NEAR-ULTRAVIOLET MULTI-EXCITATION HYPERSPECTRAL IMAGING OF LIVE REEF CORALS

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Abstract—Fluorescence in corals arises from multiple sources, including fluorescent proteins in host tissues and chlorophyll in symbiotic algae. Their emission spectra overlap, and relative intensities depend on the excitation wavelength. This study investigates near-ultraviolet multi-excitation hyperspectral imaging (ME-HSI) for characterizing fluorescence in live corals and resolving their spectral components. Coral specimens were imaged at multiple excitation wavelengths, with fluorescence emission recorded from 450 to 950 nm. The measured spectra revealed differences among corals in emission peak positions, relative fluorescence intensities, and excitation-dependent spectral profiles. Multivariate curve resolution–alternating least squares (MCR-ALS) was applied to individual excitation datasets to resolve overlapping fluorescence components and reconstruct their spatial distributions. The results showed that the excitation wavelength with the clearest separation of fluorescence components differed among specimens. The resolved spectra and component maps revealed differences in the distribution of host-associated fluorescence, chlorophyll fluorescence, and other signals within the corals. The approach was further applied to investigate fluorescent changes induced by experimentally induced bleaching. MCR-ALS analysis revealed changes in fluorescence component distributions as bleaching progressed. These results demonstrate the importance of excitation wavelength selection for characterizing coral fluorescence and establish ME-HSI combined with MCR-ALS as an approach for examining the spectral and spatial characteristics of live corals.

Index Terms—coral fluorescence, multi-excitation hyperspectral imaging, MCR-ALS, excitation selection, spectral unmixing

1. INTRODUCTION

Coral fluorescence originates from several sources, including fluorescent proteins (FPs) and chlorophyll, present in host tissues and symbiotic algae, respectively. These compounds have distinct excitation and emission characteristics, which determine the fluorescence observed under different illumination conditions. Host FPs exhibit a range of spectral properties, producing differences in fluorescence among coral species, color morphs, and tissue regions [1], [2]. The measured fluorescence can contain overlapping signals from several sources, making their individual spectral characteristics and spatial distributions difficult to distinguish.

Fluorescence hyperspectral imaging (fHSI) provides spatially resolved emission spectra, enabling the examination of fluorescence across a specimen. This technique has been used to characterize coral fluorescence and investigate changes associated with thermal stress. Zawada and Jaffe [3] examined fluorescence changes during heat-induced bleaching, while Teague et al. [4] applied fHSI to living coral specimens under two different excitation wavelengths. These studies demonstrated the use of fHSI to examine the spectral signatures from coral hosts and their symbiotic algae. However, the measurements were limited to selected excitation conditions and did not systematically investigate how excitation wavelength affects the separation of overlapping fluorescence components across different corals.

The choice of excitation wavelength is particularly important for corals as their fluorescent compounds differ in excitation characteristics. An excitation wavelength that produces a strong signal from one fluorescent source may provide limited information about others. Consequently, the spectral information obtained from a specimen can change substantially with excitation wavelength. Bollati et al. [5] demonstrated the use of two excitation wavelengths to distinguish fluorescent pigments in coral reef organisms. Their method combined selected fluorescence channels into color images for pigment discrimination. A systematic comparison of hyperspectral emission data acquired across multiple excitation wavelengths can provide further information about the spectral characteristics of individual corals and the conditions under which their fluorescence signals can be separated.

Multi-excitation hyperspectral imaging (ME-HSI) combines spatially resolved emission spectra acquired under different excitation wavelengths. The resulting datasets can be analyzed using chemometric methods as multivariate curve resolution–alternating least squares (MCR-ALS), which unmixes the spectral overlapping components of the data retrieving their spectral profiles and their relative spatial distributions [6]. Applying this analysis to each dataset obtained under each excitation wavelength may provide a means of examining how excitation wavelength affects the resolved spectra and component maps. Since coral specimens differ in their fluorescence characteristics, the excitation conditions providing clear separation of their components may also differ. Identifying these differences is important for characterizing fluorescence across coral species and selecting suitable excitation wavelengths for subsequent measurements.

In this study, near-ultraviolet ME-HSI was applied to characterize fluorescence in live corals. Emission spectra were recorded from 450 to 950 nm under multiple excitation wavelengths to examine differences in fluorescence among specimens and their dependence on excitation wavelength. MCR-ALS was applied independently to individual excitation datasets to resolve overlapping spectral components and reconstruct their spatial distributions. The analysis focused on determining how excitation wavelength affects component separation and identifying the excitation conditions that provide the clearest results for each coral. The approach was subsequently applied to investigate changes in fluorescence components during experimentally induced bleaching.

2. MATERIALS AND METHODS

2.1. Coral specimens

Ten live coral specimens (Fig. 1) were obtained from a local aquarium fish store and were placed into an aquarium filled with seawater. An automatic light system was used to maintain a photoperiod of 8 hours of light per day, with a temperature controller set at 25°C. A water filter was placed inside the aquarium and % salt water was used to refill it every week.

The specimens included corals with different visible coloration and fluorescence characteristics, comprising two *Discosoma spp.* color morphs (red and blue), three *Sinularia brassica* specimens, *Lobophytum pauciflorum*, *Caulastrea furcata*, *Porites sp.* (the Grinch), *Montipora digitata*, and *Stylophora* (milka).

Montipora digitata and *Stylophora* (milka) were subsequently selected for the thermal stress experiment. The water

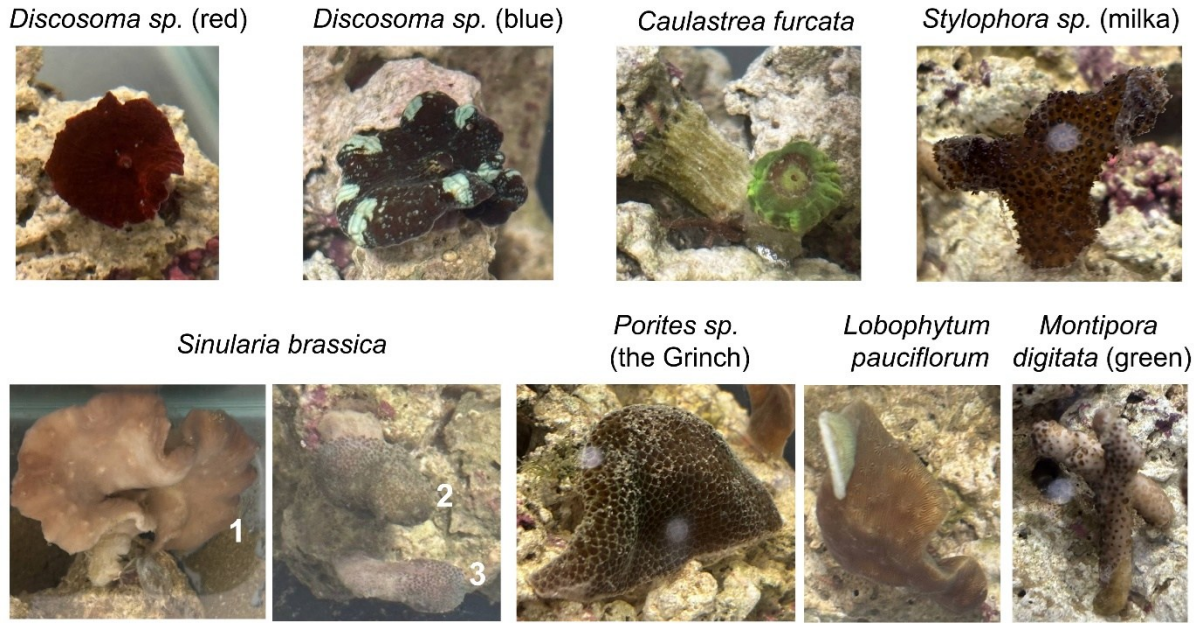


Fig 1. Photographs of the coral species used in this study. Numbers 1–3 indicate the three Sinularia brassica specimens.

temperature was increased from 25 to 30 °C to induce bleaching and the fluorescence was monitored over three consecutive days.

2.2. ME-HSI data acquisition

The hyperspectral system used for the acquisition of fHSI data contained the following components:

- A Nuance EX imaging system (*PerkinElmer*), which collects fluorescence emission from 450 to 950 nm at 10 nm intervals (51 spectral channels). A 540 nm longpass filter (deep yellow #12, Celestron) was placed in front of the camera when the sample was excited with 460 nm, to avoid interference from reflected light.
- A multiLED source (*WheeLED, Mightex*) which includes narrow band LEDs with emissions centered at range 365, 385, 400, 415 and 430 nm (10 nm FWHM). As a background light emission in the visible range was previously observed for the 365 to 415 nm LEDs, a 425 nm shortpass filter was coupled to the light source when using those excitation wavelengths. Additionally, a white LED coupled to a narrow band filter centered at 460 nm (10 nm FWHM) was used to add an excitation wavelength in the visible range.

The images were taken pointing the light source and the camera to the aquarium, in a 90° geometry, while keeping the imaging room completely dark.

2.3. Data preprocessing and spectral comparison

A morphological mask was created for each HSI cube obtained under a different excitation wavelength illumination. The mask was created by manually selecting the area covered by the coral specimens while discarding the background pixels. For each specimen and excitation wavelength, the masked 3D cube was then collapsed into a two-dimensional matrix (pixel x emission wavelength), containing one 51-value emission spectrum per valid pixel. Spectra were normalized pixel-by-pixel using L1 normalizationA mean normalized spectrum was then calculated from all the pixels from each specimen–excitation dataset.

For the bleaching experiment, the same preprocessing procedure was applied to the hyperspectral images of *Montipora digitata* and *Stylophora* (milka) acquired at 400 nm excitation on Days 1, 2, and 3. Each day's spectra were extracted using the corresponding specimen mask and normalized at the pixel level. For each species, the three pixel-by-wavelength matrices were concatenated vertically to construct a single dataset.

2.4. Independent MCR-ALS for the specimen survey

For the initial coral characterization, each specimen–excitation dataset was analyzed independently. The resolved spectral profiles and component scores were examined to compare the separation of fluorescence signals at different excitation wavelengths. Component scores were reconstructed into spatial maps using the original pixel coordinates. Pixel-wise root mean squared reconstruction errors were calculated to assess the agreement between the measured and reconstructed spectra. For the bleaching experiment, a C-augmented matrix was created by combining the three days' datasets for each of two corals' species. In that way, the MCR analysis resulted in a common set of spectral profiles and their concentration maps across days. The resulting component scores were reconstructed separately for each day to examine changes in their spatial distributions throughout the experiment.

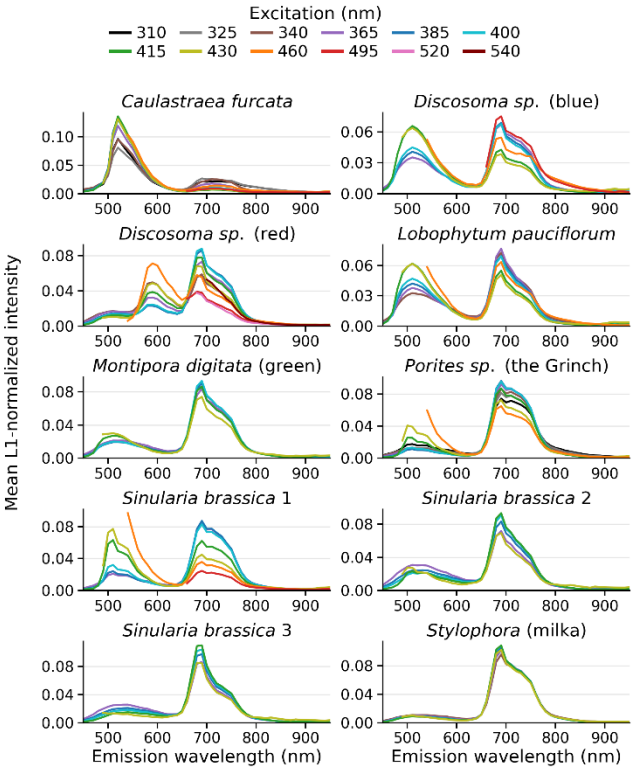


Fig. 2. Mean normalized fluorescence emission spectra of ten coral specimens acquired at different excitation wavelengths.

3. RESULTS

3.1. Coral fluorescence spectra

The mean L1-normalized fluorescence spectra differed among the ten coral specimens (Fig. 2). The main emission peaks occurred between approximately 500–550 nm and 680–720 nm, with substantial differences in their relative intensities. The two *Discosoma* color morphs, *Lobophytum pauciflorum*, and *Sinularia brassica* 1 exhibited fluorescence in both spectral regions. In contrast, *Montipora digitata*, *Stylophora* (milka), and *S. brassica* 3 showed predominantly red emission, with comparatively weak fluorescence between 500 and 600 nm. *Caulastrea furcata* exhibited strong emission near 520 nm and a relatively small red peak.

Changing the excitation wavelength altered the obtained spectra within individual specimens (Fig. 2). These differences were particularly evident in the two *Discosoma* color morphs, *L. pauciflorum*, *Porites*, and *S. brassica 1*, where the relative intensities of the two spectral regions changed with excitation. The three *S. brassica* specimens also differed in their emission profiles and responses to excitation. *Montipora digitata* and *Stylophora* (milka) showed comparatively small changes in spectral shape,

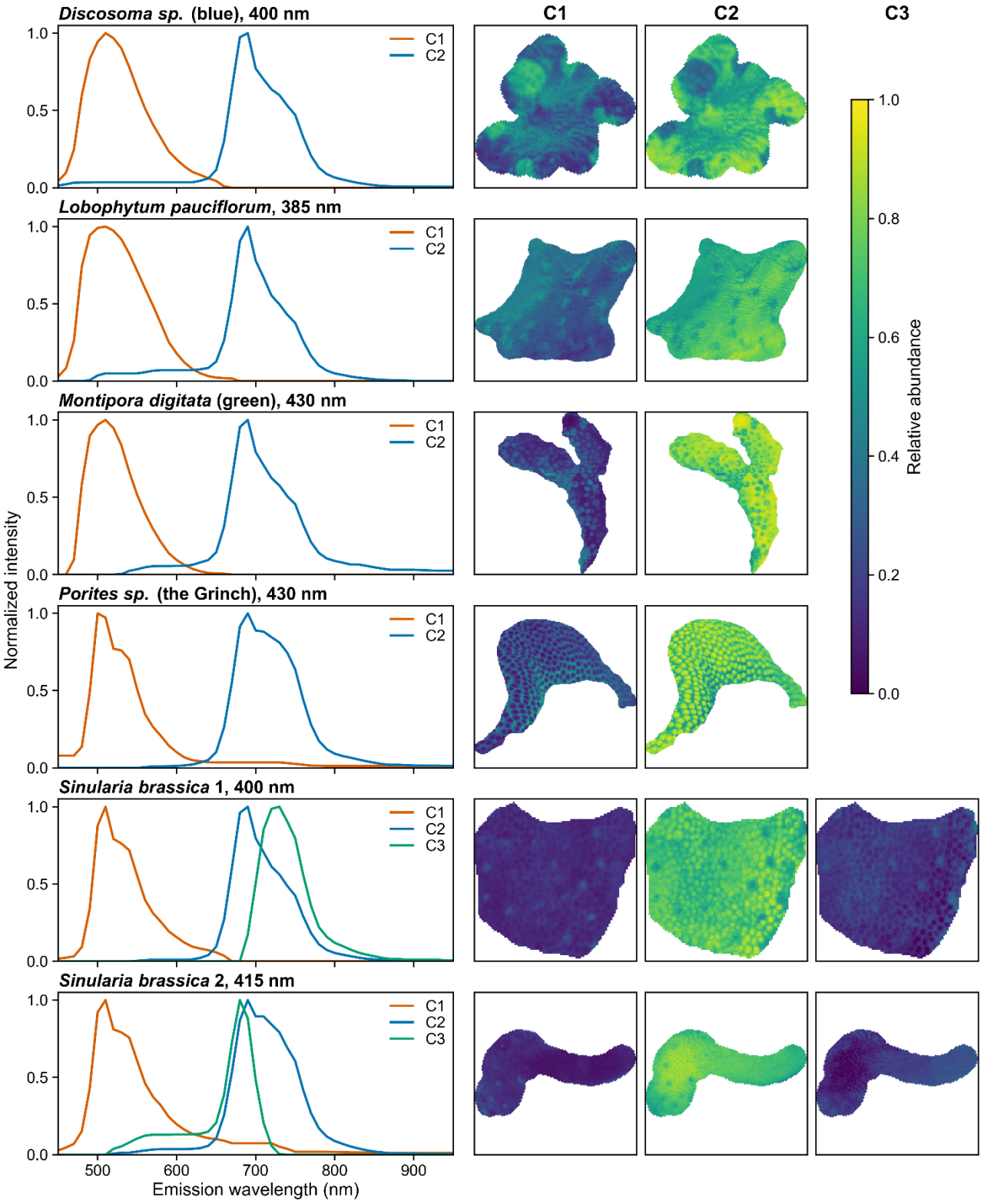


Fig. 3. MCR-ALS component maps and resolved emission spectra of selected coral specimens at the indicated excitation wavelengths.

with red emission remaining dominant across the measured excitation wavelengths.

3.2. MCR-ALS

MCR-ALS resolved two main spectral components in most of the selected coral datasets (Fig. 3). The first exhibited emission maxima between approximately 500 and 550 nm, while the second peaked near 680–700 nm. These spectral regions are consistent with fluorescence from coral host FPs and symbiont chlorophyll, respectively. The two components were recovered in *Discosoma blue*, *Lobophytum pauciflorum*, *Montipora digitata*, and *Porites*. Three-component models were obtained for the two *S. brassica* specimens, resolving an additional signal within the red fluorescence region. The overlapping spectral profiles suggest that the measured red emission may contain more than one fluorescent source, although their chemical identities cannot be established from MCR-ALS alone.

The component maps showed differences in the relative distribution of fluorescence signals across the specimens (Fig. 3). The component associated with red emission occupied large portions of the coral tissue, while the component emitting near 500–550 nm showed different spatial patterns depending on the specimen. In *S. brassica*, the additional component exhibited a spatial distribution distinct from the other two components.

The examples shown in Fig. 3 were selected from the full set of coral datasets as those in which MCR-ALS yielded the most clearly resolved components. For each specimen, the excitation wavelength presented is the one that produced the most distinct component spectra and maps. This wavelength differed among specimens: 385 nm for *Lobophytum pauciflorum*, 400 nm for *Discosoma* blue and *S. brassica* 1, 415 nm for *S. brassica* 2, and 430 nm for *Montipora digitata* and *Porites*. These differences support the use of specimen-specific excitation selection when resolving overlapping coral fluorescence signals.

3.3. Coral bleaching

The components maps changed during experimentally induced bleaching in both *Montipora digitata* and *Stylophora* (milka) (Fig. 4). Component 1, with its main emission maximum near 690 nm, showed high relative scores on Days 1 and 2, followed by a decrease on Day 3. Component 3, with emission centered near 720–730 nm, showed a similar decline. In contrast, Component 2, which exhibited emission near 500–550 nm and an additional peak near 690 nm, increased in relative abundance as bleaching progressed.

The spatial changes corresponded to the visible changes in the coral specimens, which became pale by Day 3. The decrease in the red-emitting components is consistent with changes in chlorophyll-associated fluorescence during bleaching, while the increase in Component 2 may indicate a change in the relative spectral composition of the remaining fluorescence.

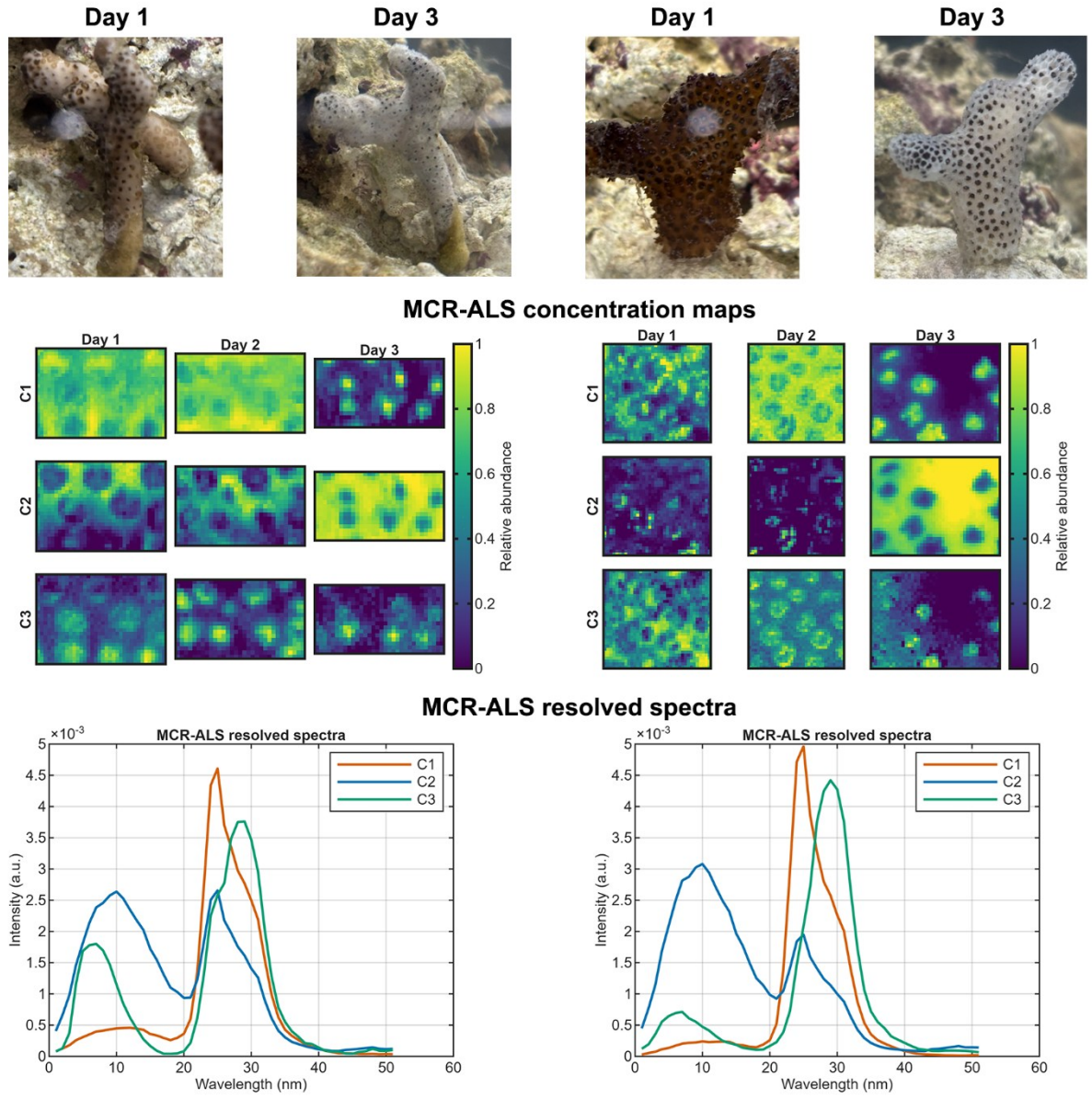


Fig. 4. Fluorescence changes during experimentally induced bleaching in Montipora digitata (left) and Stylophora milka (right).

4. DISCUSSION

The fluorescence spectra differed among coral specimens, reflecting variation in their fluorescent compounds and relative signals. These differences were observed even among specimens of the same species. The excitation wavelength also affected the measured spectra and the separation of overlapping signals by MCR-ALS. The excitation conditions providing the clearest component separation differed among corals, emphasizing the importance of examining multiple excitation wavelengths during fluorescence characterization.

The resolved spectral profiles were consistent with fluorescence from host pigments and symbiont chlorophyll. Their corresponding maps revealed differences in the spatial distribution of these signals. The additional components recovered in some specimens suggest that the measured fluorescence may contain several overlapping sources. However, their chemical identities cannot be established from MCR-ALS alone.

The bleaching experiments demonstrated the application of this approach to monitoring changes in coral fluorescence. The relative scores of red-emitting components decreased as bleaching progressed, accompanied by changes in the distribution of the remaining signals. Since the spectra were L1-normalized, these changes describe the relative fluorescence composition and cannot establish absolute changes in fluorophore concentration.

Overall, ME-HSI provides information about the excitation dependence of coral fluorescence, while MCR-ALS resolves overlapping signals and their spatial distributions. Their combination supports the characterization of different coral specimens and the examination of fluorescence changes during thermal stress.

5. CONCLUSION

Near-ultraviolet ME-HSI revealed differences in fluorescence among live coral specimens and their dependence on excitation wavelength. MCR-ALS resolved overlapping fluorescence components and their spatial distributions, with the excitation wavelength providing the clearest separation differing among corals. The approach was further applied to examine changes in relative fluorescence composition during experimentally induced bleaching. These findings demonstrate the value of combining multiple excitation wavelengths with spectral decomposition for characterizing coral fluorescence.

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REFERENCES

[1] N. O. Alieva et al., “Diversity and evolution of coral fluorescent proteins,” PLoS ONE, vol. 3, no. 7, Art. no. e2680, 2008, doi: 10.1371/journal.pone.0002680.

[2] C. H. Mazel and E. Fuchs, “Contribution of fluorescence to the spectral signature and perceived color of corals,” Limnol. Oceanogr., vol. 48, no. 1, pt. 2, pp. 390–401, 2003, doi: 10.4319/lo.2003.48.1_part_2.0390.

[3] D. G. Zawada and J. S. Jaffe, “Changes in the fluorescence of the Caribbean coral Montastraea faveolata during heat-induced bleaching,” Limnol. Oceanogr., vol. 48, no. 1, pt. 2, pp. 412–425, 2003, doi: 10.4319/lo.2003.48.1_part_2.0412.

[4] J. Teague, J. Willans, M. J. Allen, T. B. Scott, and J. C. C. Day, “Hyperspectral imaging as a tool for assessing coral health utilising natural fluorescence,” J. Spectral Imaging, vol. 8, Art. no. a7, 2019, doi: 10.1255/jsi.2019.a7.

[5] E. Bollati, C. D’Angelo, D. I. Kline, B. G. Mitchell, and J. Wiedenmann, “Development of a multi-excitation fluorescence (MEF) imaging method to improve the information content of benthic coral reef surveys,” Coral Reefs, vol. 40, pp. 1831–1847, 2021, doi: 10.1007/s00338-021-02101-1.

[6] A. de Juan and R. Tauler, “Multivariate curve resolution: 50 years addressing the mixture analysis problem—A review,” Anal. Chim. Acta, vol. 1145, pp. 59–78, 2021, doi: 10.1016/j.aca.2020.10.051.