Spatial and temporal variation in spectral reflectance: Are seagrass species spectrally distinct?

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Abstract
The spectral signatures of the seagrasses Zostera capricorni, Posidonia australis, and Halophila ovalis were investigated to determine whether species could be discriminated by remote sensing. The spectral reflectance of fouled and unfouled leaf samples collected from marine and brackish habitats at three estuaries in southeastern Australia were measured in the field with a spectroradiometer during each season in 1999 and 2000. Seagrass species were spectrally distinct regardless of whether the leaves were fouled by epibionts and despite spatial and temporal variability in the reflectance of each species. The visible wavelengths that penetrate water coincide with the regions of maximum absorption and characteristic reflectance by plant photosynthetic and accessory pigments. Strong and consistent differences in reflectance between species were recorded in the green wavelengths at 530–580 nm with additional discrimination in the regions 520–530 nm and 580–600 nm and at the red chlorophyll absorption trough at 686–700 nm. Species discrimination should be possible in the remote sensing of benthic aquatic vegetation using a hyperspectral sensor that has narrow bands centered on pigment-related spectral features in the visible wavelengths. The detection of statistically significant differences in intraspecific reflectance associated with the year, season, estuary, and habitat of sample collection suggests a potential for monitoring seagrass health and estuarine water quality. This study has produced the first spectral library of aquatic plant species to take into account the range of spectral variability expected for the species under natural conditions. The results provide a sound basis for future mapping of seagrass species in Australia.

Recent developments in sensors and processing methodologies have led to an increase in the use of hyperspectral imagery for environmental applications such as the mapping and monitoring of the coastal zone. High spectral and spatial resolution imagery provides researchers with the potential to map vegetation to species level, provided the plant species under study are spectrally distinct.

A basic assumption of mapping by remote sensing is that the features of interest in an image reflect or emit light energy in different and often unique ways (e.g., Harrison and Jupp 1990; Lillesand and Kiefer 1994). Remote sensing classification procedures are usually based on numerical techniques that group map pixels by their spectral response across the available wavebands (i.e., by their multivariate statistical parameters) (Jensen 1996). There would be no point in trying to map objects that are not spectrally different at the most basic level, no matter how much time is spent on processing or preprocessing or how advanced the classification or image extraction techniques are.

The assumption that individual plant species have unique spectral signatures has, however, been questioned (Price 1994). First, there is an overall qualitative similarity in the spectral response curves of green plant species (Kleshnin and Shul’gin 1959), although quantitative spectral differences between species have been observed for plant leaves (e.g., Gates et al. 1965; Gausman 1982; Datt 2000) and canopies (Gong et al. 1997; Yu et al. 1999). Second, reflectance from vegetation is governed by a small number of physical and physiological parameters and these can vary for a species or within an individual plant over space and time. It is therefore useful to determine whether the observed reflectance differences between plant species are not only statistically significant, but consistent (e.g., over different seasons or in different habitats), before they can be generally applied with success in remote sensing species mapping.

In studies investigating the discrimination of terrestrial plant species, the largest differences in reflectance have been recorded in the near infrared (NIR) and short infrared wavelengths (e.g., O’Neill et al. 1990; Borregaard et al. 2000). Remote sensing of benthic aquatic plants, however, is limited to the visible wavelengths where light penetrates the water column and can be reflected back to a sensor. Although pure water absorbs light to some extent in shorter wavelengths, significant attenuation occurs at wavelengths beyond 680 nm (Kirk 1994). In coastal waters, spectral scattering and absorption by phytoplankton, suspended organic and inorganic matter, and dissolved organic substances further restrict the light passing to, and reflected from, the benthos (Dekker et al. 1992). Hence, efforts to discriminate between aquatic plant species must concentrate on pigment-related spectral features within the visible wavelengths.

The light-harvesting pigments for photosynthesis in the
seagrasses are the same as for all higher plants; chlorophylls \(a\) and \(b\) (Chl \(a\) and Chl \(b\)) and a range of xanthophylls and carotenoids that constitute the carotenoids. Because all angiosperms share basically the same physiology and biochemistry, the wavelength position of pigment absorption features does not differ significantly between species (Curran 1989). However, the relative concentrations of photosynthetic pigments and the presence of accessory pigments does vary among taxa. The depths and widths of pigment absorption troughs and the position and magnitude of reflectance peaks can be quite different among species (Ustin et al. 1993). Hence, differences in the spectral reflectance of terrestrial plant species have been recorded in the visible wavelengths (e.g., Gong et al. 1997; Kumar and Skidmore 1998; Yu et al. 1999; Datt 2000). Remote sensing has been even more successful in identifying and quantifying phytoplankton taxa in algal blooms (Richardson et al. 1994; Richardson 1996; Aguirre-Gómez et al. 2001). Algal classes can be identified by the characteristic absorption features (Foppen 1971) of the specific photosynthetic and accessory pigments each class contains (Rowan 1989).

The relative concentrations of photosynthetic and accessory pigments will also vary within a plant species because of genetic variation, seasonal cycles, stage of growth, health, or environmental conditions (Pérez-Llorens et al. 1994; Dawson and Dennison 1996; Longstaff and Dennison 1999; Alcoverro et al. 2001). Chromatic acclimation of pigments can occur in an individual aquatic plant grown under changing conditions of water depth or clarity (Dawes 1998). In addition, the epibionts that grow on the surface of aquatic plants can mask the reflectance to some extent while contributing their own absorption and reflectance features to the spectral response. Seagrass epibionts, for example, include a diverse array of microalgae, bacteria, juvenile macroalgae, and sessile invertebrates such as tubeworms and bryozoans. However, it is the algae that are the most prominent and have the greatest effect in remote sensing. Fifty-six algal species were recorded by May et al. (1978) in a survey of the leaf epiphytes of Zostera capricorni and Posidonia australis in two estuaries in New South Wales. Algal epiphytes can be quite sensitive indicators of environmental conditions; hence, epiphyte biomass and species composition on a single seagrass species will change with location and can also vary over time (May et al. 1978). Spectral response in the visible wavelengths is therefore variable for any seagrass species over space and time. Intraspecific variability might increase the chance of spectral overlap with other species and make spectral discrimination using remotely sensed image data difficult or impossible.

Hyperspectral imaging data offers the high spectral resolution required for the detection of visible reflectance differences in the pigment content of plants. For example, the compact airborne spectrophotographic imager (CASI, Itres Instruments Inc.) has been applied in the delineation of seagrass beds (Mumby et al. 1997a), the estimation of seagrass standing crop (Mumby et al. 1997b), and the mapping of subtidal and intertidal seagrass and macroalgal communities (Zacharias et al. 1992; Bajjouk et al. 1996; Jupp et al. 1996; Anstee et al. 2000). Alberotanza et al. (1999) mapped seagrass and macroalgal beds using the airborne Daedalus multispectrairal infrared and visible imaging spectrometer (MIVIS). Promising results have also been obtained using HyMap (Hyperspectral Mapping; Integrated Spectronics) imagery to map beds of Zostera spp. and Posidonia spp. in South Australia (Dunk and Lewis 2000). Although these studies have been relatively successful in mapping the extent of taxonomically quite different groups of plants, few published studies have been able to accurately delineate co-occurring species.

If species are to be mapped accurately by remote sensing, differences in the spectral signatures of seagrass species need to be distinct and consistent over seasons, geographic locations, and changing environmental conditions. Hence, it is necessary to characterize the range of signatures expected for any species under natural conditions if the signatures are to be used in spectral libraries for quantitative spectral analysis (Price 1994). Indeed, spectral libraries are a fundamental part of many hyperspectral image classification and radiative transfer modeling procedures.

The main aim of this field-based study was therefore to determine whether consistent differences were apparent in the spectral response of the three common seagrass species of southeastern Australia. The patterns of reflectance associated with epibiont cover and the year, season, estuary, and habitat of sampling were characterized from detailed field spectroradiometer measurements to compare the magnitude of interspecific and intraspecific variability in the spectral signatures of the seagrass species. Accurate remote sensing of seagrasses to species level will only be possible if the species prove to be spectrally distinct despite variation within each species.

This study will also lead to the development of the first spectral library of aquatic vegetation to characterize the natural levels of both spatial and temporal variation in the regional spectral response of the plant species involved. Spectral libraries such as this could substantially improve the accuracy of vegetation species maps when applied in remote sensing image classification.

A further objective of this work was to determine the location and width of wavebands that might be practically applied in the remote sensing of benthic aquatic plants. These wavelengths subsequently can be targeted to improve the efficiency and accuracy of image data collection and processing. The information could influence decisions about the type of remote sensing scanner best suited to the mapping of seagrasses.

Methods

The spectral response of the three common species of seagrass that occur in the estuaries of southeastern Australia—eelgrass Z. capricorni, strapweed P. australis, and paddleweed Halophila ovalis—were investigated over a 2-yr period between May 1999 and January 2001. To assess the range of spectral variability that can be found in each species, reflectance from fresh seagrass leaves, both with and without their characteristic leaf epibionts, was sampled during each season from three estuaries. In addition, samples were examined from marine-dominated habitats near the mouths of each estuary and brackish habitats in the backwaters of each estuary.
Study sites—Three estuaries on the southeast coast of New South Wales, Australia, were selected to represent the range of conditions under which seagrasses grow in this region (Fig. 1). Port Hacking on the southern edge of Sydney (151°7′E, 34°4′S) is a tidally flushed, drowned river valley. Much of the catchment for this estuary lies in the Royal National Park that adjoins its southern shores, although the northern side is heavily urbanized. Lake Illawarra, ~80 km south of Sydney near Wollongong (150°50′E, 34°32′S), is a shallow barrier estuary that shows little tidal influence. The catchment of Lake Illawarra is heavily urbanized and has a history of agricultural and industrial land use. In contrast, St. Georges Basin is a tidal barrier estuary that lies approximately 200 km south of Sydney (150°37′E, 35°8′S) in a relatively pristine forested catchment area, although residential development has increased over recent years.

Not all seagrass species were found at both habitats within each estuary. \textit{Z. capricorni} is an adaptable seagrass that occurs in both the marine-dominated estuarine mouths and inlets and in the brackish habitats on the inland side of all three estuaries. However, \textit{P. australis} does not occur under brackish conditions and does not grow in Lake Illawarra at all. Similarly, \textit{H. ovalis} was not present at the brackish study site in Port Hacking although it did occur in this habitat at St. Georges Basin. \textit{H. ovalis} was not observed at either Lake Illawarra study site, although it does occur in this estuary.

Field sampling of spectral reflectance—Sampling was carried out once during each season at each of the marine and brackish habitats (study sites) within each estuary. For each seagrass species, leaf samples were haphazardly collected from an area of ~200 m² and sorted into leaves with epibionts \((n = 5)\) and those occurring naturally without epibionts \((n = 5)\). The spectral signature of each sample was immediately measured in the field with a FieldSpec FR spectroradiometer (Analytical Spectral Devices) that records radiance in the visible–infrared wavelengths. Leaves were kept wet with seawater throughout the sorting and measurement processes. To achieve a pure signal, leaves were piled on a matt black background in multiple layers (at least seven leaf layers deep [O’Neill et al. 1990] but usually several times that) to overfill the field of view of ~5 cm diameter. Leaf samples were scanned from a height of ~5 cm and from as close as possible to nadir above the sample without shad-
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Owing it, using a handheld 25° field of view foreoptic point-ed toward the direction of the sun whenever off-nadir (zenith angle 0–30°, azimuth angle 180°). Sample reflectance was calculated immediately after measurement of radiance from a 99% white Spectralon panel (Labsphere) under the same viewing and illumination geometry. Spectral averaging of 10–30 spectra per sample was performed to ensure optimal signal-to-noise ratio. Sampling was carried out on cloud-free days wherever possible within 3 h of solar noon. Given that spectra were collected on a moving boat under changing field conditions, every practical effort was made to standardize the viewing and illumination geometry while maximizing the signal from the sample in each case. The correction procedures described below compensate for less than perfect measurement conditions when applied to a homogeneous target such as the leaves of a single seagrass species.

The total number of samples collected for each species was not equal because all species did not occur at all sites and because leaves with epibionts were occasionally difficult to locate in brackish habitats. Hence, the total sample sizes were 230 unfouled Z. capricorni, 209 fouled Z. capricorni, 79 unfouled P. australis, 78 fouled P. australis, 104 unfouled H. ovalis, and 89 fouled H. ovalis.

**Correction of spectra**—To focus on the wavelengths most useful for the remote sensing of benthic plants, only the visible–NIR wavelengths in the range 430–900 nm were included from the spectral output available from the FieldSpec FR. Figure 2 summarizes the data preparation, correction, and analysis procedures undertaken in this study.

Data were converted from relative to absolute reflectance by multiplying the reflectance spectrum of each sample by the actual calibrated reflectance spectrum of the Spectralon reference panel. Each sample spectrum was then corrected for nonlinear additive and multiplicative scatter effects using a piecewise multiplicative scatter correction technique (PMSC) (Isaksson and Kowalski 1993). Multiplicative scatter correction applies a simple mathematical correction to the reflectance value at each wavelength of the sample spectrum using offset and slope values estimated by linear least squares regression of that sample spectrum against a standard spectrum (Isaksson and Kowalski 1993). In PMSC, linear regressions are fitted to local wavelength regions using a moving window of specified length (Isaksson and Kowalski 1993). The “standard” spectrum applied in PMSC to sample spectra of each seagrass species was the mean spectrum of all samples of that species collected during the study. A window size of 100 nm was selected after investigating the influence of a range of window sizes (20, 30, 50, 75, 100, 150, 200, and 235 nm) which approximates linear multiplicative scatter correction (MSC) on the correction of a test dataset of 30 field spectra of Z. capricorni (Lake Illawarra, January 1999). The purpose of PMSC correction was to ensure that the field-measured reflectance spectra were comparable regardless of sampling date, illumination conditions, or sample geometry (Datt 1998).

**Data analysis**—A single-factor analysis of variance was applied at each wavelength over the range 430–900 nm to indicate the wavelengths where significant reflectance differences between seagrass species occurred. Analysis of variance was only performed on fouled leaves because they are more abundant in the upper canopy of seagrass meadows and are therefore more likely to dominate the field of view when imaged by an airborne or satellite sensor. For the purpose of assessing the merit of PMSC correction and to demonstrate that significant results were not simply an artifact of the correction procedure, analyses of variance were performed on both the raw uncorrected and PMSC corrected datasets. Prior to each analysis, the data at each wavelength were visually assessed for normality, although in general it is reasonable to assume a normal spectral response distribution for any homogeneous target material (Lillesand and Kiefer 1994). The data at each wavelength were also examined for homoscedasticity using Cochran’s C test (Winer 1971). At wavelengths where variances were heterogeneous, a square root transformation was applied to the data. If transformation failed to homogenize the variances, analysis was performed on the untransformed data. Where significant differences were found in the analysis of variance, multiple comparisons were made using the Tukey test for unequal sample sizes (Zar 1984). All univariate analyses were performed in S-Plus (MathSoft).

Multivariate techniques were used to compare the magnitude of intraspecific variation in spectral reflectance with interspecific variation. Reflectance differences within each seagrass species associated with the estuary, habitat, year, and season of sample collection were also examined by multivariate statistical analysis. Analysis was performed on the PMSC-corrected data using all samples collected during the study (i.e., all species both with and without epibionts) and on individual species subsets of the fouled and unfouled leaf samples. The datasets were reduced to include only the 430–700-nm range after preliminary analyses suggested that NIR wavelengths overpowered the contribution of visible wavelengths in the similarity matrix. Because NIR wavelengths are absorbed by water, it was important to ensure that any reflectance differences detected between factors were based only on the wavelengths that could be applied to remote sensing through a water column.

An association matrix was generated for each dataset using Bray–Curtis similarity and applied in nonmetric multidimensional scaling (nMDS) to produce two-dimensional ordination plots (Clarke 1993). Data were not transformed or standardized prior to generation of similarity matrices.

To test the hypotheses that the spectral reflectance of seagrass species differs, a nonparametric two-way analysis of similarity (ANOSIM) (Clarke and Warwick 1994) was performed on the full dataset of samples from all species, both with and without epibiont foulers. ANOSIM utilizes the Bray–Curtis similarity matrix to compare ranks of between-group to within-group similarities using a randomization test of significance in order to test the null hypothesis that the a priori groups do not differ (Clarke and Warwick 1994; Anderson 1999). One-way ANOSIM tests were used to investigate differences within each species of seagrass associated with the factors: year, season, estuary, and habitat. Pairwise permutation tests followed each ANOSIM to examine where
Fig 2. Network diagram summarizing the data preparation, data correction, and statistical analysis procedures undertaken in this study. PMSC, piecewise multiplicative scatter correction; nMDS, nonmetric multidimensional scaling; ANOSIM, analysis of similarity; SIMPER, similarity percentages; Z.c., Zostera capricorni; P.a., Posidonia australis; H.o., Halophila ovalis; +, leaves fouled by epibionts; −, unfouled.
the significant differences between the levels of the factors lay.

Similarity percentage (SIMPER) procedures were also applied to each dataset to identify the wavelengths that contributed most to the significant differences between species or factor groups (Clarke and Warwick 1994). The SIMPER algorithm determines the relative contribution that each wavelength (in this case) has made to the average similarity within a group and the average dissimilarity between groups. If a wavelength consistently contributes to within-group similarity between pairs of samples, as well as to between-group dissimilarity between pairs of samples, then its percentage contribution to similarity is high and it can be considered a good discriminating wavelength (Clarke and Warwick 1994). All multivariate analyses were done using the PRIMER 5 software (Plymouth Marine Laboratory).

Results

Spectral differences between seagrass species—The potential for spectral discrimination of unfouled seagrass species was apparent even before correction of the reflectance data to remove the effects of illumination and sample geometry. Differences were apparent in the relative magnitude of reflectance of green and NIR wavelengths by each species. It was somewhat more difficult to establish reflectance differences between species when the seagrass leaves were fouled by epibionts (Fig. 3B). Despite large overlaps in the standard deviations of the raw spectral curves, there were significant differences in the reflectance of fouled leaf samples (Fig. 3C; $F_{1,2,378} > 6$, $p < 0.002$ for all wavelengths, Fig. 3D; Cochran’s $C$ is not significant for wavelengths 530–900 nm; $0.01 < p < 0.05$ for wavelengths 472–529 nm; $p < 0.01$ for wavelengths 430–471 nm). However, there were only a few short wavelength regions where all three species were significantly different from each other (Fig. 3C). $P$. australis reflected consistently more light than the other two species in the shorter (430–520 nm) and NIR (700–900 nm) wavelengths, whereas $Z$. capricorni was significantly less reflective than the other two species over much of the green to red wavelength range (545–580 nm, 605–700 nm).

PMSC greatly reduced the variability in the spectral signatures of each species by removing albedo effects caused by the varying illumination and geometry of samples measured under constantly changing field conditions. The PMSC-corrected spectral reflectance curves (mean ± SD) for the three seagrass species were clearly separated across the green (500–600 nm), near red (600–650 nm), and NIR (700–900 nm) wavelengths for leaf samples without (Fig. 3E) and with epibionts (Fig. 3F). There was a highly significant difference between species for the fouled seagrass samples (Fig. 3G; $F_{1,1,2,371} > 24$, $p < 0.001$) at all wavelengths. The significant heteroscedasticity suggested by Cochran’s $C$ test for many of the wavelengths (Fig. 3H) does not greatly effect the significance of the result. At those wavelengths where square root transformation of the data generated homogeneous variances, $F$ values were reduced only very slightly (Fig. 3G). Hence, Type I errors are unlikely to have had any measurable effect given the strong significance of the results. A posteriori multiple comparisons indicated a significant difference in the spectral reflectance of all three seagrass species over many of the visible and NIR wavelengths (Fig. 3G).

Multivariate (nMDS) ordination of all PMSC-corrected seagrass samples, including leaves with and without epibionts (Fig. 4), added support to the results of univariate analysis. There was a clear separation of the seagrass samples into species groups along the primary ordination axis (global $R = 0.926$, $p = 0.001$), with all species significantly different in pairwise tests at $p = 0.001$. Within each species cluster, there was a gradation of samples from fouled to un-fouled along the second ordination axis (global $R = 0.901$, $p = 0.001$). Therefore, although leaf fouling has a significant influence on the spectral response within a seagrass species, it is apparent that the presence of epibionts does not affect the spectral discrimination between species.

Spectral differences within seagrass species: Spatial and temporal effects—$Z$. capricorni: There was no significant difference between years in the spectral response of fouled $Z$. capricorni (Fig. 5A; global $R = −0.003$, $p = 0.59$). The reflectance of this species was significantly affected by the season (global $R = 0.019$, $p = 0.027$), estuary (global $R = 0.125$, $p = 0.001$), and habitat (global $R = 0.302$, $p = 0.001$) in which leaves were sampled (Fig. 5B–D). A clear pattern of spectral separation was apparent in the distribution of marine and brackish samples across the first ordination axis, with little overlap between these habitats (Fig. 5D). On the other hand, although the ANOSIM revealed significant differences ($p = 0.001$) between all estuaries in pairwise comparisons (Table 1), the ordination plot comparing the reflectance of $Z$. capricorni leaves according to the estuary of sampling did not reveal a pattern of spectral separation across either nMDS axis (Fig. 5C). Similarly, no pattern of separation was evident in the nMDS ordination plot according to the season of sampling (Fig. 5B), and in fact, only spring and summer samples showed a significant reflectance difference in pairwise tests ($p = 0.022$). Inspection of the third ordination axis of three-dimensional nMDS plots did not aid further in the separation of sample groups.

It is important to note that ANOSIM is sensitive to heterogeneous multivariate dispersions among groups. Hence, a rejection of the null hypothesis that “the groups do not differ” may be due to a difference between group dispersion rather than (or in combination with) a difference in central location of the groups (Anderson 1999). Multivariate dispersion appears to correspond to some measure of the overall variability in spectral reflectance of a group of seagrass leaf samples. This would suggest that groups that differ only on the basis of their multivariate dispersion would probably not be discriminated by image classification procedures. For example, the spectral signatures (mean ± SD) of fouled $Z$. capricorni leaf samples from marine and brackish habitats show small but discrete differences in the magnitude of their green reflectance and red absorption (Fig. 6A), which could potentially be detected by remote sensing. On the other hand, the spectral signatures (mean ± SD) of fouled $Z$. capricorni leaves from different estuaries overlap to such an extent that spectral discrimination is unlikely (Fig. 6B).
Fig. 3. Mean ± SD spectral signatures of three seagrass species and the wavelengths where significant reflectance differences between species occurred. For raw uncorrected reflectance data: (A) spectra of unfouled leaf samples, (B) spectra of fouled leaf samples, (C) results of ANOVA and Tukey test at each wavelength for fouled leaf samples, (D) results of Cochran’s C test for homogeneity of variance of fouled leaf samples. For PMSC-corrected reflectance data: (E) spectra of unfouled leaf samples, (F) spectra of fouled leaf samples, (G) results of ANOVA and Tukey test at each wavelength for fouled leaf samples, (H) results of Cochran’s C test for homogeneity of variance of fouled leaf samples. Wavelength regions where $F$ values are represented as a solid line in (C) and (G) indicate that all species were significantly different from each other in pairwise tests. $F$ values represented as circles are regions where only one species was significantly different from the others; the species is labeled below the circles. Crosses represent $F$ values at select wavelengths after square root transformation of data. Solid lines in (D) and (H) indicate Cochran’s C values for untransformed data; dotted lines are for square root–transformed data. Tabulated critical $p$ values are denoted by horizontal reference lines.
Given that fouling did not affect the distinct spectral differences between seagrass species, it was not surprising that the presence or absence of epibionts generally had little influence on the reflectance differences within a species. Unfouled Z. capricorni leaf samples showed very similar patterns of spectral grouping according to year, season, estuary, and habitat of sample collection to those of the fouled leaf samples (Table 1).

*Posidonia australis:* There was no significant grouping of fouled *P. australis* leaf samples by the year of sample collection based on spectral reflectance in the 430–700-nm wavelength region (Fig. 7A; global $R = -0.017$, $p = 0.906$). Significant seasonal groups were observed (global $R = 0.076$, $p = 0.003$), with the summer sample group different from all other seasons and the autumn group different from samples collected in spring (Table 1). However, there was no apparent separation of seasonal groups across either ordination axis in the nMDS plot (Fig. 7B). Fouled *P. australis* were significantly grouped by estuary (global $R = 0.149$, $p \leq 0.001$). Some degree of separation in the reflectance of samples collected from Port Hacking and St. Georges Basin could be observed across the second axis of the ordination plot (Fig. 7C).

The patterns in reflectance associated with season and estuary for unfouled *P. australis* leaf samples were consistent with the results for fouled *P. australis* (Table 1). In contrast to fouled samples, unfouled *P. australis* leaves were significantly grouped by year (global $R = 0.129$, $p \leq 0.001$),
Table 1. Summary of ANOSIM and pairwise test results to determine within-species differences in the spectral response of seagrass leaves over the visible wavelengths (430–700 nm) associated with the year, season, estuary, or habitat of sample collection. “Separation” indicates a pattern of spectral separation in the nMDS plot. Factors that “overlap” are unlikely to be spectrally distinct.

|                | Year†   | Year Global R | Pattern | Season‡  | Season Global R | Pattern | Estuary§   | Estuary Significant pairwise | Habitat||   | Habitat Global R | Pattern |
|----------------|---------|---------------|---------|----------|-----------------|---------|------------|-------------------------------|-----------|-----|-----------------|---------|
| Z. capricorni  | Fouled  | −0.003 ns     | Overlap | 0.019*   | Overlap spr–sum* | 0.125*** | Overlap LI–PH*** | LI–SG*** | PH–SG***                   | 0.302*** Separation |
|                | Unfouled| −0.008 ns     | Overlap | 0.022**  | Overlap win–spr* | win–sum* | spr–sum**  | LI–PH*** | LI–SG*** | 0.249*** Separation |
| P. australis   | Fouled  | −0.017 ns     | Overlap | 0.076**  | Overlap win–sum** | aut–spr** | aut–sum* | spr–sum* | aut–spr* | aut–sum** | 0.149*** Separation | NA     | NA |
|                | Unfouled| 0.129***      | Separation | 0.044*   | Overlap | 0.071** Separation | NA | NA |
| H. ovalis      | Fouled  | 0.210***       | Separation | 0.029 ns | Overlap aut–spr* | win–aut* | win–spr** | win–sum*** | aut–spr** | aut–sum*** | spr–sum*** | −0.083 ns Overlap | NA     | 0.173*** Overlap |
|                | Unfouled| 0.129***       | Separation | 0.229*** | Separation | 0.028 ns Overlap | NA | 0.117*** Overlap |

Values are significant at the *p < 0.05, **p < 0.01, and ***p < 0.001 levels. ns, not significant.

† Year factors are 1999 and 2000 for investigation over a 2-yr period between May 1999 and January 2001.
‡ Season factors are winter (win), spring (spr), summer (sum), and autumn (aut).
§ Estuary factors are Lake Illawarra (LI), Port Hacking (PH), and St. Georges Basin (SG).
|| Habitat factors are marine and brackish.

although only a weak pattern of separation between year groups could be observed in the nMDS ordination plot.

Halophila ovalis: The spectral reflectance of fouled H. ovalis was significantly affected by the year (global R = 0.210, p ≤ 0.001) and habitat (global R = 0.173, p ≤ 0.001) from which the seagrass leaves were sampled. There was some degree of spectral separation between the samples from 1999 and 2000 across the primary axis of the nMDS ordination (Fig. 8A). In contrast, the marine and brackish samples were interspersed over the nMDS plot and could not be separated in any direction (Fig. 8D). There was no signifi-

![Fig. 6](image_url)

**Fig. 6.** The mean ± SD spectral signatures of fouled Zostera capricorni leaf samples from (A) marine and brackish habitats and (B) three estuaries. Reflectance has been graphed across the visible wavelengths only.
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Fig. 7. nMDS plots of the visible spectral response of fouled *Posidonia australis* leaf samples associated with (A) year, (B) season, and (C) estuary of sample collection.

Fig. 7. nMDS plots of the visible spectral response of fouled *Posidonia australis* leaf samples associated with (A) year, (B) season, and (C) estuary of sample collection.

Significant grouping of fouled samples according to season (Fig. 8B; global $R = 0.029, p = 0.068$) or estuary (Fig. 8C; global $R = -0.083, p = 0.883$).

The results of multivariate analysis of unfouled *H. ovalis* leaf samples were consistent with those for fouled leaves when investigating intraspecific reflectance differences associated with year, estuary, and habitat of sampling (Table 1), but this was not the case for the season of sample collection. The reflectance of unfouled *H. ovalis* leaves was strongly affected by season (global $R = 0.229, p \leq 0.001$) with samples from all seasons significantly different from all others (Table 1). A transition in reflectance from summer to winter could be observed in the distribution of samples across the primary and, to some extent, the secondary ordination axes of the nMDS plot. Patterns in the distribution of spring and autumn samples were less easy to detect because these groups were variable and tended to intersperse with the summer and winter samples. Because autumn reflectance lay more in the direction of the summer samples, this did allow for some separation from spring samples.

Wavelength selection for remote sensing of seagrass species—SIMPER analysis was used to select the wavelengths that contributed most to the dissimilarity in reflectance between pairs of species or between significantly different pairs of year, season, estuary, and habitat groups within each species. No single wavelength contributed more than 1% to the dissimilarity between species or 2% to the dissimilarity between factors within a species. The range of wavelengths offering discrimination between and within seagrass species proved to be similar for all species combinations irrespective of the presence of epibionts. Hence, individual SIMPER results were pooled to demonstrate the value of each wavelength in the detection of any spectral differences between (Fig. 9A) and within species (Fig. 9B).

Strong and consistent differences between seagrasses occurred across the green wavelengths 530–580 nm, with additional discrimination in the regions 520–530 nm, 580–600 nm, and 686–700 nm (Fig. 9A). The wavelengths 580–606 nm were of particular importance in the discrimination of fouled *P. australis*, whereas *Z. capricorni* and *H. ovalis* could be separated on the basis of their absorption of the red wavelengths 665–680 nm.

The red (637–700 nm) and green (522–574 nm) wavelengths were particularly effective at discriminating within-species differences (Fig. 9B). Maximum separation by year, season, estuary, or habitat for any species occurred across the wavelength regions 549–556, 649–686, and 692–700 nm. The wavelengths 430–438, 496–505, and 580–614 nm contributed only to reflectance differences in fouled *P. australis* leaves. The region between 588–602 nm was, however, very important in the discrimination of estuary and seasonal differences in this species. Similarly, important reflectance differences within fouled *Z. capricorni* were observed across the wavelengths 550–570 nm.

Discussion

*Spectral discrimination of seagrass species*—The three common seagrasses of southeastern Australia were clearly spectrally distinct over wide regions of the visible wavelengths regardless of the presence of leaf epibionts and despite small but significant within-species variability related to the year, season, estuary, or habitat of sample collection. This research has produced the first comprehensive spectral library of aquatic plant species that takes into account the range of spectral variability expected for the “pure” reflectance of each species under natural conditions. Libraries such as this supply appropriate reference spectra for many
hyperspectral classification and radiative transfer modeling procedures and assist greatly in image waveband selection. Because the three seagrasses are spectrally distinct at the level of leaf reflectance, there is a solid baseline from which remote sensing of these species can begin.

The optimal wavelengths for the discrimination of seagrass species (530–580 nm) and some other important regions of spectral separation (520–530 nm, 580–600 nm) conveniently lie within the range of wavelengths that are least attenuated by coastal waters (Fig. 10). The red wavelengths also penetrate shallow water sufficiently to allow detection of the chlorophyll absorption features of different species (665–680 nm). In southeastern Australia, seagrasses rarely grow deeper than 3 m, and the meadows, although small, are usually dense and monospecific. It should therefore be possible to map the seagrasses of this region to species level in all but the most turbid of estuaries.

**Advantages of PMSC correction**—Although significant differences between species were observed in raw spectral

![Fig. 8](image1.png)

**Fig. 8.** nMDS plots of the visible spectral response of fouled *Halophila ovalis* leaf samples associated with (A) year, (B) season, (C) estuary, and (D) habitat of sample collection.

![Fig. 9](image2.png)

**Fig. 9.** Sum percentage contribution made by each visible wavelength toward the significant dissimilarities (A) between seagrass species and (B) between significantly different year, season, estuary, and habitat groups within each seagrass species. Totals include only the percent contribution made by wavelengths contributing to the upper 50% of dissimilarity between groups in pairwise SIMPER analyses. Results for fouled and unfouled data were pooled.
content could also benefit from the use of this simple trans-
plant growth, health, and productivity by estimating pigment
[Wang et al. 1998]).
Physics' modular airborne imaging spectrometer, MAIS
borne visible–infrared imaging spectrometer AVIRIS [Rich-
continuous hyperspectral imagery (e.g., NASA JPL's air-
image data and can therefore be used for spectral matching
classiﬁcation. PMSC-corrected spectra can be resampled or
binned to match the resolution of any narrow or broadband
classiﬁcation. PMSC offers a means of generating spectral libraries
from ﬁeld-based spectral measurements that are equivalent
to those collected under controlled laboratory conditions. In
addition, because PMSC-corrected spectral signatures retain
the shape and magnitude of reﬂectance of the raw spectral
curves, they are most suitable for use in spectral libraries
that will be applied as reference data for modeling or image
classiﬁcation. PMSC-corrected spectra can be resampled or
binned to match the resolution of any narrow or broadband
image data and can therefore be used for spectral matching
classiﬁcation of noncontinuous imagery, as well as contin-
uous hyperspectral imagery. In contrast, although derivative
analysis has proven valuable for identifying spectral features
characteristic of different species of corals, macroalgae
(Hochberg and Atkinson 2000), wetland plants (Wang et al.
1998), eucalypts (Kumar and Skidmore 1998), and phyto-
plankton (Richardson et al. 1994; Aguirre-Gómez et al.
2001), derivatives can only be used in the classiﬁcation of
continuous hyperspectral imagery (e.g., NASA JPL's air-
borne visible–infrared imaging spectrometer AVIRIS [Rich-
ardson et al. 1994] or the Shanghai Institute of Technical
Physics' modular airborne imaging spectrometer, MAIS
[Wang et al. 1998]).
Hyperspectral remote sensing procedures to determine
plant growth, health, and productivity by estimating pigment
content could also beneﬁt from the use of this simple trans-
formation. Datt (1998) found that simple linear MSC im-
proved estimation of the chlorophyll content of eucalypt
leaves using single-wavelength reﬂectance, ﬁrst derivatives,
and simple reﬂectance ratios. The PMSC method is generally
superior to MSC, particularly where scatter variation is large
in comparison to chemical variation and where the spectra
have relatively broad and strongly nonselective peaks (Is-
aksson and Kowalski 1993). These conditions are typical of
the spectral signatures recorded from plants, particularly
those collected in the ﬁeld where illumination conditions are
continually changing over time and space.

Determinants of reﬂectance—Seagrass leaf structure and
pigment content: Maximum separation in the spectral reﬂec-
tance of the three seagrass species occurred at NIR wave-
lengths (720–900 nm) where differences between species at
the leaf level can be explained by differences in surface qual-
ity (Woolley 1971) and internal structure (Gausman et al.
1969; Woolley 1971). For example, the size, arrangement,
and abundance of leaf internal air canals called lacunae
(Tomlinson 1980) vary considerably among seagrass species
and are likely to have an important inﬂuence on the reﬂect-
ivity of leaves.

NIR reﬂectance could be very useful for mapping inter-
tidal seagrasses exposed at low tide or for detecting floating
mats of leaves and algae, but these wavelengths are rapidly
attenuated by water (Fig. 10). Of greater importance are the
visible wavelengths that can be used to remotely sense ben-
thic vegetation through a water column. There were consist-
tent differences in the visible spectral response of the three
seagrass species that can be attributed to constant differences
in the relative concentrations of photosynthetic and acces-
sory pigments in their leaves. The broad region of blue ab-
sorption is the sum of the absorption features of both Chl
a and Chl b and a range of carotenoids that extend absorp-
tion to shorter wavelengths of the visible spectrum (Gausman
1982). Seagrass species differed signiﬁcantly in their capac-
ity for blue light absorption, but the differences were much
smaller and might be more difﬁcult to detect remotely than
the differences that occurred in other visible wavelengths. P.
australis and Z. capricorni could be distinguished from H.
ovalis on the basis of total chlorophyll content by the
strength of their red light absorption in the broad trough
centered near 675 nm. However, the spectral regions where
seagrass species are most distinct are those associated with
their reﬂectance of green and near-red light rather than their
absorption troughs. The magnitude of green and red reﬂec-
tance is based not only on the lack of absorption of these
wavelengths, but on the total and relative concentrations of
chlorophylls, carotenoids, and accessory pigments. Large
concentrations of any predominant pigment, including chlo-
rophyll, will reduce reﬂectance at all visible wavelengths, as
well as deepening and broadening the absorption features
associated with that pigment (Curran et al. 1991). A decrease
in chlorophyll concentration relative to carotenoid content or
a sufﬁcient increase in the concentration of carotenoids or
accessory pigments might mask the green color of chloro-
phyll and increase reﬂectance in wavelengths dependant on
the color of the dominant pigment (Curran et al. 1991). Pre-
liminary pigment analysis has shown Z. capricorni to con-

![Graph](image-url)
tain high concentrations of the photoprotective accessory pigments anthocyanins (unpubl. data). Anthocyanins absorb the wavelengths between 500 and 550 nm and reflect across a wide region near 600–640 nm (Gausman 1982). In southeastern Australia, the red colors of the anthocyanins are most obvious in the immature leaves of *Z. capricorni* but are retained as a dark bronze color in the adult leaves. It is likely that *Z. capricorni* requires the photoprotection offered by anthocyanins because it grows at shallower depths than the other two seagrasses (Dawson and Dennison 1996). Because of the combined influence of relatively high concentrations of both chlorophyll and anthocyanin pigments, the reflectance of *Z. capricorni* lacks a distinct green peak and is significantly lower than that of *P. australis* and *H. ovalis* across all visible wavelengths. Proportionately high levels of carotenoids in *H. ovalis* (unpubl. data) can be observed in the yellow color of immature and senescent leaves, although chlorophyll appears to mask these pigments in the green adult leaves. *H. ovalis* reflects less green light and more red light than *P. australis*, apparently because of higher carotene levels and lower chlorophyll content.

**Influence of epibionts:** Because mature seagrass leaves are rarely observed in temperate Australian estuaries without a moderate to heavy growth of epibionts, the spectral reflectance of fouled leaf samples can be considered to be most applicable to the development of spectral libraries for remote sensing purposes. Epiphytes might be beneficial to seagrasses if they reduce dessication damage by trapping water or increase the levels of nutrients available to the seagrass (Penhale and Smith 1977). However, fouling is most often detrimental to the seagrasses because shading by epiphytic algae interferes with photosynthesis (e.g., Sand-Jensen 1977; Fong et al. 2000). Epibionts are most abundant at the tops of the oldest leaves, whereas younger leaves at the center of the seagrass plant are more likely to be bare (May et al. 1978). Differences observed in the spectral signatures of fouled and unfouled seagrass samples can therefore be related to differences in the age and vigor of the leaves, as well as to the contribution that algal epiphytes make to the spectral response.

Fouled and unfouled seagrass leaves did display significantly different patterns of reflectance, but the clear spectral differences between seagrass species were not diminished by the presence of epibionts. Fouling did not change the comparative magnitude of reflectance for any of the species over the visible–NIR wavelengths, although it did have an influence on the shape of their spectral curves. Epibionts tended to reduce seagrass reflectance at the green peak while increasing variability in the reflectance of green wavelengths. The red chlorophyll absorption trough was deepened, but the most obvious effect of fouling was to increase reflectance in the 575–630 nm region.

The spectral responses of the seagrass species were not affected equally by the presence of epibionts. Fouling changed the spectral signature of *H. ovalis* much less than it did for *Z. capricorni*, and particularly for *P. australis*, in which epibionts produced a distinctive reflectance peak centered around 590 nm. Although leaf epiphytes are not host specific, certain algal taxa can be associated with particular seagrass species because of leaf size, shape, and turnover time (Borowitzka and Lethbridge 1989). *P. australis* supports a higher diversity of epiphytic algae because its leaves are stronger and retained by the plant for a longer time. The coralline alga *Corallina officinalis* was a ubiquitous epiphyte of *P. australis* leaves, often covering 80–90% of the leaf surface of mature leaves. This red algae would be most responsible for the near-red reflectance peak observed in the spectral signature of fouled *P. australis* because of the carotenoids and biliproteins that distinguish the Rhodophyta (Rowan 1989). In addition, significant quantities of brown algae (Phaeophyta) and diatoms (Bacillariophyceae), both distinguished by the carotenoid fucoxanthin which absorbs in green wavelengths, and other red algal species would have contributed to the strength of this reflectance peak. *Z. capricorni* supports a lower diversity of epibiont species than *P. australis* because of its smaller blade size. The cover and composition of algal species on the leaves is also more variable because this seagrass grows under a wider range of environmental situations. Coralline algae and other red and brown algal macrophytes were abundant on *Z. capricorni* leaves in marine habitats. Under brackish conditions, epibiont cover varied from almost no fouling to a heavy load of predominantly diatoms with infrequent occurrences of large quantities of green algae (Chlorophyta). The overall influence of fouling on the spectral response of *Z. capricorni* is similar but less pronounced than for *P. australis* (i.e., raised levels of red reflectance, enhanced green light absorption, and a small but distinctive reflectance peak centered around 590 nm). In contrast, the small, soft leaves of *H. ovalis* support low densities of only a few small epibiont species, notably diatoms.

**Intraspecific variability in reflectance**—Intraspecific differences were observed in the spectral signatures of seagrass leaves collected from different years, seasons, estuaries, and habitats in southeastern Australia. Interannual changes were apparent in the spectra of *H. ovalis* and unfouled *P. australis*, whereas distinct seasonal changes could only be observed in the spectral response of fouled *H. ovalis*. The estuary of sample collection clearly affected the spectral signatures of *P. australis*. For the adaptable seagrass *Z. capricorni*, leaf samples from marine and brackish sites proved to be spectrally distinct. Spatial and temporal variations in light, and nutrient availability, water temperatures, salinity levels, and the degree of water movement around plants influence growth, photosynthesis (Hillman et al. 1989), and therefore the spectral response of seagrasses. The ability of a seagrass species to adapt and maintain photosynthetic vigor under a range of different environmental conditions will also influence the direction of spectral change and the level of variability observed in the reflectance of that species.

Declines in photosynthetic rate because of environmental stress are often accompanied by reductions in chlorophyll pigments, increases in various carotenoids, changes to the Chl *a*/*b* ratio (Demmig-Adams and Adams 1992), and in-
increases in nonphotosynthetic accessory pigments (Barker et al. 1997). For example, seagrass plants exposed to low light intensities have higher total chlorophyll content (Alcoverro et al. 2001) and lower Chl/ab ratios (Longstaff and Denison 1999). Total chlorophyll content can be higher in seagrasses growing in relatively eutrophic areas compared to those at oligotrophic sites (Alcoverro et al. 2001). Anthocyanins are often transient and can be induced by a variety of environmental stresses, including exposure to high levels of ultraviolet-B and visible light (Mancinelli 1985), cold temperatures, and stress (Chalker-Scott 1999).

Environmental conditions will also influence the biomass and species composition of epibionts that contribute to the spectral reflectance of seagrass leaves. For example, many algal epiphyte species cannot tolerate extreme fluctuations in salinity and water temperatures (King 1981) that commonly occur in the shallow backwaters of estuaries. May et al. (1978) noted that higher pollution levels reduced the diversity of algal epiphytes on P. australis and Z. capricorni and suggested certain "indicator" species of clean and less than pristine environments. The sensitivity of epibionts to environmental conditions might contribute to spectral differences within a species, but they do not allow for any further spectral separation that is not already provided by differences within the plant leaves themselves. The intraspecific reflectance differences associated with year and season are masked by their presence.

**Waveband selection and application**—Although it appears that the three seagrass species could be discriminated on the basis of their green reflectance alone, the signals sensed from an airplane or satellite will contain different information from that collected by ground spectroradiometry. This will mainly be due to scattering and absorption of light in the atmosphere and water column, the reflectance of light from the water surface, the density and geometry of the seagrass canopy, and the background effect of the substrate. Hence, there are further challenges for researchers in applying the spectral response of field-measured leaf samples to real mapping situations. It would be an advantage to utilize as many of the distinct spectral features that characterize species as is practical when selecting remote sensing imagery.

An appropriate band set for remote sensing should target the range of absorption and reflectance features that characterize each species, whether fouled or unfouled. For discrimination of the seagrass species of southeastern Australia estuaries, a band set could include narrow (5–15 nm) bands centered around the following.

1. The major peaks of reflectance and troughs of absorption for the seagrass photosynthetic and accessory pigments (500, 550, 620, and 675 nm). An alternative is to place two bands near the shoulders of wide peaks or troughs to gain more information (e.g., 530 and 560 nm for the green peak).
2. The major peaks of reflectance and troughs of absorption for characteristic epiphyte photosynthetic and accessory pigments (575, 590, and 640 nm).
3. At least one region where separation between species is poor, to use as a reference wavelength where possible (e.g., 440 nm).
4. One NIR waveband to assist in discrimination of plant matter at the surface of the water (e.g., floating seagrass and algal mats).

The actual number and width of the bands might not be critical (Thomson et al. 1998), but bands should not be wider than the peak or trough they represent and must not overlap with the spectral information provided by other features. Successful mapping of intertidal macroalgae has been performed using as few as five wavebands (Zacharias et al. 1992). If the spectral response of the plant species expected in a remote sensing mapping project have not already been characterized (by ground radiometry, for example), it might be better to collect image data across a larger number of narrow bands (Bajjouk et al. 1996; Kumar and Skidmore 1998). Feature reduction techniques can then be applied during processing, if necessary.

The specific selection and placement of narrow wavebands require a programmable imaging spectrometer, such as the CASI, or a sensor with a suitably narrow spectral resolution and appropriate band centers, bandwidths, and interband center distances. Rollings et al. (1993) recommended using the CASI after pilot studies indicated its suitability for the remote sensing of benthic cover, water quality, and water depth. Because this airborne hyperspectral sensor can also provide image data of high spatial resolution, it is well suited for mapping the often small and patchy seagrass meadows that occur in the estuaries of New South Wales. Fixed band sensors such as HyMap, AVIRIS, and Hyperion (NASA TRW Inc.) (with bandwidths of 15, 9, and 10 nm, respectively) will perhaps be less suitable unless their band positioning coincides with the relevant visible spectral features, despite the large number of bands they offer.

The accuracy of benthic vegetation maps will improve with the use of spectral libraries that describe the natural variability in the reflectance of the species involved. This type of spectral data is particularly effective when used in conjunction with spectral modeling techniques (e.g., Jupp et al. 1996; Anstee et al. 2000). Species discrimination in the remote sensing of vegetation is therefore achievable as long as the species under study are spectrally distinct over space and time. The methodology employed in this study demonstrates a repeatable approach for assessing the capability for spectral discrimination of aquatic or terrestrial vegetation species.

Intraspecific differences in seagrass reflectance might be much more difficult to detect by remote sensing because these differences are very small. The significant differences in field-measured spectra associated with estuary, habitat, season, and year of sampling do suggest some potential for the monitoring of seagrass health, water quality, and therefore estuary condition. However, whether such small reflectance differences can be detected by remote sensing will depend on a wide range of factors, including the signal:noise ratio and the spatial, spectral, and radiometric resolution of the sensor; the clarity, depth, and constituents of the water column and atmosphere; and the density and homogeneity of the seagrass meadow itself. For example, Lubin et al. (2001) modeled...
up-to-atmosphere spectral radiance at the air–water interface and the top-of-atmosphere near-nadir radiance from in situ reflectance spectra for coral species, sand, and algae over a range of water depths to test the utility of Landsat TM satellite data for coral reef mapping. Satellite imagery was not able to detect the spectral features that were successfully used to distinguish coral species in ground or aircraft data (Lubin et al. 2001). In contrast, Anstee et al. (2000) and Jupp et al. (1996) have been able to map seagrasses and other benthic plant species using radiative transfer models in conjunction with airborne imagery. Future research will involve subjecting the library of PMSC-corrected seagrass spectral signatures to radiative transfer modeling of atmospheric and water column effects for comparisons with the range of signatures observed in airborne and satellite image datasets.

**References**


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