hyperspectral detection of stress in seagrass

Suzanne Fyfe[1,2] and Sharon Robinson[1]

[1] Institute for Conservation Biology and [2] GeoQuest, University of Wollongong, NSW, Australia

monitoring seagrass 'health'

Seagrass meadows are extremely important for the ecological functioning of coastal and estuarine ecosystems. However, coastal waterways and their catchments have not been well managed under the pressure of agricultural, residential, commercial and industrial development (West 1997). As a result, unprecedented seagrass declines have been reported in temperate and tropical regions throughout the world (e.g. Robblee *et al*. 1991; Short *et al*. 1996). Growing awareness of the importance of seagrasses has necessitated better management and a requirement for regular monitoring of this resource in Australia (Ward *et al*. 1998) and many other parts of the world.

Currently seagrass meadows are monitored by remote sensing of meadow distribution and extent, or field sampling of shoot density, biomass, species composition and other biophysical variables. In each case, significant biomass is lost before a decline in the condition of the meadow can be detected. It may be possible to detect physiological stress in seagrasses prior to the onset of die back by monitoring seagrass biochemistry and/or photosynthetic performance in the field or by remote sensing.

potential for detection of stress

The recent development of new high spectral, spatial and radiometric resolution airborne and satellite-based sensors has significantly increased the potential for monitoring stress in vegetation by remote sensing. Remote sensing offers a rapid, non-invasive and cost effective alternative to biochemical methods of obtaining information on plant photosynthesis, pigment content, nutrition and stress. Hence, a vast array of hyperspectral reflectance and derivative indices have been developed and tested to predict the biophysical characteristics (e.g. Malthus *et al*. 1993; Thenkabail *et al*. 2000) and physiological status (Peñuelas and Fillela 1998) of agricultural crops.

Seagrasses are marine angiosperms which respond to stress in much the same way as terrestrial higher plants (Dawson and Dennison 1996). The primary cause of seagrass die back is the sedimentation and eutrophication of waterways which effectively shades the plants and reduces photosynthesis and growth. High light stress leads to photoinhibition and a reduction in photosynthetic efficiency. Toxic pollutants, inorganic carbon and nutrient deficiencies, desiccation, and changes in pH, salinity and temperature exacerbate photoinhibition so that plants display the physiological symptoms of high light stress even under natural levels of irradiance.

aims

- to identify the biochemical changes that occur in *Zostera capricorni* leaves after exposure to high and low light stress
- to investigate whether these pigment-based 'stress indicators' can be spectrally detected
- to test existing and develop new hyperspectral indices that may be applied to predict physiological stress in seagrasses as an early warning of potential die back.

methods

1. laboratory light stress experiments

Mature *Zostera capricorni* (eelgrass) turves were stabilized in tanks in the lab under controlled environmental conditions for 1 month prior to treatment. Shade cloth covers were applied to one half of each paired replicate tank to apply shade treatment in the low light (LL) stress experiment (PAR: controls 168±49 μmol m^{-2} s^{-1}, low light 39±12 μmol m^{-2} s^{-1}; n=12) and to the controls in the high light (HL) stress experiment (PAR: controls 238±127 μmol m^{-2} s^{-1}, high light 661±190 μmol m^{-2} s^{-1}; n=24). Experiments were run for approximately 3 months until die back was almost complete in low light treatments, however, data collection focused on the initial weeks of treatment to identify 'early warning' spectral symptoms of stress.

Replicate leaf samples were cut from treatments at 3 day intervals for the first 2-3 weeks of treatment and assessed for:
- Photosynthetic efficiency (F_v:F_m) measured from dark-adapted leaves using a PAM-2000 (Walz) chlorophyll fluorescence meter
- Spectral reflectance (430-900 nm) measured under lab conditions with a Fieldspec FR spectroradiometer (ASD) and PMSC corrected (according to methods of Fyfe 2003)
- Concentration of chlorophylls *a*, *b* and 9 carotenoids analyzed by high performance liquid chromatography (HPLC method modified from Gilmore and Yamamoto 1991)
- Anthocyanin content measured by spectrophotometry (differential pH method of Fuleki and Francis 1968)
- % Carbon, % Nitrogen and C:N ratio determined with an elemental analyzer (Carlo Erba NCS1500) (low light experiment only)
- Above and below ground dry plant biomass (low light experiment only)

2. assessing potential stress indices

The pigment ratios selected as optimal indicators of light stress in seagrass (chl *a*:*b*, VAZ:T chl, VAZ:T car, Z:VAZ), as well as total chlorophyll content and photosynthetic efficiency (F_v:F_m) were correlated against a range of published narrow band indices, 'optimized' narrow band NDVI's and several new indices developed in this study (Table 2). Wavelengths applied in the indices were restricted to the visible range 430-700 nm since these wavelengths can penetrate through water and can be used in the remote sensing of submerged vegetation. Data from the high and low light stress experiments were combined for use in correlation and regression analysis.

3. developing predictive regression equations

Indices providing consistently high correlation with one or more of the pigment based stress indicators or with F_v:F_m were regressed against these indicators to develop a predictive equation for estimating light stress in seagrasses. A range of linear and non linear equations were applied to generate the best possible regression fit.

Table 2. Candidate hyperspectral indices

Published and modified indices:
- PRI = $(R_{531}-R_{570})/(R_{531}+R_{570})$ (Gamon *et al*. 1992)
- NPCI = $(R_{680}-R_{430})/(R_{680}+R_{430})$ (Peñuelas *et al*. 1993)
- green edge position (Peñuelas *et al*. 1993)
- GGFN = $(D_{GE}-D_{minG})/(D_{GE}+D_{minG})$ (Peñuelas *et al*. 1993)
- Green NDVI = $(R_{495}-R_{550})/(R_{495}+R_{550})$
- PSNDc (Blackburn 1998) modified to $(R_{495}-R_{470})/(R_{495}+R_{470})$
- CABI = $(R_{495}/R_{682}) - (R_{495}/R_{643})$
- Optimised HL and LL stress NDVIs utilizing the most significantly different wavelengths from lab experiments as index wavelengths and the least significantly different wavelengths from experiments as reference wavelengths
- Optimised pigment ratio NDVIs (e.g. Tchl NDVI) utilizing the wavelengths best-correlated with each pigment ratio as index wavelengths and the least-correlated wavelengths as reference wavelengths

Indices developed in the current study:
- RORG = $(R_{682}/R_{550}) - (R_{682}/R_{635})$
- BGBO = $(R_{495}/R_{550}) - (R_{495}/R_{635})$

Index wavelengths were taken from regions of significant reflectance change in light stressed Z. capricorni, reference wavelengths were regions where no change occurred.
- Orange edge 1 position; max 1st derivative between 600-650 nm
- Orange edge 2 position; max 1st derivative between 651-690 nm
- Orange edge 2 value; value of max 1st deriv. between 651-690 nm

results

1. laboratory light stress experiments

Declines in the 'health' of light stressed *Z. capricorni* (apparent as declines in photosynthetic efficiency and/or growth) were accompanied by significant and characteristic changes in leaf biochemistry and in the spectral reflectance measured from the leaves (Table 1).

Table 1. Summary of the significant (p < 0.05) short term (2-3 weeks) physiological and spectral responses observed in high and low light stressed *Z. capricorni*

	LOW LIGHT STRESS	HIGH LIGHT STRESS
health	— photosynthesis ↓ growth	↓ photosynthesis — growth
pigments	↓ chl *a*:*b* ↓ Z and A	↑ T car:T chl ↑ VAZ:T chl ↑ VAZ:T car ↑ Z:VAZ ↑ Z
spectral response	↑ green R ↓ orange R ↑ NIR R	↓ green R & GE → ↑ orange R ↓ red R & RE ←

increase, ⇩ decrease, - no change, ⇨ red shift, ⇦ blue shift, NIR near infrared, chl chlorophyll, car carotenoid, V violaxanthin, A antheraxanthin, Z zeaxanthin, T total, R reflectance.

Low light stressed *Z. capricorni* leaves displayed significantly higher green and NIR reflectance and stronger absorption of far green-near red (590-650 nm) wavelengths after 13 days of treatment (Figure 1), with significant differences in visible wavelengths apparent after only 7 days. High light stress had the opposite effect on spectral response; after 13 days green reflectance was significantly reduced while reflectance between 590-650 nm increased. In addition, narrowing of the chlorophyll absorption trough resulted in a significant blue shift of the red edge. The green edge was shifted toward longer wavelengths due to lower green peak reflectance.

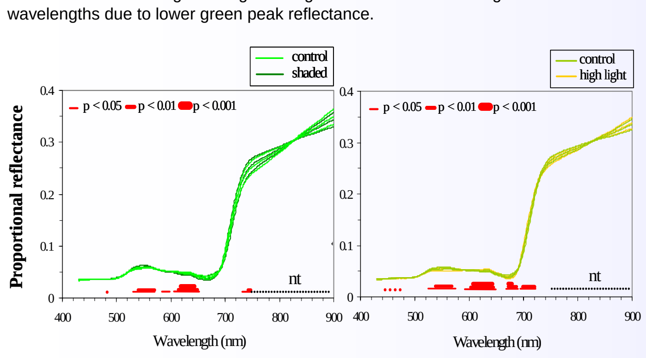


Figure 1. Mean + SD spectral signatures of (A) control grown versus low light stressed and (B) control grown versus high light stressed *Z. capricorni* leaves after 13 days of treatment.

2. applicability of lab results to the field

The pigment ratios identified as indicators of light stress in lab experiments displayed strong and consistent linear relationships with the total daily irradiance received by the seagrass leaves, regardless of whether they were grown in the lab or under natural conditions in the field, e.g. chlorophyll *a*:*b* (r^2 = 0.803) and VAZ:total carotenoids (r^2 = 0.968; Figure 2). Spectral reflectance at wavelengths 550 and 685 nm decreased with increasing daily PAR (r^2 = 0.386 and r^2 = 0.498 respectively), while reflectance at 630 nm increased with daily PAR (r^2 = 0.431) , although none of the relationships were strongly linear.

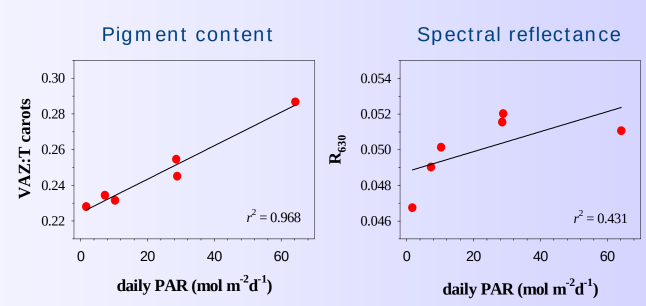
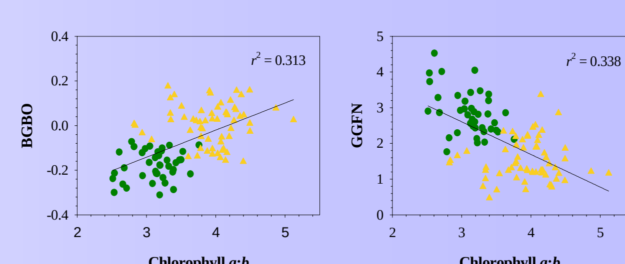


Figure 2. Influence of daily integrated PAR on mean ratio of selected pigments and reflectance at selected wavelengths for lab and field grown *Z. capricorni*. Data are the means of LL, control and HL lab, and winter and summer field samples.

3. optimal seagrass stress indices

The hyperspectral reflectance indices that correlated well with a range of seagrass stress indicators were the BGBO,GGFN, RORG and green edge position. These indices performed substantially better than established plant stress indices such as the PRI and red edge position. Based on their subsequent performance in regressions with chl *a*:*b*, VAZ:T chl and VAZ:T car, the BGBO and GGFN were considered the best indices for prediction of light stress in *Z. capricorni* leaves. Highest coefficients of determination were achieved in the prediction of chlorophyll *a*:*b* (Figure 3).



Chl *a*:*b* vs.	Regression equation	r^2	*F*	*p*	RMSE	Power
BGBO§	$y = 0.121x - 0.506$	0.313	45.147	<0.0001***	0.102	1.0000
GGFN§	$y = -0.915x + 5.350$	0.338	50.515	<0.0001***	0.724	1.0000

§ Durbin-Watson < 1.5: residuals are not independent.

Figure 3. Regression of chlorophyll *a*:*b* content of *Z. capricorni* with the BGBO and GGFN indices. Yellow = data from HL stress expt, green = data from LL stress expt.

4. test on independent field data set

The ability of BGBO to predict chlorophyll *a*:*b* (i.e. light stress) in seagrasses was tested against field sampled *Z. capricorni*, *Posidonia australis* (strapweed) and *Halophila ovalis* (paddleweed) leaves. Spectral reflectance was measured in the field with the Fieldspec FR spectroradiometer using methods detailed in Fyfe (2003) and the pigment content of samples subsequently analyzed by HPLC. The BGBO was calculated from uncorrected spectral reflectance data and applied to the inverted regression equation from Figure 3 (x = [y + 0.506]/0.121) to estimate the chlorophyll *a*:*b* of the field samples.

The measured chlorophyll *a*:*b* content of samples explained 47.6 % (RMSE = 0.917) of the variation in chlorophyll *a*:*b* values predicted by the BGBO (Figure 4). However, predicted chl *a*:*b* was not statistically equivalent to measured chl *a*:*b* (t = 5.513, p < 0.0001, df = 49) because of considerable variation around the regression line (21.5% of the mean).

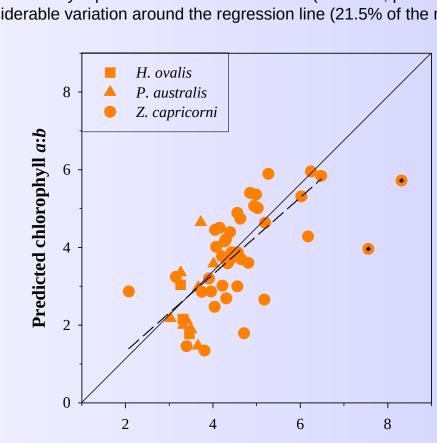


Figure 4. Relationship between measured chl a:b and chl a:b predicted by the BGBO for field sampled seagrasses. Data outliers are marked with a cross. Dashed line is regression line of measured vs. predicted.

conclusions

- Characteristic changes in leaf biochemistry indicated a decline in the 'health' of light stressed seagrasses and led to significant changes in the spectral reflectance measured from the leaves.
- Relationships between the total irradiance received by the leaves, leaf biochemistry and leaf spectral responses were consistent across lab grown and field sampled seagrasses.
- There is some potential for the spectral monitoring of stress in seagrass meadows but results are yet to be tested on remotely sensed image data. Reflectance changes indicative of stress were highly significant but of small magnitude so operational remote sensing needs to account for the attenuating effects of the atmosphere, water depth and water quality.
- The BGBO was the best hyperspectral index tested here for the prediction of light stress in seagrasses. The BGBO is simple, versatile and unlike the PRI or the GGFN, can be applied to single wavelengths or waveband data.

acknowledgements

Funding and/or support for this project were provided by:
- Australian Postgraduate Award
- Australian Research Council small grant
- Institute for Conservation Biology, University of Wollongong
- GeoQuest, University of Wollongong
- NSW Fisheries Research Institute
- CSIRO Land and Water, Environmental Remote Sensing Group

references

Blackburn, G., 1998. *RS. Environ*., **66**:273-285.
Dawson, S., and W. Dennison, 1996. *Mar. Biol*., **125**:629-638.
Fuleki, T., and F. Francis, 1968. *J. Food Sci*., **33**:78-83.
Fyfe, S., 2003. *Limn. Oceanogr*., **48**:464-479.
Gamon, J., *et al*., 1991. *RS. Environ*., **41**:35-44.
Gilmore, A., and H. Yamamoto, 1991. *J. Chromatog*., **543**:137-145.
Malthus, T., *et al*., 1993. *RS. Environ*., **46**:204-212.
Peñuelas, J., and I. Filella, 1998. *Trends Plant Sci*., **3**:151-156.
Peñuelas, J., *et al*., 1993. *RS. Environ*., **46**:110-118.
Robblee, M., *et al*., 1991. *Mar. Ecol. Progr. Ser*., **71**:297-299.
Short, F., *et al*., 1996. *Aquat. Bot*., **27**:41-57.
Thenkabail, P., *et al*., 2000. *RS. Environ*., **71**:158-182.
Ward, T., *et al*., 1998. Australia: State of the Environment Environmental Indicator Report, CSIRO Div. Mar. Res., 85 pp.
West, R., 1997. *Proc. Qindgdao Workshop*, Uni. Wollongong.