An investigation of protein variation in *Ulva lactuca* and *Ulva rigida* under high nitrogen culture conditions.

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ABSTRACT

In the light of findings that abalone achieve higher growth rates when fed on high protein diets, this study attempts to increase the protein content of two macroalgae under culture conditions. *Ulva lactuca* and *U. rigida* were grown in saturating NH_4^+ - N (1 mM) and PO_4^{3-} P (70 μM) concentrations. Of the two, protein levels of *U. lactuca* showed the greatest increase over time. Natural populations had a content of 3.7 % of dry weight. This increased significantly ($p < 0.05$) to a maximum value of 4.5 % after 8 days growth in nutrient enriched conditions. The protein content of *U. rigida* increased significantly from 3.3 % to 3.9 % after 6 days of growth in nutrient enriched conditions. An interspecies comparison was made using the intertidal species *Porphyra capensis*, *Ulva capensis* and *Gracilaria gracilis*. The highest protein content was found in *P. capensis* (11.5 %), followed by *U. capensis* (3.0 %) and *G. gracilis* (2.7 %). Results indicate the suitability of *P. capensis* as a high-protein abalone fodder. Furthermore, results suggest that the protein content of abalone fodder may be optimized under aquaculture conditions.

INTRODUCTION

Japan and China are the highest consumers of abalone products (80 % of global production) (Redmayne, 1991). In 1994, the vast majority of locally-cultured abalone was exported to the Far East where differences exist in consumer demand for abalone. Premium prices are attained by large sized species, while comparatively inferior products may be canned and are marketable in various countries. South African-cultured *H. midae* (Linnaeus) is a highly desirable species which fetches comparatively high prices in the Hong Kong market (Oakes and Ponte, 1996). Figures for 1993 show that South Africa produced 600 tons of *H. midae* which fetched US$ 25 000 per ton. Demand and prices have increased since this figure was released (Oakes and Ponte, 1996).

Abalone consume as much material as is needed to fulfill energetic requirements and maximum growth is achieved by increasing the protein content of their diet. Neori et al. (1999) showed that two species of abalone (*Haliotis tuberculata* and *H. discus* hannai) had significantly higher growth rates and better food conversion ratios when fed seaweed with a high protein content. Britz and Hecht (1997) found that the growth rates of *H. midae* peaked when fed on an artificial diet containing 44 % protein while optimal fodder protein was lower (34 %) for smaller sized abalone. The macroalgal diet of abalone has a protein content typically less than 20% (Nisizawa *et al.*, 1987) and inadequate protein is accompanied by weight loss in abalone. A fodder with a high amount of protein would be marketable and desirable specifically in terms

of promoting abalone growth. There has been an exponential rise in the amount of kelp harvested for supplying abalone farms on the south African west coast. In this area, farmed abalone are mostly fed a combination of harvested kelp and artificial feed. Artificial feed is provided in pelleted form in which protein is derived from animal and plant extracts. Although costly, these pelleted feeds have a higher protein content than seaweeds and they can be formulated to match the amino acid profile of *H. midae* (e.g. Knauer *et al.*, 1996). They are also considered necessary in intensive abalone culture due to problems relating to supply of fresh seaweed (Britz *et al.*, 1994). The problem of supply could be reduced by aquaculture of seaweeds on abalone farms and a number of abalone farms in the Western Cape (which currently feed mainly fresh kelp) are interested in the prospect of growing other seaweeds as additional feed.

While interspecific differences in protein levels are evident, it is also possible to attain intraspecific variation in protein content. *U. lactuca* Linnaeus 1753 is a palatable alga which has a variable protein content dependent on its growth conditions. Mai *et al.* (1994) found that *U. lactuca* was comparatively poor in relation to the nutritional requirements of abalone. Despite this, an increase in the protein content of *U. lactuca* improves its nutritional value to abalone. Shpigel *et al.* (1999) reported that growth in high ammonium (NH_4^+) conditions improved protein content from 12 % to 44 % of dry weight. There was also an associated increase in energy content from 12 kJ g^{-1} to 16 kJ g^{-1}. These high and low protein diets were fed to abalone. The high protein diet improved growth rate of *H. tuberculata* by 43 % and that of *H. discus hannai* by 110 %. Furthermore, the food conversion ratio was improved, meaning that less material was consumed for a given weight increase.

In a study relating to the effects of sewerage and drainage inputs into coastal and estuarine waters, Waite and Mitchell (1972) studied the effects of ammonia (NH_3) and phosphate (PO_4^{3-}) supply on the productivity of *Ulva lactuca*. Maximum growth was achieved at 42.6 μM NH_3 and 15.5 μM PO_4^{3-} and optimum response depended on the combined effect of both nutrients. *U. fenestrata* Postels & Ruprecht shows a reduction (53 % - 91 %) in uptake of either nutrient when one supplement is withheld from the culture solution (Bjornsater & Wheeler, 1990). Bongers (1956) reported that NH_3 was more stimulatory to growth than nitrate (NO_3) since N supplied as NH_3 is already in an assimilatable form. However, it is also been shown that high levels of NH_3 are toxic to *U. lactuca* and suppress growth (Waite and Mitchell, 1972).

Optimum uptake of nutrients may be temperature-specific. Harlin *et al.* (1979) reported that NH_4^+ uptake was favoured by *Gracilaria* sp. and that uptake was enhanced by increasing temperature from 15 °C to 20 °C (60.5 $\mu mol.gww^{-1}.d^{-1}$). This was not the case for *Ulva lactuca*, which showed a slight decrease in uptake at 20 °C. Growth rates of the ulvoids in non nutrient limited culture varies with temperature. Duke (1985) reported a ten-fold variation in growth rate of *U.curvata* (Kutz.) de Toni between 5 °C and 20 °C. Bjornsater and Wheeler (1990) reported a hyperbolic relationship between growth rate and tissue N for *U. fenestrata*. A maximum growth rate of 16 $\%.d^{-1}$ was achieved at 13 ± 2 °C , and the corresponding rate of nitrate uptake was 149 $\mu mol.g^{-1}.d^{-1}$ (Duke et al., 1989). A winter accumulation of N in many seaweeds may imply that temperature limits growth rates to a greater extent than uptake rates (Duke et al., 1989). Nitrate uptake is a limiting step in terms of metabolism, and protein increases with increasing nitrate supply (Lopez-Figueroa & Rudiger, 1990). Macroalgae grown in culture conditions often show an increase in

protein levels when the seawater volume exchange rate is increased (Rosen *et al.*, 2000).

Nitrate uptake is light dependent in *Enteromorpha* (Harlin, 1978) while for *M. integrifolia*, nitrate uptake is faster in the dark than in light (Wheeler and Srivastava, 1984). Light also stimulates synthesis and activation of the enzyme nitrate reductase (Azuara & Aparicio, 1983).

Seasonality of nutrient availability or a pulsed nutrient supply leads to an uncoupling of nutrient uptake and growth. Cellular reserve pools of N may accumulate in response to supply of inorganic N in excess of growth demands (Hanisak, 1983), and the abundance and form in which this N is stored may vary among species as well as with the type of inorganic N available to the macroalga. The N uptake capacity of a seaweed is a direct function of its surface area-to-volume ratio (Rosenberg & Ramus, 1984) while N storage capacity varies inversely with SA:V ratio. Thus a species with a high SA:V would have growth rates correlated with N supply, while those with a low SA:V may show an uncoupling of growth rate from N availability. The ulvoids are a good example of a species with a high SA:V ratio.

This study will include a comparison of protein content in naturally-growing populations of selected, potential feed species of seaweed. Thereafter, an attempt will be made to increase protein content under high ambient N culture conditions in local *Ulva* species. An ability to increase the protein content of an alga (especially one which is already used as abalone fodder) would optimize its nutritional value and improve the growth rate of abalone.

METHODS

For an initial test of protein analysis methods, fresh material was gathered and analysed at U.C.T. laboratories. *Ulva capensis* Areschoug 1851 and *Porphyra capensis* Keutzing 1843 was collected from Kommetjie at low tide on 23 July 2001. *Gracilaria gracilis* (Stackhouse) Steentoft, Irvine *et* Farnham had been collected from Saldahna Bay and was maintained in tanks until analysis.

Ulva rigida C. Agardh 1823 was collected at low tide from an attached population in Simon's Town harbour on 11 September 2001. In the laboratory, samples were divided among three tanks each containing 25 litres of filtered sea water (2 μm) to give a stocking density of ca 1.6 g dry weight /L and supplied with a constant air supply. The tanks were kept in a growth room set at 16:8 hours light:dark cycle (cool white fluorescent tubes providing an irradiance of 80-90 $\mu mol.m^{-2}.s^{-1}$) and were maintained at ca 13 °C for the duration of the experiment. The seaweed was left to acclimate over a period of 7 days. During this period the culture medium was not replaced. The intention was to deplete the seawater nitrogen concentration and, in so doing, to minimize the protein content before initiation of the experiment. Thereafter, seaweed was placed in a N-enriched solution. The N source was supplied in the form of NH_4SO_4. The ES enrichment solution (L. Provasoli, pers. comm.; Appendix A) was added to seawater at 20 mL/L to provide the seaweed with a concentration of 1mM N and 70 μM P. This was based on the level of enrichment used by Naldi and Wheeler (1999). Water was changed once a day to compensate for nutrient depletion. An initial analysis of protein was made of material from the collection site. A second

measurement was taken after 7 days in unenriched seawater. Thereafter, cultured samples were collected at regular intervals over a period of 7 days. Three replicates were taken at each sampling time.

For the second experiment, free-floating *U. lactuca* was collected at low tide from Simon's Town harbour on the 4 September 2001. Fresh material was returned to the laboratory and was left to acclimate (conditions mentioned above) over a period of 48 hours. After this, the water was replaced and enriched with ES solution (as above). Samples were taken at the time of collection, after acclimation and at two day intervals thereafter. The final sample was drawn after 10 days of nutrient enrichment.

Sample material was removed at the end of the dark period. Fresh weight was measured directly after removal from the tanks (samples were rinsed in distilled water and excess water was removed by blotting). Samples were then dried at 60 °C for 24 hours in a Labotec Term-O-Mat oven and then weighed. The dry material was ground in a mill to pass through a screen with aperture 1 mm and were stored in air-tight containers at –15 °C until analysis. Before analysis, samples were ground with a pestle and mortar with liquid nitrogen in order to maximize cell lysis.

EXTRACTION PROCEDURE

The extraction of protein follows the method used by Fleurence *et al.* (1995) with a few modifications. Out of a variety of different techniques, this technique was found to be the most efficient at protein extraction and has been used on *Hypnea* spp. , *U. lactuca*, *U. rigida*, and *U. rotundata*.

Approximately 3 g of algal material for each sample was used in the extraction procedure. There was an initial aqueous extraction in which the material was suspended in 60 mL deionized water (1:20 w/v). The suspension was gently stirred overnight at 25 °C and then centrifuged at 10 000 x g and 4 °C for 20 min. The supernatant was then collected and the algal material was re-suspended in a 60 mL solution of NaOH (0.1M) and mercaptoethanol (0.5% v/v). The suspension was stirred at room temperature for 2 hours before centrifugation under the conditions mentioned above. The supernatant was collected and combined with that of the initial extraction and was stored at 4 °C. The second part of the protocol was repeated 7 times. Samples were frozen at -12 °C until protein quantification.

Protein was quantified using the Bradford method (Bradford, 1976). The algal extractant was first neutralized by adding HCl. 3 ml of Bradford Reagent was added to 100μL of the extractant (disposable plastic cuvettes inverted x3). The shift in absorbance from 465 nm to 595 nm was measured after 20 minutes using a Spectronic 20 Genesys spectrophotometer. Protein concentration was calculated by comparison with standard solutions of known protein concentration based on Bovine Serum Albumin (BSA) using the extracting solution of NaOH and 2-mercaptoethanol as a blank. All standards and samples were prepared in duplicate. The absorbance reading was converted to protein concentration of extractant ($\mu g.mL^{-1}$). Protein content was then expressed as percentage of dry weight of sample material (Appendix C).

NOTES ON THE METHOD

The main modification to the procedure is in the length of extraction time and in the number of extraction repetitions. In the original technique (Fleurence *et al.*, 1995) the extraction mixture was stirred for a period of 1 hour and the protocol was repeated 6 times. In a subsequent experiment, the duration of extraction was increased to 2 hours and the protocol was repeated 5 times (Wong and Cheung, 2001). In the present study, the time number of extractions was increased to 7 since there was still a small amount of protein being extracted after 6 repetitions. These modifications were aimed at maximizing the proportion of total protein extracted from the samples. However, although it is expected to increase the proportion, the extraction technique does not recover the total protein content of the seaweed. Due to limitations in the amount of material that could be cultured, the amount of dry material was decreased from 10 g to 3 g; however, the relative extracting volumes were maintained and should not have lead to any discrepancies in the results.

STATISTICAL ANALYSIS

Due to the small sample size, data was analysed using Kruskal-Wallis non-parametric analysis of variance. Least Squares Difference (LSD) was used as a *post hoc* test for comparison of treatment means. Results were significant at $p < 0.05$.

RESULTS

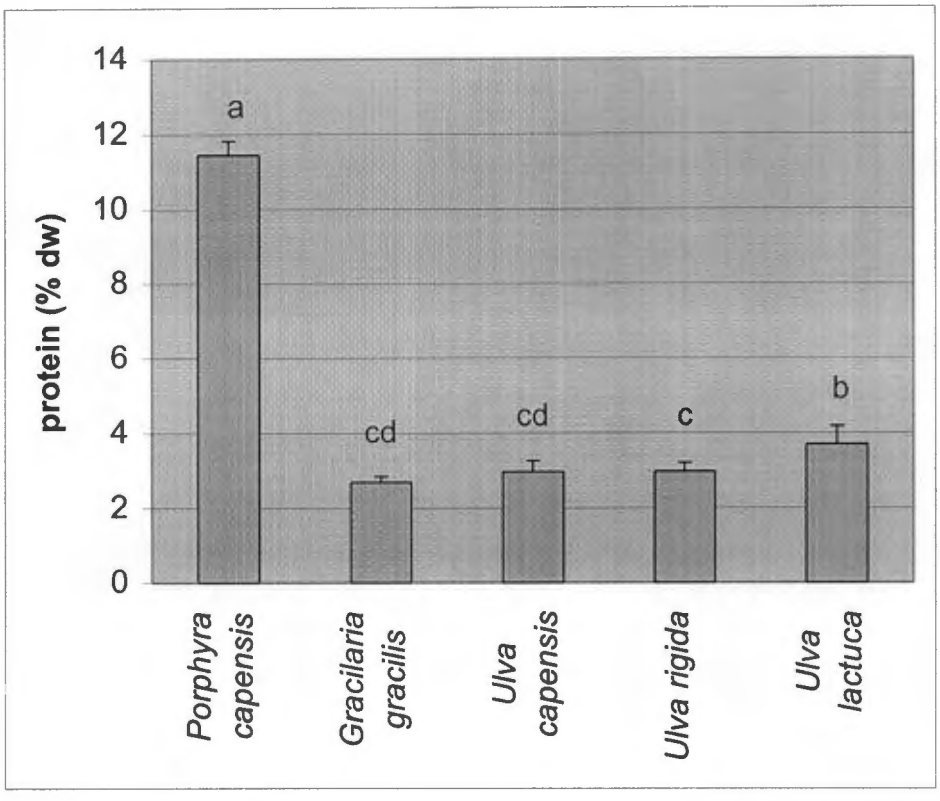

Fig. 1: Graph of protein content of various seaweed species

Whiskers show standard deviation
a,b,c,d: Different letters indicate statistically significant differences ($p < 0.05$)

Table 1: Summary table of protein content of different species

Different letters indicate statistically significant differences ($p < 0.05$)

Species	Mean (% dw)	Standard Deviation
P.capensis	11.45 a	0.38
G.gracilis	2.68 cd	0.15
U.capensis	2.97 cd	0.29
U.rigida	2.98 c	0.24
U.lactuca	3.70 b	0.48

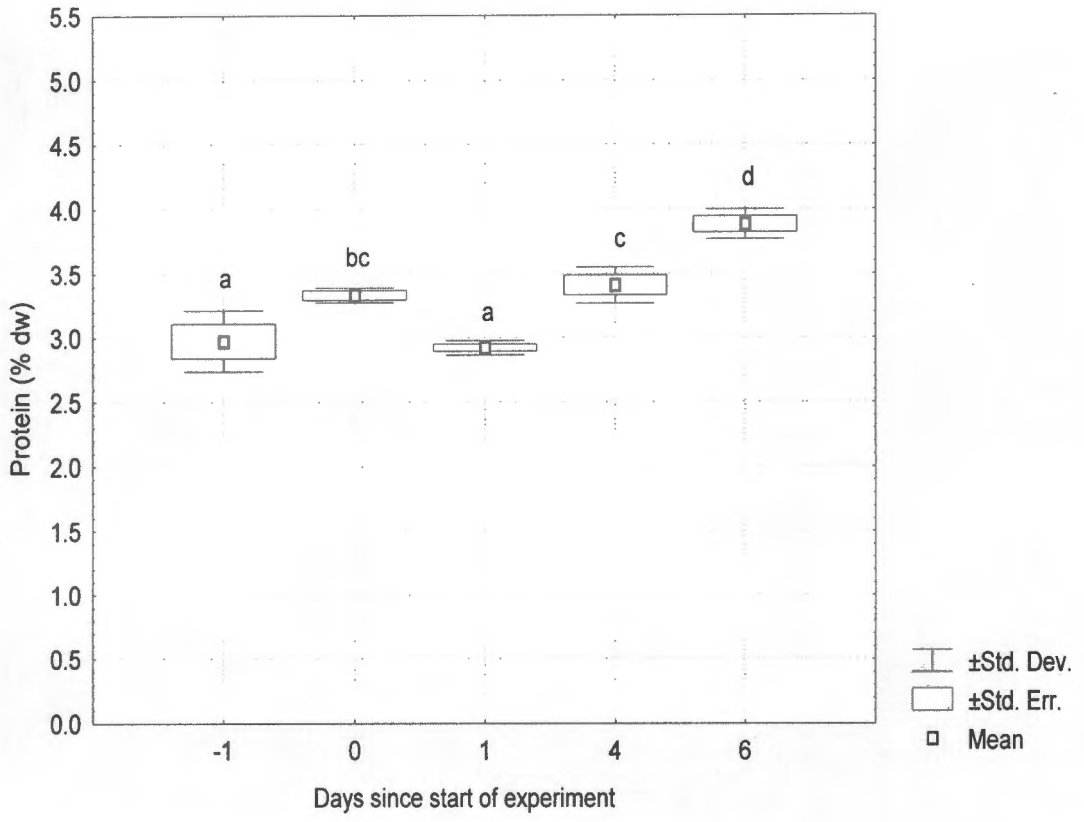

Fig. 2: Graph showing protein content of *U. rigida* for Experiment 1.

Different letters indicate statistically significant differences ($p < 0.05$)

Table 2: Summary table for protein content of *U. rigida* (Experiment 1)

Different letters indicate statistically significant differences ($p < 0.05$)

Days since start of experiment	Mean protein (% dw)	Standard Deviation
-1	2.98 a	0.24
0	3.33 bc	0.06
1	2.92 a	0.06
4	3.41 c	0.14
6	3.88 d	0.11

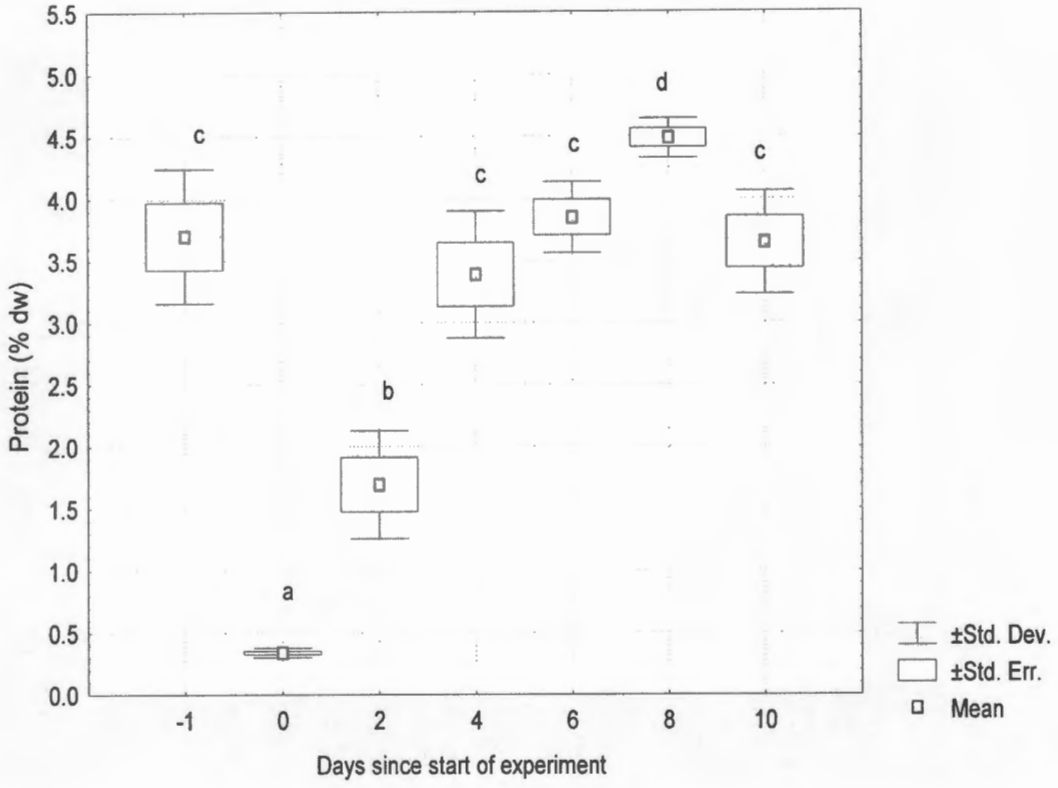

Fig. 3: Graph showing protein content of *U. lactuca* for Experiment 2

Different letters indicate statistically significant differences ($p < 0.05$)

Table 3: Summary table for protein content of *U.lactuca* (Experiment 2)

Different letters indicate statistically significant differences ($p < 0.05$)

Days since start of experiment	Mean protein (% dw)	Standard.Deviation
-1	3.70 c	0.48
0	0.34 a	0.03
2	1.69 b	0.38
4	3.39 c	0.45
6	3.85 c	0.25
8	4.49 d	0.14
10	3.64 c	0.30

In the interspecies comparison (Fig. 1, Table 1), the highest protein content was found in *Porphyra capensis* with a protein content of 11.45 % dw. The ulvoid species had much lower protein contents. *Ulva capensis* and *Ulva rigida* showed very similar values of 2.97

% and 2.98 % respectively. *Ulva lactuca* had a significantly higher content of 3.70 %. The lowest protein level was found in *Gracilaria gracilis* (2.68 %) and this was not significantly different from that of *U. capensis* or *U. rigida*.

In the first enrichment experiment, *U.rigida* sporulated on the first day of nutrient enrichment. The empty sporulated tissue was removed before analysis for protein. Results show a general trend of increasing protein over the duration of the experiment (Fig. 2, Table 2). Results suggest that *U. rigida* had a significantly higher protein content after 7 days growth in nutrient-poor conditions. After one day of nutrient enrichment, protein content had decreased significantly to its initial 'pre-starvation' value. After 4 days of enrichment, protein had increased significantly to 3.41 %. The maximum content of 3.88 % was observed after 6 days. Protein increased significantly over the 6 days of enrichment from 3.33 % to 3.88 % dw. Over this period the protein content increased by 16.5 %.

Figure 3 and Table 3 give the results of the second experiment. *U. lactuca* collected from Simon's Town had a 3.70 % protein level. This decreased to 0.34 % after 2 days growth in depleted ambient nutrient conditions. After 2 and 4 days of enrichment, protein had increased significantly to 1.69 % and 3.39 % respectively. There was an increase to 3.85 % (not significant) after 6 days and a further significant increase to 4.49 % after 8 days. Thereafter, protein levels dropped significantly to 3.64 % after a further 2 days growth in nutrient rich conditions. The protein level of starved material increased over ten-fold to a maximum protein content after 8 days.

DISCUSSION

The current literature employs a few different techniques for quantification of protein. This fact must be kept in mind when attempting a comparison of measured values among cited experiments. The Kjeldahl method is the most frequently used for determining total crude N. The N concentration is converted to protein content by a factor of 6.25 (based on the protein N content of 0.16 $g.g^{-1}$). Although this seems to be a commonly accepted conversion factor, it includes N not in the form of protein (i.e. intracellular reserve pools of N). Therefore the Kjeldahl method would tend to overestimate the actual protein content. The consequences of this may differ among algal species which have specific N storage capacities. Protein tends to be the most important storage form of N in seaweeds, however there are differences in the relative amounts among species. Naldi and Wheeler (1999) conducted an experiment on the effects of N enrichment on total N of *U. fenestrata* in which external NH_4^+ was supplied at a saturating concentration of 1 mM (this concentration was used in the current study). It was found that protein was the most important storage form of N and ranged from 43 % - 66 % of total N. Furthermore, enrichment caused protein to increase more than the other N pools (41% - 89% of total N increase). The remaining N was allocated to cellular pools of nitrate, ammonium, free amino acids and chlorophyll.

As this project is aimed at increasing the nutritional benefit to abalone, it is important that the actual available protein constituent is assessed. Thus it was decided to employ a more direct method of quantification namely the Bradford method. This method involves staining the protein constituent with Coomassie Brilliant Blue which

causes a shift in the absorption maximum of the dye. The procedure for protein extraction is the most successful of an array of techniques which were tested on *Ulva* spp. (Fleurence *et al.*, 1995). This technique recovered roughly one third of the total protein (compared before and after the extraction using the Kjeldahl method). Therefore when comparing the results of this experiment with those reviewed, one could attempt a rough conversion by multiplying up these figures by a factor of 3. This is a guideline comparison, as the effectiveness of the technique is also dependent on the seaweed species being tested. (With reference to the total protein content of each species, extraction yielded 27 % of total protein from *U. rigida* and 36 % of total protein from *U. rotundata;* Fleurence *et al.*, 1995) . This may lead to difficulties in comparing protein content among species; however, the within- species comparisons will provide a clear indication of protein variation relative to control values. The effect of experimental conditions on relative protein variation is apparent from these results.

Previous studies have shown that the highest natural protein levels tend to occur in certain rhodophytes, while phaeophytes generally have the lowest protein levels (Fleurence, 1999). Protein content of *Porphyra tenera* Kjellman ranges between 33 % and 47 % of dry weight (Indegaard and Minsaas, 1991; Nisizawa *et al.*, 1987) while *U. lactuca* has protein levels ranging between 10 % and 21 % of dry weight (Castro-Gonzales et al., 1996; Smith and Young, 1954). In the current study, *U. lactuca* obtained a maximum value of 4.49 % (after nutrient enrichment). Wong and Cheung (2001) reported a 7.11 % crude protein content for *U. lactuca*. The same extraction technique was used, however a different quantification technique was employed (N was measured using an auto-analyser and converted to a protein content by 6.25 conversion factor). Since the values obtained in this study are lower it can be

suggested that the difference is due either to discrepancies in the quantification technique used, or else the difference was due to a seasonal dependence on protein content. Since the extraction procedure recovered approximately one third of total protein, the results obtained in this study fall within the range of protein values for *U. lactuca* (as measured by the Kjeldahl method) of 10 % - 21 % (Castro-Gonzales et al., 1996; Smith and Young, 1954).

Amongst the ulvoids, *U. lactuca* has relatively low protein content – *U. pertusa* contains 20 % to 26 % of dry weight protein (Nisizawa *et al.*, 1987). Thus protein content differs even within the same genus. In the current study, *U. rigida* had the lower content of the two *Ulva* spp. tested. Both species were collected from the same area and it is likely that they would have been exposed to the same level of nutrient enrichment. This could have led to the conclusion that differences in measured protein content were due to interspecific variation; however, the *U. rigida* had been growing in the intertidal zone while *U. lactuca* was free-floating. *U. rigida* would have been exposed during low tide and therefore the two seaweeds may have differed with respect to total nutrient uptake over time.

In the current study, *Gracilaria gracilis* had a surprisingly low protein content in comparison with other rhodophytes. Smit et al. (1997) found a protein value of 4.15 % of dry weight for *G. gracilis*. The quantification technique used was a direct measurement of protein, similar to that used in the current study, therefore results of the two studies should be comparable. The low measured values are probably due to the fact that the material had been growing in tanks prior to analysis, which may have resulted in a loss of stored protein.

Phenolic compounds and cell wall polysaccharides are substances that interfere with protein extractability (Jordan and Vilter, 1991; Fleurence *et al.*, 1995). Since algal species differ with respect to their relative content of these substances, the protein extractability should be species dependent. The extraction technique chosen for this study had been used both on *Ulva* spp. and the rhodophyte *Hypnea* sp. Protein extractability was reported to be higher for the *Hypnea* sp. compared with *Ulva* spp. (Wong and Cheung, 2001), therefore it seems unlikely that the low protein content found for *Gracilaria* was due to less efficient extraction. High levels of phenolic compounds also hinder the digestion of proteins and this is especially true of brown algae (Horie *et al.*, 1995).

U. rigida released spores early in the enrichment period. N enrichment stimulates vegetative reproduction in *Ulva* spp., while low levels of nutrients may also cause the release of gametes (DeBoer, 1981). Therefore it is possible that the sporulation was triggered by the sudden change in ambient nutrient concentrations. Alternatively, the alga may have been on a sporulation cycle which coincided with the experiment. This would probably have caused a lowering of the protein content and is a possible explanation as to why protein was seen to decrease after one day of enrichment. It is more likely that the unexpected values at 0 and 1 day after enrichment were caused by a change in the extraction protocol. In these two samples, the amount of dry material recovered was less than the standard 3 g used for the remaining samples with the result that only 1 g of dry material was subjected to protein extraction. The volume of extraction solution was reduced proportionally and underwent the full extraction process, thus in theory there should not have been any effect relative to the remaining samples. It seems that the extraction of protein is sensitive to the initial mass of sample material. The experiment was continued to give an indication of the relative

protein increase over time. Although the protein content remains low compared to other species, *U. rigida* grown in culture showed a significant increase over time. Over the course of the three last sampling intervals, protein increased by 33 %. The experiment was terminated because of insufficient amounts of material but it is evident that at the last sampling time the protein content was still increasing and it would be interesting to see at what point the protein of this species reaches a maximum. *U. rigida* was dried down to 10.5 % of fresh weight while *U. lactuca* was dried down to 5.9 % of fresh weight. This means that abalone would consume smaller quantities of *U. rigida* to fulfill a given energy requirement.

The second experiment using *U. lactuca* indicates that this seaweed experiences high N growth conditions at the sampling site. Once the material had been starved, it took three days of growth in saturating N and P to recover the protein content initially present. The protein decrease in starved material was substantial (a 91 % reduction of protein over the course of 2 days). This dramatic reduction is not surprising as Nasr *et al.* (1968) reported a 20-fold decrease in specific amino acids of nutrient-starved *U. lactuca*. Furthermore, the speed with which protein was lost illustrates the low N storage capacity of a high SA:V ratio species (Rosenberg and Ramus, 1982). Unfortunately, there is a factor leading to interference in the results obtained at the 8 day and 10 day sampling. There was a technical problem with the refrigeration system resulting in an increase in temperature from 13° C to 21° C for the last two sampling times. This may have caused the drop in protein concentration after 10 days, since it has been noted that uptake of NH_4^+ by *U. lactuca* is greater at 15 °C than at 20 °C (Harlin *et al.*, 1979).

The area where this algal material was collected indicates that these relatively high protein levels may be due to a high supply of N from eutrophic conditions in the harbour. It has been observed that growth response of *U. lactuca* in the culture condition may be different according to the area from which the material was collected (Steffensen, 1976). This relates to differences in the pre-adapted growth conditions, and in particular temperature.

An effect which may have influenced the results occurs in the culturing condition. The algal material was not maintained at a constant stocking density since the density decreased as the experiment progressed. Thus there may have been less light limitation in samples drawn at the end of the experiment. The decrease in stocking density may also have acted to increase the relative amount of nutrients available per surface area of material; however, since NH_4^+ and PO_4^{3-} were supplied at saturating levels, this effect should be negligible. If these factors did have any effect it would emphasize the difference in protein content between the beginning and end of the experiment.

Protein was measured on a dry weight basis; however, the different species were subjected to the same drying conditions and thus there may have been a difference with respect to the water content of the sample dry material at the time of analysis. The consequence is that those species with a comparatively low SA:V ratio may have had an underestimated protein content in comparison with those that lost more moisture during the drying period. A future improvement would include drying material to constant weight before analysis.

Different species of abalone have different amino acid requirements. Mai *et al.* (1994) reported that the two species of abalone; *Haliotis tuberculata* and *Haliotis discus* hannai, had similar amino acid profiles. The amino acid profile of the algivore gives an indication of its requirements for growth. Thus a diet for cultured abalone should aim to match the correct combination of amino acids. An assessment of the specific pattern of amino acids and total protein content of potentially viable algae should indicate their suitability as fodder. Providing a diet which contains the correct combination and quantity of amino acids may appear to be the optimum fodder; however, several factors interfere with this simple relation. Firstly , some substances decrease protein digestibility (phenolic compounds) and this effect is evident when comparisons are made between abalone fed on different diets. Some algal species may have a relatively low content of particular amino acids and this may limit growth of abalone supplied with a single source of protein. The range of required amino acids could be provided by a mixed diet of a variety of different seaweeds. Certain amino acids have an adverse affect in abalone when supplied in excessive amounts. Effects negate optimal abalone output and include a depression in appetite and lower growth rates (Harper *et al.*, 1970).

The interspecies comparison indicates that *P. capensis* is by the most promising candidate as a high protein abalone fodder. It would be beneficial to assess suitability of *Porphyra* in terms of its amino acid composition (its ability to offer essential amino acids in the correct proportion). Alternatively, *Porphyra* could be used in conjunction with other seaweeds so as to increase the array of amino acids provided to the abalone. *Porphyra* should be adaptable to aquaculture conditions since several species are cultivated on a large scale in Japan and China (Okazaki, 1971). Of course

the most important factor to consider is whether abalone accept *Porphyra* as a food source.

U. lactuca showed a rapid accumulation of protein in response to saturating ambient N and P. This illustrates the correlated nutrient uptake and conversion in an alga having a high SA:V ratio. The physiological response makes *U. lactuca* a suitable candidate for manipulation of protein under aquaculture conditions. *U. lactuca* has been grown successfully under tank culture conditions (DeBusk *et al.*, 1968) and a number of local abalone farms are currently experimenting with cultured *Ulva* as fodder. Moreover, these results suggest the potential for biofiltering. This involves integration of seaweed and abalone culture, whereby effluents from abalone culture provide the macroalgae with a supply of nutrients. In removing the nutrients, seaweeds filter the seawater and allow it to be recycled into abalone tanks. Furthermore, the seaweeds are used as fodder for the abalone. High growth rates of *U. lactuca* have been achieved in such a system as well as efficient seawater filtration (80 % removal of ammonia) Neori *et al.* (1997).

Aquaculture of seaweed by abalone farmers increases the availability of a fresh supply of feed. Furthermore, this study has shown that it is possible to maximize the protein content seaweed under controlled conditions. These benefits support the prospect of seaweed aquaculture by abalone farmers.

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APPENDIX A

Table A1: Components of ES-enrichment
(made up to 1 L volume with distilled water)

Tris buffer	3.333 g
$(NH_4)_2SO_4$	3.303 g
Na_2 glycerophosphate	1.071 g
Fe-solution	166.5 mL
PII metals	166.5 mL
Vitamin B_{12} (10 000X)	0.1 mL
Thiamine (10 000X)	0.1 mL
Biotin (1 000X)	1.0 mL

Table A2: Components of PII metal solution

Na_2EDTA	1.000 g
H_3BO_3	1.140 g
$FeCl_3 \cdot 6H_2O$	0.049 g
$MnSO_4 \cdot H_2O$	0.164 g
$ZnSO_4 \cdot 7H_2O$	0.022 g
$CoSO_4 \cdot 7H_2O$	0.0048 g
Distilled water	1 000 mL

Table A3: Components of Fe-solution

$Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$	3.51 g
Na_2EDTA	3.00 g
Distilled water	1 000 mL

APPENDIX B

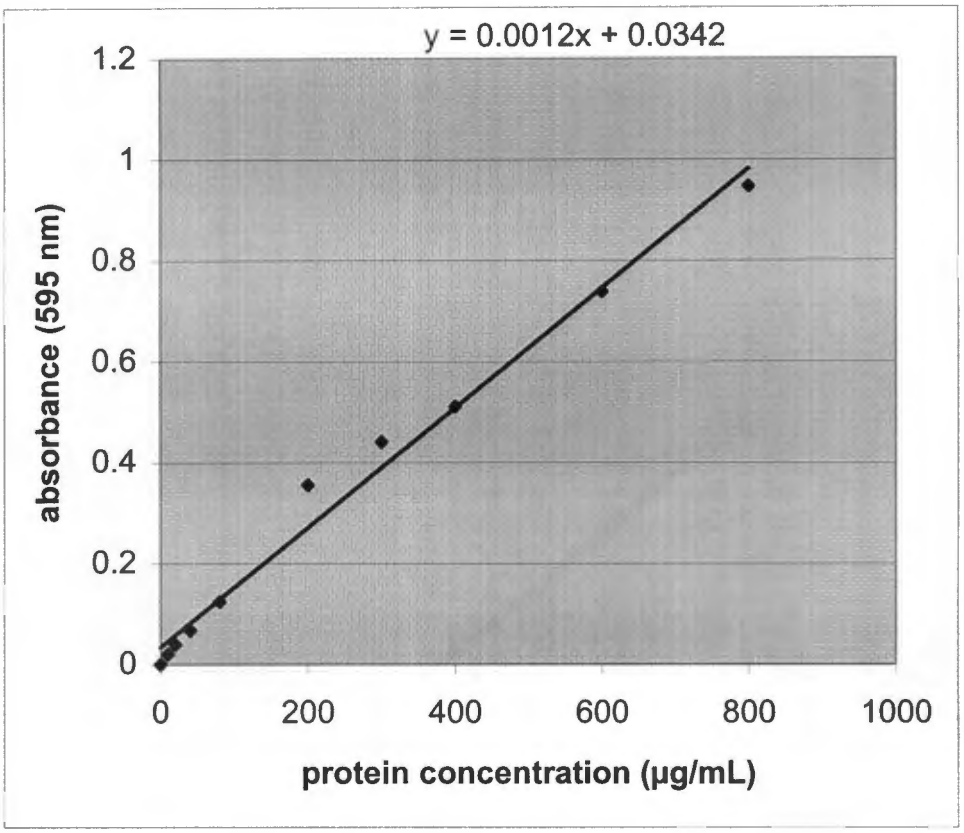

Fig. B1: Colour response curve of Bradford reagent to protein (Bovine Serum Albumin) standards

APPENDIX C

Table B1: Measurements of absorbencies and calculated protein concentrations of extracts for sampled macroalgae.

species	sample dry weight (g)	volume of extractant (mL)	absorbance (595nm)	protein conc. ($\mu g.mL^{-1}$)	protein (% dw)	absorbance (595nm)	protein conc. ($\mu g.mL^{-1}$)	protein (% dw)	average protein (% dw)
P.capensis	3.0033	540	0.688	599.7273	10.7832	0.716	625.1818	11.2409	11.0121
P.capensis	3.0018	540	0.733	640.6364	11.5245	0.752	657.9091	11.8353	11.6799
P.capensis	3.0036	540	0.769	673.3636	12.1060	0.714	623.3636	11.2071	11.6566
U.capensis	3.0146	540	0.231	184.2727	3.3008	0.225	178.8182	3.2031	3.2520
U.capensis	3.0004	540	0.211	166.0909	2.9892	0.21	165.1818	2.9729	2.9811
U.capensis	2.4242	540	0.154	114.2727	2.5455	0.167	126.0909	2.8087	2.6771
G.capensis	3.0268	540	0.209	164.2727	2.9307	0.198	154.2727	2.7523	2.8415
G.capensis	3.0239	540	0.196	152.4545	2.7225	0.19	147	2.6251	2.6738
G.capensis	3.0148	540	0.19	147	2.6330	0.178	136.0909	2.4376	2.5353
U.rigidaT=-1	3.0042	540	0.228	181.5455	3.2632	0.222	176.0909	3.1652	3.2142
U.rigidaT=-1	3.019	540	0.212	167	2.9871	0.21	165.1818	2.9546	2.9708
U.rigidaT=-1	3.0222	540	0.207	162.4545	2.9027	0.187	144.2727	2.5778	2.7403
U.rigidaT=0	1.0686	180	0.253	204.2727	3.4409	0.244	196.0909	3.3030	3.3720
U.rigidaT=0	1.0949	180	0.24	192.4545	3.1639	0.257	207.9091	3.4180	3.2910
U.rigidaT=0	1.0886	180							
U.rigidaT=1	1.0919	180	0.236	188.8182	3.1127	0.219	173.3636	2.8579	2.9853
U.rigidaT=1	1.0952	180	0.228	181.5455	2.9838	0.214	168.8182	2.7746	2.8792
U.rigidaT=1	0.9902	180	0.204	159.7273	2.9035	0.204	159.7273	2.9035	2.9035
U.rigidaT=4	2.8164	540	0.212	167	3.2020	0.219	173.3636	3.3240	3.2630
U.rigidaT=4	2.9887	540	0.239	191.5455	3.4609	0.234	187	3.3787	3.4198
U.rigidaT=4	2.3793	540	0.197	153.3636	3.4807	0.203	158.8182	3.6045	3.5426
U.rigidaT=6	2.95	540	0.249	200.6364	3.6727	0.29	237.9091	4.3549	4.0138
U.rigidaT=6	2.6544	540	0.22	174.2727	3.5453	0.252	203.3636	4.1371	3.8412
U.rigidaT=6	2.4986	540	0.23	183.3636	3.9629	0.213	167.9091	3.6289	3.7959
U.lactucaT=-1	3.0881	540	0.31	229.8333	4.0190	0.342	256.5	4.4853	4.2521
U.lactucaT=-1	3.0262	540	0.257	185.6667	3.3131	0.266	193.1667	3.4469	3.3800
U.lactucaT=-1	3.0082	540	0.262	189.8333	3.4077	0.27	196.5	3.5274	3.4675
U.lactucaT=0	3.0174	540	0.057	19	0.3400	0.053	15.66667	0.2804	0.3102
U.lactucaT=0	3.0281	540	0.056	18.16667	0.3240	0.063	24	0.4280	0.3760
U.lactucaT=0	1.7156	540	0.047	10.66667	0.3357	0.047	10.66667	0.3357	0.3357
U.lactucaT=2	3.0282	540	0.169	112.3333	2.0032	0.187	127.3333	2.2707	2.1369
U.lactucaT=2	3.0134	540	0.13	79.83333	1.4306	0.137	85.66667	1.5351	1.4829
U.lactucaT=2	2.7835	540	0.126	76.5	1.4841	0.123	74	1.4356	1.4599
U.lactucaT=4	3.0648	540	0.304	224.8333	3.9614	0.292	214.8333	3.7852	3.8733
U.lactucaT=4	3.0091	540	0.255	184	3.3020	0.256	184.8333	3.3169	3.3095
U.lactucaT=4	2.5667	540	0.2	138.1667	2.9068	0.208	144.8333	3.0471	2.9770
U.lactucaT=6	3.0733	540	0.316	234.8333	4.1262	0.317	235.6667	4.1408	4.1335
U.lactucaT=6	3.0099	540	0.29	213.1667	3.8244	0.281	205.6667	3.6898	3.7571
U.lactucaT=6	2.7089	540	0.255	184	3.6679	0.253	182.3333	3.6347	3.6513
U.lactucaT=8	3.0375	540	0.332	248.1667	4.4119	0.361	272.3333	4.8415	4.6267
U.lactucaT=8	3.0626	540	0.338	253.1667	4.4639	0.322	239.8333	4.2288	4.3463
U.lactucaT=8	2.9882	540	0.328	244.8333	4.4244	0.336	251.5	4.5449	4.4846
U.lactucaT=10	3.0075	540	0.258	186.5	3.3486	0.269	195.6667	3.5132	3.4309
U.lactucaT=10	3.0022	540	0.289	212.3333	3.8192	0.294	216.5	3.8941	3.8567