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Spectral definition of the macro-algae *Ulva curvata* in the back-barrier bays of the Eastern Shore of Virginia, USA

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We have developed methods to determine the visible (VIS) to near-infrared (NIR) spectral properties of thalli and epiphytes of bloom-forming and green macrophyte *Ulva curvata* in back-barrier lagoons in Virginia, USA. A 2% increase in NIR thalli reflectance from winter to summer (ca. 9.5%) matched the drop in summer NIR transmittance (ca. 90%). In contrast, summer and winter VIS reflectance (reaching 6%) were nearly identical while winter transmittance (ca. 85%) was 10–20% higher. NIR absorption remained at 5% but VIS absorption increased by 10–20% from winter to summer. Replicate consistency substantiated the high transmittance difference indicating thallus composition changed from summer to winter. Epiphytes increased thallus reflectance (<ca. 4%) and decreased transmittance (<ca. 10%) and exhibited broadband VIS and NIR absorptions in summer and selective peaks in winter. A simulation coupling water extinction with thallus reflectance and transmittance found seven submerged thalli maximized the surface reflectance enhancement (ca. 2.5%).

1. Introduction

Our work aims to support a large-scale and long-term monitoring programme on marine macrophytes in the back-barrier island lagoons along the seaside of Virginia's Eastern Shore (figure 1). By developing new optical techniques to assess the status of the dominant macrophytes, we hope to be able to advance the detection and mapping of these macrophytes throughout the Delmarva Peninsula lagoons. *Ulva curvata* (hereafter, *Ulva*) is amongst the most abundant algae throughout Virginian lagoons and the sister species (*Ulva* spp.) are known to dominate in temperate, shallow water lagoons throughout the world (in particular, under nutrient-enriched conditions); thus, our first specific objective was to define its spectral properties.

From a remote-sensing perspective, the reflectance of macrophytes is the key property to quantify for mapping. Factors of illumination, atmosphere or satellite sensors

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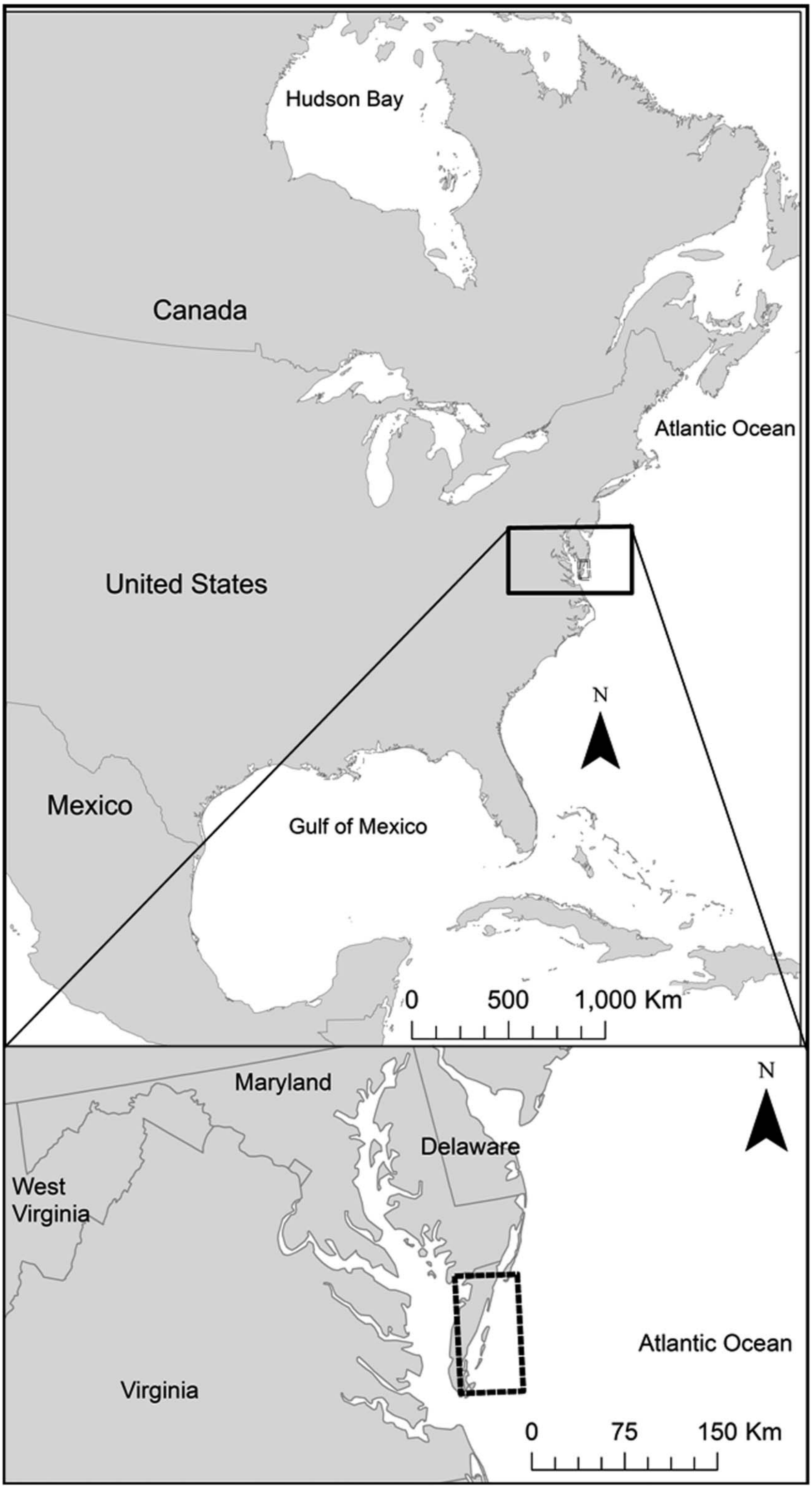

Figure 1. The box on the lower figure locates the study area on the seaside of Virginia's eastern shore that includes the Delmarva Peninsula lagoons.

aside, diminished and variable spectral contrast lowers the ability to detect macrophyte presence and map macrophyte differences based on type or biophysical factors. Water turbidity attenuates detection at depth and limits the spectral contrast between the overlying water and macrophyte (Dekker *et al*. 2005, Kutser *et al*. 2006). Similarly, inorganic materials and detritus that settle onto the macrophytes (referred to as fouling) and surface organic films (hereafter referred to as epiphytes) alter the surface obscuring an accurate representation of the unobstructed macrophyte reflectance (Malta *et al.* 2003, Kutser *et al.* 2006). As in water turbidity, foreign surface materials lower the spectral contrast of the macrophyte diminishing the performance of field-validated and deterministic remote-sensing mapping.

Performance can be improved when *a priori* spectral information is used to stratify the landscape into spectral components (e.g. Ji *et al*. 1992, Dekker *et al*. 2005, Brockmann and Stelzer 2008). Without *a priori* information, mapping success depends on the conditions at the time of data collection and the ability to compile sufficient field observations pertinent to the range of environmental conditions existent at that instant in time. Strategic mapping requires repeatable classifier protocols based on *a priori* spectral information that are adjustable to varying environmental conditions. Preferably, spatial information extracted from the same data stream would be used to adjust protocols and provide detection limits for conditions at the time of collection.

In order to build a strategic mapping program, an accurate rendition of *Ulva* thalli spectral properties is required. This rendition would start with the expected thallus response (e.g. reflectance, transmittance) and its expected variability. Those basic thallus spectral properties could then be modified by established fouling and epiphyte spectral influences and subsequently aggregated to simulate the common physical structure of thalli in the lagoon environment. Finally, these aggregated responses could be adjusted by different depths of submergence and water turbidities or by different durations of surface exposure at low water stands. This basic spectral information would provide an expectation of *Ulva* thalli spectral contrast in different lagoon environments and, in turn, advance field-validated remote-sensing detection and deterministic macrophyte mapping. Both advances will ultimately limit the number of required field measurements and improve the determination of macrophyte type and condition.

Reflectance spectra inferred from measurements of macrophytes above the water surface or on thalli laid out on a flat plate have provided an extensive spectral library (Kutser *et al*. 2006). In order to quantify thallus reflectance, however, the combined returns from multiple layers submerged within the water column or used in flat-plate thalli recordings must be corrected to a single thallus layer (Ramsey *et al*. in press). This is particularly pertinent to the two-cell-thick thallus (figure 2). In layered thalli recordings, the number and magnitude of the contribution layers largely depend on the thallus transmittance. The higher the thallus transmittance at a selected wavelength, the potentially higher the number of contributing layers.

While transmittance is necessary to correct reflectance recordings of layered thalli, knowledge of thallus transmittance is also necessary to estimate the contribution and condition of successively lower thallus layers in submerged thalli. In the water column, the thalli are structured in layers, with progressively deeper layers containing higher concentrations of pigments due to shading (Malta *et al*. 2003). Adequate knowledge of transmittance will aid in mapping macrophyte biomass and in determining the macrophyte condition and trends. Transmittance measurements are also more directly relatable to thallus condition than reflectance measurements. Thallus surface reflections, as a portion of the obtained reflectance, do not contain information about

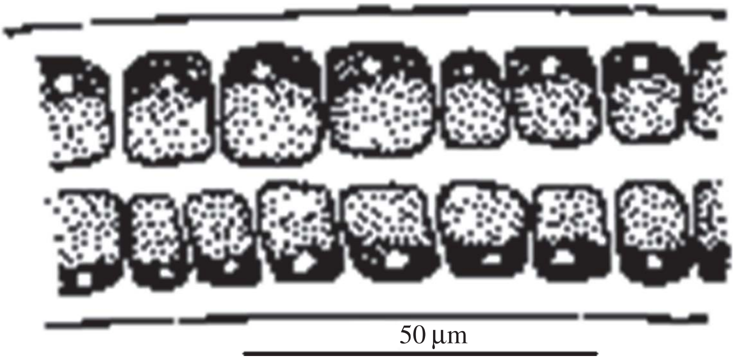

Figure 2. An illustration of an *Ulva curvata* leaf cross-section showing the two cell layers (adapted from Humm, Harold J. *The Marine Algae of Virginia*. p. 216. © 1979 by the Rector and Visitors of the University of Virginia).

the internal structure and turgidity, while transmittance is an integrated product of the internal optics and surface transmissions.

Assessment activities that include direct spectral monitoring of macrophyte condition also require measurements that supply thalli pigment information via calculations of absorption (Malta *et al*. 2003). Thallus absorption can be estimated from transmittance and reflectance measurements per wavelength (λ) as

$$\%\,\text{Absorption}\,(\lambda) = 1 - [\%\,\text{Reflectance}\,(\lambda) + \%\,\text{Transmittance}\,(\lambda)]. \tag{1}$$

Thallus absorption in the visible (VIS) wavelength region is the optical feature most closely aligned with pigments and concentration changes (e.g. Ramsey and Rangoonwala 2005). Spectral signatures based on selective absorption provide unique indicators of pigment types contained in the thallus (Malta *et al*. 2003) and, thereby, are potent determinants of the macrophyte type and condition.

In order to obtain accurate and consistent thallus diffuse reflectance, transmittance and, by combination, absorption, we developed new and standardized measurement techniques. In that development, we tested the sensitivity and influence of thallus drying on measurement consistency and the presence and absence of epiphytes. In addition, we tested the ability to discern epiphyte pigment composition in order to reveal epiphyte identification and aid environmental assessment monitoring (Malta *et al*. 2003). As part of the lagoon monitoring programmes, we applied the standardized techniques to provide the thallus mean spectral response and variance in the summer and winter seasons. With this basic spectral information, we provided an expectation of the *Ulva* mat spectral contrast at a single depth of submergence and water lagoon turbidity. That mapping simulation coupled an estimate of the water extinction coefficient derived from a secchi measurement with the reflectance and transmittance of submerged layered thalli mats.

2. Methods

Ulva thalli were collected during the summer (September 2007) and winter (January 2008) seasons. Summer samples were collected along an inland tidal bank exposed at low tide (figure 1, box outline). Winter samples were collected while the thalli were submerged in the water column. To minimize changes in thallus optics due to possible plant stress, the summer and winter samples were kept wet and aerated under cool conditions until spectral measurements were taken. Most summer *Ulva* thalli were discarded due to an abundance of small pinholes. All measurements were completed within 2.5 days of sample collection. Fluorescence contributions were not considered.

2.1 *Spectral recording equipment*

For single-thallus recordings, we used a LI-COR 1800-12 integrating sphere attached to a Spectron Engineering SE590 radiometer (LI-COR, Inc. 1984, Spectron Engineering, Inc. no date). The LICOR sphere has a 14.5 mm diameter sample port illuminated by a lamp with a 11.4 mm diameter beam. To ensure a spectrally stable output, at least 15 s were allowed for lamp warm-up prior to a new measurement. In all measurements, the source lamp was turned off at the end of each set of spectral measurements to minimize lamp warming of the thallus samples. The radiometer processes light energy from 400 to 1100 nm in 252 bands centred ca. every 2.6 nm (nominal spacing) and each with a 10 nm bandwidth (Markham *et al*. 1995).

The methods described in Ramsey and Rangoonwala (2004, 2005) as adapted from Daughtry *et al*. (1989) were used for collecting transmitted and reflected diffuse light intensities and reference recordings for each thallus sample. Reflectance of the repacked barium sulfate reference is reported to be 0.98 at 680 nm (LI-COR, Inc. 1984). During the single-thallus recordings, measurements were recorded for sphere-wall reflectance factor and dark-current calculations. These results were used to correct reflected and transmitted light measurements prior to normalization by the reference recording. These corrected and normalized light measurements became the thallus reflectance and transmittance spectra. Thallus reflectance measurements followed the definition for directional hemispherical reflectance as defined by Nicodemus *et al*. (1977).

On average, three to five replicate measurements were obtained for each measurement. Single-thallus absorption spectra were calculated from the set of reflectance and transmittance sample spectra (equation (1)). The single-thallus error estimate was calculated by propagation of the error associated with each input variable used in the reflectance and transmittance calculation. The average reflectance, transmittance and absorption spectra for summer and winter thallus were calculated as the mean of all single-thallus sample means per season. In the same way, the seasonal mean errors were calculated as the variance about each summer and winter overall mean.

2.2 *Mount construction and sample selection*

The two-cell-thick summer and winter thallus samples were mounted on a support design adapted from Ramsey and Rangoonwala (2005) (figure 3). The specialized design provided support for thallus samples covering the entire inner mount (figure 3). Only undamaged thalli without grazer scars, bite-marks or grazer-induced holes were used. The addition of damaged samples would have dramatically increased the reflectance variability.

2.3 *Summer thallus sample preparation*

Before beginning our summer spectral measurements, we conducted preliminary tests to define whether spectral measurements were sensitive to the sample preparation

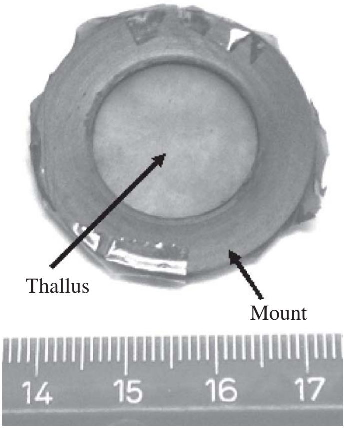

Figure 3. The specialized mount constructed to hold the thin thalli firm during the spectral measurements. The ruler tick marks are in mm.

method or the time between mounting and measurement. First, each thallus sample was rinsed under gently flowing distilled water to remove fouling and excess surface water was allowed to evaporate before initial spectral measurements commenced. To qualify how delaying the measurements beyond the point of excess surface water evaporation (in effect, advancing thallus drying) influenced the spectral results, we repeated recordings every 15 to 30 minutes after initial recordings on three separate samples. Second, to determine to what extent epiphytes influence spectral measurements, we removed the samples from the mount for cleaning and subsequent remeasurement. Cleaning entailed softly rubbing the leaf between two fingers under gently flowing distilled water in order to remove surface epiphytes. After fouling and epiphyte removal, spectral measurements were then obtained from 15 summer thallus samples at the moment of surface water dissipation.

2.4 *Winter thallus sample preparation*

Summer preparation tests showed that both measurement delay and removal of epiphyte film affected spectral properties. To further quantify those results, we performed spectral measurements of five additional thallus samples from the winter collection. As with the summer samples, all winter thallus samples were first rinsed in distilled water to remove fouling before being mounted. All spectral recordings were obtained only after evaporation of excess water from the thallus surface.

In the first set of measurements, five thallus samples without epiphyte film removal were entered into a series of measurements to estimate the effects of elapsed time since surface water dissipation on spectral reflectance and transmittance. On each thallus sample, up to five sets of spectral recordings were sequentially repeated every 15 to 30 minutes after the initial dissipation of surface water.

After the completion of these delay measurements on the samples without epiphyte film removal, each of the samples was removed from the mount, the epiphyte film removed and the sample remounted for subsequent measurements. The set of sequential time-delay measurements was again repeated every 15 to 30 minutes. After epiphyte removal and during the time-delay experiment, two of the five original samples were discarded because of tears appearing within the sample. As replacements, two additional samples were added and the progressive set of measurements taken before and after epiphyte removal initiated. In contrast to the original five samples, however, these samples were not removed from the support for epiphyte removal prior to the second set of measurements. This method of epiphyte removal provided inconsistent spectral results and, consequently, measurements associated with these additional samples were discarded. The three pairs of reflectance, transmittance and absorption spectra associated with winter samples before and after epiphyte film removal were differenced. These differences were used to estimate epiphyte spectral properties and to infer their contributions to the thallus spectral properties. Three similar sample pairs from the summer collection were also used to estimate summer epiphyte contributions.

2.5 *Surface reflectance of submerged Ulva thalli*

In order to approximate the detection limit of submerged *Ulva* mats, water extinction estimates were coupled to thallus reflectance and transmittance. The extinction-coefficient estimates were derived from vertical Secchi measurements in a tidal river connected to the lagoon system during the summer *Ulva* collections. The Secchi depth (SD) of 0.85 m, used in the light-extinction estimation, was obtained at an optically deep point in the river (no bottom reflectance contributions) on the shady side of a pier when the sun elevation was 51°. Although SDs contain uncertainty due to changing environmental conditions, the operator and other factors, the estimation of the extinction coefficient (K) from SD by using the empirical relationship $K = 1.7/\mathrm{SD}$ (1.7 is unitless) was shown to be commonly applicable in freshwater and seawater (Idso and Gilbert 1974). A form of the Beer–Lambert law was used to relate the estimated extinction coefficient to the light attenuation from an intensity just below the water surface of I_0 to an intensity of I_Z at a depth of Z as

$$I_Z/I_0 = \mathrm{e}^{-KZ}. \tag{2}$$

The depth of the first thallus layer (Z) was set at 0.45 m and SD was set at 0.85 m ($K = 2.0\ \mathrm{m}^{-1}$) for the simulation. The same expression was used to transfer the reflected light at depth upwards to just below the water's surface. This simplistic derivation includes multiple sources of errors, including the different geometries in the downward versus upward light distributions and, in the measurement of SD, the specific applicability of the empirical 1.7 value (Idso and Gilbert 1974). These calculations, however, are only intended to provide instructive limits to detection of submerged *Ulva*, particularly when accounting for the layered nature of *Ulva* thalli.

The reflectance from the layered thalli was computed from flat plate recordings of sequential additions of thallus layers (Ramsey *et al.* in press). The single-thallus reflectance and transmittance spectra of a typical thallus sample were entered into a relationship derived by Lillesaeter (1982) that estimated the multiple reflectance and transmittance contributions to the resultant radiation recorded at the top thallus layer. In our approximations, neither scattered light additions from below nor light attenuations and additions from lagoon water between the thallus layers were considered. Sky light contributions to the water-surface reflections were not used in these simulations. In addition, a water volume reflectance spectrum based on radiometer measurements collected at the same place and near the same time as the secchi measurement that was used in this simulation was included for comparison.

Downwelling sunlight irradiance, measured with a handheld radiometer (Ramsey *et al.* 1992) at the time of the SD recordings, was transmitted through the water surface (0.978 transmittance factor) and then to the depth (0.45 m) of the thallus layer(s) with equation (2). The resultant irradiance, reflected upwards from the combined thallus layers at depth, was then transmitted again to just above the water surface by using equation (2) (again including a 0.978 transmittance adjustment). The irradiance above the surface contributed by the thallus layer(s) was divided by the downwelling sunlight irradiance at the top water surface, providing a reflectance estimate. That reflectance estimate solely corresponded to the contribution of a single thallus layer or multiple thallus layers at depth. Also, by substituting the unwashed–washed reflectance difference calculated at each sequential thallus layer addition for the unwashed resultant reflectance spectra, the surface reflectance associated solely with the epiphyte was simulated.

3. Results

3.1 *Thallus sample cleaning*

Single-thallus transmittances of winter samples after epiphyte removal tended to be higher than before removal, with spectral differences ranging from nearly zero to near 5% transmittance (figure 4(*a*)). In one sample, transmittance differences reached around 10% in the blue wavelengths (400–500 nm). All transmittance spectra were nearly aligned in the red and red-edge wavelengths around 660–720 nm.

Winter samples devoid of epiphyte film exhibited lower reflectance spectra than the same samples without epiphyte removal above 500 nm (figure 4(*b*)). Reflectance differences between before and after epiphyte removal ranged from 0.5% to 3% reflectance near 550 nm and then tapered to about zero near 660 nm. As with transmittance

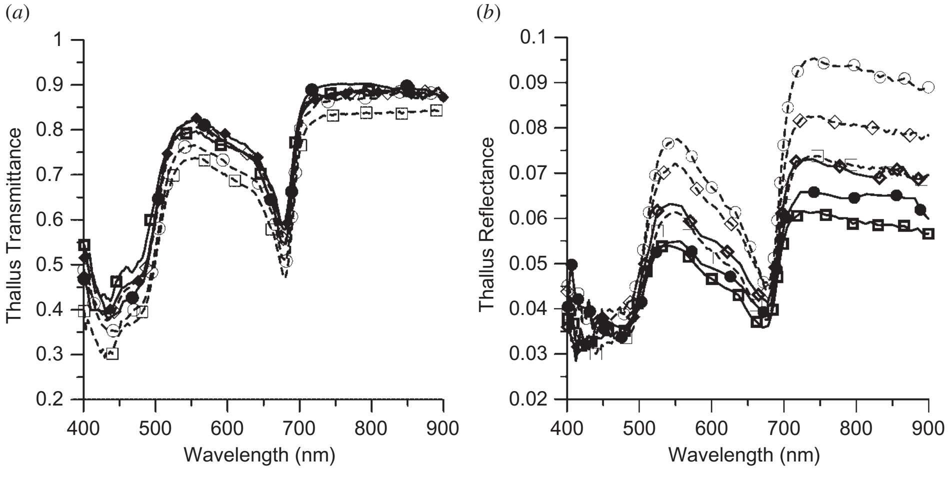

Figure 4. Epiphyte removal experiment. (*a*) Transmittance and (*b*) reflectance of winter thallus samples are shown as solid lines and symbols after epiphyte removal and as dashed lines and open symbols before epiphyte removal, respectively. The same symbol designates the same sample in (*a*) and (*b*).

spectra, all sets of reflectance spectra nearly overlapped in the red and red-edge wavelength region. Before- and after-removal differences again increased in the near-infrared (NIR) (here 700–900 nm) from about 1% to 3% reflectance for individual samples.

3.2 *Measurement time delay*

Results of the time-delay experiments found transmittance spectra of samples without epiphyte removal grouped around 3–5% lower, in comparison to the initial spectra, in two sample sets and nearly unchanged in the third (figure 5(*a*)). In two of the three sample sets including spectra without epiphyte removal, reflectance reached between

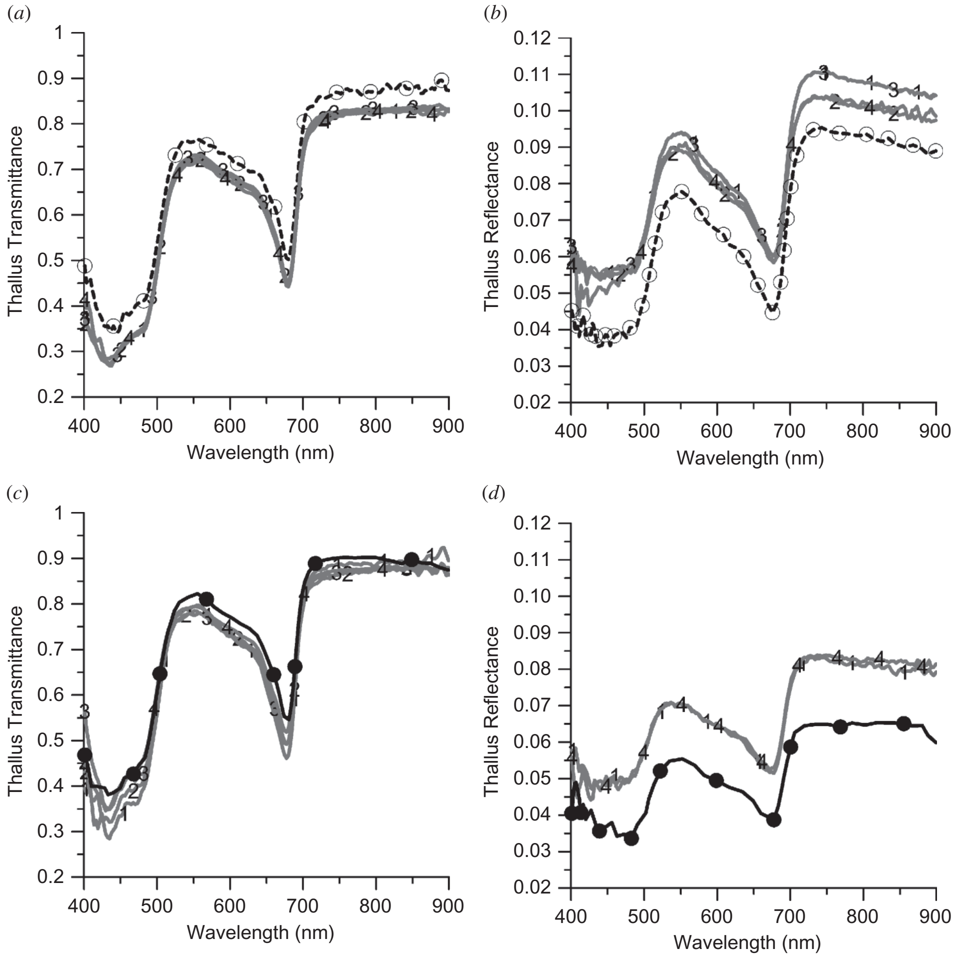

Figure 5. Time-delay experiment results for one of three sample sets. (*a*) Transmittance and (*b*) reflectance of single winter thallus samples before epiphyte removal, (*c*) transmittance and (*d*) reflectance for the same samples after epiphyte film removal. Initial spectral measurement are shown (1) before epiphyte removal – dashed lines and open circles, and (2) after epiphyte removal – solid lines and circles. Grey lines represent spectral results after time delay since the initial spectra were obtained. The associated numbers signify increasing time delays. Some spectra were eliminated because of high measurement noise or sample damage.

1.5% and 2% higher, while the third reflectance spectra grouped 4% and 5% higher than the initial spectra (figure 5(*b*)). In samples after epiphyte removal, transmittances decreased from 1% to 5% with delay in measurement (figure 5(*c*)) and reflectance spectra closely grouped from 1.5% to 3% higher than the initial spectra in all three sample sets (figure 5(*d*)). Results of the time-delay experiment showed that initial and delayed reflectance spectra were more closely grouped after epiphyte film removal than without epiphyte removal.

3.3 *Epiphyte spectral properties*

Epiphyte reflectance spectra exhibited somewhat similar shapes between summer and winter (the difference of before and after epiphyte spectra). Broad minima were centred in the blue and red (600–700 nm), a broad maximum was centred in the green (500–650 nm) and an elevated plateau was defined in the NIR (figure 6(*a*)). Although

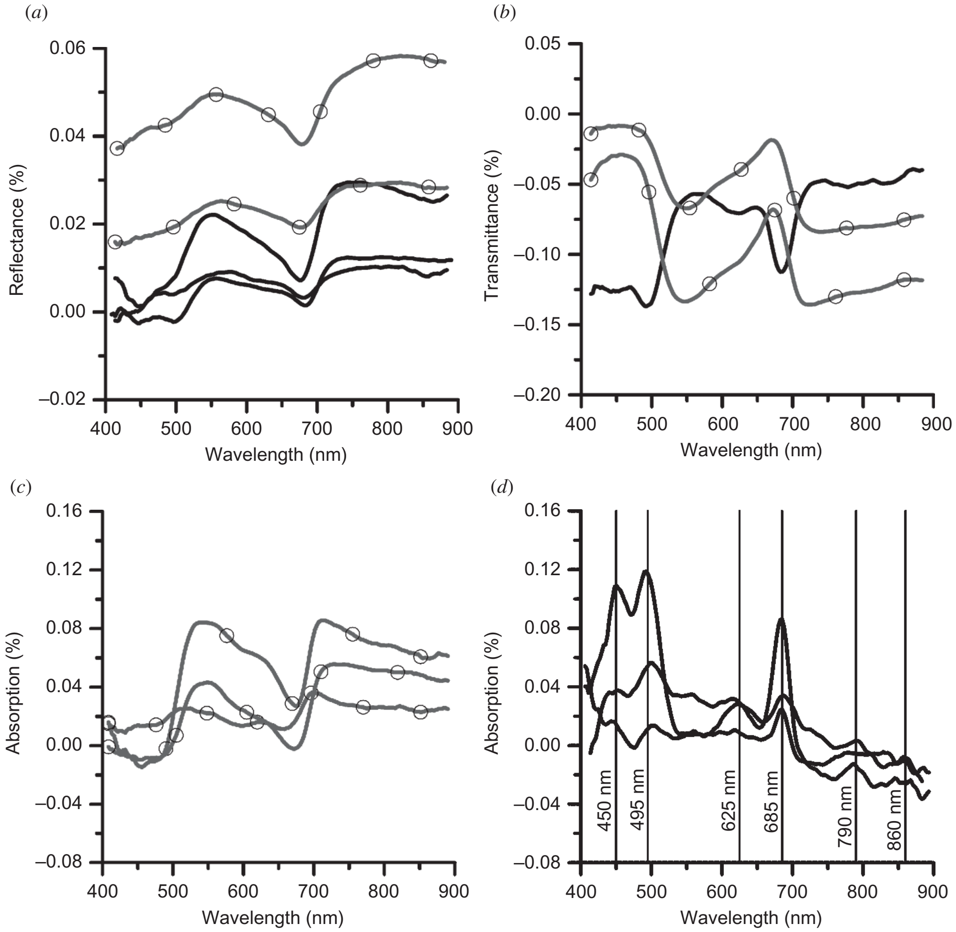

Figure 6. Surface epiphyte (*a*) reflectance, (*b*) transmittance, (*c*) absorption in summer and (*d*) absorption in winter samples. Grey lines and open circles denote summer samples while black lines denote winter samples. For clarity, reflectance and transmittance lacking spectral features and near zero were not included.

clearly apparent, these spectral features were not distinct, except possibly in one winter reflectance spectrum. Reflectance magnitudes of summer epiphyte samples tended to be higher than those of winter samples. Transmittance spectra of summer epiphyte samples indicated that the epiphyte surface layer broadly depressed transmittance starting in the green and extending into the red band (500–650 nm) and again in the NIR (figure 6(*b*)). One winter spectrum, however, exhibited nearly the reverse transmittance pattern. In contrast to the summer spectra, this single winter spectrum indicated that surface epiphyte depressed transmittance broadly in the blue and moderately in the high red bands.

The epiphyte absorption spectra of summer samples largely complemented the transmittance spectra showing broad absorption centred on the green extending to middle-red and the NIR absorption plateau (figure 6(*c*)). The summer absorption spectra had noticeable minima in the blue and high red and a shoulder peak near 630 nm. The winter spectra contrasted highly with the summer absorption spectra. Although absorption magnitudes varied widely, distinct peaks were noticeable in all three winter epiphyte spectra (figure 6(*d*)). These peaks were in the blue at 450 and 495 nm, in the red at 625 and 685 nm and more subtly in the NIR at 790 and 860 nm.

3.4 *Thallus spectral means and variation between summer and winter samples*

The seasonal mean and variance (represented by standard errors) exhibited differences between the summer and winter samples (figure 7(*a*)–(*c*)). Slight differences in the mean VIS reflectance spectra of summer and winter samples were well within calculated variances, while the mean summer NIR reflectances were 2–3% higher than those of the winter, well above the less than ±0.5% seasonal variances. The NIR transmittance difference mirrored the NIR reflectance difference, although in the opposite direction. Seasonal variances were well below the 2–3% NIR transmittance difference. In the VIS, the overall mean transmittance of summer samples was 10–20% lower than those of the winter samples. Again, the variance about the seasonal means was less than ±1% transmittance. As expected, the mean thallus absorption was highest in the blue and red wavelength regions. In the VIS, a broad absorption minimum was centred at 550 nm in the green wavelengths. The absorption mean difference between summer and winter samples was 10–20% absorption. The NIR exhibited the least absorption, averaging around 5% in both summer and winter.

3.5 *Surface reflectance of submerged Ulva thalli*

The simulated water-surface reflectance spectra exhibited a progressive increase from 1% to 3% at 550 nm with the sequential addition of thallus layers (figure 8). The simulation was based on an unwashed single-thallus reflectance and transmittance, the two-way light attenuation through the water column based on equation (2) and the predicted reflectance of thallus layers. After seven thallus layers, there was no noticeable change in above-surface reflectance. In comparison, the maximum surface reflectance contribution from the seven-layer free-floating thalli mat was of the same order as the observed water volume reflectance in the green (3.4% at 551 nm) and red wavelength regions but around 1.5% lower in the blue (figure 8).

The epiphyte reflectance spectra, derived from the same *Ulva* thallus sample used in the layered simulation, exhibited a lower and different spectral pattern than exhibited by the *Ulva* thallus layer(s). After addition of two thallus layers, the broad epiphyte

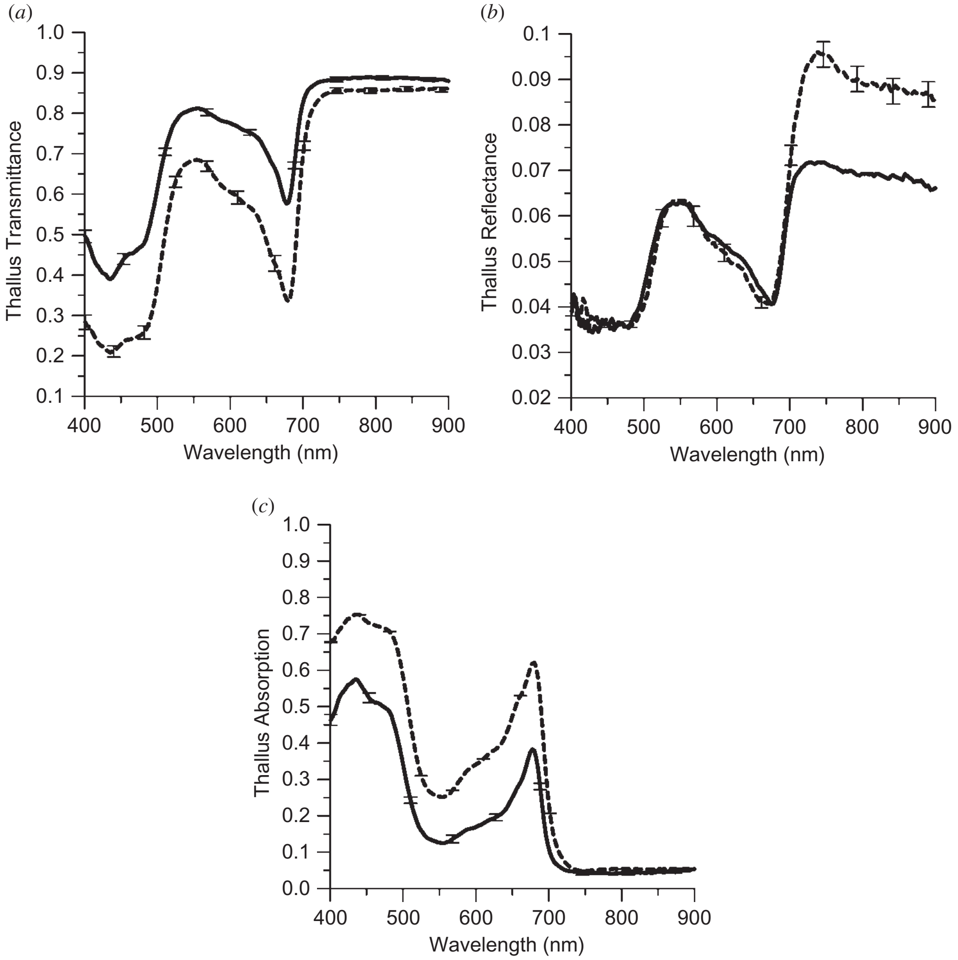

Figure 7. Average thallus transmittance, reflectance and absorption spectra of summer (dashed line, $n = 15$) and winter (solid line, $n = 5$) samples. Error bars denote one standard deviation about the mean.

reflectance band centred nearer 575 nm reached a peak of 0.3% and subsequently decreased with additional layers. The low epiphyte magnitudes were accompanied by a higher relative-noise component.

4. Discussion

Surface epiphyte film removal prior to spectral measurements dramatically changed the reflectance and transmittance spectra magnitudes in most samples. The magnitude of change depended on the spectral influence of the epiphyte. Although the final spectra between samples still varied, removal of the surface film tended to produce more consistent results than did non-removal results. In addition, epiphyte removal while the sample remained in the mount produced less consistent results than removing the sample from the mount for epiphyte removal. While an explanation was not identified,

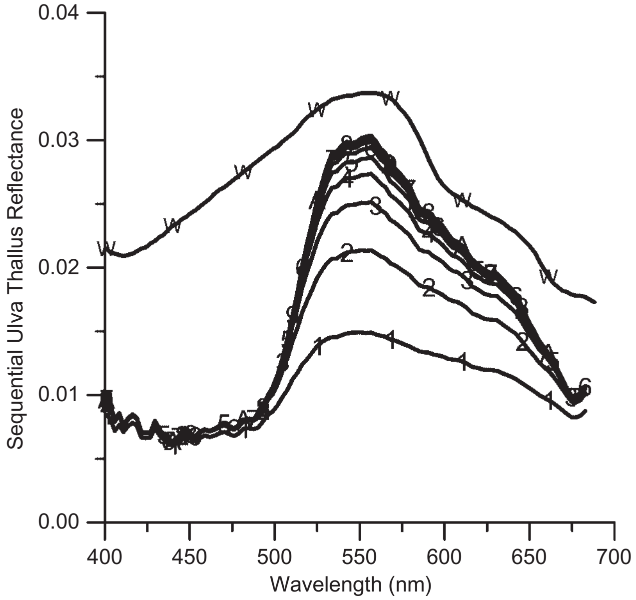

Figure 8. Estimated surface reflectance due to submerged *Ulva* thalli. Numbers refer to the sequential addition of thallus layers (e.g. one layer '1'). Reflectance spectra and associated symbols from 7 to 10 (designated with an 'A') layers overlay and are not clearly distinguishable. The water volume reflectance obtained from thalli free and optically deep water is designated with a 'W'. Reflectance estimates are most appropriate at 550 nm.

removing the thallus from the sample support most likely guaranteed a more thorough elimination of the epiphyte surface film.

Even though less varied after epiphyte removal, spectral variability still existed, thus providing a more valid rendition of thallus spectral variety within the summer and winter seasons. Reflectance differences between single-sample means reached 1.5% reflectance in the VIS to NIR (VNIR) wavelengths in both the summer and winter samples. Transmittance differences between single-sample means in both the summer and winter seasons tended to be lower in magnitude than reflectance differences, even though transmittance magnitudes could be 10 or more times higher than reflectance. This higher consistency may be due to the lack of thallus surface reflections in the transmittance versus their inclusion in the reflectance measurements.

The spectral difference between samples before and after surface film removal was an indication of the spectral influence of the epiphyte covering. The film cover increased the reflectance (>500 nm) and decreased the transmittance throughout the spectral range. Differences between spectra were used to estimate epiphyte contributions to the thallus reflectance, transmittance and absorption spectra. Three of the six spectra of epiphyte reflectance and transmittance indicated little influence on the thallus spectral properties. Of the remaining spectra, epiphyte surface films on summer and winter samples tended to broadly increase reflectance magnitudes in the green to the mid-red and the NIR plateau. These same spectra exhibited blue-band and red-band (~675 nm) minima. As expected from the epiphyte reflectance, transmittance spectra of summer samples indicated that epiphyte depressed thallus transmittance broadly in the green and middle-red and NIR. One winter transmittance spectrum, however, indicated nearly the reverse.

Epiphyte absorption spectra of summer samples tended to follow the summer reflectance and transmittance spectra: relatively broad and high-absorption bands in the green to mid-red and the NIR plateau. This broad absorption from about 500 to 650 nm closely reproduced the shapes of two of the three absorption spectra observed by Malta *et al*. (2003). These same two spectra also exhibited a minimum in the red region; however, neither included a blue minimum nor a shoulder peak near 630 nm as observed in our spectra. A third spectra observed by Malta *et al*. (2003) exhibited a broad blue maximum, a broad green minimum and a red peak from about 650 to 680 nm. Absorption spectra of winter samples exhibited the same absorption maxima and minima but with higher detail. Two prominent peaks are defined in the blue maximum at 450 and 495 nm, indicative of many chlorophyll, carotene and bacterium pigments (Gitelson *et al.* 1997). A green minimum is clearly defined, as well as an additional absorption peak near 625 nm and a prominent peak near 685 nm, often indicative of chlorophyll-*a* and accessory pigments (Gitelson *et al*. 1999a). Although more subtle, two additional peaks are evident at 790 and 860 nm, which are absorption regions normally indicative of bacteria (Gitelson *et al*. 1999b, Zaar *et al*. 2003).

Delaying spectral measurements after mounting thallus samples and dissipation of surface water tended to decrease transmittance and increase reflectance. Samples before and after epiphyte removal responded similarly; however, spectral changes brought about by the measurement delay associated with samples after epiphyte removal were more likely less than 2% rather than up to the 4% differences exhibited by samples without removal. In all but one sample, most change occurred in the first 30 minutes after the excess water had dissipated from the thallum. Changes in the transmittance magnitudes were most often nearly the same as their complementary reflectance changes; however, given the high difference in magnitudes, the relative change in reflectance was over a magnitude higher than the relative change in transmittance.

Single-thallus samples were processed following methods that were determined to provide the highest consistency and the most accurate estimates of spectral properties. Differences between the reflectance means of summer and winter samples were contained solely in the NIR. Changes in reflectance related to internal structure are normally sought to provide plant status information, such as turgidity and maturity. In the summer and winter thallus samples, the higher summer NIR reflectance transformed into an almost equally lower summer NIR transmittance. This nearly equal but opposite change is fairly common in leaves in the NIR. The equal but opposite difference culminated in constant and equal summer and winter NIR absorption magnitudes.

Not reproduced in the reflectance spectra means was the much higher VIS transmittance of winter samples; this was from 10% to 20% higher than that of summer samples. The VIS region is most influenced by the absorption characteristics and concentrations of pigments and accessory materials. Although not exact, transmittance changes in the VIS are normally reflected in the VIS reflectance spectra as well. The lack of reflectance difference between summer and winter samples may indicate high absorbance of pigments in the two cell layers. Strong absorption could largely limit reflectance to the surface of the first cell layer but transmitted light would interact with the two cell layers and the internal leaf structure. In this case, transmittance would be modified by thallus internal structural changes while reflectance would not. Thallus transmittance would in turn solely control the absorption decrease as observed in the summer versus winter samples. Although the reason for the high change in VIS transmittance not accompanied by a complementary change in the VIS reflectance cannot be clearly determined, it is clear that if detecting a change in thalli condition solely relied on reflectance, then this change would not have been noticed.

The high transmittance of the thallus has direct implications within natural conditions. Malta *et al*. (2003) found that five to seven thallus layers are typical when thalli are free-floating in the water column. Each subsequently deeper layer would experience irradiance (light) that has been diminished and spectrally filtered via the overriding layers and the intermediate water optical properties. In consequence, upper layers would experience a different light field than lower layers with each extreme potentially constraining growth (Malta *et al*. 2003). The ability to define the thallus transmittance provides a better prediction of light transfer through the water column containing thallus layers and, in turn, better linkage between the returned light field measured at the above-water-surface sensor and the thallus structure.

Considering the repeatable precision of correcting for atmospheric influences, water-surface reflections and other factors, the surface reflectance spectra simulated for one thallus layer (~1% at 550 nm) would most likely lie below the detection limit within the variability of lagoon water turbidities. The maximum increase of 3% in surface reflectance was from seven thallus layers at 0.45 m. The same number of layers that defined the maximum *Ulva* contribution to the above-water-surface reflectance matched the number of typically observed free-floating thallus layers (Malta *et al.* 2003). The maximum increase of 3% at 550 nm was of the same order of magnitude as water volume reflectance obtained in optically deep lagoon waters without *Ulva* thalli. While increasing the surface reflectance, the *Ulva* thalli eliminated contributions from the water column below the *Ulva* layers. By using the same extinction coefficient used in the submerged *Ulva* thalli surface reflectance contributions, we estimated 80% of the water volume reflectance would be contributed from the top 0.45 m of the water column (two-way attenuation). At 550 nm, a 3% contribution from *Ulva* mats at 0.45 m below the water surface would combine with the water volume reflectance above 0.45 m to increase the observed water-surface reflectance from 3.4% up to 5.7%. A similar calculation estimated a less than 0.5% increase in the blue wavelength region and a progressively decreasing absolute contribution from *Ulva* thalli to surface reflectance with increasing wavelengths in the red region. The actual balance between eliminating deeper water volume returns and surface reflectance enhancement by the *Ulva* thallus layers, however, would depend on the relative absorption and scattering strengths of water column. As stated previously and demonstrated by the less than 0.3% epiphyte contribution to the surface reflectance, determination of epiphyte type and pigment composition, as an aid to environmental monitoring, is limited to laboratory analyses of field samples.

5. Conclusion

We found that the removal of surface films (epiphytes) on the thallus surface and the time delay between sample preparation and spectral measurements impacted the spectral magnitudes by up to 4%. Epiphyte removal prior to the spectral measurements reduced magnitude differences associated with time delay to less than 2%. To avoid surface epiphyte influences and differences caused by measurement delay, we removed the surface epiphyte and initiated spectral recordings at the time of surface water dissipation.

The overall reflectance means of summer and winter samples differed by 2–3% in the NIR, while VIS differences were insignificant within the maximum mean errors of ±0.5%. The higher summer NIR reflectance nearly equalled the decrease in NIR transmittance thus representing a fairly normal result in the NIR. Thallus absorption in the NIR was nearly equal and low at around 5% in both seasons. Even though VIS reflectance spectra did not change from summer to winter, VIS transmittance of thallus increased by 10–20%. The VIS transmittance increase dominated the summer to winter absorption decrease. Although the reason the thallus reflectance did not exhibit a similar pattern was not clearly determined, the change in absorption indicated that the thallus composition and structure changed from summer to winter. This change would not have been detected in mapping submerged *Ulva* thalli via measurements taken above the water surface.

The influence of surface epiphytes on thallus spectral properties could either impede or enhance the mapping of *Ulva* condition. With both the summer and winter samples, surface epiphytes tended to broadly increase thallus reflectance (<ca. 4%) and decrease transmittance (<ca. 10%). Within these broad VNIR trends, the magnitude and selectivity of changes were highly variable. In summer samples, epiphyte absorption spectra tended to follow thallus reflectance and transmittance spectra. In the late winter samples, epiphyte absorption spectra displayed prominent peaks in the blue and red (indicative of many chlorophyll, carotene and bacterium pigments) and, although more subtle, in NIR regions normally indicative of bacteria. Although differences existed and our spectra included higher detail, similar epiphyte absorption spectra shapes were observed in a study of *Ulva* spp. mats (Malta *et al*. 2003). Due to the high water absorption in the NIR, epiphyte features were not reproducible on simulations of submerged *Ulva* thalli contributions to the water-surface reflectance. Epiphyte feature monitoring may be more useful when mapping exposed thalli and when conducting environmental surveys to assess the status of *Ulva*.

The thallus transmittance also had implications for understanding and mapping the dynamics and layered structure of the thalli. Although VIS reflectance and transmittance from one to 10 thallus layers within the water column were simulated, the spectral selectivity of the downward irradiance illuminating the sequentially lower thallus layers related to pigment concentrations and type were not within the scope of this study. Even so, the simulation did indicate that the *Ulva* contribution to surface reflectance was limited to around 3% at 550 nm and as observed by Malta *et al*. (2003) to around seven thallus layers. This maximum reflectance magnitude of 3% approached the green reflectance magnitudes of surface-water volume in optically deep and *Ulva*-free lagoon waters. Accounting for the elimination of water volume backscatter below the submerged mats, a 3% reflectance contributed by *Ulva* thalli was estimated to increase the above-surface reflectance by up to 2.7%. Surface reflectance enhancements progressively decrease with wavelength increase in the red and were less than 0.5% in the blue. Although surface reflectance would be most likely to be enhanced by the presence of *Ulva* thalli, the magnitude of that enhancement would depend on the relative absorption and scattering strengths of the lagoon water at the time of the remote-sensing data collection.

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