RESEARCH PAPER:

Estimation of Foliar Pigment Concentration in Floating Macrophytes using Hyperspectral Vegetation Indices

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Abstract. Foliar pigment concentrations of chlorophylls and carotenoids are important indicators of plant physiological status, photosynthesis rate and net primary productivity. Although the utility of hyperspectral derived vegetation indices for estimating foliar pigment concentration has been documented for many vegetation types, floating macrophytes have not been assessed despite their ecological importance. This study surveyed 39 wetland species (12 floating macrophytes (FM), 8 grasses/sedges/rushes (GSR) and 19 herbs/wildflowers (HWF)) to determine whether foliar pigments concentrations could be estimated from hyperspectral reflectance. Hyperspectral reflectance of samples was recorded using an ASD FieldSpec3 Max portable spectroradiometer with the plant probe attachment or via a typical laboratory setup. A semi-empirical relationship was established using either a linear, second-degree polynomial or logarithmic function between 13 candidate vegetation indices and Chl-a, Chl-b, Car and Chl-a+b pigment concentrations. Vegetation indices R-M, CI-Red and MTCI were strongly correlated with foliar pigment concentrations using a linear fitting function. Chl-a+b and Chl-b concentrations for all samples were reasonably estimated by the R-M index (R^2 : 0.66 and 0.64), although Chl-a and Car concentration estimates using CI-Red were weaker (R^2 : 0.63 and 0.51). Regression results indicate that pooled samples to estimate individual foliar pigments were less correlated than when each type of vegetation type was treated separately. For instance, Chl-a+b was best estimated by CI-Red for FM (R^2 : 0.80), MTCI for HWF (R^2 : 0.77) and R-M for GSR (R^2 : 0.67). Although floating macrophytes feature unique adaptations to their aquatic environment, their foliar pigment concentrations and spectral signatures were comparable to other wetland vegetation types. Overall, vegetation indices that exploit the red-edge region were a reasonable compromise that had good explanatory power for estimation of foliar pigments across the sampled wetland vegetation types with CI-Red the best suited index for floating macrophytes.

Keywords: floating macrophyte, chlorophyll, vegetation indices, pigment concentration estimation, wetland vegetation types

1.0 Introduction.

Foliar photosynthetic pigments are the dominant influence upon the amount of solar radiation absorbed by leaves. Of these, chlorophyll-a (Chl-a) and chlorophyll-b (Chl-b) absorb the greatest fraction of photosynthetically active radiation and supply the majority of the energy for the reaction of photosynthesis (Ustin et al. 1998). Hence, foliar concentrations of Chl-a and Chl-b (Chls) often control the carbon fixed per unit radiation intercepted and are strongly related to net primary productivity (Dawson et al. 2003). Other important photosynthetic components include the carotenoids (Cars), which are essential structural components of the photosynthetic antenna and reaction centre complexes (Bartley and Scolnik 1995). Cars have dual photosynthetic and photoprotective roles, coincidentally contributing energy to photosynthesis and dissipating excess energy through the xanthophyll cycle to protect the reaction centres.

Since foliar concentrations of Chls and Cars are linked to the photosynthesis rate (Daughtry et al. 2000) quantifying their concentration at the leaf and canopy levels has a range of applications including indicating the plants physiological status (le Maire et al. 2004). Foliar Chls concentration also provides an indirect estimate of plant nutrient status since their molecular structure incorporates a large proportion of total leaf nitrogen (Moran et al. 2000). Quantifying individual pigment concentrations are also valuable, since individual pigments respond differently to changing environmental conditions. For instance, the ratio of Chl-a to Chl-b increases during leaf senescence which is induced by the decline in the daily photoperiod (Fang et al. 1998). Hence, individual measures of foliar pigment concentrations can provide useful insights into plant–environment interactions (Richardson et al. 2002).

Traditional wet chemistry techniques for measuring foliar pigment concentrations require destructive sampling involving extracting pigments with a solvent and subsequent spectrophotometric analysis. These procedures are costly, laborious and hence can only provide limited spatial coverage (Gitelson et al. 2005). Alternatively, measures of reflected radiation are a non-destructive method for quantifying pigment concentration due to the unique spectral absorbance features of foliar pigments manifesting as the dominant constituents of leaf reflectance. Many studies have shown that surface reflectance of intact leaves are directly correlated with leaf chemical composition (Jacquemoud et al. 1996) and surface reflectance is often used as indicators of photosynthetic function in the remote sensing of vegetation (Field et al. 1994). Provided hyperspectral data of sufficient resolution is available (usually bandwidths of 10nm or less (Broge and Mortensen 2002)), Chls and Cars concentration can be estimated for a diverse range of vegetation types based on the establishment of a semi-empirical relationship between laboratory measured pigment concentration and hyperspectral reflectance.

As hyperspectral data volumes are quite large and contain many collinear bands, the whole hyperspectral spectrum is often reduced to a smaller feature space. Feature selection approaches have included principal components (Yao and Tian 2003), factor analysis (Coops et al. 2002), artificial neural networks (Chen et al. 2007) and stepwise multiple regression (Osborne et al. 2002). In addition, vegetation indices exploiting two to four bands are often used as they are computationally simple and are reasonably applicable to estimate foliar pigments at the leaf scale (Sims and Gamon 2002, Gitelson et al. 2003, le Maire et al. 2004), and canopy scale (Thenkabail et al. 2002, Dash and Curran 2004), while NDVI also outperformed a partial least squares regression model based on the whole spectrum for winter wheat (Hansen and Schjoerring (2003).

Floating macrophytes are an important component of wetland ecosystems as they foster biological diversity and their high growth rates provide an abundant foodstuff that many species

depend upon (Chapman et al. 2001). They also filter excess nutrients and thus reduce the onset and severity of eutrophication. Certain species also hyperaccumulate toxic trace elements and thus play a considerable role in wastewater treatment, biomonitoring of pollutants and phytoremediation (Outridge and Noller 1991, Rahman and Hasegawa 2011). Conversely, floating macrophytes can also interfere with biogeochemical processes, ecological functioning and human utilization of water bodies as a consequence of their prolific productivity (Kateregga and Sterner 2007). For instance, dominant stands of *Trapa natans* often induce hypoxic conditions. Reduced dissolved oxygen concentrations cause stress for juveniles in key breeding grounds (Burlakova and Karatayev 2007) and may induce increased atmospheric emission of carbon dioxide and methane, becoming a net greenhouse gas source over the long term (Pierobon et al. 2010). However, a comprehensive examination of floating macrophytes as regulators of trace gasses has not been conducted and many questions regarding their biogeochemical affects remain.

In addition to their varied importance, floating macrophytes feature a variety of unique morphological adaptations to the aquatic environment. Many of these adaptations reflect the species different ecological niches, nutrient acquisition strategies or are related to buoyancy or protection from wave action. These adaptations influence the spectral reflectance at the species level, where considerable variation has been documented between floating macrophytes (Anderson 1995). For instance, Khanna et al. (2011) exploits biophysiological differences to differentiate between three mat-forming floating macrophytes in the Sacramento–San Joaquin Delta. In spite of the species expressing a wide range of phenotypic variability, a decision trees classifier could distinguish each species with 71–88% accuracy due, in part, to variations in short-wave infrared, red edge index and near-infrared reflectance. Tilley et al. (2003) also documents differing average leaf hyperspectral reflectance in the emergent macrophytes *Typha latifolia* and *B. frutescens*, where the blue and red wavebands had a strong negative correlation to water column total ammonia. The negative correlation indicates there was more chlorophyll present when ammonia levels were higher and that *B. frutescens* responded more strongly than *T. latifolia*, likely related to its greater nutrient uptake ability. Aside from plant response environmental stimuli, reflectance is also sensitive to specialized plant adaptation's (e.g. waxy leaf surfaces in desert plants increase reflectance to guard against photo-inhibition of photosynthesis (Robinson et al. 1993)) and can vary independently of pigment concentration (Sims and Gamon 2002). Hence, floating macrophyte adaptations to the aquatic environment may introduce confounding factors that cause both hyperspectral reflectance and pigment concentrations to vary to a greater degree between floating macrophyte species than between species of other vegetation types.

Despite their unique traits, floating macrophytes have received less attention than other vegetation types, perhaps due to the logistical challenges posed by the wetland environment (Lee and Lunetta 1996). The utility of several vegetation indices for foliar pigment estimation has been documented for many other types of vegetation including crops (Zarco-Tejada et al. 2005), forests (Sampson et al. 2000) and heterogeneous grasslands (Darvishzadeh et al. 2008). Nonetheless, previous research is primarily empirical. The developed relationships are unique to a specific vegetation type under defined environmental conditions and are thus non-transferable. Consequently, to meet the requirements related to estimation accuracy and consistency, there is a need for the identification of specific vegetation indices that are sensitive exclusively to floating macrophytes. In addition, it is desirable to avoid the need for a semi-empirical relationship for each wetland vegetation type. However, no studies have assessed the difference in explanatory power between hyperspectral reflectance and pigment concentrations for all wetland species or separate wetland vegetation types.

1 In this study, we assess several vegetation indices derived from laboratory based
2 hyperspectral data for their explanatory power to estimate laboratory extracted foliar pigment
3 concentration from all species and independently for various wetland vegetation types. This
4 study seeks to address two questions: (1) to what extent do floating macrophyte pigment
5 concentrations differ from other wetland vegetation types, and (2) which vegetation indices can
6 best estimate floating macrophyte foliar photosynthetically active pigment concentrations (Chl-a,
7 Chl-b, Car, Chl-a+b).

8 9 **2.0 Methodology**

10 11 ***2.1 Site Description and Sample Collection***

12
13 Leaves from 39 wetland species (12 floating macrophytes (FM), 8 grasses/sedges/rushes
14 (GSR) and 19 herbs/wildflowers (HWF)) were collected from two plant nurseries and from two
15 field sites near Toronto, Ontario (Table 1). Site one was Cranberry marsh (43.844 °N, 78.965
16 °W), a 40 hectare provincially significant coastal wetland along the northern section of the Lake
17 Ontario shoreline between Ajax and Oshawa. Although a coastal wetland, Cranberry Marsh
18 water levels were controlled prior to 2001. After 2001, a low-high water level cycle was re-
19 established to promote regeneration of the vegetation community. Site two was the wetlands at
20 the outflow of the Rough River into Lake Ontario near Rouge Beech Park (43.794 °N, 79.121
21 °W) along the border of Toronto and Pickering. The outflow of the Rough River meanders
22 through dense cattails, creating several pockets of slow moving water where various floating
23 macrophytes have taken advantage of the stagnant conditions. In addition to the specimens
24 collected in the field, various invasive and native species that were not present at the field sites
25 were acquired from two local plant nurseries in Brampton and Ajax. Multiple samples were
26 taken for certain species due to leaf age, presence at multiple sites or due to heterophyllous
27 morphologies. In total 48 samples were acquired, 17 FM, 9 GSR and 22 HWF.

28 Field work was conducted August 4th for Cranberry Marsh and August 11th and 18th for
29 Rough Park during the summer of 2012. Plant nursery samples were collected the same day.
30 Samples collected in the field and from plant nurseries were packaged in an opaque plastic bag
31 with air holes and stored in a cooler for transportation from the fields to the laboratory for
32 subsequent in-vivo measurements. All samples were processed within 24 hours. Samples were
33 washed with distilled water and dried with a paper towel to remove any dirt and debris from the
34 vegetation.

35 36 ***2.2 Hyperspectral Measurements***

37
38 Leaf level spectral measurements were taken in the lab from 10 leaves of each sample
39 with five replicate measurements, using a FieldSpec3 Max portable spectroradiometer
40 (Analytical Spectral Devices, Inc., Boulder, CO). The FieldSpec3 uses one 512 element Si
41 photodiode array to measure between 350-1000 nm and two InGaAs photodiodes to measure
42 between 1000-2500 nm with an approximate spectral resolution of 3 nm @ 700 nm and 10 nm @
43 1400/2100 nm. Hyperspectral measurements were taken using the plant probe and leaf clip
44 attachments to control the illumination geometry and standardized the measurement conditions.
45 The sample was illuminated by the plant probe's halogen lamp (3.825 Volt, 4.05 Watt) which
46 was internally set at a constant distance resulting in field of view of 10mm. Prior to each

reflectance measurement, the FieldSpec3 was optimized and the radiance of the illuminated back face panel without sample was recorded for normalization of the target measurements.

Hyperspectral measurements of *Azolla filiculoides*, *Salvinia molesta*, *Lemna minor* and *Spirodela polyrhiza* could not be taken by the plant probe due to the small leaf size (*Wolffia arrhiza*: 0.5 mm to *Salvinia molesta*: 2 cm). For these species, hyperspectral measurements were taken using an ASD Pro Lamp (14.5 Volt, 50 Watt) designed for indoor lab diffuse reflectance measurements and the FieldSpec3 fore optic, both placed at 20 cm height at a 45° angle off nadir in perpendicular positions facing a target black surface. Each species was placed in a standard container filled with distilled water such that 100% areal coverage two times the fore optic field of view was achieved. The sample container was rotated and the vegetation mixed prior to each of 10 measurements with five replicates per measurement. Prior to each reflectance measurement, the radiance of a Spectralon white reference panel (Labsphere, Inc., North Sutton, NH) was recorded for normalization of the target measurements. To ensure the compatibility between the plant probe and the lab setup methods, hyperspectral measurements using both methods were conducted for all samples obtained from Cranberry Marsh. A visual comparison indicated that the lab setup yielded comparable spectral signatures to the plant probe approach.

Recorded spectra were processed using the RS3 software (Analytical Spectral Devices, Inc., Boulder, CO). Splice correction was applied to remove artifacts associated with detector differences at 1000 and 1800nm. The recorded spectra were averaged for each sample.

2.3 Derivation of Vegetation Indices

Of the >40 published vegetation indices, a subset of the available indices that are sensitive to pigment concentration and generally unresponsive to leaf area index and soil background were selected for this study (Table 2). Care was taken to select indices that exploit different spectral regions such as the position of the red-edge, the reflectance change at the red-edge, the depth of chlorophyll absorption in the visible region, the contrast between red and near infrared (NIR) regions and reflectance in the visible region due to xanthophylls cycle pigments. All selected hyperspectral vegetation indices were calculated from mean reflectance.

2.4 Foliar Pigment Extracts

For pigment determination, two extracts per sample were processed (n=96). Leaves were removed from the stem, roots and remaining plant matter with a scalpel. Leaves were cut into small pieces and 120 mg fresh weight was extracted in 100% acetone (0.7 mL) and 30 mg sodium bicarbonate. Samples were mixed in a vortex for 20 minutes and centrifuged at 14,000g for six minutes to separate the natant from the pellet. After two hours on ice in an opaque box, the natant was extracted and the pellet re-extracted in acetone a further two times for a combined extraction of 2.1 mL in 100% acetone. Chl-a, Chl-b, Car and Chl-a+b were then quantified using a spectrophotometer tuned to the absorbance of the liquid extracts at 470, 645 and 662 nm. Between three and six replicate spectrophotometer reading per sample were taken. The pigment concentrations were calculated using the specific absorption coefficients provided by Porra et al. (1989). The Chl-a, Chl-b, Car and Chl-a+ b concentration per extract was calculated as the mean of all replicates. The concentration per sample was taken as the mean of the two extracts.

2.5 Statistical Analysis

To test whether Chl-a, Chl-b, Car and Chl-a+b could be estimated regardless of vegetation type, regression was performed on all sample data (class ALL) for each of the 13 vegetation indices. Regression was additionally performed for all samples belonging to the three vegetation types (Table 1). In all cases, linear, second-degree polynomial and logarithmic functions were fitted to the measured pigment concentration as the dependent variable and the hyperspectral vegetation index as the independent variable. The fitted functions were obtained by the least square adjustment method using the R software package and coefficient of variation of the root mean square error (cvRMSE) were coincidentally calculated to evaluate the explanatory strength of the fitted function. The vegetation indices that yielded the lowest cvRMSE and highest R^2 were deemed preferred for foliar pigment concentration estimation. cvRMSE was selected as it less sensitive than root mean square error to variations in the range and foliar pigment concentrations are of different scales.

3.0 Results

The range of pigment concentration for ALL samples is 0.017-0.306 ug/mg for Chl-a, 0.008-0.228 ug/mg for Chl-b, 0.006-0.099 ug/mg for Car and 0.025-0.512 ug/mg for Chl-a+b. Foliar concentrations of Chl-b and Car are typically lower than Chl-a in most plant species due to their secondary and auxiliary roles in photosynthesis (Blackburn 1998). All foliar pigment concentrations are highly correlated. Chl-a concentration has a R^2 of 0.91 with Chl-b using a linear fitting function and a R^2 of 0.90 with Car and a R^2 of 0.97 with Chl-a+b respectively.

Mean foliar concentrations of individual pigments vary between vegetation types (Table 3). Although FM and HWF pigment concentrations have a similar range and variability (Figure 1), FM has the lowest mean pigment concentration for all pigment types, while HWF has the highest mean Chl-a and Car concentrations. GSR has the highest mean Chl-b and Chl-a+b concentration. However, pigments concentrations of GSR are more variable than FM or HWF, with the strongest variability in GSR Chl-b concentration (mean 0.078 ug/mg, standard deviation 0.076 ug/mg). The variability is partially due to certain GSR species that have high Chl-a concentrations compared to all other species and correspondingly high Chl-b concentrations. Hence, the mean Chl-a/Chl-b ratio is considerably lower for GSR (2.78) when compared to FM (3.07) and HWF (3.03), which are very similar considering the Chl-a/Chl-b ratio varies considerably.

Reflectance spectra clearly display the major absorption features indicative of chlorophyll with the typical green peak around 550nm, red-edge around 675 nm, sill in the NIR around 750 nm and subsequent NIR plateau. Standard deviation of all samples mean reflectance indicates that samples primarily varied in the NIR (Figure 2). Variability in reflectance was minimal around 675 nm and increased along the red-edge as well as approaching the green peak. The regions of variability correspond as expected from samples varying in pigment concentration. FM species tended to have the lowest reflectance in the visible and green peak regions (e.g. Water Smartweed spectra curve in Figure 2). In terms of the red-edge regions FM species generally have a similar slope to HWF species. The slope in the red-edge is less pronounced in GSR species which tended to have high reflectance in the visible region and low to average NIR reflectance. There is considerable overlap in the shape of spectral signatures between vegetation types. Furthermore, reflectance varied considerable between species belonging to a vegetation type. For instance, Marsh Pepper Smartweed has a high NIR reflectance for a member of its vegetation type (Figure 2).

3.1 Relationships between vegetation indices and foliar pigment concentration

For a given vegetation index, the performance of the three tested fitted functions are similar (Table 4). Compared to the linear regression, a log fitted function generally resulted in a decrease in R^2 . In contrast, utilizing a second-degree polynomial yields a slightly improved correlation in all cases. At minimum the increase in R^2 is 0.01 to a maximum of 0.18, although a ~ 0.04 improvement is average. The fitted curve often approximates a straight line with a weakly curved shape (Figure 3(a iii)). Thus, the gain in performance is likely due to better estimation of the values at the poles of the pigment concentration range, as is evident in the case of ALL Chl-b (Figure 3(a ii)). However, the number of data points is often sparse in the curves of the regression line and insufficient to support second-degree polynomial regression. Given that previous studies have shown a linear relationship when the chlorophyll concentration range of the samples is moderate (Gitelson et al. 2003, Gitelson et al. 2005), the linear regression is more sound, contrary to the increase in performance from a second-degree polynomial.

NDVI and HNDVI vegetation indices performed poorly for all groups regardless of pigment type (Table 4). NDVI and HNDVI likely performed poorly in predicting leaf chlorophyll content as they are partially based on NIR scattering which likely varies between species as a function of the number of cell-water interfaces within the leaf spongy mesophyll. Similarly, NGAI and OSAVI also consistently poor as they are partially based on the contrast between the NIR plateau and the green peak or red-edge. For the group of species under investigation, the PRI index is also not well correlated to any pigment concentration, possibly since the index is sensitive to rapid changes in the relative levels of xanthophyll cycle pigments (Gamon et al. 1997) and photosynthesis was not controlled post harvesting. Moderately performing vegetation indices include DD, REP, CI-Green, CARI and MCARI. Their R^2 values tend to range between the mid 0.5's and mid 0.7's depending upon the vegetation type. For instance, REP correlated with Chl-a concentration well for HWF (R^2 : 0.738) but only reasonably for FM (R^2 : 0.542). The indices that consistently perform high for each vegetation type across all pigment types are R-M, CI-Red and MTCI.

Overall the R^2 values between the derived hyperspectral vegetation indices and pigment concentrations for ALL samples ranged from 0.51 to 0.66 (Table 5). The R-M and CI-Red indices fitted with a linear function performed best for ALL samples with Chl-a+b and Chl-b having the strongest relationship with the former and the slightly more scattered Chl-a and Car by the latter. The performance of these indices are negligibly greater than other top performing indices such as CI-RED, MTCI.

Regression results indicate that pooled samples to estimate individual foliar pigments is less correlated than when each type of vegetation type is treated separately (Table 5). When estimated per vegetation type, the fitted functions have greater R^2 values between the tested vegetation indices and the individual foliar pigments concentrations. The only exception is the Chl-b correlation is slightly lower for GSR than all pooled sampled (R^2 ALL: 0.64, GSR: 0.55), although the relative error is comparable (cvRMSE All: 0.230, GSR: 0.215), suggesting the fitted functions are approximately equal in performance. The vegetation index that yielded the best fit varies between vegetation types (Table 5). FM foliar pigments are best estimated by indices that exploit the red-edge. In all cases of FM foliar pigment estimation, CI-Red has a slight performance improvement over MTCI and R-M, on the order of 0.02 to 0.09 R^2 . The second best performing index does vary by pigment type, with MTCI better for Chl-a and Chl-a+b and R-M better for Chl-b and Car. MTCI is also the best estimator of HWF pigment concentration for all pigments except Chl-b to which MCARI offers a 0.08 R^2 improvement. Lastly the correlation

1 between GSR and pigment concentrations is generally lower due to the low number of data
2 points and larger concentration range. GSR is best estimated by R-M which consistently offers a
3 slight improvement over R-M and MTCI. The second best index is consistently CI-Red followed
4 closely by MTCI for all pigment types.

5 6 **4.0 Discussion**

7
8 FM pigment concentrations are subtly different from other wetland vegetation types.
9 Floating macrophyte species tend to have pigment concentrations less than terrestrial herbs,
10 wildflowers and grasses. However, the difference between vegetation types is not as stark as has
11 been reported between arctic shrubs (Fletcher et al. 2012) and bushland or forest vegetation types
12 (Blackburn 2007). Chl-b concentrations varied the most between vegetation types, with FM
13 constrained to a narrower range and lower concentration than HWF or GSR (Figure 4). In
14 general all pigment concentrations for GSR are highly variable. This variability may not be
15 related to GSR biochemistry, but due to environmental factors. Nutrient availability and water
16 stress can cause pigments concentrations to vary (le Maire et al. 2004). Notably the summer of
17 2012 was unusually warm and samples obtained from the two field sites are from exposed soils
18 that are typically inundated. In contrast, HWF Chl-b variations appear related to differences in
19 photosynthetic machinery. Increased levels of Chl-b are a shade adaption that permits more
20 trapping of light energy (Kitajima and Hogan 2003). Thus, the variable Chl-b concentration is
21 likely due to the inclusion of sun and shade species within the HWF vegetation type. Floating
22 macrophytes are rarely shaded and the photosynthetic photon flux density at which
23 photosynthesis is saturated is higher than $1400 \mu\text{mol m}^{-2}\text{s}^{-1}$ (Tsuchiya 1991). Hence, floating
24 macrophyte species are well adapted to full sunlit conditions and thus may expend minimal
25 resources on shade adapted photosynthetic machinery.

26 The variable pigment concentrations between wetland vegetation types suggest that
27 adaptations to ecological niches play a role in determining foliar biochemistry. However, the
28 specialized adaptations of floating macrophyte species to the aquatic environment did not cause
29 abnormal reflectance or pigment concentrations. The hair-like strands covering the bristly
30 surface of *Salvinia* fronds may have confounded the reflectance measurement, as hairs are
31 known to increase reflectance in the visible region and occasionally the NIR region (Slaton et al.
32 2001) though no such effect could be distinguished. The only species that displays any sizeable
33 disproportionality between reflectance and pigment concentration is Water Lettuce. Reflectance
34 of Water Lettuce is higher than its pigment concentration indicates. Since Water Lettuce
35 buoyancy is provided by its thick, air-filled leaves (the largest of any sampled species) the
36 pigment concentration is likely reduced as these cavities consume a greater portion of the total
37 weight.

38 Overall the CI-Red vegetation index fitted with a linear function performs best for
39 estimating floating macrophyte pigment concentrations. The R-M index follows closely in
40 performance. Both indices perform well because of their derivation from the slope of the red-
41 edge. The major calculation difference is that CI-Red determines the red-edge slope from a wider
42 range of bandwidth approaching the red-edge inflection point and the NIR sill, whereas R-M is
43 determined squarely within the red-edge. Although, both CI-Red and R-M perform well for
44 estimating HWF pigment concentrations, the MTCI index achieves a slight 0.02 to 0.10 better
45 R^2 . The sole exception for MTCI is for Car concentration for HWF. For this pigment, MCARI
46 outperforms the linear MTCI (R^2 : 0.545 versus 0.468). In summation, the results suggest that
47 vegetation indices based on the red-edge tend to outperform those involving the green peak.

Indices involving the green peak (NGAI) or slightly offset of the green peak (PRI) tend to perform poorly while mixed NIR and green peak indices (CARI and MCARI) tend to perform reasonably well for certain vegetation types. In the case of Chl-a estimation, CARI and MCARI performed on par with MTCI for HWF, reasonably well for ALL, mixed for FM (CARI R^2 : 0.705, MCARI R^2 : 0.294) and poor for GSR (Table 4). In comparison CI-green, also involving the green peak, performs more consistently regardless of group or pigment. The R^2 value of CI-green is ~ 0.04 higher for each pigment compared to MTCI for HWF (e.g. MTCI R^2 : 0.771 for Chl-a+b versus CI-green R^2 : 0.733).

While this study documents good performance in foliar pigment estimation, extrapolating from the leaf to canopy scale involves a number of confounding factors and previous attempts to estimate canopy pigment concentrations have shown poor signal propagation between the leaf and canopy scale (Yoder and Pettigrew-Crosby 1995, Jacquemoud et al. 1996, Asner 1998, He and Mui 2010). For floating macrophytes the largest confounding factor is the likelihood of variable water inundation. The spectral signatures from leaves covered by an increasingly thick water layer will exhibit decreasing reflectance in the NIR plateau. Although plant NIR reflectance is typically flat between 800-1400nm, small water absorption affects in 970 and 1180 nm can be present to a degree depending upon the internal water content. The addition of a surface water layer increases their effects on the spectral curve sharply, reminiscent of the typical strong absorption effects of water at 1500 and 2000nm. Kisevic et al. (2011) showed that leaf reflectance at 980nm dropped approximately 50% from a wet leaf with the addition of a 7mm thick water layer. Furthermore, a 2mm thick water layer reduced reflectance beyond the 1500nm mark to near zero.

Other common confounders are variations in leaf area index, orientation of leaves, canopy ground coverage, presence of non-leaf elements, areas of shadow and soil/litter surface reflectance (Blackburn 2007). However, many of these vary minimally within floating macrophyte stands. Floating macrophytes usually arrange their leaves horizontally, or near the water surface with a few leaf layers (Tsuchiya 1991). Only certain species have considerable three dimensional features. In addition, leaf area index variations are minimal compared to the range of terrestrial species (floating: 1.1-2m²m⁻², terrestrial 3-13m²m⁻² (Tsuchiya 1991)) and Jakubauskas et al. (2000) successfully assessed the density of *Nuphar polysepala* due to the high contrast between the vegetation and water background. Considering floating macrophytes can occur in simple arrangements, there are good prospects for extrapolating leaf level measurements to the canopy scale. However, further research is required to determine whether leaf level relationships are scalable to the canopy.

5.0 Conclusions

This study demonstrates that grouping various wetland vegetation types can achieve reasonable accuracy when estimating foliar pigment concentration. Avoiding the need to develop a semi-empirical relationship for each wetland vegetation type is an attractive approach, especially when computation simplicity is favored above overall accuracy. If accuracy is favored, then the correlations between vegetation indices and Chl-a, Chl-b, Car and Chl-a+b are generally improved by their separation. These findings suggest that floating macrophyte foliar biochemistry is subtly distinct from other vegetation types and these differences are primarily detectable along the red-edge. Hyperspectral observation of leaf level reflectance is a promising tool for assessing the foliar biochemistry of floating macrophytes since it is a quick and reliable

1 method that considerably reduces the effort need to work in this already challenging
2 environment.

3
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5
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Table 1. List of species sampled by vegetation type.

Common Name	Scientific Name	Type	Sample Source
Sweet Flag	<i>Acorus calamus</i>	GSR	Cranberry Marsh
Canada Anemone	<i>Anemone canadensis</i>	HWF	
Nodding Bur Marigold	<i>Bidens cernua</i>	HWF	
Marsh Marigold	<i>Caltha palustris</i>	HWF	
3-Leaved Solomon's Seal	<i>Maianthemum trifolium</i>	HWF	
Marsh Timothy	<i>Muhlenbergia glomerta</i>	GSR	
Common Reed	<i>Phragmites australis</i>	GSR	
Lady's Thumb	<i>Polygonum persicaria</i>	HWF	
River Bullrush	<i>Scripus fluviatilis</i>	GSR	
Beaked Sedge	<i>Carex utriculata</i>	GSR	Rouge Park
Swamp Milk Weed	<i>Asclepias incarnata</i>	HWF	
Common Milk Weed	<i>Asclepias syriaca</i>	HWF	
Bog Arum *	<i>Calla palustris</i>	HWF	
Swamp Thistle	<i>Cirsium palustre</i>	HWF	
European Frog-bit *	<i>Hydrocharis morsus-ranae</i>	FM	
Yellow Pond Lily	<i>Nuphar variegatum</i>	FM	
Marsh Pepper Smartweed	<i>Polygonum hydropiper</i>	HWF	
Pickrel Weed	<i>Pontederia cordata</i>	HWF	
Broadleaf arrowhead	<i>Sagittaria latifolia</i>	HWF	
Common Three Square	<i>Scirpus pungens</i>	GSR	
Cup Plant *	<i>Silphium perfoliatum</i>	HWF	
Greater Duckweed	<i>Spirodela polyrhiza</i>	FM	
Kidney-Leaved Violet	<i>Viola renifolia</i>	HWF	
Northern Bugleweed	<i>Lycopus uniflorus</i>	HWF	
Bog Aster	<i>Aster nemoralis</i>	HWF	
Northern Bog Goldenrod	<i>Solidago uliginosa</i>	HWF	
Common Burreed	<i>Sparganium eurycarpum</i>	GSR	
Floating Water Lily	<i>Nymphaea odorata</i>	FM	Cranberry Marsh/ Rouge Park
Water Smart Weed **	<i>Polygonum amphibium</i>	FM	
Cattail	<i>Typha latifolia</i>	GSR	
Purple Loosestrife	<i>Lythrum salicaria</i>	HWF	
Fairy Moss	<i>Azolla filiculoides</i>	FM	
Water Hyacinth	<i>Eichhoria crassipes</i>	FM	
Columbia Watermeal	<i>Wolffia arrhiza</i>	FM	
SpadderDock	<i>Nuphar luteum</i>	FM	
Yellow Floating Heart	<i>Nymphoides peltata</i>	FM	
Bog Bean	<i>Menyanthes trifoliata</i>	HWF	Plant Nursery
Water Lettuce	<i>Pistia stratiotes</i>	FM	
Common Salvinia	<i>Salvinia molesta</i>	FM	

* Significant leaf color variation was present due to leaf age. Samples were taken for new and mature leaves. ** *Polygonum amphibium* is a heterophyllous species with both floating and aerial leaf forms with different morphology and coloration. Samples were taken for both the floating and aerial leaves.

Table 2. Equations for the vegetation indices investigated by this study (R_λ indicates the reflectance of the band centered on wavelength λ)

Index	Formula	Reference
CARI	$[(R_{700} - R_{670}) - 0.2 * (R_{700} - R_{550})]$	Kim et al. (1994)
CI-Red	$(R_{780}/R_{710}) - 1$	Gitelson et al. (2003)
CI-Green	$(R_{780}/R_{550}) - 1$	Gitelson et al. (2003)
DD	$[(R_{750} - R_{720}) - (R_{700} - R_{670})]$	le Maire et al. (2004)
HNDVI	$(R_{827} - R_{668})/(R_{827} + R_{668})$	Oppelt and Mauser (2004)
REP	$700 + 40 \frac{(R_{670} + R_{780})/2 - R_{700}}{R_{740} - R_{700}}$	Guyot and Baret (1988)
MCARI	$(R_{700} - R_{670}) - 0.2 * (R_{700} - R_{550}) * (R_{700}/R_{670})$	Daughtry et al. (2000)
MTCI	$[(R_{750} - R_{710})/(R_{710} - R_{680})]$	Dash and Curran (2004)
NDVI	$(R_{820} - R_{670})/(R_{820} + R_{670})$	Rouse et al. (1974)
NGAI	$\cos^{-1} \left(\frac{a^2 + b^2 + c^2}{2ab} \right)$ $a = R_{820} - R_{554}$ $b = R_{977} - R_{554}$ $c = R_{977} - R_{820}$	Palacios-Orueta et al. (2006)
OSAVI	$1.16 * (R_{800} - R_{670})/(R_{800} + R_{670} + 0.16)$	Rondeaux et al. 1996
PRI	$(R_{531} - R_{570})/(R_{531} + R_{570})$	Gamon et al. (1997)
R-M	$(R_{750}/R_{720}) - 1$	Gitelson et al. (2005)

Table 3. Foliar pigment concentrations (ug/mg) derived from sample extracts.

Type	Pigment	Min	Max	Mean	St.Dev
ALL	Chl-a	0.017	0.306	0.157	0.068
	Chl-b	0.008	0.228	0.057	0.039
	Car	0.006	0.099	0.052	0.022
	Chl-a+b	0.025	0.512	0.214	0.104
FM	Chl-a	0.071	0.254	0.135	0.045
	Chl-b	0.017	0.083	0.045	0.015
	Car	0.022	0.074	0.046	0.016
	Chl-a+b	0.088	0.337	0.179	0.060
GSR	Chl-a	0.048	0.306	0.164	0.104
	Chl-b	0.017	0.124	0.078	0.076
	Car	0.015	0.099	0.053	0.033
	Chl-a+b	0.065	0.512	0.242	0.176
HWF	Chl-a	0.016	0.291	0.172	0.064
	Chl-b	0.008	0.106	0.058	0.025
	Car	0.005	0.095	0.057	0.021
	Chl-a+b	0.025	0.398	0.23	0.089

Table 4. Regression results of the three fitted function for Chl-a concentration.

Type	Index	R^2		
		Linear	Polynomial	Log
ALL	CARI	0.440	0.443	0.413
	CI-Red	0.633	0.644	0.484
	CI-Green	0.443	0.452	0.393
	DD	0.574	0.576	0.472
	HNDVI	0.035	0.147	0.058
	REP	0.511	0.512	0.420
	MCARI	0.381	0.386	0.351
	MTCI	0.615	0.635	0.455
	NDVI	0.029	0.132	0.050
	NGAI	0.170	0.193	0.145
	OSAVI	0.080	0.141	0.084
	PRI	0.070	0.083	0.044
	R-M	0.612	0.640	0.453
FM	CARI	0.705	0.706	0.652
	CI-Red	0.808	0.838	0.824
	CI-Green	0.676	0.691	0.693
	DD	0.730	0.779	0.772
	HNDVI	0.258	0.401	0.345
	REP	0.542	0.662	0.624
	MCARI	0.294	0.323	0.229
	MTCI	0.769	0.787	0.774
	NDVI	0.251	0.366	0.327
	NGAI	0.266	0.448	0.354
	OSAVI	0.313	0.451	0.399
	PRI	0.055	0.097	0.035
	R-M	0.753	0.808	0.797
GSR	CARI	0.277	0.281	0.243
	CI-Red	0.641	0.647	0.588
	CI-Green	0.466	0.467	0.439
	DD	0.572	0.575	0.524
	HNDVI	0.131	0.211	0.106
	REP	0.561	0.562	0.518
	MCARI	0.309	0.309	0.274
	MTCI	0.629	0.630	0.583
	NDVI	0.111	0.215	0.086
	NGAI	0.201	0.203	0.199
	OSAVI	0.278	0.344	0.239
	PRI	0.263	0.269	0.240
	R-M	0.702	0.711	0.643
HWF	CARI	0.729	0.752	0.567
	CI-Red	0.735	0.778	0.474
	CI-Green	0.697	0.747	0.438
	DD	0.723	0.724	0.528
	HNDVI	0.044	0.057	0.034
	REP	0.738	0.746	0.539
	MCARI	0.737	0.747	0.604
	MTCI	0.744	0.802	0.46
	NDVI	0.039	0.045	0.025
	NGAI	0.315	0.321	0.218
	OSAVI	0.003	0.012	0.001
	PRI	0.054	0.056	0.037
	R-M	0.739	0.767	0.491

Table 5. Regression results of the top performing fitted functions

Type	Pigment	Index	R ²	cvRMSE	Fitted Equation
ALL	Chl-a	CI-Red	0.63	0.235	0.45 + 7.34 * X
	Chl-b	R-M	0.64	0.230	0.33 + 4.98 * X
	Car	CI-Red	0.51	0.273	0.55 + 20.02 * X
	Chl-a+b	R-M	0.66	0.224	0.21 + 1.88 * X
FM	Chl-a	CI-Red	0.81	0.170	-0.12 + 11.83 * X
	Chl-b	CI-Red	0.76	0.190	-0.03 + 33.83 * X
	Car	CI-Red	0.73	0.200	-0.03 + 32.48 * X
	Chl-a+b	CI-Red	0.80	0.174	-0.11 + 8.84 * X
GSR	Chl-a	R-M	0.70	0.176	0.45 + 2.24 * X
	Chl-b	R-M	0.55	0.215	0.52 + 5.44 * X
	Car	R-M	0.70	0.176	0.44 + 7.03 * X
	Chl-a+b	R-M	0.67	0.186	0.47 + 1.57 * X
HWF	Chl-a	MTCI	0.74	0.217	0.00 + 12.17 * X
	Chl-b	MTCI	0.83	0.176	0.19 + 32.73 * X
	Car	MCARI	0.54	0.479	1.21 - 12.49 * X
	Chl-a+b	MTCI	0.77	0.203	0.03 + 8.97 * X

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Figure 1. Boxplots of Chl-a+b concentration per vegetation type. Dark line is the mean.

Figure 2. Reflectance of Common Burred (dot-dash line), Marsh Pepper Smartweed (dashed line), Water Smartweed (dotted line) and ALL standard deviation + 0.9 (solid line).

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Figure 3. Predicted relationship between best performing vegetation indices for four vegetation types (a) ALL (top row), (b) FM (second row), (c) GSR (third row) and (d) HWF (bottom row) against foliar pigment concentrations, (i) Chl-a (1st column), (ii) Chl-b (2nd column), (iii) Car (3rd column) and (iv) Chl-a+b (4th column). R^2 values in top left corner are for the second-degree polynomial (top) and linear fitted function (bottom).

Figure 4. Boxplots of Chl-b concentration per vegetation type. Bold line is the mean