PIGMENT ANALYSIS ON *EUCHEUMA DENTICULATUM* (COLLINS & HERVEY) AND *KAPPAPHYCUS ALVAREZII* (DOTY) CULTIVARS CULTURED AT DIFFERENT DEPTHS

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Abstract

There are three kinds of pigments directly involved in algal photosynthesis - the chlorophylls (a and b), phycobiliproteins and carotenoids. Red algae do not contain chlorophyll b and use phycobiliproteins for the capture of light energy. This study determines whether concentrations of chlorophyll a and phycoerythrin in Eucheuma denticulatum (Collins & Hervey) Kappaphycus alvarezii (Doty) cultivars cultured in Bais Bay and Olingan, Dipolog City vary with depths. Concentrations of chlorophyll a in Eucheuma denticulatum and Kappaphycus alvarezii species particularly the small varieties may not vary with depth, but, with species. Phycoerythrin concentration varies with species and depth.

Keywords: *Kappaphycus alvarezii, Eucheuma denticulatum, chlorophyll a, phycoerythrin*

Introduction

Autotrophic organisms such as the algae rely on a variety of photosynthetic pigments to capture light energy from the sun. These colored compounds are particularly diverse in the algae and their prominence in the biology of algae forms the basis in the classification of many algal divisions. There are three kinds of pigments directly involved in algal photosynthesis - the chlorophylls, phycobiliproteins and carotenoids (Goodwin, 1974; Rowan, 1989; Dring, 1990).

Chlorophylls are responsible for the capture of light energy used to drive photosynthetic electron transport. Chlorophyll *a* is very important in the reaction center. It is associated with reaction center *chlorophyll* binding proteins as well as light-harvesting antenna complexes, thus, it is found in all algae. while chlorophyll *b* is only associated with light-harvesting antenna complexes. It is found in Ulvophyceae and in other Chlorophyta and higher plants. Chlorophylls c_1 and c_2 occur in Phaeophyceae. All chlorophylls have a tetrapyrrole ring with Mg2+ chelated in the middle. In chlorophylls

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a and *b* this ring is attached to a long fatty-acid tail (phytol tail) which is very hydrophobic and is probably responsible for the tight association of the chlorophylls with the thylakoid membranes and their solubility in organic solvents while relatively insoluble in water. The typical ratio of *chlorophyll a* to *chorophyll b* in higher plants and algae is about 3 to 1. Chlorophylls *c* absorb blue light more strongly and red light less strongly than do chlorophylls *a* and *b*. Rowan (1989) reported the occurrence of chlorophyll *d* in some red algae, but is believed to have no function in photosynthesis.

The second major class of pigments are the carotenoids. They are associated with the photosynthetic membranes of all photosynthetic organisms. They mainly function in light harvesting especially in very dim spectrally unusual light available (Fork & Larkum, 1989).Like the chlorophylls, carotenoids are soluble in organic solvents and relatively insoluble in water. Carotenoids are classified into carotenes (pure hydrocarbons) and xanthophylls (contain oxygen in the terminal rings), all of which are 40-carbon compounds. They are usually red, orange or yellow but some are green, others pink, and some quite black. Carotenoids occur in all major phyla of plants. In higher plants they are found in chloroplasts and other types of plastids, called chromoplasts, in fruits and flower parts. The major carotenoids in higher plants are the ß□-carotene (a carotene) and lutein (a xanthophyll) while in all green algae and some higher plants xanthophylls, neoxanthin and violaxanthin occur.

Phycobiliproteins are characteristic pigments of red and blue-green algae, as well as Cryptophyceae. Unlike green algae and higher plants, the red algae do not contain *chlorophyll b* and use phycobiliproteins for the capture of light energy. They form particles called phycobilisomes on the surfaces of thylakoid rather than being embedded in the membranes. Phycobilisomes are composed of phycobilin chromophores (phycoerythrin, phycocyanin, and allophycocyanin) associated with proteins. The phycobilin chromophore is a linear tetrapyrrole, somewhat similar in structure to the tetrapyrrole ring portion of chlorophyll molecules, but linearized. Phycobilins do not possess a hydrophobic portion like the phytol tail of the chlorophyll molecules and therefore are water-soluble. Pycoerythrins (PE) absorb in the green region (495-570 nm) while phycocyanins (PC) absorb in the green-yellow region (550-630 nm) and allophycocyanins (APC) in the orange-red region (650-670 nm). Figure 1 presents the absorption spectra of the algal pigments.

Kappaphycus alvarezii (Doty) is found to contain carotenoids (carotene & violaxanthin), pheophytin and chlorophyll *a* (Maino, 2000) while *Kappaphycus striatum* (Schmitz) has carotenoids (carotene: α -carotene & β-carotene; xanthophylls: zeaxanthin ester, free zeaxanthin, lutein 5, cryptoxanthin, and fucoxanthin), chlorophyll *a*, pheophytin and phycobilin (Triswara, 2001).

This study determines the concentration of *chlorophyll a* and phycobilins in *Eucheuma denticulatum* (Collins & Hervey) *Kappaphycus alvarezii* (Doty) cultivars cultured in Bais Bay and Olingan, Dipolog City at different depths. It finds out whether concentration of pigments vary with depths.

Research Method and Design

Study Sites. Two farm sites were established for the experiment, one in Bais Bay (N 9° 35.966'; E 123° 9.032') on April 22, 2005 to June 3, 2005 and one in Olingan, Dipolog City (N 8° 32.838'; E 123° 19.226') in April 29, 2005 to May 28, 2005.

Experimental Set-up. The brown and green cultivars of *Eucheuma denticulatum* (Collins & Hervey) and giant brown variety of *Kappaphycus alvarezii* (Doty) were used in the study. Thirty 50-g seedlings of each variety were tied randomly on a strings at 30 cm apart (Figure1).

For the Dipolog set-up, the brown giant variety *Kappaphycus alvarezii* was used since it was the only species available in the area. The seedlings were tide randomly on the string in three different lengths - 5m, 2m and surface (6 cm) at one meter apart (Figure 2). Both cultures were terminated after 30 days.

Data Collection. Initial weights of seedlings were measured and recorded. Environmental parameters such as light intensity, temperature, salinity, and transparency were measured every two weeks.

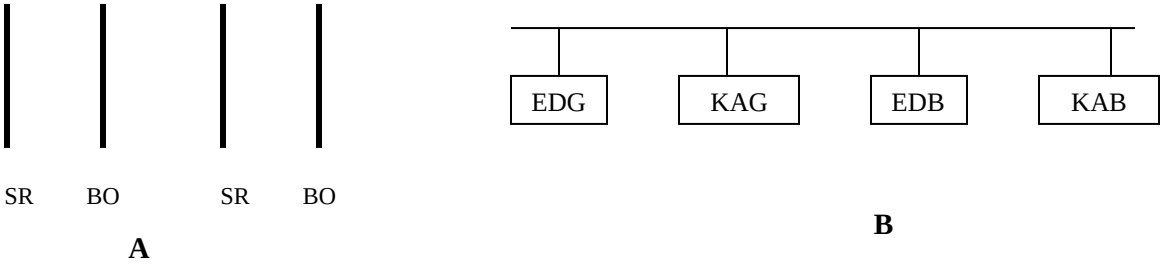


Figure 1. Experimental Design in Bais Bay. A. Depth: SR – surface; BO – bottom (2 m). B. Cultivar: EDG - *Eucheuma denticulatum* , green; EDB - *E. denticulatum,* brown***;*** KAG - *Kappaphycus alvarezii,* green; KAB – *K. alvarezii* , brown.

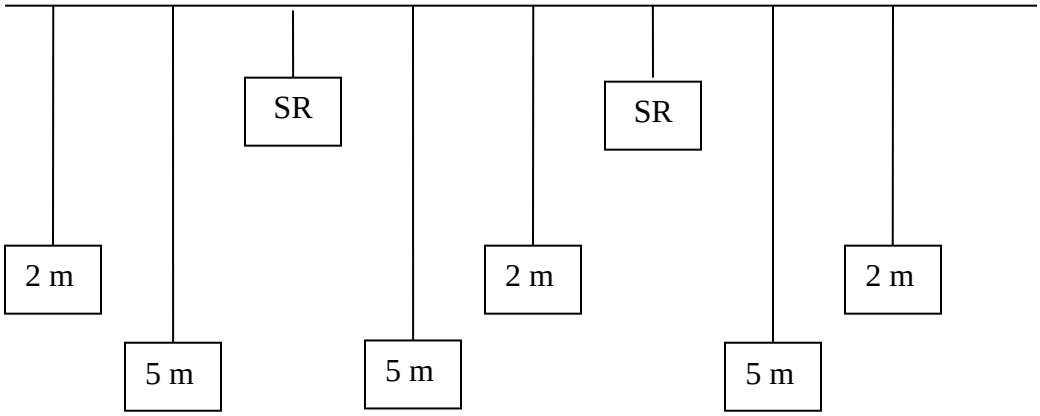


Figure 3. Experimental Design in Olingan, Dipolog City. SR – surface.

Pigment Extraction and Analysis

Chlorophyll Extraction and Analysis. About 2 g of each cultivar was finely chopped, placed in flask and added with 10 ml 100% acetone. The flask was then wrapped with aluminum foil and refrigerated overnight. The chopped and soaked thallus with acetone was ground in chilled mortar with acid-washed sand.

The pigmented solution was transferred to microcentrifuge tube and spinned at 1400 g for 2 minutes. The supernatant was decanted into a spectrophotometer cuvette and placed inside the spectrophotometer. Wavelength scanning was done to check the presence of chlorophyll *a*. Once chlo *a* was detected, the spectrophotometer was set to multiple wavelength mode with wavelengths 664 nm , 647nm and 645 nm after which the machine was ran and recorded the absorbance value for each wavelength.

Phycobilins Extraction and Analysis. Phycobilin was extracted following the protocol of Evans (1988). Same amount of thalli was used, chopped into tiny pieces and soaked with 10 ml 0.1M phosphate buffer, pH 6.8 overnight. The next day the chopped n soaked thalli together with the solvent were transferred into a chilled mortar and ground with acid-washed sand. The resulting solution was placed into eppendorf tubes and centrifuged for 2 minutes at 1000 x g.

The supernatant was poured into the glass cuvette and scanned using the spectrophotometer for the presence of phycobilins. Whenever phycobilins were present the spectrophotometer was set to multiple wavelengths mode using the following wavelengths: 645, 618, 592, 564 and 455 nm and ran. Absorbance value was then read and recorded.

The concentrations of chlorophyll *a* and phycoerythrin were then calculated based on the following formula from Jeffrey and Humphrey (1975):

$$\textit{Chlorophyll a (mg/L)} = 11.93\,(A664) - 1.93\,(A647)$$

$$\textit{Phycoerythrin (mg/L)} = [(A564 - A592) - (A455 - A592)\,0.20] * 0.12$$

To determine the concentration of the pigments per gram of algae, the following was used:

$$\frac{\textit{Concentration in mg/L} \;\; X \;\; \textit{Volume of solvent in mL}}{\textit{Weight of Algal thallus in grams}} \;\; \text{X} \;\; \frac{1000\ \mu g}{mg}$$

Statistical Analysis. Descriptive statistics, one-way and two-way analysis of variance were used to summarize and analyze the data. Graphs were constructed using Microsoft Excel.

Results and Discussion

Identification of the pigments extracted relied mainly on the peaks produced in the spectrophotometer (model U-2001) during wavelength scanning. Results revealed that chlorophyll *a* was obtained in the acetone extract with a peak at around 661-664 nm (Fig. 4-6) while the phosphate buffer extracts contained phycobilin pigment specifically phycoerythrin with peaks at approximately 499, 533 and 562 nm (Fig. 3). This is based on the fact that phycoerythrin (PE) absorbs in the green region about 495-570 nm (Lobban and Harrison,1994).

As indicated in Fig. 8, *Kappaphycus alvarezii* brown variety planted using the floating method (surface) contained the greatest concentration of chlorophyll a (mean = 17.152 $\pm$ 11.461 μg/mg) followed by *Eucheuma denticulatum* green variety (mean=6.684 + 2.24 μg/mg). Two-way analysis of variance revealed a significant difference between species [(F (3,16) = 3.685, p = 0.051)]. Post hoc comparisons using Tukey HSD test indicated that the mean concentration of *K. alvarezii* brown variety was significantly higher than that of *E. denticulatum* green variety. In terms of depth [F (1,16) = 3.005, p = 0.102] as well as the interaction between species and depth [F(3,16) = 2.372, p = 308] did not reach statistical significance which means chlorophyll concentration does not vary with depth with respect to the small varieties of *Eucheuma denticulatum* and *Kappaphycus alvarezii*.

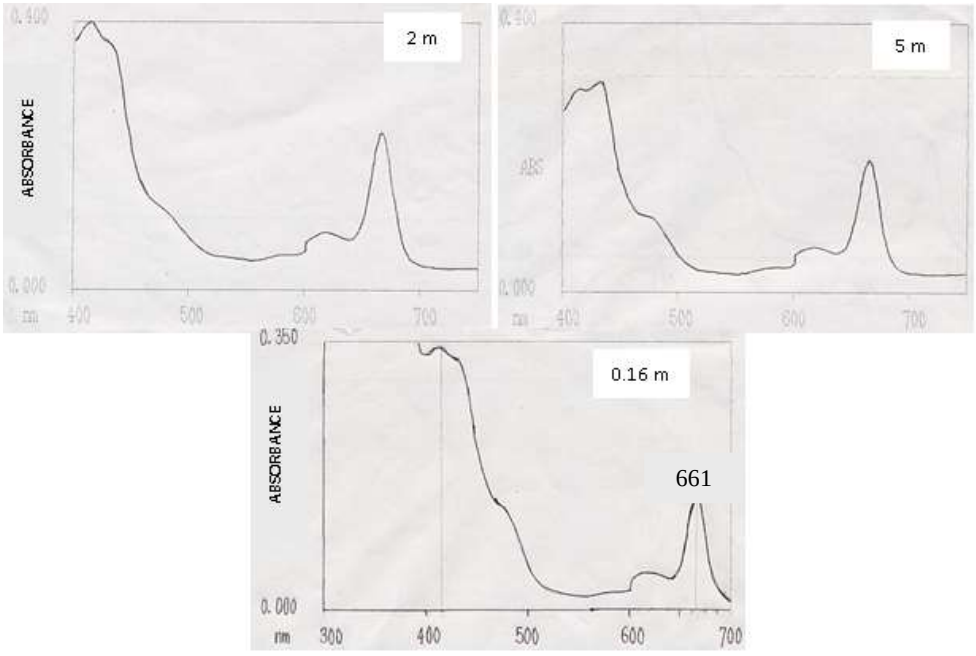


Figure 5. Absorbance curves of chlorophyll *a* from *Kappaphycus alvarezii* giant brown variety.

With regard to phycoerythrin concentration, two-way ANOVA revealed that it is significantly higher [F(3,13) = 190.860, p=0.00] in *Eucheuma denticulatum* brown

variety (4.038E-02 ± 4.32749E-03 µg/mg) in both the floating (surface) and bottom culture [F(1,13) = 7.926, p = 0.015] (Fig. 9). This indicates that phycoerythrin concentration varies with species and depth [F (3,13) = 4.266, p = 0.026].

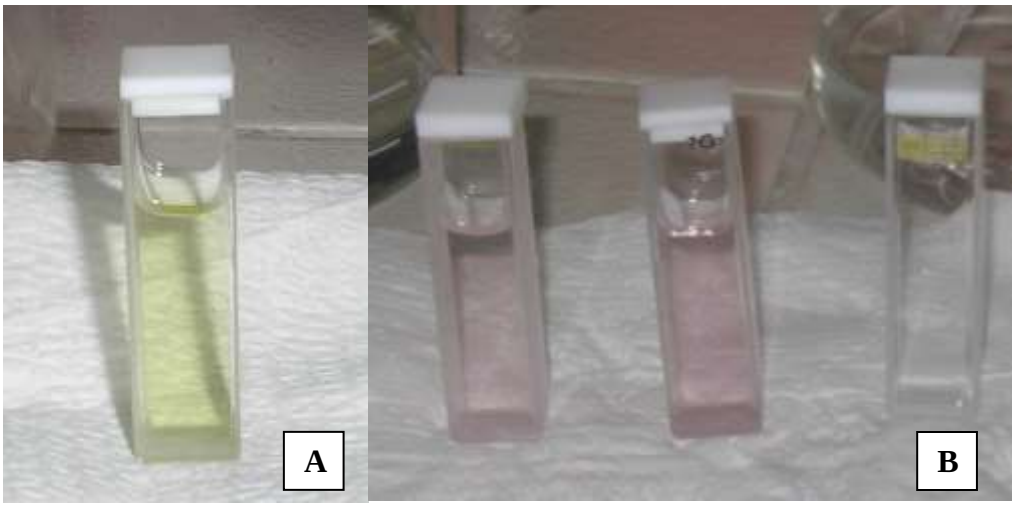


Figure 6. Pigment extracts from *Eucheuma denticulatum* and *Kappaphycus alvarezii*. A. Chlorophyll extracts in acetone. B. Phycobilin extracts in Phosphate buffer.

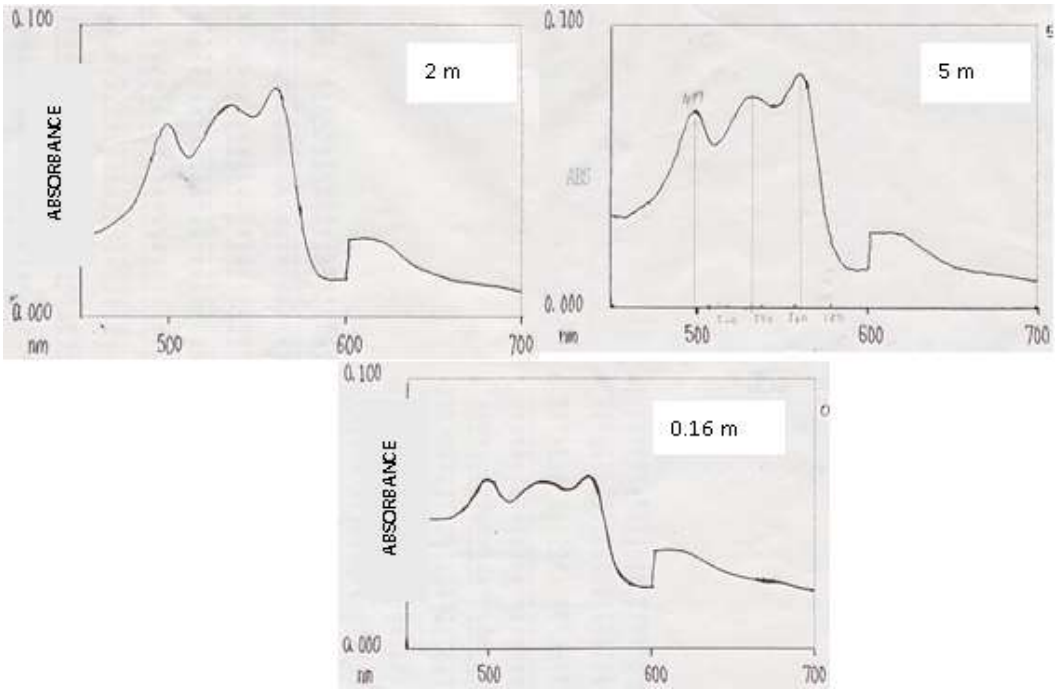


Figure 7. Absorbance curves of phycoerythrin from *Kappaphycus alvarezii* giant brown

On the other hand, a culture of *Kappaphycus alvarezii* giant variety at three different depths (0.16 m, 2 m and 5 m) in Dipolog City showed that alga planted at 2 m has the highest chlorophyll concentration, 17.18 ± 7.11 µg/mg (Table 2 and Fig. 12). However, One-way ANOVA revealed no significant difference [$F(2,7) = 0.035$, $p=0.966$]. Whereas the concentrations of phycoerythrin were significantly higher in 2 m- and 5 m-cultures [$F(2,6) = 103.044$, $p = 0.00$] (Table 2 and Fig. 10).

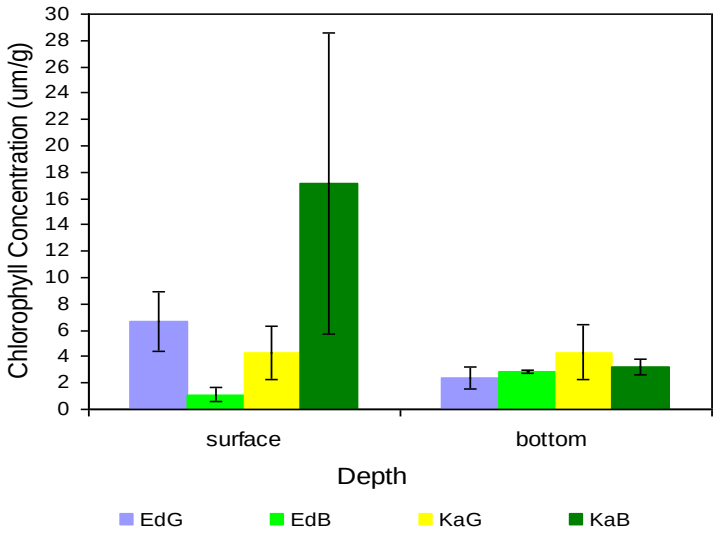


Figure 8. Concents of Chlorophyll *a* in µg/mg of *Eucheuma* spp farmed in Bais Bay at two different depths . EdG- *Eucheuma denticulatum* (Burman) green variety; EdB – *E. denticulatum* brown variety; KaG- *Kappaphycus alvarezii* (Doty) green variety; KaB - *Kappaphycus alvarezii* brown variety.

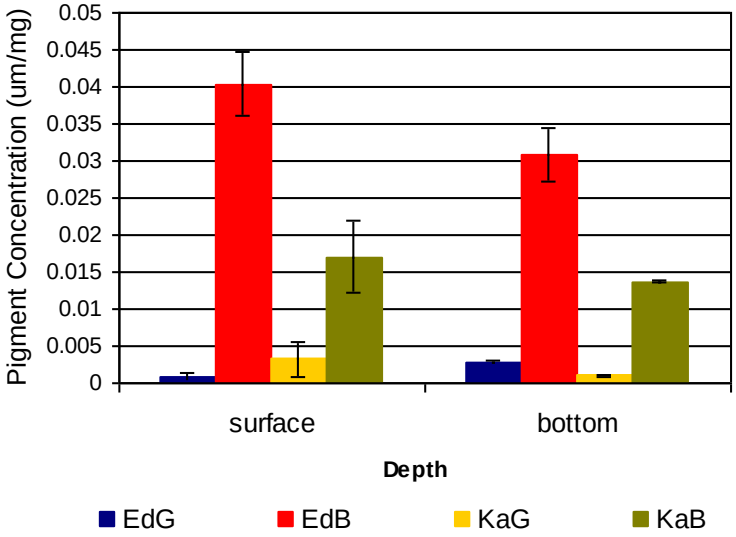
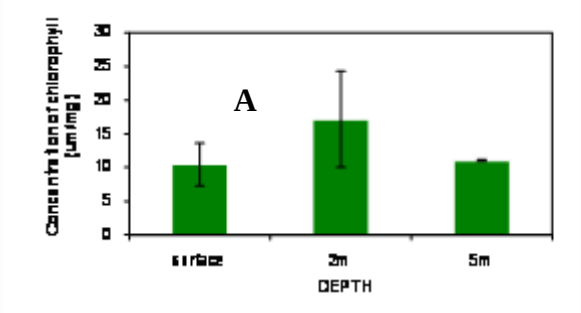


Figure 9. Concentrations of phycoerythrin in µg/mg of *Eucheuma* spp farmed in Bais Bay at two different depths . EdG- *Eucheuma denticulatum* (Burman) green variety; EdB – *E. denticulatum* brown variety; KaG- *Kappaphycus alvarezii* (Doty) green variety; KaB - *Kappaphycus alvarezii* brown variety.



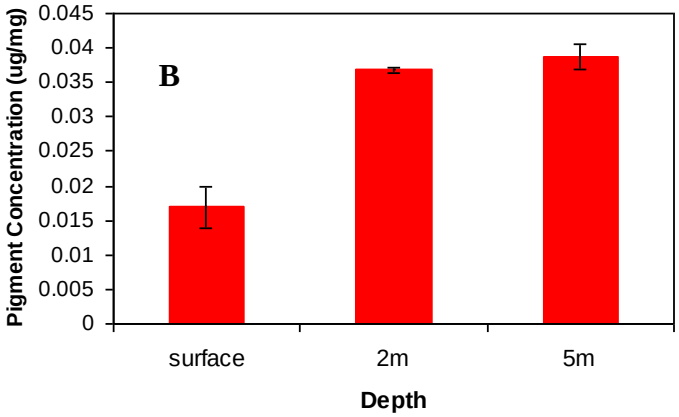


Figure 10. Pigment concentrations (µg/mg) of *Kappaphycus alvarezii* (Doty) giant variety farmed in Olingan, Dipolog City at three different depths . A. Chlorophyll *a*,. B. phycobilins. (Temperature = 30° C; Salinity = 35 ppt; Transparency = 7.8 m)

Concentrations of chlorophyll *a* in *Eucheuma denticulatum* and *Kappaphycus alvarezii* species particularly the small varieties may not vary with depth, but, with species. Phycoerythrin on the other hand, may increase with depth particularly in *K. alvarezii* giant variety. Phycoerythrin absorbs blue light which can penetrate at greater depths allowing the red alga to photosynthesize and live in such areas.

References

Dring, M.J. 1990. Light harvesting and pigment composition in marine phytoplankton and macroalgae. In: P.J. Herring, A.K. Campbell, M. Whitfield & L. Maddock (eds.), Light and Life in the Sea. Cambridge University Press. Pp. 89-103.

Fork, D.C. & A.W. D. Larkum. 1989. Light harvesting in the green alga Ostreobium sp., acoral symbiont adapted to extreme sahde. Mar. Biol. 103: 381-5.

Goodwin , T.W. 1974. Carotenoids and biliproteins. In: W.D. P. Stewart (ed.), Algal Physiology and Biochemistry. Oxford: Blackwell Scientific, pp. 176-205.

Goodwin , T.W. and E.I. Mercer. 1983. Introduction to Plant Biochemistry. 2nd Ed. Oxford: Pergamon.

Jeffrey and Humprey. 1975. *Biochem. Biophys*. Phlanz. 167: 191-194.

Lobban, C. and P. Harrison. 1994. *Seaweed Ecology and Physiology*. Cambridge University Press. pp. 129-133.

Maino, S. 2000. Analysis of photosynthetic pigment contents of *Kappaphycus alvarezii* (Doty) Doty from the coastal waters of Nain & Belang, Indonesia.Masters Thesis, Sam Ratulangi University.

Nakamura, A. and T. Watanabe. 2001. Separation and determination of minor photosynthetic pigments by reversed-phase HPLC with minimal alteration of chlorophylls. Analytical Sciences. Japan Soc for Analytical Chem. Pp. 503- 508.

Rowan, K.S. 1989. *Photosynthetic Pigments of Algae*. Cambridge University Press.

Triswara, R.N. 2001. Isolation of carotenoid pigments from *Kappaphycus striatum* (Schmitz) Doty. Masters Thesis, Sam Ratulangi University.

www.clarku.edu. 2005. Algal Pigments.