of various red algae, including *Gracilaria* (e.g. Yokoya et al., 1999, 2003).

It was not in the scope of this study to prove that plant growth hormones are the active ingredients in Kelpak[®], causing a positive effect on growth in *Gracilaria* and *Ulva*. The reasons for the increase in SGR are not understood, but it is thought that the hormonal content, particularly cytokinin, plays an important role (Feantonby-Smith & van Staden, 1983, 1987; Bradley, 1991). Other studies have looked at the effects of additions of cytokinin (Blunden & Wildgoose, 1977) and synthetic cytokinin (Finnie & van Staden, 1985) on SGR and have come to the same conclusions.

Tissue N values decreased in all treatments between the first and second runs where SGR increased. This relationship follows that described by Rosenberg and Ramus, 1982; Duke et al., 1986, 1987 and 1989a,b, with an increase in SGR causing a decrease in tissue nitrogen. This is due to the faster SGR requiring more nutrients. The increased growth with Kelpak[®], compared to the other treatments did not, however, decrease N, meaning that there is potential for using the *Ulva* in bioremediation, and as high quality abalone feed.

All experiments showed that the SGR was slightly higher during the second run compared to the first run. This may indicate that the alga has an acclimatization period to the experimental conditions before an effect can be monitored. Observations (unpublished) showed a similar acclimatization period for *Gracilaria gracilis*, a species cultivated on a pilot commercial scale at JSP. Results for *Gracilaria* are not shown as the SGR was low due to low nightly water temperatures (5.5 °C) present at the time of the study. Branching in *Gracilaria* however, appeared to correlate well with the Kelpak[®] concentrations similar to that noted in the laboratory culture (unpublished data).

Conclusions

The results of the seaweed cultivation experiments using Kelpak[®] dilutions are similar to previous findings on the effect of seaweed extract applications on certain field crops (Feantonby-Smith & van Staden, 1983). The highest SGR in the pilot commercial experiments was obtained using a concentration of 1:2500 of Kelpak[®]. The weakest concentration of 1:5000 did not change the SGR. The highest concentration (1:500) used in this study showed inhibitory effects. Thus, Kelpak[®], may have commercial potential in the seaweed mariculture industry.

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