Spectral Signatures of Macroalgae on Hawaiian reefs.

by

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ABSTRACT

In Hawai‘i, native macroalgae or “limu” are of ecological, cultural, and economic value. Invasive algae threaten native algae and coral that serve a key role in the reef ecosystem. Spectroscopy can be a valuable tool for species discrimination, while simultaneously providing insight into chemical processes occurring within photosynthetic organisms. The spectral identity and separability of Hawaiian macroalgal taxonomic groups and invasive and native macroalgae are poorly known and thus were the focus of this study. A macroalgal spectroscopic library of 30 species and species complexes found in Hawai‘i was created. Spectral reflectance signatures were aligned with known absorption bands of division-specific photosynthetic pigments. Discriminant analysis was used to explore if taxonomic groups of algae and native versus invasive algae were separable. Discriminant analyses resulted in high overall classification accuracies. Algae were correctly classified based on taxonomic divisions 96.5% of the time and by species 83.2% of the time. Invasive versus native algae was correctly classified at a rate of 93% and higher. Analyses suggest there is spectral separability of algal taxonomic divisions and native-invasive status, which could have significant implications for coastal management. This study lays the groundwork for testing spectral mapping of native and invasive algal species using current airborne and forthcoming spaceborne imaging spectroscopy.

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1. INTRODUCTION

Hawaiʻi's native marine macroalgae or "limu" have ecological, economical, and cultural value. Limu provide key food sources for many marine organisms and thereby enrich nearshore fisheries on which local communities rely (Friedlander et al., 2005; Huisman et al., 2007). Limu is also an important part of diet and ceremony for many native Hawaiians (Friedlander et al., 2005; Hart et al., 2014; Huisman et al., 2007; McDermid et al., 2019). Limu is an integral part of coral reefs, which in turn, provide many of the previously mentioned benefits. Additionally, coral reefs are estimated to annually contribute 1.2 billion US dollars to the state of Hawaiʻi associated with tourism and protect coastlines from erosion and flooding (Ferrario et al., 2014; Spalding et al., 2017; Storlazzi et al., 2019).

Both coral and native algae are biocultural keystone organisms that compete for space, but in healthy systems, the two lifeforms are usually well-balanced. Healthy coral-dominated reefs generally have low macroalgal cover, with algae present in areas where herbivores cannot easily reach them (Huisman et al., 2007; Smith et al., 2002). There are also instances of natural and healthy native algae-dominated reefs with low coral cover (Vroom et al., 2006). Two important factors that could cause a coral-dominated or macroalgae-dominated habitat are herbivore activity and nutrient levels (Jouffray et al., 2015; Littler & Littler, 1984; Rasher et al., 2012; Smith et al., 2010). Anthropogenic activities have changed these drivers by increasing nutrients with land-based sources of pollution and decreasing herbivory with fishing pressure (Friedlander et al., 2005; Jouffray et al., 2015; Smith et al., 2002). These human-mediated alterations have resulted in dramatic ecosystem-level regime shifts from coral to macroalgae dominated benthic composition (Smith et al., 2002).

Invasive macroalgae compound other anthropogenic stressors on coral reefs and native algae. Invasive macroalgae are non-native algae that have been introduced to native ecosystems, often casuing detrimental impacts. They typically adapt well to habitats degraded by human activity, grow quickly, and are less desired by herbivorous fish than native algae (Conklin & Smith, 2005; Huisman et al., 2007; Neilson et al., 2018; Smith et al., 2002). These characteristics allow invasive algae to overgrow and kill corals and outcompete native algae, reducing diversity by creating monocultures (Conklin & Smith, 2005; Huisman et al., 2007; Neilson et al., 2018; Smith et al., 2002). Invasive algae are capable of creating harmful benthic phase shifts from coral to algae-dominated systems and in some cases from sand to mud (Conklin & Smith, 2005; Foster et al., 2019; Neilson et al., 2018; Smith et al., 2002). Approximately 20 species of invasive marine algae have been introduced to Hawai‘i since the 1950s and at least one quarter are considered to be invasive (Carlton & Eldredge, 2009, 2015; Department of Land and Natural Resources, 2003; Smith et al., 2002).

All algae can be taxonomically classified into divisions. Taxonomic divisions of algae include red (Rhodophyta), green (Chlorophyta), brown (Ochrophyta), and blue-green algae or cyanobacteria (Cyanophyta). There are about 519 species of red (355), green (102), and brown (62) marine algae described in the Hawaiian Islands (Tsuda, 2014). It is unknown how many species of benthic cyanobacteria are found in Hawai‘i but at least seven species are described (Huisman et al., 2007). Most invasive algae in Hawai‘i belong to the red division and a few belong to the green division. The division names associated with the morphological taxonomic classification of algae may suggest color categorization but the intraspecific variation of visible color within taxonomic divisions can be large. Physical taxonomic distinctions and characteristic photosynthetic pigments define the groups.

As primary producers, algae have photosynthetic pigments that allow them to harvest sunlight to synthesize chemical energy. The taxonomic algal divisions have characteristic pigments, and some divisions have pigments unique to those divisions. The green algal taxonomic division is the only one to contain Chlorophyll b (Chl b) and the brown algal taxonomic division is the only one to contain Chlorophyll c (Chl c) (Chao Rodríguez et al., 2017; Huisman et al., 2007). Both cyanobacteria and red algae contain photosynthetic accessory pigments phycocyanin and phycoerythrin (Chao Rodríguez et al., 2017; Huisman et al., 2007).

The photosynthetic pigments found in algae most strongly absorb light at documented wavelengths in the visible spectrum (Chao Rodríguez et al., 2017). This photosynthetic absorption activity of an alga can be recorded in a spectral signature. Algal spectral signatures show the absorption or reflectance of the individual measured along the visible spectrum and are indicative of the chemistry occurring within the alga. Because the different taxonomic divisions of algae have characteristic photosynthetic pigments and these pigments are expressed differently in the spectra, different species are likely to have characteristic spectral signatures (Chao Rodríguez et al., 2017; Huisman et al., 2007). Several studies have indicated that marine macroalgae can be spectrally separable by taxonomic division (Chao Rodríguez et al., 2017; Olmedo-Masat et al., 2020). A step further would be to spectrally identify algae species, which has been accomplished in terrestrial plants (Falcioni et al., 2020).

Spectral species identification would allow us to categorize species as invasive or native if the biogeographic status of those species is known. However, differences in life strategy between native and invasive algae may also allow us to spectrally distinguish these categories independently of algal species. We are unaware of any studies on invasive and native macroalgal spectral separability. However, Hawaiian terrestrial native, introduced, and invasive plant species spectral differences have been delineated and used in invasive mapping efforts (Asner et al., 2008).

The spectral differentiation of taxonomic groups and invasive and native algae can be useful for spectral classification. We likely can extract even more information about these designations by looking at their spectral signatures, which are indicative of algal chemical processes. Algal taxonomic groups could have specific adaptations that allow them to survive in certain habitats that are reflected in their spectral signature. Invasive algae, which is known to grow quickly and outcompete native algae, may show areas of light absorption that are advantageous to those invasive characteristics.

The spectral differentiation of algae is a fundamental requisite to future classification across broad geographic regions when applied to remote sensing. Traditional field studies can produce detailed information but they often are inadequate to survey large geological areas (Li et al., 2019). When paired with field studies, remote sensing can provide a comprehensive solution to surveying large areas. For example, rugosity to 22 m depth and live coral distribution to 16 m depth were mapped using imaging spectroscopy of nearshore marine environments in the Hawaiian Islands (Asner et al., 2020, 2021; Kutser et al., 2020; Thompson et al., 2017). Hawaiian corals have also been mapped to the species level in one ecosystem using imaging spectroscopy (Drury et al., 2022). The distribution of algal cover in Hawaiʻi has not yet been mapped or studied in detail. Documentation of the distribution of algal taxonomic groups, ideally to species level, would be important in driving management decisions such as designating a native algae limited-take area. Distribution and density maps of native and invasive algae could also help to prioritize areas for place-based control.

Here we measured the spectral reflectance of 30 representative species or species complexes of Hawaiian marine macroalgae with the aim to 1) create a spectral library of Hawaiian marine macroalgae, 2) explore the spectral separability of Hawaiian marine macroalgae by taxonomic groups, and 3) explore the spectral separability of Hawaiian marine macroalgae by invasive or native status. These steps will add to macroalgal spectral knowledge and establish baseline spectral data for future Hawaiʻi-based studies.

2. MATERIALS AND METHODS

2.1 Macroalgae Hyperspectral Reflectance Sampling

Macroalgae were sampled from the shoreline and shallow reefs of Miloliʻi and Pāpā Bay on Hawaiʻi Island, and Maunalua and Kāneʻohe Bay on Oʻahu Island, Hawaiʻi USA. The habitats of the algae sampled ranged from intertidal to subtidal, with a maximum collection depth of 10.6 m. Depending on the habitat, walking the shoreline, snorkeling, and SCUBA diving were used to perform roving surveys to collect spectral signatures of macroalgae and cyanobacteria. Spectral signatures were collected in June and July of 2021 and July and August of 2022.

Spectroscopic readings were gathered either *ex-situ* or *in-situ*. For *ex-situ* measurements, algae were collected in Whirl-paks® with seawater and brought back to field stations close to each collection area. Algae samples were placed into a plastic tub coated with matte black paint and submerged in clean seawater to measure spectral reflectance properties. *Ex-situ* measurements were particularly helpful for intertidal species that likely could not be measured where they were growing because of shallow water depth and wave action. For *in-situ* measurements, divers using either SCUBA or snorkel took measurements of the algae where it was growing on the reef.

Sample reflectance spectra were measured using a FieldSpec® HandHeld 2 Spectroradiometer Pro in a waterproof case with the light receiving end of the attached fiberoptic cable mounted to a dive light. Incident radiance was measured using a Spectralon® panel for the reflectance calculation (the ratio between the radiance of the sample and the incident radiance) performed by the spectroradiometer software.

Algal samples were identified to the lowest taxonomic level possible using the best morphological data available and cryptic species were avoided. For the few samples that could not be identified to the species level without further analysis, descriptive names were used in place of genus and species.

Spectral reflectance readings were taken from a total of 30 species or species complexes for a total of 604 samples (Table 1). Spectral samples included species from the cyanobacteria (Cyanophyta), red algae (Rhodophyta), green algae (Chlorophyta), and brown algae (Ochrophyta) divisions. Samples also included species classified as invasive, native, and with unknown biogeographic status. Turf was sampled and listed as a complex of divisions since it likely contains many different species of algae

Table 1. *Macroalgal Species and Spectral Readings by Division and by Invasive, Native, and Unknown Biogeographic Status.*

The number of unique species is given with the number of spectral readings in parentheses. Dashes indicate no samples were taken. Details of the species sampled are provided in Appendix A, Table A1. Complex here is turf algae.

Division	Invasive	Native	Unknown	Total
Cyanobacteria	-	2 (50)	-	2 (50)
Red	4 (90)	5 (71)	2 (6)	11 (167)
Green	1 (32)	8 (165)	-	9 (197)
Brown	-	7 (161)	-	7 (161)
Complex	-	-	1(29)	1(29)
Total	5 (122)	22 (447)	3 (35)	30 (604)

2.2 Data Processing and Analysis

2.2.1 Data Cleaning and Standardization

The spectrum for each reading were averaged every consecutive 5 nm to mirror previous studies, reduce noise, and retain important spectral signature characteristics that might be lost with a smoothing filter (Olmedo-Masat et al., 2020). The brightness index was calculated and used to brightness-normalize the readings to eliminate cross-spectrum brightness effects as well as overall deviations due to illumination conditions of different measurement regimes (Feilhauer et al., 2010).

Spectral readings were visually inspected and areas at the beginning and end of the spectra were cut to reduce noise, resulting in readings ranging from 427-702 nm. Anomalous reads were excluded from the data set analyzed and the totals in Table 1 reflect only those samples used. Anomalous reads included flat reads that did not follow the pattern of the majority for that species, reads that fell far from the mean reflectance signature of the species, and reads containing false peaks around 475 nm believed to be caused by water column artifacts.

Spectral signatures were graphed by species, taxonomic division, and invasive and native status. Spectral characteristics were visually inspected and described. A mixture of Microsoft Excel, R version 4.1.3 (R Core Team, 2022), and JMP® Pro 16 (SAS institute Inc., 2022) was used for initial data cleaning and visualization.

2.2.2 Discriminant Analysis

To explore the ability to distinguish algal taxonomic groups and native and invasive status, methods such as hierarchical clustering analysis (HCA) were investigated. Hierarchical clustering analysis using Ward's method will cluster observations that are most similar together in a bottom-up fashion (Kent, 2015). The smaller clusters will be averaged to determine the next similarity between clusters. This method is strongly biased toward producing clusters with the same number of observations and is sensitive to outliers (SAS Institute Inc., 2022). Because the sample sizes of the taxonomic groups and native and invasive categories were uneven and the clusters from this analysis would be biased to contain equal numbers of observations, we decided that HCA may not be the best methodology to explore the separability of these groups.

We then explored the data set using discriminant analysis. Discriminant analysis uses known categories to identify characteristics of continuous variables that indicate membership to that category. This is a step further than simply clustering the observations using spectral similarity, this analysis maximizes the differences between known categories (SAS Institute Inc., 2022). Quadratic discriminant analysis (QDA) is commonly used when the within-group covariance matrices vary (SAS Institute Inc., 2022). Each group had a varying sample size and thus covariance matrices, so QDA was selected. Quadratic discriminant analysis was performed in JMP® Pro 16 (SAS institute Inc., 2022).

Three analyses were performed with the QDA categorical variables assigned as algal taxonomic divisions, algal species, and as native and invasive algae by division. Algal turf reflectance was not included in the discriminant analysis of taxonomic divisions since turf is less than one centimeter in height, is difficult to define taxonomically with the naked eye, and is likely to be a combination of divisions. A total of 21 species or species complexes were included in the QDA exploring species or species complex separability, including algal turf. Algal species that had less than 20 samples were excluded from the species discriminant analysis. Since divisions are likely separable, we investigated the difference in each division of native and invasive species. For all analyses, continuous variables were the brightness-normalized reflectance of samples at wavelengths from 427-702 nm for a total of 56 continuous variables.

Discriminant analysis can be used to develop a model with a training set of data, validate that model, and test the model to assess the fit of the model and the level of misclassification that occurs throughout each set. Each spectral sample was randomly assigned to training, validation, or test sets at a rate of 60%, 20%, and 20% respectively. A low misclassification rate shows the categorical model is correctly predicting the membership of observations and indicates that there is separability of categories. The entropy R^2 value indicates the fit of the model, with a value of one indicating a perfect fit. This analysis used subsets of our limited data to explore the separability of groups and further studies would be needed to have a model capable of delineating all taxonomic groups and invasive and native algae present in Hawaiian waters.

3. RESULTS

3.1 Spectral Signatures of Algae

3.1.1 Spectral Signatures of Algae by Taxonomic Division

The mean brightness-normalized spectral signatures of each algal division had characteristic peaks and absorption wells (Figure 1, original spectra can be seen in Appendix A, Figure A3). Bands reported are an average of reflectance readings spanning 5 nm, so should be interpreted with a ±2.5 nm. Brown algae, red algae, and cyanobacteria had spectral peaks around 600 nm and 650 nm. Green algae were characterized by a single peak with a local maximum at 562 nm. A peak unique to red algae had a local maximum at 552 nm. While the species' spectral signatures generally followed taxonomic division characteristics, there was variation between species and some characteristics apparent in species were muted in division mean spectral signatures. Turf algae, assumed to be a complex of divisions, displayed peaks at 600 nm and 650 nm that were common to brown algae, cyanobacteria, and red algae. The characteristic spectra by species can be found in Appendix A, Figure A1.

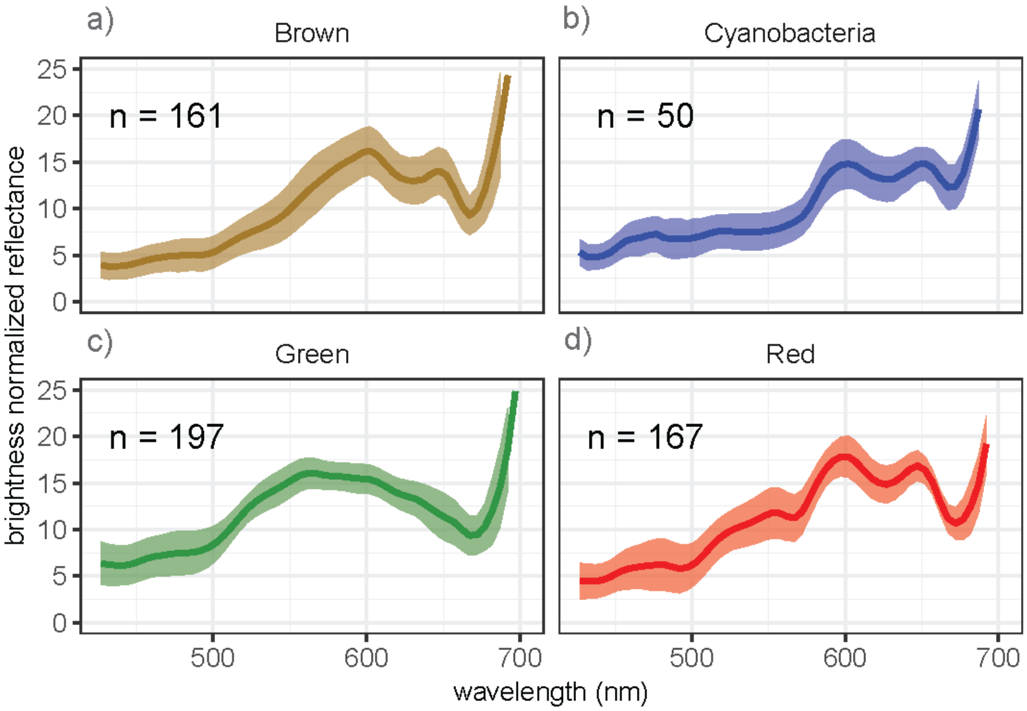

Figure 1. *Brightness Normalized Spectral Signatures by Algal Taxonomic Divisions: a) Brown (Ochrophyta), b) Cyanobacteria (Cyanophyta), c) Green (Chlorophyta), and d) Red (Rhodophyta).* The mean spectral reflectance of each division is shown by the solid line. The shaded bands show the standard deviation. n indicates the number of samples per division.

3.1.2 Spectral Signatures of Invasive and Native Algae

The green and red algal taxonomic divisions were the only divisions to contain invasive algae. The invasive and native means of those divisions are shown in Figure 2. The spectral characteristics of each taxonomic division are present in both invasive and native algae belonging to that division.

Red invasive algae have a mean reflectance that is higher than the red native algae from 427 – 587 nm. The mean reflectance of red invasive algae is lower than red native algae from 592 – 702 nm. The standard deviation of red invasive and native groups overlaps from 427 – 487 nm and 527 – 702 nm indicating there is likely not a significant difference between invasive and native red algae at those wavelengths. The standard deviation of the invasive and native red algae does not overlap from 487 – 527 nm.

The mean reflectance of invasive green algae is lower than native green algae from 512 – 602 nm and higher than native green algae from 427 – 507 nm and 612 – 702 nm. The standard deviation of invasive and native green algae do not overlap from 552 – 577 nm and 667 – 672 nm.

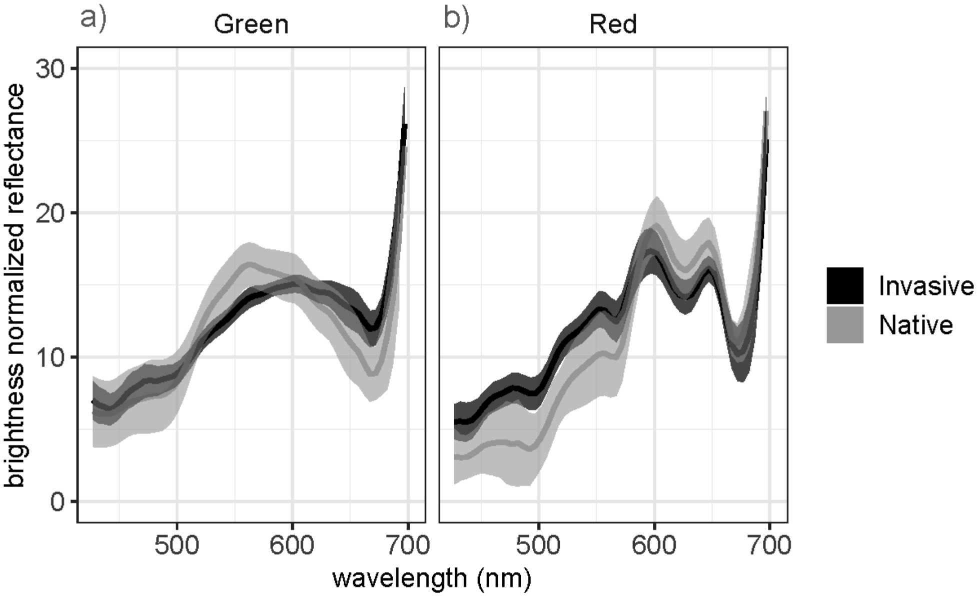

Figure 2. *Brightness Normalized Spectral Signatures of Invasive and Native Algae*: a) green (Chlorophyta), native (n=165), invasive (n=32) and b) red (Rhodophyta), native (n=71), invasive (n=90). The mean spectral reflectance is shown by the solid line. The error bands show the standard deviation. n indicates the number of samples

3.2 Discriminant Analysis

3.2.1 Spectral Separability of Algal Taxonomic Divisions

Discriminant analysis (QDA) with algal taxonomic divisions as known categories had low levels of misclassification and indicates there is separability of the divisions. The discriminant analysis algal division score summary can be seen in Table 2. The training set had a misclassification rate of 0.3% and an entropy $R^2 = 0.95$. Validation and testing sets had a higher misclassification rate of 3.5% and a lower entropy R^2 of 0.23 and 0.29, respectively. This indicates that the validation and testing sets were less well fit than the training sets, but they still had a correct classification rate of this data subset at over 96%.

Brown algae and cyanobacteria were most misclassified as each other in this analysis. Brown samples were misclassified as cyanobacteria at a rate of 2.5%, and cyanobacteria were misclassified as brown algae at a rate of 6%. Green algae samples were misclassified as belonging to red (0.5%) and brown (0.5%) taxonomic divisions.

Table 2**.** *Algal Division Discriminant Analysis Score Summary for Training, Validation, and Test Sets.*

Source	**Count**	**Number Misclassified**	**Percent Misclassified**	**Entropy R^2**
Training	345	1	0.3	0.95
Validation	115	4	3.5	0.23
Test	115	4	3.5	0.29

Figure 3 illustrates the clustering occurring in one of three possible canonical relationships that are incorporated into the classifications of algal taxonomic divisions. The visualization of canonicals shows the clear clustering of different taxonomic divisions and illustrates the slight overlap between divisions. Cyanobacteria are clustered in the middle of the other three clusters and are most misclassified as brown algae in the algal division QDA. For the canonical scatterplot matrix with canonicals 1-3 resulting from this QDA, please refer to Appendix A, Figure A4.

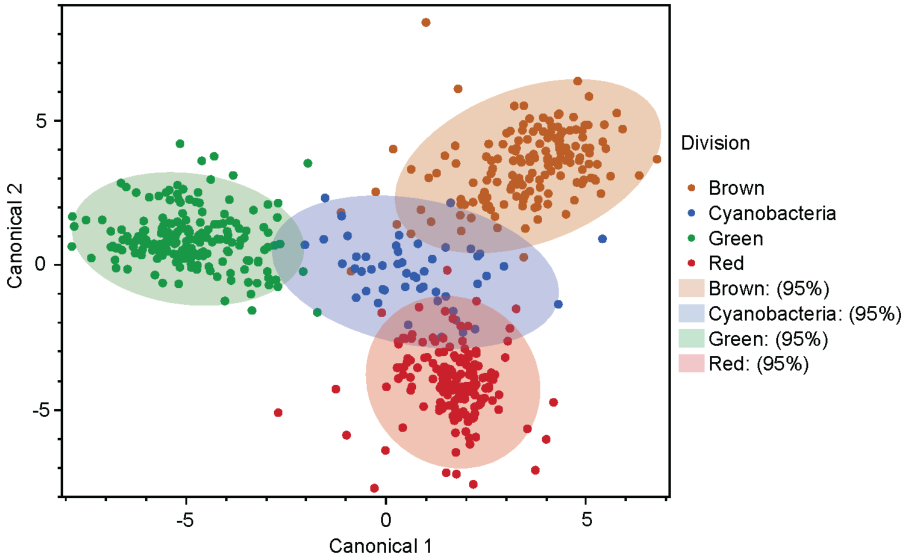

Figure 3. *Canonical Plot of Canonical One and Canonical Two from Quadratic Discriminant Analysis of All Samples with the Algal Divisions as Known Categories.* Points indicate samples and ellipses contain 95% of each algal division.

3.2.2 Spectral Separability of Algal Species

Discriminant analysis with algae species or species complexes as the known categories showed a low percentage of misclassification for the training set (1.7%) and an entropy R^2 (0.98) close to one (Table 3). Validation and test sets had a higher rate of misclassification at 16.8% and 16.7% respectively. The validation and test sets had entropy R^2 values that were negative and indicate a less fit model.

Table 3. *Algal Species Discriminant Analysis Score Summary for Training, Validation, and Test Sets.*

Source	**Count**	**Number Misclassified**	**Percent Misclassified**	**Entropy R^2**
Training	348	6	1.7	0.98
Validation	113	19	16.8	-1.81
Test	114	19	16.7	-4.40

The taxonomic divisions with the highest rates of misclassifications of the species within them were brown algae (15.3%) and cyanobacteria (16%). The five algae species with the highest misclassification rates are either brown algae or cyanobacteria. The two species with the highest misclassification rates are the brown algae *Sargassum echinocarpum* (41.6%) and *Lobophora variegata* (26.7%). Cyanobacteria *Symploca hydnoides* (20%), brown alga *Dictyota acutiloba* (13.8%), and cyanobacteria *Leptolyngbya crosbyana* (12%) misclassification rates followed. Brown algae species were most misclassified as other species of brown algae (8.9%) but also were misclassified as turf and species in the other three divisions. Cyanobacteria were most misclassified as turf (6%), but also were misclassified as other species of cyanobacteria, and species belonging to the brown and red taxonomic divisions.

Turf and red algae species had mid-level misclassification rates. Turf was misclassified (6.9%) as species belonging to red and brown algal taxonomic divisions. Species in the red algae division that were misclassified (5.2%) were primarily invasive algae. Red algae were misclassified as other species of red algae, cyanobacteria, and brown algae. All red invasive algae were misclassified as native species.

The taxonomic division with the lowest misclassification rate of species was the green division (1.1%). The two algae with the lowest misclassification rates are the green invasive alga *Avrainvillea lacerata* (3.1%) and the green native alga *Dictyosphaeria versluysii* (3.3%). Both species were misclassified once as green alga *Halimeda opuntina*. No other green algae species were misclassified, but brown alga *Sargassum echinocarpum* was misclassified once as green invasive alga *Avrainvillea lacerata* (4.2%).

Almost half of the misclassifications were species misclassified as different species of the same division (47.8%) and 20.5 % of the misclassifications involved turf which likely has species of more than one division. Invasive algae were misclassified 6.8% of the time and made up 18.2 % of the misclassifications. All invasive algae misclassifications were classified as native species and only one native alga was misclassified as invasive. Native algae were misclassified at a rate of 7.9% and made up 77.3% of the misclassifications. Misclassified species were most classified as one of four species: cyanobacteria *Symploca hydnoides*, turf, brown algae *Turbinaria ornata*, and red algae *Dichotomaria marginata.* Full misclassification details can be found in Appendix A, Table A2.

3.2.3 Spectral Separability of Invasive and Native Algae

Discriminant analysis with invasive and native algae as known categories had a very low rate of misclassification by division (Table 3), with only one native green alga misclassified as an invasive green alga. No other misclassifications occurred and all entropy R^2 values were 1 except for the green algae validation set with an entropy R^2 of 0.65. An entropy R^2 value of 1 indicates a perfectly fit model.

Table 4. *Native and Invasive Algae Discriminant Analysis Score Summary for Training, Validation, and Test Sets.*

Division	**Source**	**Count**	**Number Misclassified**	**Percent Misclassified**	**Entropy R^2**
Green	Training	118	0	0	1
Green	Validation	38	1	2.63	0.65
Green	Test	41	0	0	1
Red	Training	97	0	0	1
Red	Validation	33	0	0	1
Red	Test	31	0	0	1

4. DISCUSSION

4.1 Spectral Signatures of Algae

4.1.1 Spectral Signatures of Algae by Taxonomic Division

Spectral reflectance patterns of algal taxonomic divisions followed characteristics observed in previous studies and lined up with absorption bands of photosynthetic pigments characteristic to the divisions (Chao Rodríguez et al., 2017; Douay et al., 2022; Olmedo-Masat et al., 2020). All algal taxonomic division spectral signatures had an absorption well with a minimum reflectance at 667 - 672 nm that corresponds to chlorophyll a (Chl a) (Chao Rodríguez et al., 2017). Chl a is common across all algal taxonomic divisions. Brown algae, cyanobacteria, and red algae divisions had reflectance peaks at 600 nm and 650 nm and green had a singular peak around 560 nm in agreement with findings from previous studies (Douay et al., 2022; Olmedo-Masat et al., 2020; Slonecker et al., 2021). These characteristic reflectance peaks are due to the different photosynthetic pigments present in each division.

The brown algal division is the only division known to contain Chlorophyll c (Chl c), and the characteristic absorbance well with a local minimum at 632 nm we observed corresponds to the absorption of that pigment (Chao Rodríguez et al., 2017; Olmedo-Masat et al., 2020). While the brown algal division mean spectral signature does not show peaks around 570 nm found in previous studies, most of the brown algae species means showed peaks in that area (Appendix A, Figure A1) (Douay et al., 2022; Olmedo-Masat et al., 2020). The 570 nm peak in brown algae could be indicative of absorption by Chl c in the 582-596 nm range (Chao Rodríguez et al., 2017).

Red algae and cyanobacteria have similar reflectance peaks as brown algae with local maxima at 600 nm and 650 nm. However, the photosynthetic pigments responsible for these peaks differ. Unlike brown algae, red algae and cyanobacteria do not contain Chl c. They both contain phycocyanin, which brown algae lack, that is responsible for the reflectance peaks at 600 nm and 650 nm. Phycocyanin has an absorption band at 608-628 nm which could account for the absorbance well minimum at 627 nm observed in cyanobacteria and red algae (Chao Rodríguez et al., 2017; Huisman et al., 2007). Both cyanobacteria and red algae showed an absorbance well at 492 nm associated with phycoerythrin (Chao Rodríguez et al., 2017; Olmedo-Masat et al., 2020). Red algae showed a unique peak at 552 nm linked to an absorbance well at 567 nm that corresponds to phycoerythrin absorption at 556 – 581 nm (Chao Rodríguez et al., 2017). Douay et al. (2022) found a peak unique to red with a local maximum at 515 nm that we did not observe. However, the peak with a local maximum at 552 nm we observed shows increased reflectance starting before 515 nm.

4.1.2 Spectral Signatures of Invasive and Native Algae

Invasive algae spectral signatures had similar division characteristics as native algae belonging to the same division, but the reflectance of native and invasive algae within the same division differed (Figure 3). While the reflectance standard deviation of invasive and native algae often overlapped, there were certain wavelengths that the standard deviation did not overlap which could indicate areas of distinction between the groups.

Invasive red algae had higher reflectance than native red algae and standard deviations did not overlap from 487 – 527 nm, wavelengths at which phycoerythrin absorbs light. This indicates that native algae are absorbing more light at these wavelengths than invasive algae. There may be some physiological advantage for red invasive algae to reflect more light at these wavelengths, but this remains unknown at this time.

The mean reflectance of invasive green algae differed from native green algae reflectance at different wavelengths. The invasive green algae reflectance was lower than the native green algae without standard deviation overlap at 552 – 577 nm. These wavelengths correspond to the characteristic peak of green algae influenced by chlorophyll a (Chl a) and chlorophyll b (Chl b). The native green algae peak maximum is at 557 nm, while the invasive green algae peak maximum is at 597 nm. Previous studies have suggested higher absorption by Chl b will narrow and shift the peak to the shorter wavelengths (Olmedo-Masat et al., 2020). This also may explain why the invasive green algae have higher reflectance from 667 – 672 nm. As only one species of invasive green algae was sampled, more data are needed to confirm these results.

Invasive algae are known to adapt to degraded environments, grow quickly, and outcompete native algae. We found that the mean spectral reflectance of invasive algae is higher and lower at certain wavelengths than native algae and there are certain wavelengths at which native and invasive algae are distinct. There may be an advantage in absorbing more or less light at different portions of the spectrum that explain the invasive behavior that certain algae exhibit.

4.1.3 Spectral Signature Considerations

These are the first spectral signatures documented and published specific to Hawaiian macroalgal species to our knowledge. However, 30 species or species complexes represent a small portion of the diversity present in Hawaiʻi (Tsuda, 2014). Further collection of more Hawaiian macroalgae species spectra could add to the current library and create a more comprehensive baseline. Future studies could better capture intraspecific, phenological, and spatial variation to increase the robustness of the library.

4.2 Discriminant Analysis

4.2.1 Spectral Separability of Algal Taxonomic Divisions

Discriminant analysis (QDA) with algal divisions as the categorical variables indicated a low misclassification percentage throughout training, validation, and test sets not surpassing four percent. The entropy R^2 decreased in validation and test sets indicating a decrease in model fit. However, small entropy R^2 values are common with discriminant models due to uncertainty in the predicted probabilities (SAS Institute Inc., 2022).

Brown algae and cyanobacteria were most confused with each other in the discriminant classification. Both brown algae and cyanobacteria have peaks at 600 nm and 650 nm and lack the peak unique to red found with a mean maximum at 552 nm. These spectral similarities likely play into why they are commonly misclassified but the chemical compounds behind these matching peaks differ.

Previous algal spectral separability studies, to the authors' knowledge, have not included cyanobacteria in the analysis. This is likely because cyanobacteria are protists, whereas the other divisions are eukaryotes. We included cyanobacteria because it is a photosynthetic benthic species that grows in the same habitat as traditional algae divisions. As they inhabit similar niches and have spectral characteristics similar to brown algae, it is important to include Cyanobacteria in future spectroscopy studies.

To explore the spectral separability of cyanobacteria, brown, green, and red algal divisions we used discriminant analysis and known categorical data were input into the model built. We also included intraspecific variation by inputting all readings into the QDA instead of species means. Douay et al. (2022) and Olmedo-Masat et al. (2020) did not use categorical input and instead used multiscale bootstrap resampling and Hierarchical Cluster Analysis as a bottom-up approach. Olmedo-Masat et al. (2020) found this approach was sufficient to identify macroalgal divisions but used species medians that did not consider intraspecific variation. Douay et al. (2022) found that brown and red divisions could not be differentiated with the bottom-up methodology when including intraspecific variation. Our findings indicate that it may be valuable to include known categorical input into models to determine the separability of algal divisions.

4.2.2 Spectral Separability of Algal Species

Discriminant analysis-based classification accuracies at algal species or complexes levels were lower than classification accuracies at algal taxonomic division levels. Nonetheless, species-level discriminant analysis still performed well, with an accuracy exceeding 83%. Almost half of the misclassifications were species confused as other species of the same division, likely due to similarities in photosynthetic pigments within each division. The brown algae with the highest misclassification rate, *Sargassum* *echinocarpum*, was misclassified 90% of the time as other species of brown algae that share similar color, structure, and intertidal habitat (Huisman et al., 2007). This shows that misclassification may not only be influenced by the common taxonomic division photosynthetic pigments, but also by algal structure and habitat.

Twenty percent of misclassifications were associated with turf. Because turf is likely a combination of divisions and species, it is not surprising that a high percentage of misclassifications involve turf. Turf was most misclassified as brown algae *Lobophora variegata*. The spectral signatures of turf and *L. variegata* share peaks at 600 nm and 650 nm and an absorbance well minimum at 500 nm. *L. variegata* is slightly calcified, and although it belongs to the brown algal taxonomic division, it sometimes looks red in color. The *L. variegata* we sampled is usually found on shaded surfaces and was found growing adjacent to the turf we sampled.

Native and invasive algae species in discriminant analysis using algal species as categorical variables yielded similar accuracies of 92.1% and 93.2%, respectively. Although more invasive algal species were mistakenly reported as native species than native algae species reported as invasive species, invasive algae were not misclassified at a rate higher than the native algae species. Half of the invasive algae species misclassified were classified as other species belonging to the same division, following the larger trend of misclassification.

4.2.3 Spectral Separability of Invasive and Native Algae

Discriminant analysis (QDA) of samples with invasive and native algae as the categorical variables by division only yielded one misclassification of a native green alga as an invasive green alga (3.1%). These results differ from the algal species QDA, where

6.8% of invasive algae were misclassified as native algae and were primarily comprised of red taxonomic division misclassifications. This analysis of invasive and native algae was grouped by division, so the potential for misclassification of the invasive algae as other species belonging to different divisions was excluded. This analysis also was run at a larger scale than the algal species QDA, only including taxonomic division and native and invasive status in the input. Perhaps the species distinction highlighted in the algal species QDA allowed similarities between certain native and invasive red species to be more apparent than when looking at native reds as a whole division. While there were misclassifications in both QDA analyses, there still was a correct classification rate of invasive algae at 93% or higher.

This invasive and native algae by taxonomic division QDA indicates that the classification of invasive and native algae is possible at this resolution. This supports the findings of Asner et al. (2008) in which Hawaiian terrestrial native and invasive species were spectrally delineated. Asner et al. (2008) found that invasive and native trees were best distinguished by a combination of canopy reflectance from 1125 – 2500 nm and absorption features in the 400 – 700 nm range. Here we only assessed the visible wavelengths for which marine spectroscopy is limited due to water absorption of longer wavelength energy.

4.2.4 Discriminant Analysis Considerations

Samples used for the validation and test sets were subsets of all the data collected and contained the same species used for the training set. Because the test sets were not data containing different species of algae, one cannot assume that the model would perform with the same level of misclassifications if data with different species was inputted. This analysis was simply an exploratory investigation of the separability of algal groups in the samples obtained. As more spectra of Hawaiian macroalgae are collected they should be used to develop a more robust discriminant model.

5. CONCLUSION

We created a spectral library of 30 Hawaiian marine macroalgae species or species complexes. The algal taxonomic division signatures generally followed the characteristics found in previous studies and were aligned with the photosynthetic pigment absorption characteristic of the divisions. Discriminant analysis (QDA) with taxonomic divisions as the categorical variables showed a high accuracy equal to or greater than 96%. Algal species QDA showed a lower accuracy rate than algal taxonomic division QDA, but still amounted to over 83%. Spectral signatures of native and invasive algae followed taxonomic division characteristics and differences between native and invasive species allowed us to discriminate between these categories 93%- 100% of the time. The high rate of correct classification indicates that there is spectral separability between algae belonging to different taxonomic divisions and between native and invasive algae by division.

This study lays the groundwork for further research involving the spectral signatures of Hawaiian marine macroalgae. If taxonomic groups are spectrally separable with high accuracy, *in situ* spectral measurements could be used for the identification of those groups difficult to identify in the field. Additionally, these findings of the spectral separability of taxonomic divisions and native and invasive macroalgae could be applied to remote sensing studies that could be transformative for marine management.

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APPENDIX A

SUPPLEMENTARY TABLES AND GRAPHS

Table A1. *Sampled Macroalgal Species Details.*

Division	**Invasive, Native, or Unknown**	**Island**	**Site**	**Genus**	**Species**	**Method**	**Number of Individuals**
Brown	Native	Hawai‘i	Miloli‘i	*Sargassum*	*echinocarpum*	*ex-situ*	23
Brown	Native	Hawai‘i	Miloli‘i	*Sargassum*	*obtusifolium*	*ex-situ*	24
Brown	Native	Hawai‘i	Miloli‘i	*Turbinaria*	*ornata*	*ex-situ*	24
Brown	Native	Hawai‘i	Papa	*Chrysocystis*	*fragilis*	*in-situ*	24
Brown	Native	Hawai‘i	Papa	*Lobophora*	*variegata*	*in-situ*	27
Brown	Native	Hawai‘i	Papa	*Lobophora*	*variegata*	*ex-situ*	3
Brown	Native	Hawai‘i	Papa	*Padina*	*australis*	*ex-situ*	1
Brown	Native	Hawai‘i	Papa	*Sargassum*	*echinocarpum*	*ex-situ*	1
Brown	Native	Hawai‘i	Papa	*Sargassum*	*obtusifolium*	*ex-situ*	1
Brown	Native	Hawai‘i	Papa	*Turbinaria*	*ornata*	*ex-situ*	1
Brown	Native	O‘ahu	Maunalua	*Dictyota*	*acutiloba*	*ex-situ*	29
Brown	Native	O‘ahu	Maunalua	*Padina*	*australis*	*ex-situ*	3
Complex	Unknown	Hawai‘i	Papa	Turf	spp.	*in-situ*	23
Complex	Unknown	Hawai‘i	Papa	Turf	spp.	*ex-situ*	6
Cyanobacteria	Native	Hawai‘i	Papa	*Leptolyngbya*	*crosbyana*	*ex-situ*	25
Cyanobacteria	Native	Hawai‘i	Papa	*Symploca*	*hydnoides*	*in-situ*	16
Cyanobacteria	Native	Hawai‘i	Papa	*Symploca*	*hydnoides*	*ex-situ*	9
Green	Invasive	O‘ahu	Maunalua	*Avrainvillea*	*lacerata*	*ex-situ*	32
Green	Native	Hawai‘i	Miloli‘i	*Chaetomorpha*	*antennina*	*ex-situ*	5
Green	Native	Hawai‘i	Papa	*Caulerpa*	*taxifolia*	*ex-situ*	4
Green	Native	Hawai‘i	Papa	*Halimeda*	*opuntina*	*in-situ*	22
Green	Native	Hawai‘i	Papa	*Halimeda*	*opuntina*	*ex-situ*	2
Green	Native	O‘ahu	Kāne‘ohe	*Dictyosphaeria*	*cavernosa*	*in-situ*	25
Green	Native	O‘ahu	Kāne‘ohe	*Dictyosphaeria*	*versluysii*	*in-situ*	24
Green	Native	O‘ahu	Kāne‘ohe	*Halimeda*	*discoidea*	*in-situ*	25
Green	Native	O‘ahu	Kāne‘ohe	*Dictyosphaeria*	*versluysii*	*ex-situ*	6
Green	Native	O‘ahu	Maunalua	*Caulerpa*	*serularioides*	*ex-situ*	43
Green	Native	O‘ahu	Maunalua	*Dictyosphaeria*	*cavernosa*	*ex-situ*	3
Green	Native	O‘ahu	Maunalua	*Enteromorpha*	*prolifera*	*ex-situ*	3
Green	Native	O‘ahu	Maunalua	*Halimeda*	*discoidea*	*ex-situ*	3
Red	Invasive	O‘ahu	Kāne‘ohe	*Acanthophora*	*spicifera*	*in-situ*	25
Red	Invasive	O‘ahu	Kāne‘ohe	*Eucheuma*	*spp.*	*in-situ*	25
Red	Invasive	O‘ahu	Kāne‘ohe	*Eucheuma*	*spp.*	*ex-situ*	3
Red	Invasive	O‘ahu	Kāne‘ohe	*Gracillaria*	*salicornia*	*ex-situ*	3
Red	Invasive	O‘ahu	Kāne‘ohe	*Kappaphycus*	*spp.*	*ex-situ*	5
Red	Invasive	O‘ahu	Maunalua	*Acanthophora*	*spicifera*	*ex-situ*	2
Red	Invasive	O‘ahu	Maunalua	*Gracillaria*	*salicornia*	*ex-situ*	27
Red	Native	Hawai‘i	Miloli‘i	*Anfeltiopsis*	*concinna*	*ex-situ*	20
Red	Native	Hawai‘i	Papa	*Anfeltiopsis*	*concinna*	*ex-situ*	1
Red	Native	Hawai‘i	Papa	*Dichotomaria*	*marginata*	*in-situ*	19
Red	Native	Hawai‘i	Papa	*Dichotomaria*	*marginata*	*ex-situ*	3
Red	Native	Hawai‘i	Papa	*Ramicrusta*	*hawaiiensis*	*in-situ*	26
Red	Native	O‘ahu	Kāne‘ohe	Green CCA	spp_007	*ex-situ*	1
Red	Native	O‘ahu	Kāne‘ohe	Red CCA	spp_006	*ex-situ*	1

Table A1. continued

Red	Unknown	O‘ahu	Kāne‘ohe	Red Branching	spp_005	*ex-situ*	3
Red	Unknown	O‘ahu	Kāne‘ohe	Red Filamentous	spp_004	*ex-situ*	3

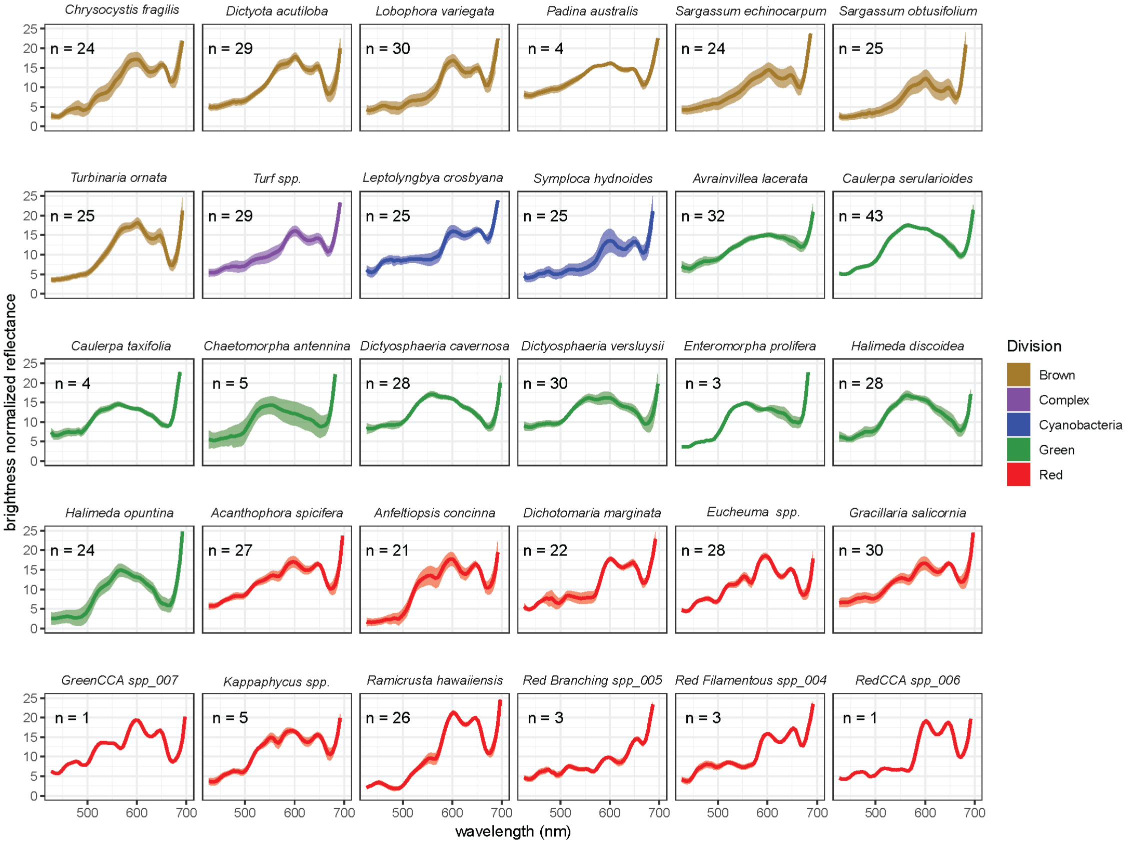

Figure A1. *Brightness Normalized Reflectance Spectra of Hawaiian Macroalgae Species.* Species means are plotted as solid lines. Standard deviation is the shaded area. The number of samples is indicated by n.

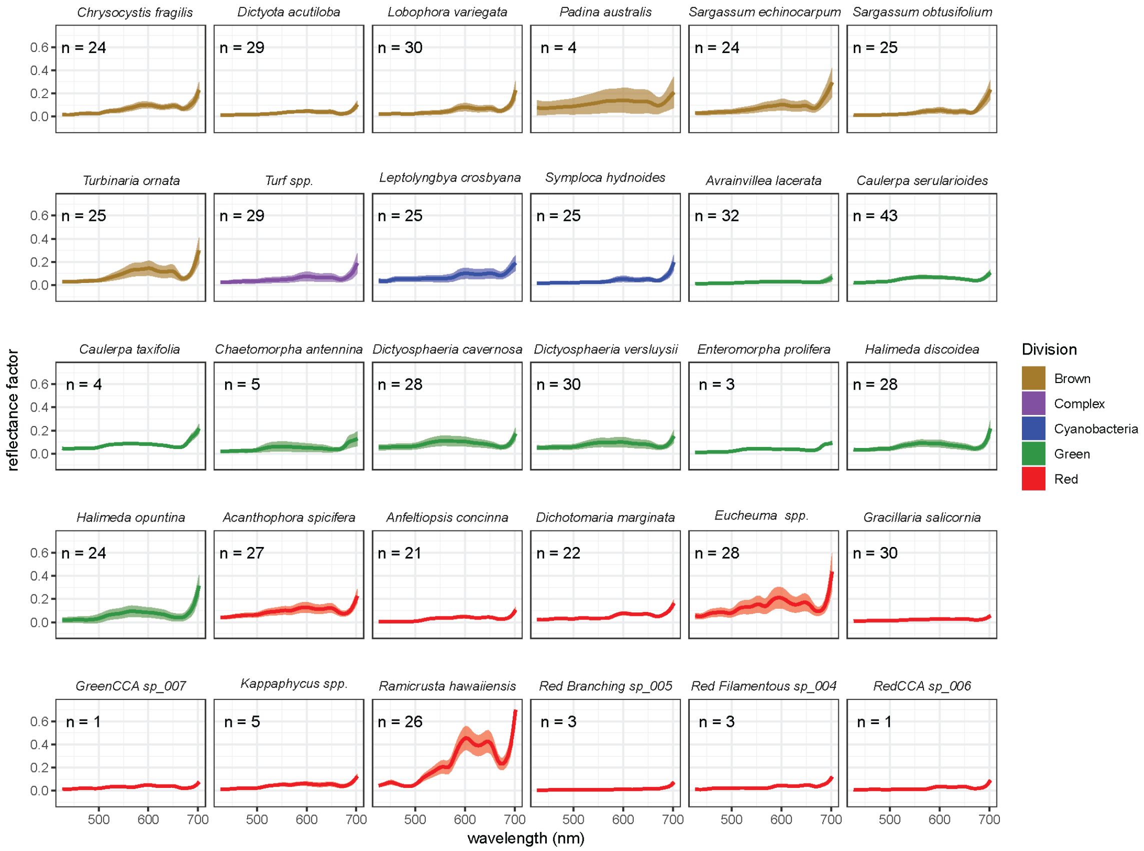

Figure A2. *Raw Reflectance Spectra of Hawaiian Macroalgae Species.* Species means are plotted as solid lines. Standard deviation is the shaded area. The number of samples is indicated by n.

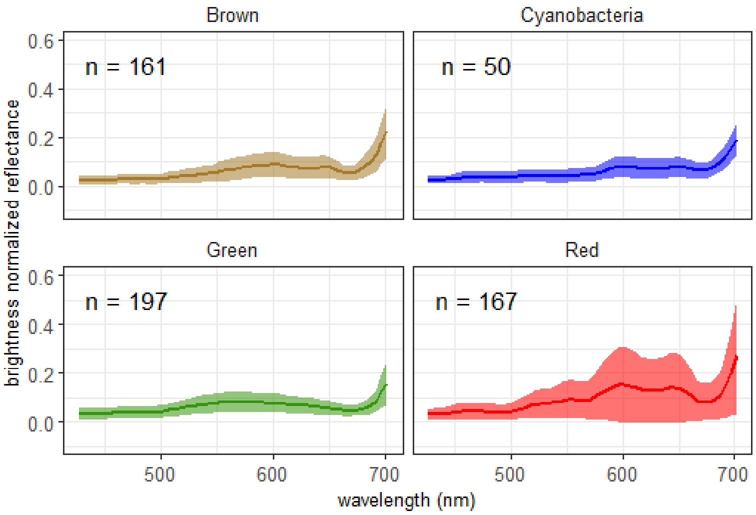

Figure A3. *Raw Reflectance Spectra of Hawaiian Macroalgae Taxonomic Divisions.* Division means are plotted as solid lines. Standard deviation is the shaded area. The number of samples is indicated by n.

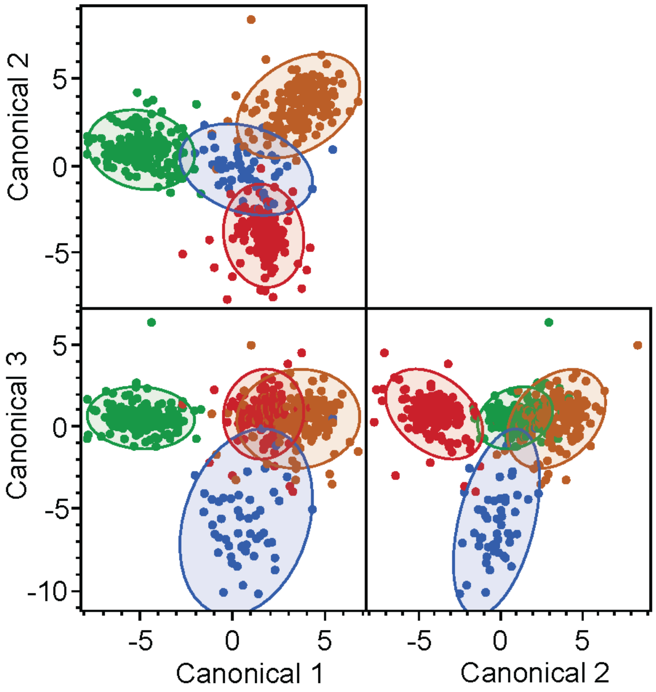

Figure A4. *Canonical Scatter Plot Matrix of Canonicals 1-3 from Quadratic Discriminant Analysis using Algal Divisions as the Known Categories.*

Table A2: *Confusion Matrix of Algal Species Discriminant Analysis Misclassifications.*

			Predicted										
			Brown					Complex	Cyanobacteria	Green		Red	
Actual			Native					Unknown	Native	Invasive	Native	Native	
Division	Biogeographic status	Species	*Dictyota acutiloba*	*Lobophora variegata*	*Sargassum echinocarpum*	*Sargassum obtusifolium*	*Turbinaria ornata*	Turf	*Symploca hydnoides*	*Avrainvillea lacerata*	*Halimeda opuntina*	*Dichotomaria marginata*	Total
Brown	Native	*Dictyota acutiloba*	0	0	1	0	2	0	1	0	0	0	4
Brown	Native	*Lobophora variegata*	0	0	0	0	0	4	2	0	0	2	8
Brown	Native	*Sargassum echinocarpum*	0	0	0	5	4	0	0	1	0	0	10
Brown	Native	*Sargassum obtusifolium*	0	0	0	0	1	0	0	0	0	0	1
Brown	Native	*Turbinaria ornata*	1	0	0	0	0	0	0	0	0	0	1
Complex	Unknown	Turf	0	1	0	0	0	0	0	0	0	1	2
Cyanobacteria	Native	*Leptolyngbya crosbyana*	0	0	1	0	0	0	2	0	0	0	3
Cyanobacteria	Native	*Symploca hydnoides*	0	1	0	0	0	3	0	0	0	1	5
Green	Invasive	*Avrainvillea lacerata*	0	0	0	0	0	0	0	0	1	0	1
Green	Native	*Dictyosphaeria versluysii*	0	0	0	0	0	0	0	0	1	0	1
Red	Invasive	*Acanthophora spicifera*	0	1	0	0	0	0	0	0	0	1	2
Red	Invasive	*Eucheuma* spp.	0	0	0	0	0	0	1	0	0	2	3
Red	Invasive	*Gracillaria salicornia*	0	0	0	0	0	0	2	0	0	0	2
Red	Native	*Ramicrusta hawaiiensis*	0	1	0	0	0	0	0	0	0	0	1
		Total	1	4	2	5	7	7	8	1	2	7	44