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Benthic coverage of the invasive tunicate *Didemnum vexillum* on Georges Bank is largely unknown. Monitoring of *D. vexillum* coverage is vital to understanding the impact this invasive species will have on the productive fishing grounds of Georges Bank. Here we investigate using reflectance spectroscopy as a method for remote identification of *D. vexillum*. Using two different systems, a NightSea Dive-Spec and a combination of LED light sources with a hyperspectral radiometer, we collected *in-situ* measurements of reflectance from *D. vexillum* colonies. In comparison to reflectance spectra of other common benthic substrates, *D. vexillum* appears to have a unique spectral signature between 500 and 600 nm. Measuring the slope of the spectrum between these wavelengths appears to be the most robust method for spectral identification. Using derivative analysis or principal component analysis, the reflectance spectra of *D. vexillum* can be identified among numerous other spectra of common benthic substrates. An optical system consisting of a radiometer, light source, and camera was deployed on a remotely operated vehicle to test the feasibility of using reflectance to assess *D. vexillum* coverage. Preliminary results, analyzed here, prove the method to be successful for the areas we surveyed and open the way for its use on large-scale surveys.

OCIS codes: 010.4450, 240.6645.

1. Introduction

Reports of an invasive tunicate, *Didemnum* sp., in coastal areas of New England date back to the late 1980s [1]. In the past there has been controversy over the exact identification and origin of the species [2,3]. However, a recent study has identified the invasive tunicate as *Didemnum vexillum* [4], first described by Kott [5] in New Zealand. The means of introduction is not well known, however, it is likely that *D. vexillum* was introduced by the aquaculture of shellfish imported from Japan [6].

Since its introduction to New England waters, *D. vexillum* has expanded throughout the Gulf of Maine. It has been observed colonizing both inshore and offshore habitats, including the prized fishing grounds of Georges Bank. *Didemnum vexillum* was first observed on Georges Bank in 2002 and has since colonized large areas of the benthos [7]. Using

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photographic surveys of Georges Bank (2004–2005) it was estimated to cover 50%–90% of hard substrates over a 230 km^2 survey area [1].

Didemnum vexillum is an invasive ecosystem engineer and may pose a threat to the Georges Bank ecosystem. *Didemnum vexillum* is able to form dense mats over hard substrates, even cobble bottoms. These mats are predicted to have numerous negative impacts including: smothering benthic organisms, reducing settlement of larvae, reducing shelter for juvenile fish, and preventing groundfish from feeding on benthic organisms [7]. Since the introduction of *D. vexillum*, the benthic species composition on Georges Bank has been noticeably altered [4].

In order to understand the impacts *D. vexillum* will have on Georges Bank, it is important to monitor its distribution. Photographic surveys are currently the method of choice for mapping *D. vexillum* coverage. However, current surveys could be improved by employing a simpler and more rapid method of identification. A possible solution is to identify *D. vexillum* using reflectance spectroscopy. Different substrates typically reflect light in a unique way based on their composition and roughness [8,9]. Using multispectral and/or hyperspectral radiometers, differences in the reflectance spectrum can be used to identify different substrates.

This technique has been used successfully to remotely identify substrates in numerous fields of study. Substrates on earth as well as other planets have been reliably identified based on their reflectance spectrum [10,11]. In the marine environment, reflectance has been used to identify benthic substrate composition in shallow waters [12–14].

In addition to physical studies, reflectance has also been used for biological applications. It has been successfully used to assess the health of crops from a remote location [15,16]. Off the coast of Washington, eelgrass beds have been identified from an autonomous underwater vehicle (AUV) using benthic reflectance [17]. We hypothesized that similar methods could be used to identify *D. vexillum* on Georges Bank. Benthic reflectance spectra from Georges Bank could be collected using a radiometer mounted on an AUV. If the reflectance spectrum of *D. vexillum* were unique, it would provide a rapid and accurate method of identification.

Large-scale surveys using reflectance require automated substrate identification. This can be accomplished using numerous techniques [18]. In many applications, identification can be accomplished by simply calculating reflectance ratios using carefully chosen wavelengths [16,17]. In other cases, derivative analysis can amplify even subtle features in the reflectance spectrum [19].

To investigate the reflectance from *D. vexillum* mats, we compared benthic reflectance spectra of *D. vexillum* with numerous other substrates. From this, we were able to identify unique features in the *D. vexillum* reflectance spectrum, and used these features to develop an algorithm for automated detection. Last, we deployed an optical system on a remotely operated vehicle (ROV) to test the feasibility of identifying *D. vexillum* from a remote platform.

2. Methods

Two days of fieldwork were conducted in June 2010 to collect *in-situ* reflectance data. Divers at the Darling Marine Center in Walpole, ME, collected reflectance spectra from various substrates including *D. vexillum*. Spectra were collected using both a NightSea DiveSpec [20] and a Satlantic Hyperspectral Ocean Color Radiometer (HyperOCR). Additional spectra were collected in a flowing seawater lab using the DiveSpec. When using the DiveSpec, reflectance measurements were collected approximately 5 cm from the substrate under illumination from the built in LEDs. Substrates measured with the HyperOCR were illuminated using an LED Aquasun dive light. The dive light helped to enrich incident light in blue and red wavelengths (which are attenuated by the water column). LED illumination was chosen for efficiency and spectral stability over time. Reflectance measurements using the HyperOCR were made roughly 1 m from the substrate.

During *in-situ* data collection, the HyperOCR was connected by a power and communications cable above the sampling location, where data were logged continuously on a laptop computer. The radiometer lens cap was used as a delimiter between samples. Using notes taken by the divers and the delimiter between samples, the dataset was organized according to substrate. Substrates measured with the DiveSpec were identified by headings entered by the diver.

We applied corrections to our radiance measurements to account for both changes in the ambient light field and for attenuation of light by suspended particles between the radiometer and the substrate. This was achieved by referencing our measurements to the radiance spectrum from a surface of known reflectance. Reflectance from a Spectralon plaque, calibrated to diffusely reflect 95% of incident light, was frequently measured with the HyperOCR. The DiveSpec was corrected using a Spectralon plaque calibrated to reflect 99% of incident light. The reflectance spectrum from every substrate measured was normalized by the area under the spectrum, and then divided by the reflectance spectrum of the closest Spectralon plaque measurement. Area normalization was essential to account for any changes in the illumination intensity between standard and substrate measurements. The corrections applied to the spectra are described by Eq. 1:

$$\tilde{R}(\lambda) = \left(\frac{L(\lambda)}{\int_{425\ \mathrm{nm}}^{700\ \mathrm{nm}} L(\lambda)\mathrm{d}\lambda}\right) \Big/ \left(\frac{L^*(\lambda)}{\int_{425\ \mathrm{nm}}^{700\ \mathrm{nm}} L^*(\lambda)\mathrm{d}\lambda}\right), \quad (1)$$

where $L(\lambda)$ is the radiance measured from the substrate, $L^*(\lambda)$ is the radiance measured from the Spectralon plaque, and $\tilde{R}(\lambda)$ is the area normalized reflectance. The HyperOCR measures radiance from

350 to 800 nm, however the spectra were truncated to 425–700 nm because of noise in the ultraviolet and near infrared. The light sources used in this study did not provide adequate light at these wavelengths, which lead to low signal to noise ratios in these regions.

In December 2011 the ROV Reef Explorer II was deployed at the Sunset Bay Marina in Hull, MA. It was outfitted with a forward facing HyperOCR and LED light source. The ROV was also equipped with a forward facing camera. A Spectralon plaque was lowered into the water to allow for *in-situ* measurement of its reflectance. Following measurement of the Spectralon plaque, the ROV collected reflectance spectra from *D. vexillum* encrusted dock pilings and from benthic mud and algae. Spectra were normalized and referenced to the Spectralon plaque using the same method described earlier [Eq. (1)]. Area normalized reflectance spectra were matched to images from the camera by timestamp. This allowed for identification of the substrate that the spectrum was collected from.

The fraction of the camera's field of view occupied by the dock piling (that was of known diameter) was used to calculate distance between the radiometer and the piling. Using the distance and the radiometer acceptance angle, a circle representing the radiometer field of view was overlaid on each image (Fig. 1). Spectra of the Spectralon plaque were easily distinguishable from all other spectra. The images that corresponded to these spectra were used to estimate the most likely offset angle of the radiometer in relation to the optical axis of the camera. The offset angle was used to adjust the position the circle representing the radiometer field of view.

3. Results

A. Darling Marine Center

Reflectance spectra from ten different colonies of *D. vexillum* were recorded in the lab and *in-situ* using the DiveSpec (http://misclab.umeoce.maine.edu/research/SpecIndex/spec_index.htm). The reflectance spectra all showed a characteristic shape with high reflectance near 600 nm. The variation in the reflectance spectrum between colonies was small. The variation was smallest near 525 nm (Fig. 2A).

Reflectance spectra from seven different *D. vexillum* colonies were collected *in-situ* using the HyperOCR. All reflectance spectra were similar in shape and showed little overall variation. However, there was larger uncertainty in the short and long wavelengths (Fig. 2B). In comparison to spectra collected with the DiveSpec, the orange peak at 600 nm was less defined.

Compared to reflectance spectra of the most common substrates we encountered, the reflectance spectrum of *D. vexillum* is most unique between 500 and 600 nm. Reflectance spectra of many inorganic substrates (e.g., mud, sand, shells) appear relatively featureless in comparison to *D. vexillum* [12] (Fig. 3A). In comparison to biological substrates, the reflectance spectrum of *D. vexillum* still maintains unique features. Even in comparison to another unidentified species of tunicate, the slope of the spectra between 500 and 600 nm is considerably different (Fig. 3B).

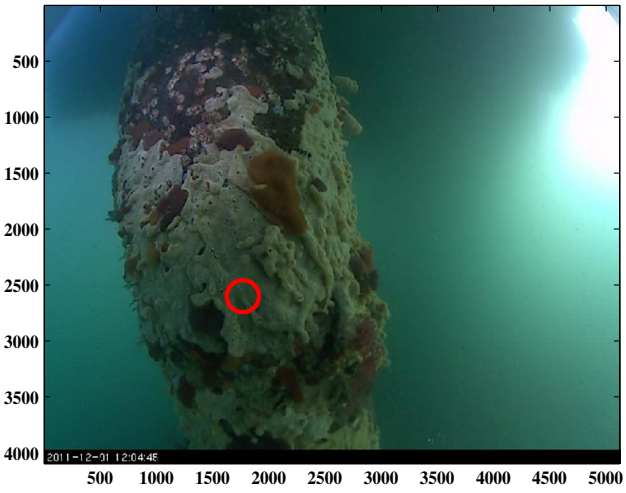


Fig. 1. (Color online) Image of *D. vexillum* taken by the ROV. The red circle marks the field of view of the radiometer. Axis labels denote pixel number.

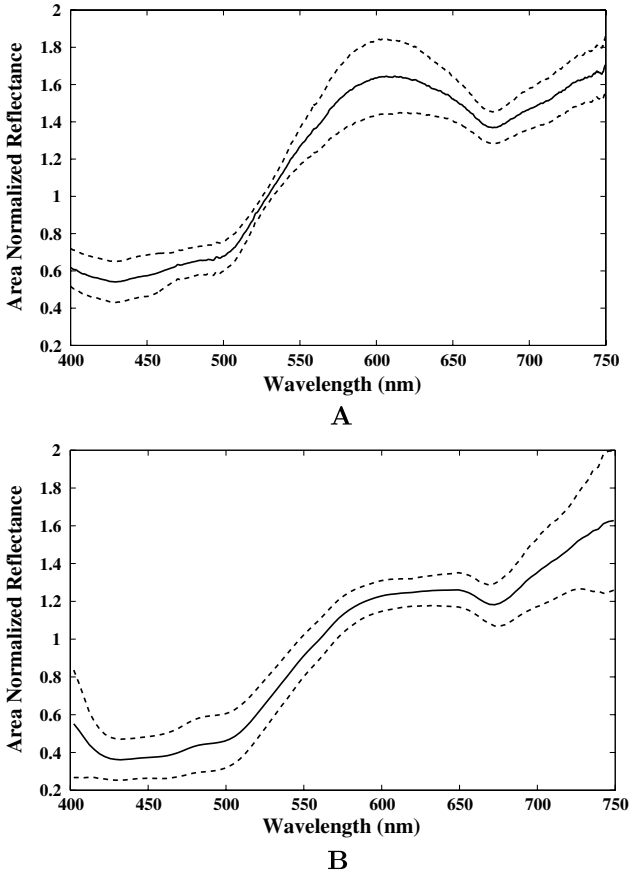
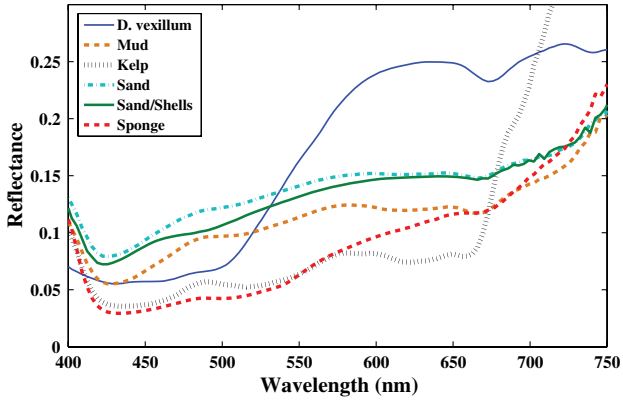


Fig. 2. (A) Mean *in-situ* reflectance spectra for *D. vexillum* using the DiveSpec ($n = 10$) and (B) the HyperOCR ($n = 7$). All spectra are referenced to a 95% reflectance standard. Dashed lines show 95% confidence interval.



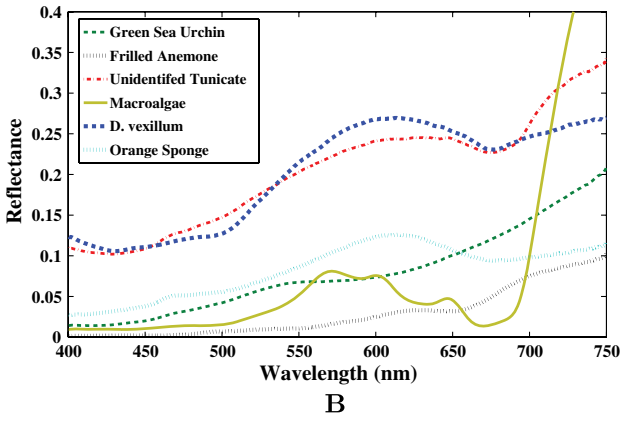


Fig. 3. (Color online) Comparison of *D. vexillum* spectra with other common substrates measured using (A) the HyperOCR and (B) the DiveSpec. Spectra are not area normalized and have been left as fractional reflectance ($L(\lambda)/L^*(\lambda)$) to allow for easier visual comparison.

The first derivatives of the reflectance spectra collected at the Darling Marine Center were used to generate a detection algorithm. Area normalized spectra [Eq. (1)] were smoothed using a 15 nm moving average filter. The first derivative of the smoothed spectrum was calculated using a 3 nm interval (resolution of the HyperOCR) then interpolated to every nanometer between 425 and 700 nm. The first derivative of the *D. vexillum* spectrum was consistently higher between 510 and 520 nm in comparison to all other spectra collected (Fig. 4). The average first derivative value from 510 to 520 nm was used to assign each spectrum a score value:

$$S = \left(\frac{\sum_{510\ \mathrm{nm}}^{520\ \mathrm{nm}} \tilde{R}'(\lambda)}{11}\right) * 1000, \qquad (2)$$

where $\tilde{R}'(\lambda)$ is the first derivative of the area normalized reflectance spectrum with respect to wavelength and S is the score value. The mean score value for *D. vexillum* spectra measured by the HyperOCR was 9.41 ± 1.35 ($\alpha = 0.05$, $N = 7$). No other substrate achieved a score value within this confidence interval. The highest score value recorded from a substrate other than *D. vexillum* was 4.82. This score value was from a species of sponge.

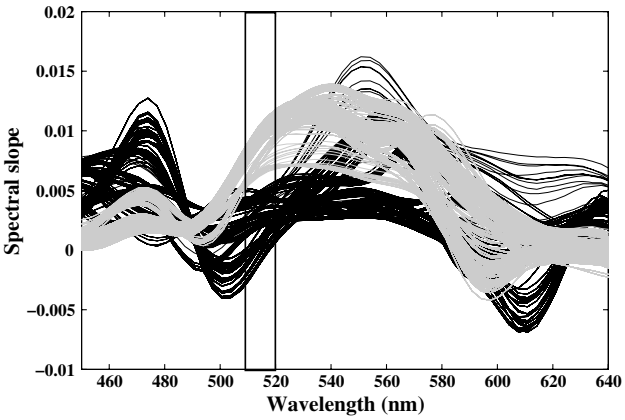


Fig. 4. First derivative spectra of *D. vexillum* (gray) and all other substrates measured (black). The box encloses the unique wavelengths (510–520 nm) that were used in the detection algorithm. Inside the box there is large separation between *D. vexillum* spectra and all other spectra collected.

B. Sunset Bay Marina

Approximately 700 spectra were collected during the hour deployment of the ROV. The ROV collected images and spectra from dock pilings covered in *D. vexillum* and various species of macroalgae. Spectra of open water and benthic mud were also collected. Score values were calculated for all spectra collected [Eq. (2)] and were used to test the effectiveness of the identification algorithm. All spectra with a score value within 1.5 standard deviations of the mean score value were classified as *D. vexillum* (9.41 ± 2.99).

Using this method, 155 spectra out of the 700 spectra collected were classified as *D. vexillum*. Based on images taken by the ROV, only two out of the 155 spectra were false positives. These two spectra were taken while the ROV was scanning across a section of the dock piling containing macroalgae. There were ten *D. vexillum* spectra that were missed by the detection algorithm (false negative). These spectra were either collected at a range greater then 1.3 m or the *D. vexillum* colony being measured was in direct sunlight. Overall, the algorithm was able to discriminate between *D. vexillum* and other substrates 98% of the time.

4. Discussion

The reflectance spectrum of *D. vexillum* was found to have characteristics sufficient to uniquely differentiate it among the spectra of other organisms and substrates we collected. For the areas surveyed in this study, the detection algorithm was able to accurately identify *D. vexillum* based on its reflectance signature. This has shown that *D. vexillum* can be detected using little computational effort. This approach can have many advantages, including faster processing of data and real time detection of *D. vexillum*. Real time detection onboard the AUV also opens up the possibility for adaptive feature mapping [21].

Our dataset includes only a small portion of the substrates likely to be encountered on Georges Bank. Collection and comparison of more reflectance spectra in the future will determine if our algorithm must be revised. If we find the detection accuracy unsatisfactory, higher order statistical methods could be employed. Principal component analysis (PCA) has been used for spectral identification of numerous substances and substrates [22–24]. This type of analysis can also be used to generate a more robust method for spectral identification of *D. vexillum*. All the substrates measured with the HyperOCR during fieldwork at the Darling Marine Center can be identified using a PCA conducted on the first derivative of the spectra between 490 and 557 nm (Fig. 5). In addition to presence or absence of *D. vexillum*, this could provide additional information about benthic habitat on Georges Bank.

The results of this study open the way for larger scale optical surveys of *D. vexillum* on Georges Bank. A downward facing radiometer and light source mounted on an AUV, such as the Odyssey IV [25,26], could be used to collect benthic reflectance spectra on Georges Bank. Using the methods described in this study, the data collected would provide a rapid and accurate assessment of *D. vexillum* coverage. However, the success of this method relies on a few key assumptions that must be addressed.

In shallow waters, *D. vexillum* has been observed to show seasonal variation in colony size and color [27]. This has been attributed to a decline in heath associated with lower water temperatures. However, we did not find significant changes in the reflectance spectrum. In our study, the *D. vexillum* reflectance spectra collected in December corresponded well with the reflectance spectra collected in June. However, seasonal variation in *D. vexillum* health and color has not been observed on Georges Bank [27], possibly due to more stable water temperatures.

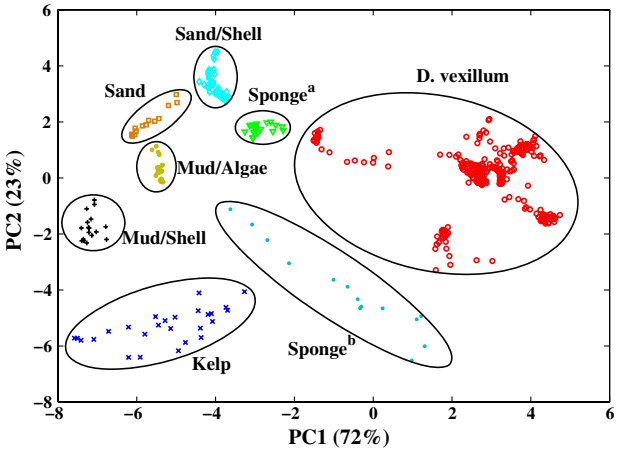


Fig. 5. (Color online) Score plot from a PCA of first derivative spectra from 490 to 557 nm. The first two principal components are plotted, accounting for 95% of the variation within the data. All substrates are easily distinguishable. The two distant *D. vexillum* groups were spectra collected under low illumination.

Settlement of other organisms on the surface of *D. vexillum* mats poses a potential problem for the detection method. Absorption of light by pigments of fouling organisms would alter the reflectance spectrum, masking the signal from *D. vexillum*. However, *D. vexillum* mats observed on Georges Bank appear to have clean surfaces, free of other organisms [27]. This may be due to compounds produced by *D. vexillum*. Many tunicate species, including members of the *Didemnum* genus, have been found to produce anti-predatory chemical deterrents [28]. Such chemical deterrents would reduce fouling and ensure an uncontaminated reflectance signal from the surface of the mat.

Another potential issue is changes in the optical properties of the water between the radiometer and the substrate. Our method assumes that they are constant from the time the Spectralon plaque is measured to the time a spectrum is collected. During the ROV deployment, the Spectralon plaque was measured at the beginning of the deployment and used to correct all spectra that were later collected. Thus, changes in the optical properties of the water and sample illumination (i.e., shaded versus full sunlight) over the course of the experiment likely contributed to false negatives.

The detection algorithm uses wavelengths from 510 to 520 nm, where the variability in absorption is mostly due to phytoplankton [29]. During surveys, the phytoplankton community structure is likely to change in space and time. This will cause a change in the optical properties of the water that, if not accounted for, will make it harder to distinguish between substrates. Changes in the ambient light field would lead to similar errors. This is important because the light field in the ocean is variable and depends on a number of oceanographic and atmospheric conditions [30]. Thus, when using an AUV platform, the time and space between Spectralon plaque measurements and collection of spectra should be minimized.

There are numerous methods that could be used to correct for changes in substrate illumination. Two radiometers could be mounted on the AUV, one pointed at the seafloor and other pointed at a Spectralon plaque (similar to what was used in Moline *et al.* [17]). Both the seafloor and the Spectralon plaque would be illuminated using the same type of LEDs. A second, and calibration independent, solution would be to use a mechanical system to move a single radiometer. The radiometer could be alternated between two positions, one pointing at the seafloor and one pointing at a Spectralon plaque mounted on the AUV. This could be further simplified by pointing the radiometer at a rotating mirror. Changing the angle of mirror would determine whether the radiometer receives a signal from the seafloor or the Spectralon plaque. This method could also be used to scan the seafloor for increased spatial coverage.

The results of this study show that the reflectance spectrum of *D. vexillum* can be successfully identified

from a remote platform. Using the methods in this study, the distribution of *D. vexillum* on Georges Bank could be mapped rapidly and accurately. Ability to detect and map *D. vexillum* will help determine the ecological ramifications of the *D. vexillum* colonization. The reflectance data collected by the surveys could also be used for a number of additional studies including mapping of benthic habitat on Georges Bank.

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