Robertson-Andersson, D.V., et al. (2009). A Rapid assessment of tissue nitrogen in cultivated *Gracilaria gracilis* (Rhodophyta) and *Ulva lactuca* (Chlorophyta). AFRICAN JOURNAL OF AQUATIC SCIENCE, 34(2): 169-172

Rapid assessment of tissue nitrogen in cultivated *Gracilaria gracilis* (Rhodophyta) and *Ulva lactuca* (Chlorophyta)

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Abstract

Tissue nitrogen content and thallus colour were quantified using a rapid assessment method based on the Pantone® matt uncoated formula guide for raft-cultivated *Gracilaria gracilis* Steentoft Irvine *et* Farnham at Saldanha Bay and tank-cultivated *Ulva lactuca* Linnaeus at Jacobsbaai in 2001 – 2002. A relationship between thallus colour and tissue nitrogen, as well as a transition between green-yellows and yellow-browns that occurs between 0.8 – 1.3 mg N per g tissue (Pantone® colours 460U – 455U) for *Gracilaria* were found, with the green-yellow colour indicating nitrogen-starved material and the yellow-browns indicating nitrogen-replete material. For *Ulva* a transition between green and yellow-green occurred at a tissue nitrogen content of between 1.5 – 1.7 mg N per g tissue (Pantone® colours 585U and 583U). This relationship can be used by seaweed farmers for cultivation management as a quick guide to determine nutritional status of the seaweeds, and as an indication of protein content when the seaweeds are used as feeds.

Keywords: cultivated seaweeds, Pantone®, tissue nitrogen, thallus colour

Introduction

Variations in seaweed thallus colour are largely related to varying amounts of pigments (chlorophylls, xanthophylls, phycobilins) and their breakdown products. Studies with *Gracilaria*, for example, have showed that levels of pigment proteins are closely correlated with tissue nitrogen content (Lapointe and Ryther 1979, Hegazi *et al*. 1998). This is because the pigment phycoerythrin (a phycobiliprotein) is largely responsible for determining the red colour of the thalli, and the concentration of this

pigment changes according to the nitrogen content, causing a lightening or darkening of the thallus. In green seaweeds the main pigment proteins are chlorophylls and, in this case, changes in the concentrations of chlorophylls would cause lightening or darkening of the thallus, altering the seaweed's colour.

Thallus colour changes due to changes in tissue nitrogen content in cultured seaweeds have already been documented for *Gracilaria* spp. (Lapointe and Ryther 1979, Wilson 1999), *Caulerpa prolifera* (Forsskal) Lamouroux, *Jania rubens* (Linnaeus) Lamouroux, *Padina pavonica* (Linnaeus) Thivy (Hegazi *et al.* 1998), *Ulva rigida* C. Agardh (Lahaye *et al.* 1995), *Chondrus crispus* (Linnaeus) J. Stackhouse (Haxo and Strout 1950, Neish and Shacklock 1971, Harvey and McLachlan 1973, MacKenzie 2003), *Euchuma* spp. (Neish 2003), *Ascophyllum nodosum* (Linnaeus) Le Jolis, and several species of *Fucus* (MacKenzie 2002). Even chlorosis (yellowing due to pigment destruction) of *Ulva* thalli has been well documented (Turpin 1991, Floreto and Teshima 1998).

However, all these authors use terms such as lighter, darker, bleaching, yellowing and reddening without providing a meaningful description of colour quantification. While some phycologists (e.g. Chamberlain and Keats 1994) have quantified the colour of seaweeds, albeit for descriptive purposes, using the *Methuen Handbook of colour* (Kornerup and Wanscher 1984) for example, to our knowledge no one has attempted to quantify the relationship between tissue nitrogen content and thallus colour in seaweeds. The aim of this research was to quantify the relationship between tissue nitrogen content and thallus colour for two increasingly important commercial aquaculture species in South Africa, *Gracilaria gracilis* Steentoft, Irvine *et* Farnham and *Ulva lactuca* Linnaeus, in order to provide a means for seaweed cultivators to identify the quality of the seaweeds as a feed source for abalone, *Haliotis midae* Linnaeus, and sea urchins, *Tripneustes gratilla* Linnaeus, as well as a fast and economical means of determining seaweed nutritional status for cultivation purposes.

Material and methods

Seaweeds and sources of nitrogen

Gracilaria gracilis was cultivated using suspended cultivation in the sea on rope rafts in Saldanha Bay (17° 57' 16" E, 33° 00' 04" S, Western Cape) on the west coast of South Africa. Stocking weight was ± 30 g of seaweed to prevent self-shading and minimize competition for nutrients and the seaweeds were suspended at roughly 1 m below the surface. Netlon® lines were then tied across the frames at 0.75 m intervals parallel to the line of the prevailing wind direction. For more details on the methods and results of this type of cultivation see Dawes (1995), Anderson *et al.* (1992, 1996), and Wakibia *et al.* (2001). The nutrient regime ranged between oligotrophic in summer to nutrient rich in winter and was similar to that reported in Anderson et al (2004).

Ulva lactuca was cultivated on a commercial abalone farm in Jacobsbaai (17° 53' 12.5" E, 32° 58' 2.5" S, Western Cape) just north of Saldanha Bay in 96 L white PVC tanks (0.60 m × 0.40 m × 0.40 m). Unfiltered seawater, abalone (*Haliotis midae*) effluent and turbot (*Scopthalmus maximus* Linnaeus) effluent generated on the farm were used as the three culture media for *U. lactuca*. These were supplied at 20 volume exchanges per day (see Robertson-Andersson 2004 for a complete description of the cultivation method). The average seawater temperature on the farm was 14.6 °C (min 6 °C, max 20 °C). The experiments ran from May 2001 to August 2002.

Colour determination

A total of 2.5 kg of each of the two seaweeds were grown for two weeks in the various culture media to allow their pigment contents to acclimatize to the culture environment. For colour determination, seaweeds were placed onto white herbarium sheets, blotted dry and their colours determined under fluorescent lighting away from the influence of natural light. The colour chosen was taken as that representative of roughly 80 % of the thallus. Five thalli of each species per culture medium were selected as representative of roughly 80 % of the sample.

The Pantone® colour print formula guide for uncoated matt colours was chosen as a tool to match seaweed thallus colour. Each Pantone® colour has a print guide allowing for reproduction of the exact colours on a desktop PC or at a printer. The corresponding colour code was read from the colour guide that best matched the seaweed thallus colour.

Tissue nitrogen determination

Representative samples were collected and transported in a cool-box to the laboratory where epiphytes and epifauna were removed. The samples were then washed in deionised water to remove smaller organisms such as isopods and diatoms, oven dried at 50 °C for 70 hours and ground to 1 mm particle size using a mechanical grinder. Tissue nitrogen content from the samples of *Gracilaria* was determined by isotopic analysis at the UCT/ FRD Goldfields Light Stable Isotope Facility (Cape Town, South Africa). Samples were weighed to 1.8 - 2.2 mg, sealed in tin capsules and analysed on a Finnigan MAT 252 isotope ratio mass spectrometer with a Carlo-Erba NA 1500NC elemental analyser employed as a combustion unit. The δ^{13} C and δ^{15} N ratios, as well as total C and N percentage values were obtained simultaneously. Standards used included Merck gel and the laboratory reference gas was high purity nitrogen (99.995 %) calibrated against atmospheric N. *Ulva* total tissue nitrogen content was determined using the micro-Kjeldahl technique (Solórzano 1969).

Results

For both seaweeds a strong relationship between thallus colour and nitrogen content was visible, with the intensity of the colour indicating the more nitrogen-rich material (Figures 1, 2). In *G. gracilis* the tissue nitrogen vs. thallus colour showed a transition between green-yellows and yellow-browns that occurred between 0.8 – 1.3 mg N per g tissue of the total nitrogen (Pantone® colours 460U – 455U) (Figure 1), with the green-yellow colour indicating nitrogen-starved material and the deep browns indicating nitrogen-replete material. Similarly, in *U. lactuca* the transition between green-yellows and green appeared to occur between 1.5 – 1.7 mg N per g tissue (Pantone® colours 585U and 583U) (Figure 2).

Discussion

To our knowledge the relationship between seaweed tissue nitrogen content and thallus colour has not previously been successfully quantified. This study clearly demonstrates that thallus colour can be attributed to the total tissue nitrogen content of the thallus. In addition, the intensity of the colour denoting the algal group (green or red seaweeds) notably provides clues as to the relative amounts of nitrogen in the alga's thallus. These results are supported by Robertson-Andersson (2004) who showed that when *U. lactuca* that was a green-yellow colour (nitrogen-limited) was placed into a nitrogen-rich culture medium, the thallus colour changed from green-yellow to green, whilst the thallus nitrogen content also increased. Alternatively, when dark-green (nitrogen-replete) *U. lactuca* material was placed in a nitrogen-poor culture medium the thallus colour became yellow-green and the thallus nitrogen concentration decreased. This colour response to varying nitrogen concentrations has also been demonstrated for *G. gracilis* (see Wilson 1999, Njobeni 2006).

Since the accumulation of pigments occurs directly in response to the availability of N in excess of that required for growth (Lapointe and Ryther 1979, Hegazi *et al.* 1998), the intensity of the colour that denotes that algal group could be used as a guide to the relative levels of N in the thallus tissue, and possibly of other secondary metabolites as well. Neish and Shacklock (1971) found that growing *Chondrus crispus* in conditions of high light and low nitrogen culture media resulted in a higher yield of kappa-carageenan and higher gel strength extracts (called the Neish effect). This relationship has been confirmed for a number of cultivated agarophytes and carragenophytes, and it has been hypothesized that this ripening phenomenon may be related to nutrient storage, in particular of nitrogen (DeBoer 1979, Lapointe and Ryther 1979, Bird *et al.* 1981, Lapointe 1981, Guist *et al.* 1982, Patwary and van der Meer 1983, Rotem *et al.* 1986). It is well known that ripening conditions result in a change in the colour of *Gracilaria* (Lapointe and Ryther 1979).

In 2007 seaweeds were the largest cultured marine crop in South Africa, with a total of 1100 tons wet weight being produced (Robertson-Andersson 2007, Bolton *et al.* 2008). All of this was notably used as feed for abalone, *Haliotis midae*. The colour-scale produced in this study may be useful to mariculture farmers to assess the nutrient value of their seaweeds as a feed for cultivated abalone and sea urchins and this has important benefits for the aquaculture industry as protein-rich sources of feed are constantly being sought. Seaweed farmers wishing to use this tool would place their cultivated seaweeds on a colour chart and be able to read off the nitrogen content and this would provide them with a quick and cost-effective means of assessing their feed quality. Nevertheless, more laboratory work needs to be done to determine the exact switch-over point between nitrogen-replete and nitrogen-starved material.

Acknowledgements

Funding for this research was provided by SIDA, DEAT, NRF and the UCT. Thanks to A. McLachlan for allowing us the use of his raft and help in collecting material, to D. Kemp and C. Boothroyd who provided assistance in the field, and A.J. Smit and Prof. W.M. Stock for useful communications.

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Figure captions

Figure 1: Relationship between tissue nitrogen (% δ^{15} N) and thallus colour for *Gracilaria gracilis* (shown by Pantone ® matt colour labels). N = 165.
Figure 2: Relationship between tissue nitrogen (mg.g^{-1}) and thallus colour for *Ulva lactuca* (shown by Pantone ® matt colour labels). N = 600.

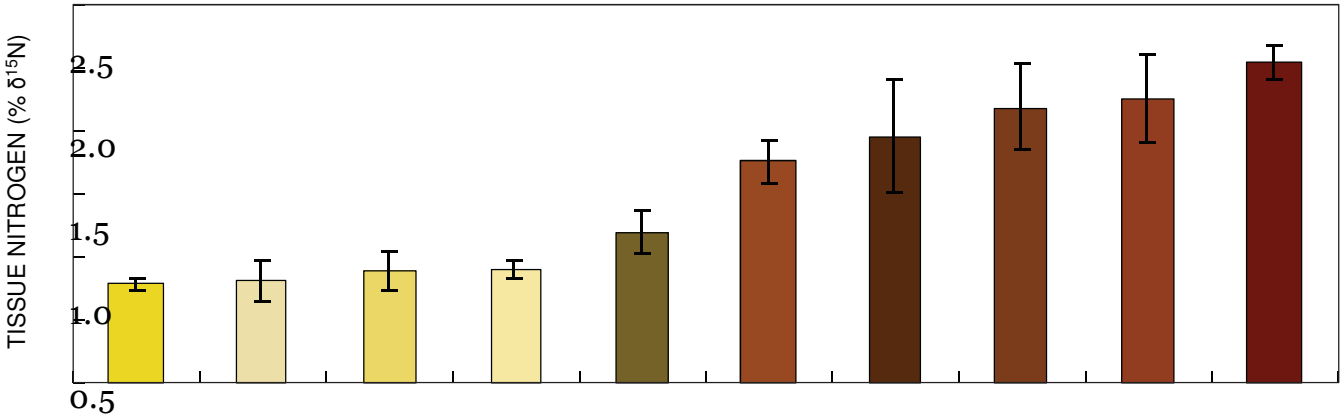

Figure 1: Relationship between tissue nitrogen (% δ15N) and thallus colour for *Gracilaria gracilis*, shown by Pantone® matt colour labels (*n* = 165). Print or

online representation of the Pantone® colours in the figure is as a precise as technical constraints permit

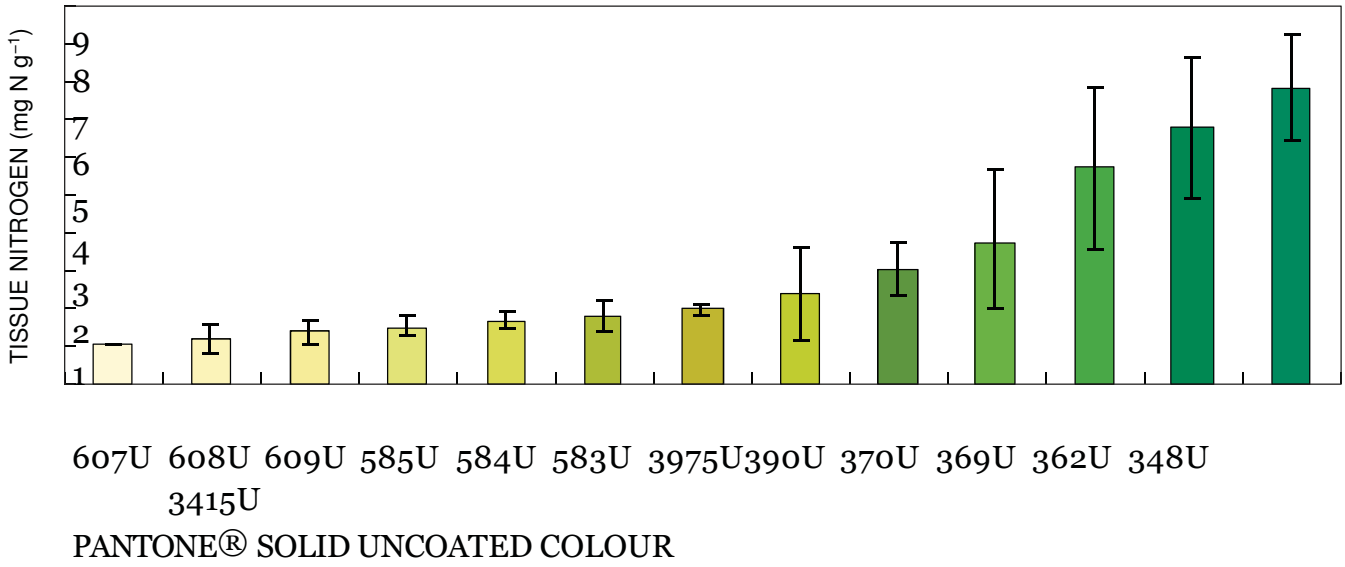

Figure 2: Relationship between tissue nitrogen (mg g^{-1}) and thallus colour for *Ulva lactuca*, shown by Pantone® matt colour labels (n = 600). Print or online representation of the Pantone® colours in the figure is as a precise as technical constraints permit