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Article in *Journal of Coastal Research* · July 2012

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Hyperspectral Reflectance Response of Seagrass (*Enhalus acoroides*) and Brown Algae (*Sargassum* sp.) to Nutrient Enrichment at Laboratory Scale

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ABSTRACT

Suwandana, E.; Kawamura, K.; Sakuno, Y.; Evri, M., and Lesmana, A.H., 2012. Hyperspectral reflectance response of seagrass (*Enhalus acoroides*) and brown algae (*Sargassum* sp.) to nutrient enrichment at laboratory scale. *Journal of Coastal Research*, 28(4), 956–963. West Palm Beach (Florida), ISSN 0749-0208.

Coastal environments are prone to nutrient contamination as a result of excessive use of fertilizers in paddy agriculture on the land. To detect nutrient increases in coastal areas, researchers have used hyperspectral reflectance response to examine some coastal plants, which have proved to be effective as bioindicators. In this study, field hyperspectral technique was evaluated as a tool to detect nutrient concentrations in two coastal plants, *i.e.*, seagrass (*Enhalus acoroides*) and brown algae (*Sargassum* sp.), taken from Banten Bay Indonesia, at laboratory scale. Although our initial experiments are still too few in elucidating an accurate relationship of nutrients and spectral signature, we are pleased to communicate that there is scientific evidence that hyperspectral measurement can be used to detect nutrient concentrations in coastal vegetation. Two types of fertilizers—urea, which contains 46% nitrogen, and triple super phosphate (TSP), which contains 14–20% soluble P₂O₅, commonly used by the local paddy farmers—were applied to both coastal plants in the aquarium experiment. The results of factorial analysis of variance (ANOVA) tests and pigment-related indices have proved that some significant differences exist in several wavelengths in response to the fertilizer treatments. This study revealed that brown algae were more sensitive to the same amount of fertilizer applied than seagrass.

ADDITIONAL INDEX WORDS: *Bioindicator, nutrient contamination, seaweed, nitrogen, phosphorus.*

INTRODUCTION

Coastal aquatic ecosystems are prone to nutrient contamination due to excessive use of fertilizers in paddy farming on land. Pollution and nutrient levels in coastal sea environments can be detected by analysing the tissue of some sea animals, *i.e.*, molluscs, fish and corals, and some submerged coastal plants, *i.e.*, seagrass and brown algae. Some studies have used coastal aquatic vegetation as a bioindicator for coastal contaminants such as nutrients (Fertig *et al.*, 2009; Sheppard *et al.*, 2008) and

heavy metals (Lytle and Lytle, 2001; Prange and Dennison, 2000). However, most studies involving heavy metal nutrients and contaminants in coastal aquatic plants are conducted using analytical chemistry methods. The application of hyperspectral analyses for aquatic plants seems to have received little attention.

Hyperspectral studies using airborne sensors or portable spectroradiometers have been increasing over the past decade, especially for land vegetation. A wide range of plant physiological parameters and structures including leaf area index, biomass, water, pigment and nutrient content, crop canopy, and density have been investigated extensively (Kawamura *et al.*, 2009; Müller *et al.*, 2008; Ray, Singh, and Panigrahy, 2010).

DOI: 10.2112/JCOASTRES-D-11-00222.1 received 10 December 2011;
accepted in revision 5 February 2012.

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Aquatic vegetation such as seagrass possesses similar organs and tissues to flowering plants on the land. However, plant tissues normally consist of roots, rhizomes, and stems that play important roles in absorbing nutrients from soil and sediment, while upper parts constitute shoots that bear several leaves in which photosynthesis takes place (Kuo and den Hartog, 2006). With similar biophysical structures, absorption ability of root system, transport systems, and photosynthesis mechanisms to land vegetation, seagrass may also have the potential to be examined under spectroradiometer analysis as an indicator plant for pollution.

A previous study investigating spectral reflectance of marine faunas was conducted by Fyfe (2003). The study concluded that three species of marine seagrasses—*Zostera capricorni*, *Posidonia australis*, and *Halophila ovalis*—collected from three estuaries in southern Australia were spectrally distinct over the wide regions of the visible wavelengths.

One important barrier in applying spectroradiometry for aquatic plants is the effect of the water column between the radiometer and the plant and the suspended particles that affect the spectral signatures of the measured plants; hence a water correction algorithm is needed for each water condition (Lu and Cho, 2011). Spectral measurement in the clearer water will produce better signatures than a water body that contains high amounts of suspended sediments (Govender, Chetty, and Bulcock, 2007; Lillesand and Kiefer, 2003). To avoid this problem, some studies focus on the observation of spectral measurement in the laboratory (dark room) by scanning fresh leaves (Yuan and Zhang, 2006), semidry leaves, or leaf powder (Serusi, 2010) of aquatic plants.

Given the possibility of detecting nutrient content in aquatic plants with hyperspectral radiometry, a laboratory experiment was conducted in this study using two coastal aquatic plants—seagrass (*Enhalus acoroides*) and brown algae (*Sargassum* sp.)—collected from Pamujan Besar Island in Banten Bay, Indonesia. This study aimed to analyse the spectral response of leaf reflectance from both species to different concentrations of nitrogen (N) and phosphorus (P) at a laboratory scale.

Chlorophyll Pigment-Related Indices

Field hyperspectroscopy with a portable spectroradiometer has been widely used to assess various biophysical properties, including pigment content and chlorophyll, crop canopy and density, leaf area index and biomass quality, and heavy metals and nutrient content, to benefit farm management. This approach could lead to the invention of new methodologies and scientific findings for aquatic vegetation (Yang *et al.*, 2002).

Among important nutrients, N and P are two elements upon which a large portion of remote sensing studies has concentrated (Pacumbaba and Beyl, 2011) because they are essential elements required for vegetation growth, particularly in photosynthetic processes (Reich and Schoettle, 1988). Deficiency of these elements may cause a plant to be physically small and slow growing. The initial symptoms of nutrient deficiency in a plant are normally seen on leaves, where they often become necrotic and lose their greenness (Uchida, 2000).

Waveband reflectance is used as an indicator of chlorophyll activity, by using differences in the relative concentrations of

photosynthetic and accessory pigments (Fyfe, 2003). Of greater importance are visible wavelengths and near infrared. Seagrass species differed significantly in their capacity for blue light absorption. However, seagrass species are most spectrally distinct in green and near-red light wavebands. The magnitude of green and red reflectance is based not only on the minimum absorption of these wavelengths, but also on the total and relative concentrations of chlorophylls, carotenoids, and accessory pigments (Curran, 1989). The wide hyperspectral wavelengths are important to detect the impact of any particular nutrient in the photosynthesis process.

To estimate nutrient concentrations and biophysical properties of a plant using a spectroradiometer, some vegetation indices have been developed with various band combinations. There are three different approaches of waveband combinations commonly used. These are the simple ratio λ_1/λ_2 , a normalized difference index $(\lambda_1 - \lambda_2)/(\lambda_1 + \lambda_2)$, and multiple regression (Müller *et al.*, 2008). These indices are categorized into three classifications, *i.e.*, structural indices, chlorophyll pigment-related indices, and red edge indices (Ray, Singh, and Panigrahy, 2010).

MATERIALS AND METHODS

In this study, two species of submerged coastal plants, seagrass (Figure 1a) and brown algae (Figure 1b), were collected from Pamujan Besar Island (106°13'00.07"E, 5°56'37.20"S) for the laboratory experiment. The island is located in Banten Bay, which is in NW Java, Indonesia. Banten Bay is one example of a coastal ecosystem that receives a continuous supply of nutrients from a large area of irrigated paddy rice fields in the catchment (Kelderman *et al.*, 2002). For many years, seagrass meadows in Banten Bay have been located in close proximity to intensive paddy-cultivated coastal plains and receive nutrient contaminants from untreated sewage and agriculture runoff (*i.e.*, P and N) coming from some rivers (Tomascik *et al.*, 1997). The concentrations of P and N in the seagrass tissues collected from Banten Bay were higher than those found in Jakarta Bay, Larymna Bay, and the Gulf of Mexico. The mean concentrations of total N and P (P_2O_5) collected from 16 stations in Banten Bay are $2.33\% \pm 0.23\%$ and $0.26\% \pm 0.03\%$ in the leaves and $1.31\% \pm 0.40\%$ and $0.22\% \pm 0.07\%$ in the corms, respectively (Suwandana, Kawamura, and Soeyanto, 2011).

Ten glass aquariums (35 × 22 × 25 cm) were prepared for this laboratory experiment. The first five aquariums were allocated for seagrass, each with three clumps of seagrass (each clump consisting of *ca.* 5–7 leaf blades). The other five aquariums were allocated for brown algae, each with five bunches of brown algae (each bunch consisting of *ca.* more than 50 leaves). Those samples were placed in the aquariums after they were filled with seawater taken from the same sampling site. Then the aquariums were kept for 3-day acclimatization at a natural water temperature with continuous aeration. After acclimatization, the first hyperspectral measurement was carried out to get the spectral signatures of the samples at the initial condition (before treatment) (day 3 [D3]).

The spectral measurement was done in dark room conditions (Figure 2), where the only light source was obtained from two

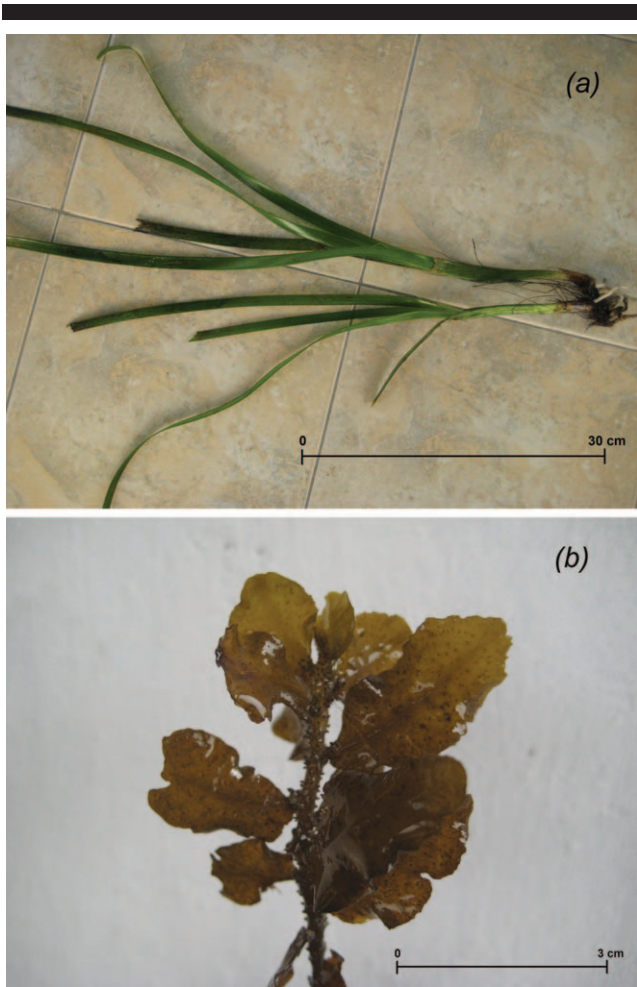


Figure 1. Samples of (a) seagrass (*Enhalus acoroides*) and (b) brown algae (*Sargassum* sp.) taken from the study area.

50-W halogen lamps (Pro-Lamp) installed at *ca.* 50 cm distance from the scanning area, at a 45° angle. A FieldSpec HandHeld Spectroradiometer with a spectral range of 325–1075 nm, including ultraviolet/visible and near infrared bands (ASD Inc., Boulder, Colorado) was employed. Calibration tests were done several times using a reference sample (matte white ceramic tile) to ensure that a constant zero radiance ($\text{W m}^{-2} \text{sr}^{-1} \text{nm}^{-1}$) was maintained during the measurement. The leaf spectra were obtained by taking three leaf blades of seagrass (or five leaf sheets of brown algae) from each aquarium, and the scanning was done five times at *ca.* 20 cm from the plants. This procedure was repeated three times by taking other leaves randomly from each aquarium. In total, 15 reflectance spectra were obtained from three different groups of leaves for each aquarium.

Two types of single-element fertilizers commonly used by the local farmers for paddy cultivation—urea that contains 46% N, and triple super phosphate (TSP) that contains 14–20% soluble $\text{P}_2\text{O}_5(\text{P})$ —were used in this study as sources of artificial nutrient enrichment. Treatments were applied just after the completion of the first spectral measurement. Four aquariums

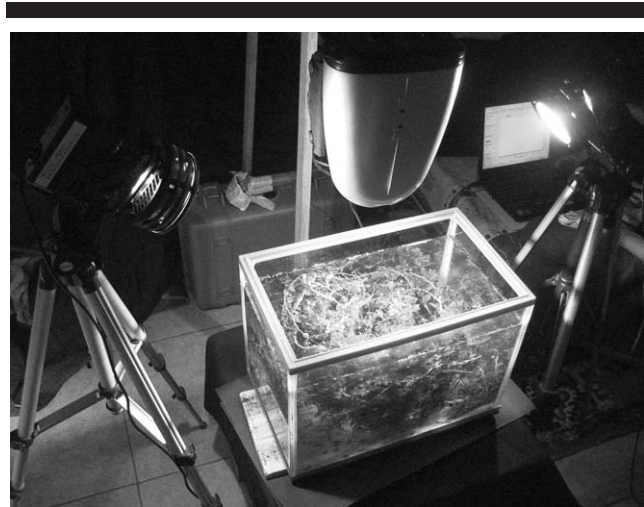


Figure 2. Spectral measurement in the dark room where the only light source was from two stable 50-W halogen lamps.

of seagrass were enriched with 1.0 g of urea (N_1), 2.0 g of urea (N_2), 1.0 g of TSP (P_1), and 2.0 g of TSP (P_2), and another aquarium of seagrass was used for control (C). Similarly, the above treatments were also applied to the five aquariums with brown algae. After being enriched with fertilizers, the aquariums were again kept at a natural water temperature with continuous aeration for another 3 days, to allow the samples to metabolize the supplied fertilizers into their tissues. The second spectral measurement was conducted on day 6 (D6) or 3 days after the fertilizer treatments were applied.

The initial reflectance spectra results were displayed using SAMS version 3.2 (University of California, Davis). Further data processing, including statistical analysis, was done using Matlab version 7.10.0.499 (Math Works Inc., Natick, Massachusetts). The first derivative reflectance (FDR) spectra were estimated by the following equation (Bruce and Li, 2001):

$$\frac{df}{d\lambda} = \frac{f(\lambda_2) - f(\lambda_1)}{\lambda_2 - \lambda_1} = \frac{f(\lambda_2) - f(\lambda_1)}{\Delta\lambda} \quad (1)$$

where $\Delta\lambda$ is the derivative window width, in which we used 2 nm based on Li *et al.* (2011), and $f(\lambda_1)$ and $f(\lambda_2)$ are the reflectance values for wavebands λ_1 and λ_2 , respectively. The spectra were further filtered using the Savitsky-Golay smoothing method (Kawamura *et al.*, 2010). In response to fertilizer treatments, the individual narrow-band reflectance spectra and FDR spectra, consisting of 15 replicates obtained for each aquarium, were analysed in Matlab using 3×2 factorial analysis of variance (ANOVA). The spectra were also analysed using two chlorophyll pigment-related indices, *i.e.*, modified chlorophyll absorption ratio index (MCARI) and transformed chlorophyll absorption ratio index (TCARI), and statistically analysed using two-way ANOVA test in SPSS version 16 (SPSS Inc., Chicago).

RESULTS

The spectral responses of seagrass and brown algae at visible wavelengths (400–700 nm) before and after fertilizer treatment

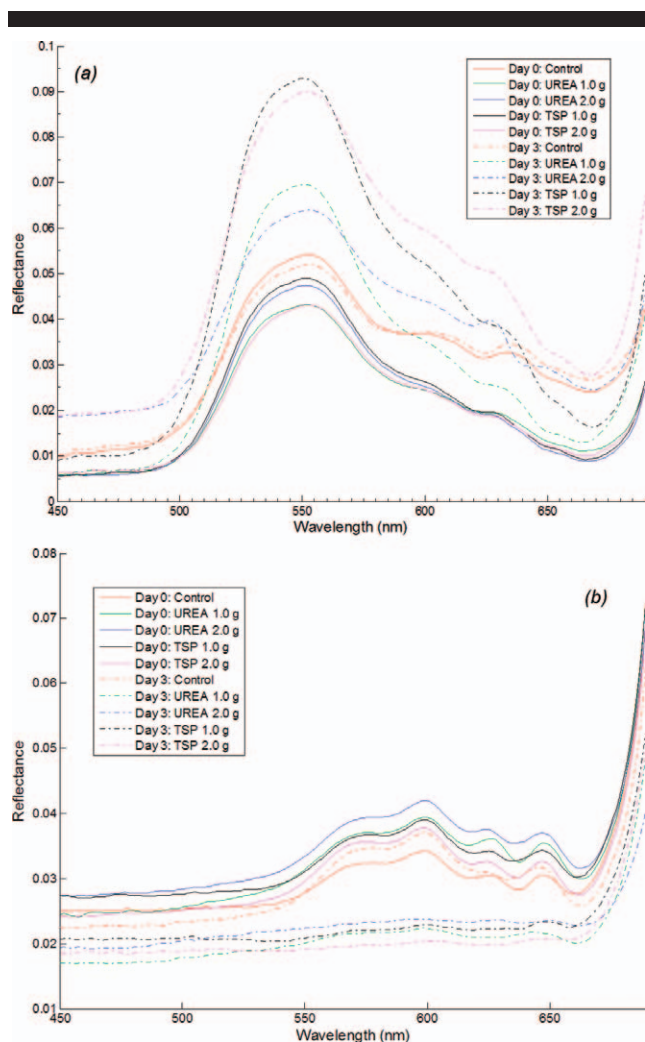


Figure 3. Leaf reflectance signature of (a) seagrass (*Enhalus acoroides*) and (b) brown algae (*Sargassum* sp.) at visible wavelengths (450–690 nm) before and after fertilizer treatment.

application are presented in Figure 3. By looking at the D3 spectra before the treatment, both species showed a different maximum range of reflectance spectra at visible wavelengths. The maximum range of reflectance spectra of seagrass at visible wavelengths between 500 and 690 nm with the peak of reflectance was reached at 550 nm (Figure 3a). Meanwhile brown algae showed a different range of reflectance spectra, where the maximum reflectance at visible wavelengths ranged from 550 to 670 and the peak of reflectance was obtained at 600 nm (Figure 3b).

In response to fertilizer treatments, both species demonstrated different sensitivity to the given nutrient concentrations. Reflectance spectra of seagrass at D6 showed a high increase in response to the nutrient enrichment in the four aquariums. Seagrass samples in the P-treated aquariums produced higher reflectance compared with the samples in the N-treated aquariums, which can be observed from the peak of reflectance spectra, especially at 500 nm, as shown

in Figure 3a. On the contrary, brown algae showed a sharp decrease in the reflectance spectra at 600 nm (Figure 3b).

Given that F statistics are values resulting from a standard statistical test used in ANOVA and regression analysis to determine whether the variances between the means of two populations are different, with the bigger value implying more different; therefore F statistics are applied in this study to see the magnitude of differences for every single wavelength. F statistics, calculated from the individual narrow-band reflectance spectra and FDR spectra of seagrass at every wavelength in response to the N, are shown in Figures 4a and b, as are the responses to P in Figures 4c and d. The F statistics of individual narrow-band reflectance spectra of seagrass were extremely high, especially within the green band (520–600 nm) and red band (630–690 nm) in response to P (Figure 4c) and within the violet-blue band (400–520 nm) and red band (630–690 nm) in response to N (Figure 4a). The FDR spectra of seagrass resulted in different F statistics, as shown in Figures 4b and d for N and P, respectively. The peaks of spectra in violet-blue, green, and narrow-red band were similar to what Fyfe (2003) elaborated in her seagrass study.

All chlorophyll pigment-related indices (MCARI in Figure 5a and TCARI in Figure 5b) used in this study showed that nutrient enrichment increased chlorophyll activity in seagrass leaves (Figure 5). Here, the seagrass samples did not show any stress symptoms in the leaf tissues after 3 days of the treatment, rather it increased chlorophyll pigment activity in response to the given nutrient concentrations. On the contrary, the same vegetation indices showed that nutrient addition caused a great decrease in chlorophyll pigment activity in the brown algae leaves in response to the given nutrients (Figure 6). It was very clear that elevated nutrient concentrations were detrimental to leaf tissue of brown algae, whereas these concentrations stimulated chlorophyll activity in seagrass.

Statistical ANOVA tests revealed that the chlorophyll-related indices applied in this study showed a significant increase of the chlorophyll activity in the seagrass leaves after the fertilizer treatments compared with the reflectance spectra at initial conditions. The chlorophyll activity increase in both P-treated aquariums (P_1 and P_2) was higher than the increase in the two N-treated aquariums (N_1 and N_2), as shown in the box-and-whisker plots (Figures 5 and 6).

DISCUSSION

The differences in reflectance spectra and range of maximum spectra given by seagrass and brown algae are believed to be related to the leaf structures and types of pigment contained in each species. Seagrasses carry the same basic complement of photosynthetic pigments, such as chlorophylls *a* and *b*, and a range of xanthophylls and carotenes that constitute the carotenoids (Fyfe, 2003); therefore they produced a high reflectance within the green band, especially at 550 nm. Meanwhile, brown algae contain pigments such as fucoxanthins and carotenes that create brown coloration in the leaves that mask the other pigments (Boney, 1966), thereby resulting in a high reflectance within green and red bands, with the peak reached at 600 nm. The results confirmed the study done by Fyfe (2003), which

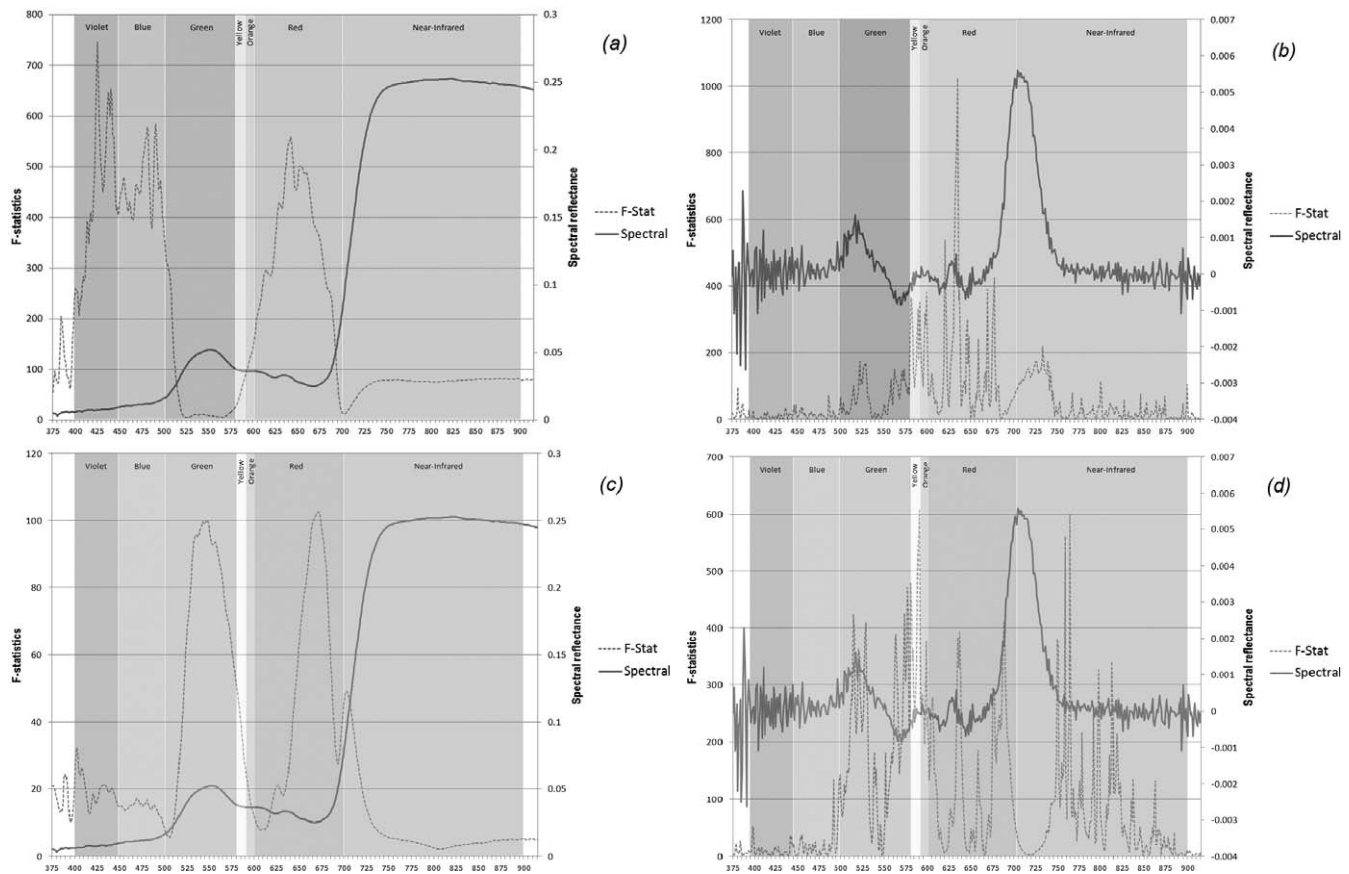


Figure 4. F statistics (factorial ANOVA) of spectral reflectance of seagrass (*Enhalus acoroides*) in response to urea (N) using (a) individual narrow-band reflectance and (b) first derivative reflectance; and in response to triple superphosphate (TSP; P) using (c) individual narrow-band spectral and (d) first derivative reflectance.

showed that visible wavelengths, especially green and near-red (520–700 nm), are the wavelength regions where the reflectance spectra from seagrass are most distinct.

In response to fertilizers, seagrass produced a significant increase in reflectance spectra, especially within the visible bands, in which generally P produced higher reflectance spectral values compared with N. For example at 550 nm, the spectra for P was significantly different from that of N ($p = 0.041$ at confidence level (α) = 0.05), but the test did not prove any difference between the given fertilizer concentrations ($p = 0.664$) after 3 days of treatment (Figure 5). However, the results could be different for each individual wavelength.

The rapid increase in magnitude of reflectance spectra of seagrass just 3 days after the treatment was applied is due to the nutrient absorption capacity of these plants, which can be achieved not only by roots but also by leaves (Stapel *et al.*, 1996). The higher increase of reflectance spectra given by the P-treated samples compared with N-treated samples may be related to the significant role of phosphate in the leaves, especially for bioenergetic processes. Photosynthetic processes in seagrass can indeed be measured and detected by

fluorometers and light reflectance devices, as discussed in more detail by Silva *et al.* (2009).

In most cases, seagrass and macroscopic algae such as brown and green algae live together at the same habitat (Dawes, 1998), and both species also exist in Banten Bay. The commercial cultivation of seaweed (brown algae) has increased enormously in Banten Bay (Kuriandewa *et al.*, 2003). The same concentrations of fertilizers may give different responses by every species in an environment, depending on the species sensitivity. In this study, the leaf condition of brown algae samples in four nutrient-enriched aquariums seemed to show a “stressful” symptom in response to the increased fertilizer concentrations. It is too early to state, but this study implies that the sudden increase of fertilizer concentrations could still support seagrass to grow at least for 3 days after the treatment, but not for brown algae. Brown algae, which normally grows quickly with a high presence of nutrients in the environment, known as eutrophication (Burkholder, Tomasko, and Touchette, 2007), showed symptoms of dying in this study. Their death, which might be due to the sudden increase of N and P in their original forms, before transforming into NO_3 , NH_4 , PO_4 , *etc.*, requires more careful consideration.

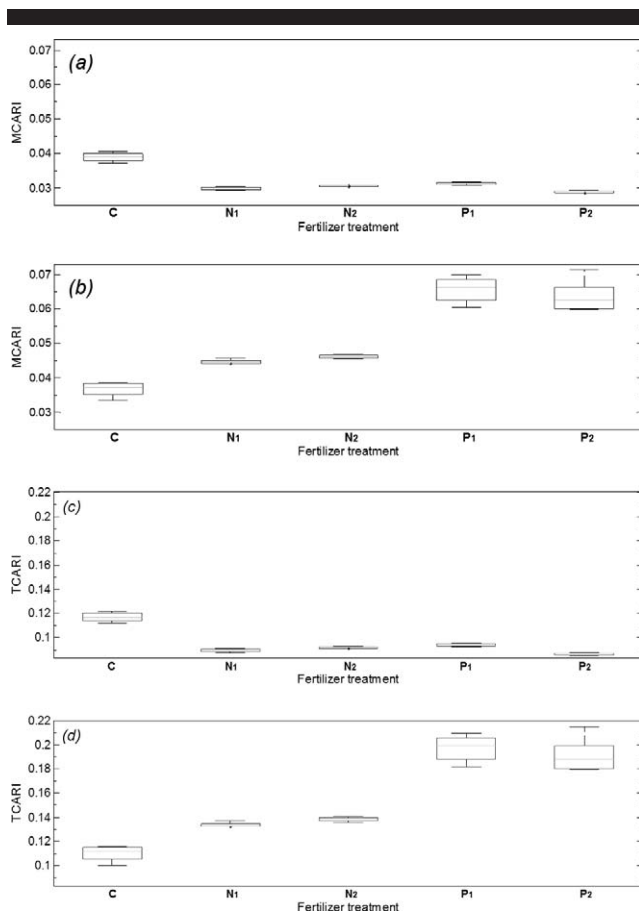


Figure 5. Box-and-whisker plots of chlorophyll pigment-related indices of spectral reflectance of seagrass (*Enhalus acoroides*) in different nutrient concentrations at a laboratory scale. MCARI: modified chlorophyll absorption ratio index (a) before and (b) after the treatment; TCARI: transformed chlorophyll absorption ratio index (c) before and (d) after the treatment. Label in x-axes: C = control, N₁ = urea (N) 1.0 g, N₂ = urea (N) 2.0 g, P₁ = TSP (P) 1.0 g, and P₂ = TSP (P) 2.0 g.

Owing to “stressful” symptoms shown by brown algae, the factorial ANOVA test was therefore performed only for the reflectance spectra given by seagrass (Figures 4a–d). An interesting finding is the large F values for individual narrow-band reflectance spectra of seagrass found within green and red channels in response to P and within violet-blue and red channels in response to N. These findings again supported the previous study done by Fyfe (2003). However, since hyperspectral results deal only with physical reflectance characteristics of the leaves, a molecular study is required to further investigate the roles of nutrients in the photosynthesis process in relation to its spectral response, especially P, which plays an important role in the formation of adenosine triphosphate during photosynthesis (Furbank and Taylor, 1995). Chlorophyll activity can be measured by fluorescence devices (Silva *et al.*, 2009); however this technical note was not sufficient to derive a robust conclusion. The FDR behaviour differed from that of individual narrow-band reflectance spectra, since background reflectance confounding was reduced in the FDR spectra.

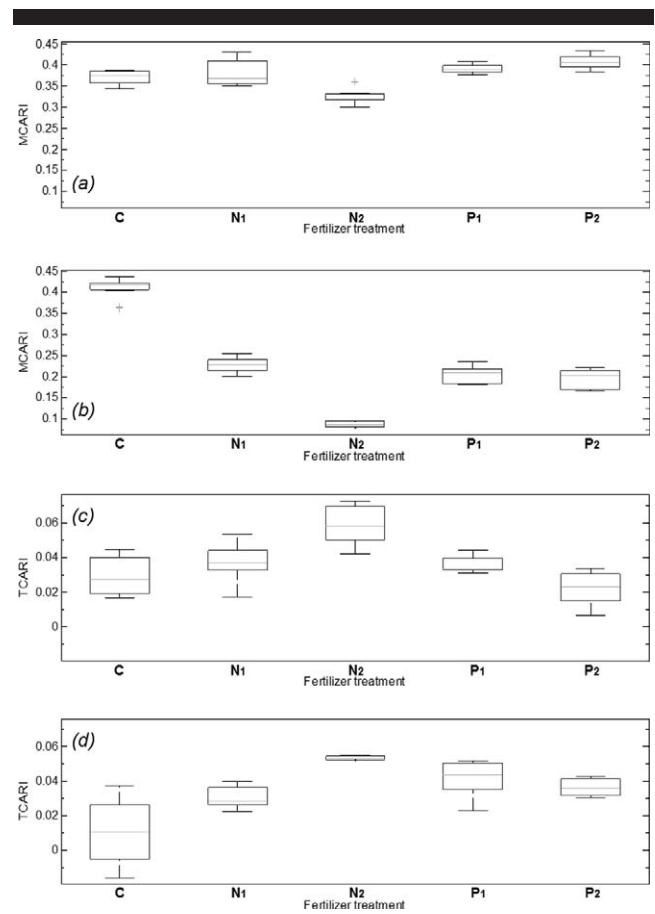


Figure 6. Box-and-whisker plots of chlorophyll pigment-related indices of spectral reflectance of brown algae (*Sargassum* sp.) in different nutrient concentrations at a laboratory scale. MCARI: modified chlorophyll absorption ratio index (a) before and (b) after the treatment; TCARI: transformed chlorophyll absorption ratio index (c) before and (d) after the treatment. Label in x-axes: C = control, N₁ = urea (N) 1.0 g, N₂ = urea (N) 2.0 g, P₁ = TSP (P) 1.0 g, and P₂ = TSP (P) 2.0 g.

The MCARI developed by Kim (1994) and simplified by Daughtry *et al.* (2000) is intended to minimize the effects of nonphotosynthetic materials on spectral estimates of absorbed photosynthetically active radiation. The MCARI equation defines the depth of the chlorophyll absorption at 670 nm relative to the reflectance at 550 nm and 700 nm. Since the spectral reflectance from this study was greatest at 550 nm in all nutrient-enriched aquariums, the MCARI indices before (D3) and after fertilizer treatment (D6) were significantly different ($p < 0.01$). The Tukey’s honestly significant difference between all treatment combinations showed significant differences ($p < 0.01$).

The TCARI, modified from MCARI by Haboudane *et al.* (2002) to compensate for the effects of background reflectance by soil and nonphotosynthetic material, showed significant difference before and after treatment ($p < 0.01$) and between nutrient treatments ($p < 0.01$), except for TCARI between N₁ and N₂ and TCARI between P₁ and P₂, which did not show any difference, with p values 0.702 and 0.160, respectively.

CONCLUSIONS

Our initial experiments are still too few to present an accurate relationship between spectral reflectance of seagrass and brown algae in response to nutrient concentrations. However, we are pleased to communicate that there is scientific evidence that hyperspectral reflectance measurement can be used to detect nutrient contaminants in the coastal aquatic vegetation more quickly than traditional analytical chemistry. Our study also shows that brown algae were more sensitive to nutrient contaminants compared with seagrass. In contrast, seagrass was more tolerant to nutrient enrichment, and the chlorophyll activity in the seagrass leaves increased significantly in response to nutrient addition. A more comprehensive study is needed to understand the spectral behaviour and the sensitivity of both species to small increments in nutrient concentrations.

ACKNOWLEDGMENTS

The authors would like to thank to the Global Environmental Leader and Global Internship Program projects of the Graduate School for International Development and Cooperation, Hiroshima University, Japan, which has funded this study. Special thanks are also addressed to Yoke F. Azhari, of the Agency for the Assessment and Application of Technology, for his assistance during the study.

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