

Using Coral Color to Indicate Coral Health in Five Caribbean Species

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ABSTRACT

Coral reefs are one of the most biodiverse and productive ecosystems on Earth, and color has been shown to indicate coral health in Australian and Hawaiian reef systems. However, no standardized method exists to quantify coral health for Caribbean corals. Therefore, a health assessment card using coral color was developed for five species of Caribbean corals to monitor coral health non-invasively. To quantify coral health, individual corals of each species were photographed in a controlled environment to develop color profiles. Simultaneously, nondestructive measurements of “health” were quantified by measuring photosynthetic efficiency (F_v/F_m) using pulse amplitude modulation (PAM) fluorometry, which determines how efficiently the symbiotic algae provides energy to the coral host. The results of this work successfully corresponded photosynthetic efficiency to coral color for five dominant species of Caribbean corals to develop a Coral Health Assessment Card for Caribbean reefs. Implementing a standardized assessment of symbiont performance can assist in monitoring changes in coral health, which can consequently be implemented into long-term and widespread monitoring projects to track overall Caribbean reef health.

KEYWORDS

Photosynthetic Efficiency, *Symbiodinium* spp., Coral Bleaching, Pulse-Amplitude Modulated Fluorometry, Health Assessment

INTRODUCTION

Coral reefs are one of the most biodiverse ecosystems in the ocean.¹ Nearly a quarter of all marine life depends on coral reefs at some point in their life cycle for various services, including shelter and food. Moreover, humans rely on reefs for their ecosystem services (e.g., fishing, tourism, coastal protection, economies),² and they also serve as a significant source of protein for more than half a billion people.³ Coral reefs are also natural barriers from waves and effectively protect tropical coasts and reef islands.⁴ However, coral reefs are declining rapidly, threatening marine biodiversity, local and global economies, communities, coastlines, and islands.² Therefore implementing rapid and effective reef management tools is key to the longevity of coral reefs by providing more information regarding the health of reefs and directing management efforts.⁵

Corals and their reefs thrive in oligotrophic waters because of their mutualistic symbiotic relationship with dinoflagellate algae symbionts (i.e., *Symbiodinium* spp.).⁶⁻⁸ *Symbiodinium* spp. reside in the coral tissue, providing color to the coral along with a large portion of their energy requirements (up to 90%), allowing corals to thrive in these nutrient-poor waters.⁹ The symbiotic relationship between corals and their symbionts may be disrupted by environmental changes (e.g., warming waters, eutrophication, acidification, etc.).^{7,10,11} This disruption is often evidenced by a loss of symbionts and, as a result, is accompanied by a loss of color or significant paling in the coral host.^{9,10} This process is known as ‘coral bleaching’ because the coral tissue becomes translucent, revealing the white coral skeleton.¹¹ A prolonged bleaching state can lead to partial or complete coral mortality,^{8,10} reduced or delayed reproduction,^{9,10} reduced calcification,¹¹ and a decreased ability to resist the invasion of competing species and diseases.¹¹ Due to the loss of color in the coral tissue, coral color can be considered a visual indicator of coral stress.^{13,14}

Since the presence of *Symbiodinium* spp. is imperative for coral health, it is important to understand the symbionts’ health and symbiosis with corals. The photosynthetic pigments of algae may decline up to 80% during periods of stress, directly influencing their photosynthetic capacity and efficiency.¹² Pulse-Amplitude Modulation Fluorometry (PAM) is a rapid, effective, and non-invasive way to quantify photosynthetic efficiency, which has been instrumental in understanding *Symbiodinium* spp. and coral holobiont health.¹⁴⁻¹⁸ For example, PAM can be used to generate a saturation pulse quenching analysis, which measures the efficiency of photosystem II,¹⁵ a minimum and maximum fluorescence value from a rapid light curve (RLC) to quantify photosynthetic efficiency from the maximum quantum yield (F_v/F_m),¹⁹ or the electron transport rate (ETR) from

photosynthetically active radiation (PAR).¹⁹ These photosynthetic analyses can be performed non-invasively on live corals and are highly indicative of the overall health and function of photosynthesis, thereby providing a health assessment of corals and their symbionts. For this study, health is defined by the photosynthetic efficiency (F_v/F_m) of the algal symbiont, *Symbiodinium* spp., which provides the coral host approximately 90% of the energy it needs to survive.⁹

Due to the rapid decline of coral reefs globally, it is crucial to understand the health of the coral holobiont for successful intervention. Coral reef health assessments are instrumental for understanding the effects of stressors facing coral reefs,²⁰ for example, over-harvesting, pollution, disease, and climate change.³ However, it is challenging to quantify coral health rapidly and non-invasively.²¹ Ongoing monitoring is often costly and labor-intensive, making traditional methods unsustainable.¹³ Traditional assessment methods, like the coral reef health index in Sangiang Island, Indonesia, require comprehensive data, time, and high levels of scientific experience.²⁰ Additionally, utilizing PAM fluorometry alone to quantify coral health is unrealistic due to the cost and time associated with the measurements.^{5, 21} For rapid, accessible assessment to inform management, coral color can be linked with quantitative measurements from PAM fluorometry to provide a visual indicator for coral health.

Color has been linked to PAM fluorometry measurements via symbiont density in previous studies from Australia and Hawai'i.^{5, 13} A color card is a tool designed to track the coral symbionts' photosynthetic capabilities over time using visual assessments of coral reefs. The color card can be held up to a coral to understand its health. The Coral Watch Chart uses a six-point color scale designed for Indo-Pacific reefs.¹³ The Hawai'i Ko'a card uses a 35-point color scale to detect changes in symbiont density in Hawaiian corals.⁵ Both cards have helped guide local management strategies,^{5, 13} but coral species differ between regions. Those color cards were developed to track and monitor bleaching in Indo-Pacific and Hawaiian reef systems, but a color card has not been developed for Caribbean species. Coral pigmentation and zooxanthellae characteristics can vary by region due to species composition and water quality differences.⁵ Therefore, a tool designed specifically for Caribbean species and their respective pigmentations is necessary for the success of efforts to manage coral reefs in the region. This card can be used for Caribbean corals in the wild or captivity. The Hawai'i Ko'a card and Coral Watch Chart were designed for corals in the wild. However, the Caribbean coral card was designed in collaboration with Texas State Aquarium, monitoring coral wellness in captivity. To support management efforts, a coral color card was developed for five Caribbean species in collaboration with Texas State Aquarium (TSA); the card drew inspiration from Coral Watch Chart and the Hawai'i Ko'a card. Implementing the Caribbean color card into management and wellness programs can benefit Caribbean corals and be a valuable educational tool. This tool can also be incorporated into coral monitoring programs, allowing for standardized, rapid, and non-invasive monitoring of coral health in the Caribbean over time. This tool provides valuable information at ease to reverse the effects of local and global climate change in one of the most biodiverse ecosystems in the world.

METHODS AND PROCEDURES

Phase 1: Card Development

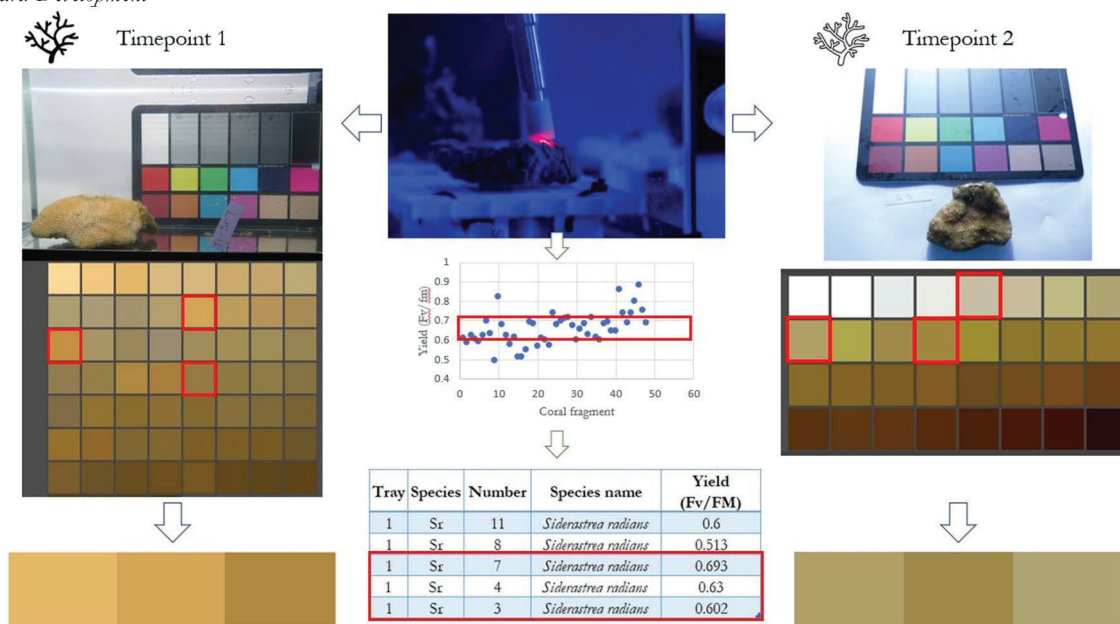


Figure 1. A flow chart showing Phase I of the methods outlined. Phase I was conducted at two time points: Timepoint 1 (Fall 2020) and Timepoint 2 (Fall 2022). Each time point followed the same order: PAM measurements were taken, corals were photographed, and images were processed in Photoshop to produce a color swatch.

Coral Husbandry

The coral species used in this study were *Siderastrea radians*, *Acropora palmata*, *Acropora cervicornis*, *Solenastrea bournoni*, and *Porites* spp. (N=5 species). Most species were acquired from the reefs in the Florida Keys National Marine Sanctuary (FKNMS-2017-041 to Texas State Aquarium).

The data collection was split into two time points: Timepoint 1 (Fall 2020), when the corals appeared healthy, and Timepoint 2 (Fall 2022) when the corals appeared unhealthy with paling coloration. In Fall 2020, corals were kept in stable and optimal growth conditions on a nursery table (318 L). Water parameters were held at optimal conditions for coral growth. The corals received a constant irradiance level (210-120 Watts m⁻²) on a 12 h on-off schedule (Al Hydra 52 LEDs). Raw seawater from Corpus Christi Bay feeds the water table, and water conditions were held at optimal salinity (34 ppt), temperature (25°C), and pH levels (8.0-8.2). However, in the Fall of 2022, a significant amount of algae grew in the nursery table, and corals appeared pale and bleached. Due to inadequate water quality testing at TSA, the water conditions were not recorded; however, it appeared to be unfavorable conditions for coral growth.

Coral Photobiology

The research approach and methodology followed similar methods developed by Siebeck et al.,(2008) and Bahr et al. (2020) for the Coral Watch Chart and Hawai'i Ko'a card, respectively. Rapid light curves (RLC) were conducted on corals using a diving-PAM (V2, Walz GmbH, Effeltrich, Germany) on each coral fragment to measure the photosynthetic efficiency (F_v/F_m) of the symbiotic algae. RLCs measure the effective quantum yield (F_v/F_m) as a function of irradiance.¹⁵ The yield shows the algae reaction to a range of light levels.¹⁵ PAM uses three types of lights: weak measuring light, saturating pulse, and actinic light.¹⁹ The weak measuring light determines the proportion of closed PSII reaction centers and finds the minimum fluorescence; the saturating pulse closes all PSII reaction centers to find the maximum fluorescence.¹⁹ Saturating pulse is used to understand the photosynthetic activity, and actinic light induces photosynthesis.¹⁹ Photosynthetic efficiency can be quantified from the maximum quantum yield (F_v/F_m), which is the minimum and maximum fluorescence value.¹⁹

Selected coral fragments (N=34) were removed from the seawater tables, placed in a small 2-gallon holding tank, and placed in a dark environment for at least a 20-minute acclimation. Dark acclimation allows all chlorophyll reaction centers to close for optimal fluorescence measurements.²² Corals were aerated with battery-powered bubblers during dark acclimation. The diving-PAM was connected to a PC running WinControl software (version 3.25) and was fitted with a red light-emitting probe (470 nm, LED, 0.05 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$, 5 Hz). Rubber surgical tubing was attached to the probe to allow measurements at a consistent distance for each coral and prevent damage to the probe and coral.

Individual corals were separated by a black partition in the acclimation tank for each RLC to prevent photosystems in non-target fragments from reacting to the strong saturating light pulse and the increasing actinic light emitted from the PAM during measurements. RLCs were performed at two unique points and a 90° angle to each coral fragment. The first probe location was selected in the dark, with subsequent locations as far from the previous as possible. This was necessary to avoid photosystem activation in areas adjacent to the probe. The mean value of the two F_v/F_m measurements was used for analysis. The change in F_v/F_m between the two time points for each species was analyzed in RStudio 4.2.2 using a paired t-test (Fig. 3).

Coral Color

The color of each coral was documented as photographic images in a controlled environment. Coral fragments were placed in aquaria filled with clean seawater immediately after PAM measurements for photographing. A digital camera (Canon G16) and an external flash mounted on a stationary stand were used to evenly illuminate and photograph each coral specimen while keeping a fixed distance and angle. Camera parameters were manually set and kept consistent across all images. Camera parameters were set at mode: M, aperture: 8, ISO: 80, and white balance: auto. Flash settings were set at mode: M and power: 1/8. A commercial underwater color reference card (DGK Color Tools WDKK Waterproof Color Chart) was placed on the back wall of the aquaria for color balancing in Adobe Photoshop CS5. Three to five images per species were selected for processing in Adobe Photoshop CS5. All images were preserved as raw image files in DNG format to retain the camera sensor's full resolution while minimizing information loss. Preserved DNG raw image files were balanced to white (90% reflectance) and neutral (gray, 18% reflectance) in Adobe Photoshop CS5 using DGK color Tools WDKK Waterproof Color Chart captured in an image as references. The histogram function allowed evaluation of the distribution of RGB values representing white and neutral to maintain constant values while avoiding over-exposure of images. Images were then converted into TIFF format for subsequent processes of color indexing and selection to establish relationships between the colors of photographs and coral fragments. Each image was white, grey, and black balanced using the curves adjustment. A represented surface area of each coral fragment was outlined, ensuring the selected area did not have shadows, bubbles, or areas of discoloration. A color table was then produced in Adobe Photoshop CS5, and the ten most frequent colors from each area were selected and saved as a color table file with .act extension, which saved

the numerical color values, such as RGB, CMYK, and HSB (hues, saturation, and brightness) (Fig. 2). These methods followed those outlined in Bahr et al., 2020.

A color swatch was produced for each species for each time point (i.e., Fall 2020 and Fall 2022). These colors represent the most frequently observed colors that best represent each fragment of the species (Table 1). A range of healthy colors was chosen to account for morphological and spatial variation in color, as bleaching is not typically a uniform response.¹² The colors from Fall 2020 are considered “healthy” and those from Fall 2022 were considered “unhealthy” colors. The colors were then oriented in a light-to-dark gradient to dark to increase ease of use.


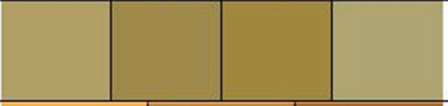



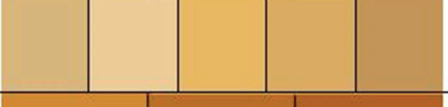

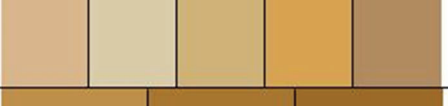

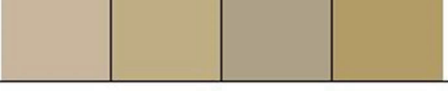
| Species | Timepoint | Average F_v/F_m ± SE | Representative Colors |
|-----------------------------|--------------------|---------------------------|--|
| <i>Siderastrea radians</i> | Fall 2020 (N=3) | 0.606 ± 0.007 |  |
| | Fall 2022 (N=3) | 0.577 ± 0.012 |  |
| <i>Acropora palmata</i> | Fall 2020 (N=3) | 0.455 ± 0.034 |  |
| | Fall 2022 (N=3) | 0.454 ± 0.029 |  |
| <i>Acropora cervicornis</i> | Fall 2020 (N=5) | 0.5698 ± 0.04 |  |
| | Fall 2022 (N=5) | 0.463 ± 0.018 |  |
| <i>Solenastrea bournoni</i> | Fall 2020 (N=3) | 0.658 ± 0.021 |  |
| | Fall 2022 (N=3) | 0.582 ± 0.02 |  |
| <i>Porites species</i> | Fall 2020 (N=3) | 0.527 ± 0.047 |  |
| | Fall 2022 (N=3) | 0.309 ± 0.019 |  |

Table 1. Detailed information regarding coral species, time point (Fall 2020 or Fall 2022), number of fragments sampled, average quantum yield (F_v/F_m) for all fragments, standard deviation, and the color swatch produced to represent the species.

Phase 2: Card Validation

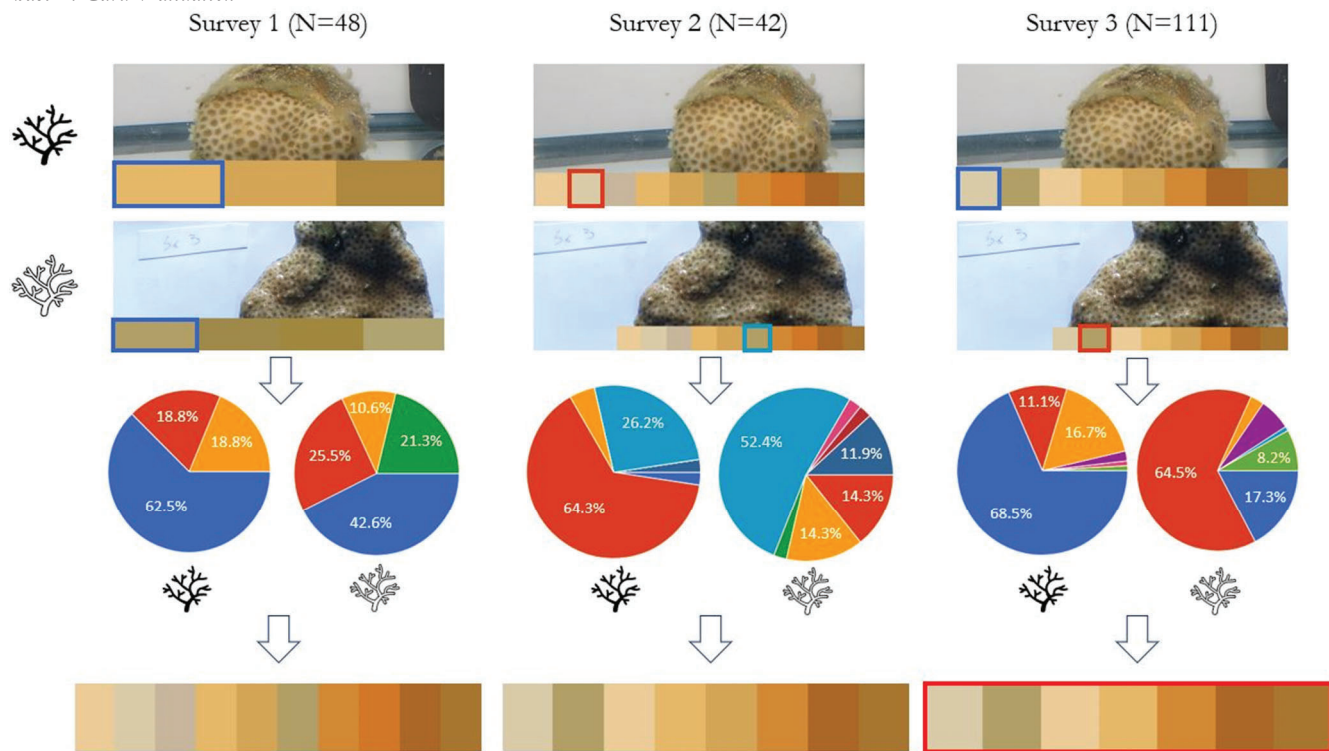


Figure 2. A flowchart showing Phase II methods process for final color card selection following three external surveys. N represents the number of observers for each survey. The pie charts represent the percentage of observers who agreed on a single color for each coral fragment pictured. The highlighted colors show which color was perceived the most for each survey. The results of each survey were used to make the final color swatch.

Quantitative Analysis

The color selection followed a quantitative and qualitative process to represent the five species using a seven-color range. For the quantitative analysis, the PAM measurements from each coral were used to find the mean quantum yield value for each species at both time points (*i.e.*, Fall 2020 and Fall 2022). The F_v/F_m values were compared across all individuals, and the three fragments from each species that best fit the mean quantum yield were selected for color analysis, as they best represented the entire species. A range of 3-5 colors was selected from the color table produced by Adobe Photoshop CS5 (Table 1), and the F_v/F_m values of the selected fragments were correlated to the corresponding color. The color values produced by the images of the coral species from Photoshop were compared with the color values on the card as the expected color values (card) compared to actual color values (coral colors from Photoshop) (Fig. 4).

Qualitative Analysis

The qualitative analysis consisted of four surveys to assess the colors of 31 corals from five species. A photograph of the swatch was placed alongside photographs of each coral on the same computer screen. Each observer independently determined the best representative color for each coral, avoiding the tips and edges of the coral. Other parameters were collected from the observers to help explain variation, including age, gender, coral familiarity, and the highest level of education. Observers were not trained before taking the survey and came from all different backgrounds (*i.e.*, ages, experience levels, educational backgrounds, etc.). The surveys were anonymous, so it is unknown how many repeat observers there were across four surveys. However, each survey was shared and communicated with similar audiences, so we assume there were repeat observers across the surveys.

Survey one (N=48 observers) had 3-5 colors specific to each individual from each species at each time point. The ten colors selected the most were used to make a swatch representing all five species. Survey two (N=42 observers) assessed the ten colors alongside all 30 coral fragments, allowing survey three (N=111 observers) to test eight colors by removing the two least selected colors. The final color swatch used all survey data to produce a 7-color card representing both time points. Survey four (N=26 observers) assessed the final color card with 14 fragments that showed disagreement among all three surveys to ensure the narrowed-down color card accurately represented all variations in pigmentation and observation (Fig. 2).

RESULTS

Quantitative Validation

Coral Photobiology

The PAM measurements show relatively healthy photosynthetic efficiency (F_v/F_m) with a low standard deviation. Table 1 shows all the individuals across the five species tested at each time point. At Timepoint 1, the average yield across species ranged from 0.52-0.65; and at Timepoint 2, the average yield ranged from 0.30-0.58 (t-test: $p < 0.001$). *Porites* spp. displayed the lowest yield (0.527 ± 0.047 and 0.309 ± 0.019) at both time points, and *S. bournoni* displayed the highest yield at both time points (0.658 ± 0.021 and 0.582 ± 0.02). This analysis of the photosynthetic efficiency by time point was correlated with the colors of the corals at that time point so that each species had a 3-5 color swatch for each time point (Table 1). For further analysis of coral color, five individuals from Timepoint 1 were chosen for analysis, so there was evenness across the time points. All species except *A. palmata* ($p = 0.4877$) significantly declined in photosynthetic efficiency between time points (t-test: *A. cervicornis* $p < 0.05$; *S. radians* $p < 0.01$; *Porites* spp. $p < 0.001$; *S. bournoni* $p < 0.01$) (Fig. 3).

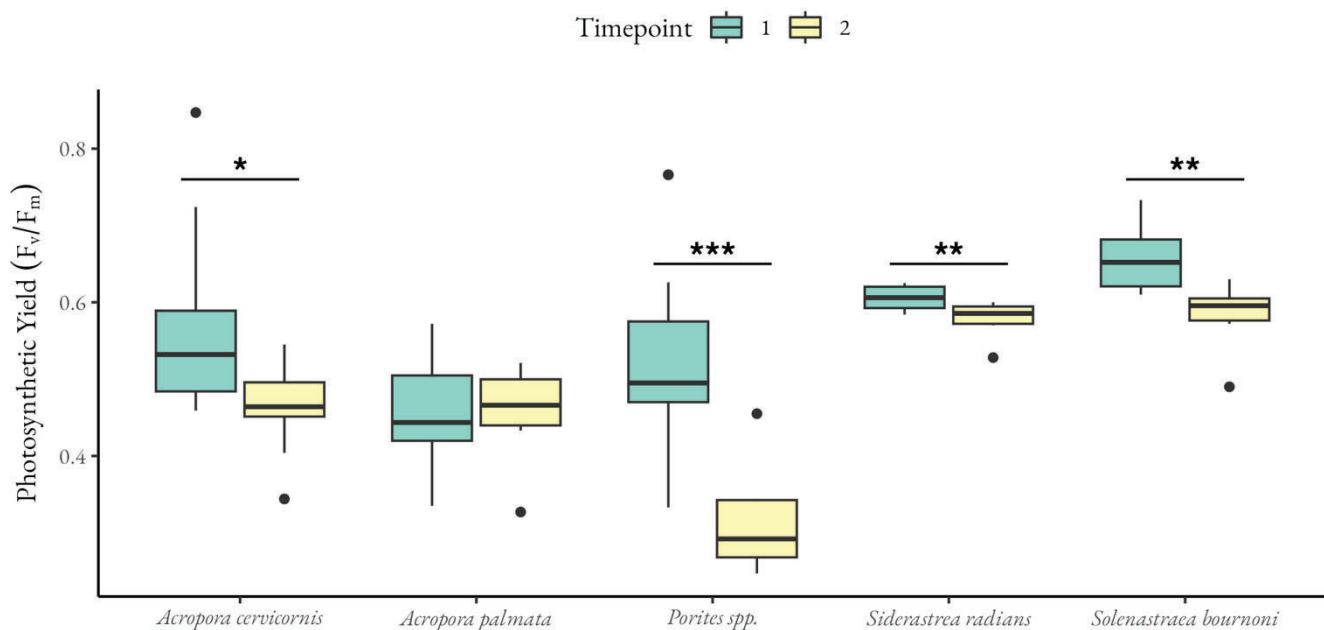


Figure 3. Box plot of the photosynthetic efficiency (F_v/F_m) measured by PAM by species across the two time points (Fall 2020 and Fall 2022). Lower and upper box boundaries represent the 25th and 75th percentiles, the line inside the box represents the median, and the lower and upper error lines represent the 10th and 90th percentiles. The filled circles represent data falling outside the 10th and 90th percentiles. Asterisks represent significance from paired t-tests using RStudio 4.2.2

Coral Color

The final seven colors in the card were correlated to the subset of 31 individuals tested. Photosynthetic yield (F_v/F_m) strongly correlated to color with reasonable variation. High variation in color was observed in color 1 because unhealthy *Porites* spp. and healthy *S. radians* well represent this color. In this case, this color health metric is species-specific, and the card should be further developed with a morphological key to determine which species is being measured. Photosynthetic yield also correlated with color, with darker colors generally having higher yield (Fig. 4A).

Following this, the color from the image of the coral extracted from Photoshop correlated to the color on the color card. Red, Green, and Blue (RGB) values and hue, saturation, and brightness (HSB) values were calculated for the colors on the card, and the five most representative colors from the corals were generated in Photoshop (Fig. 1). Colors 2, 4, 6, and 7 matched most closely for both the RGB and HSB values, while 1, 3, and 5 had slightly lower values from the images as compared to the card; however, all image values trended towards the card values. For example, colors 6 and 7 had the lowest RGB values for the images and the card (Fig. 4B) and lower brightness with higher saturation for both the images and the card (Fig. 4C).

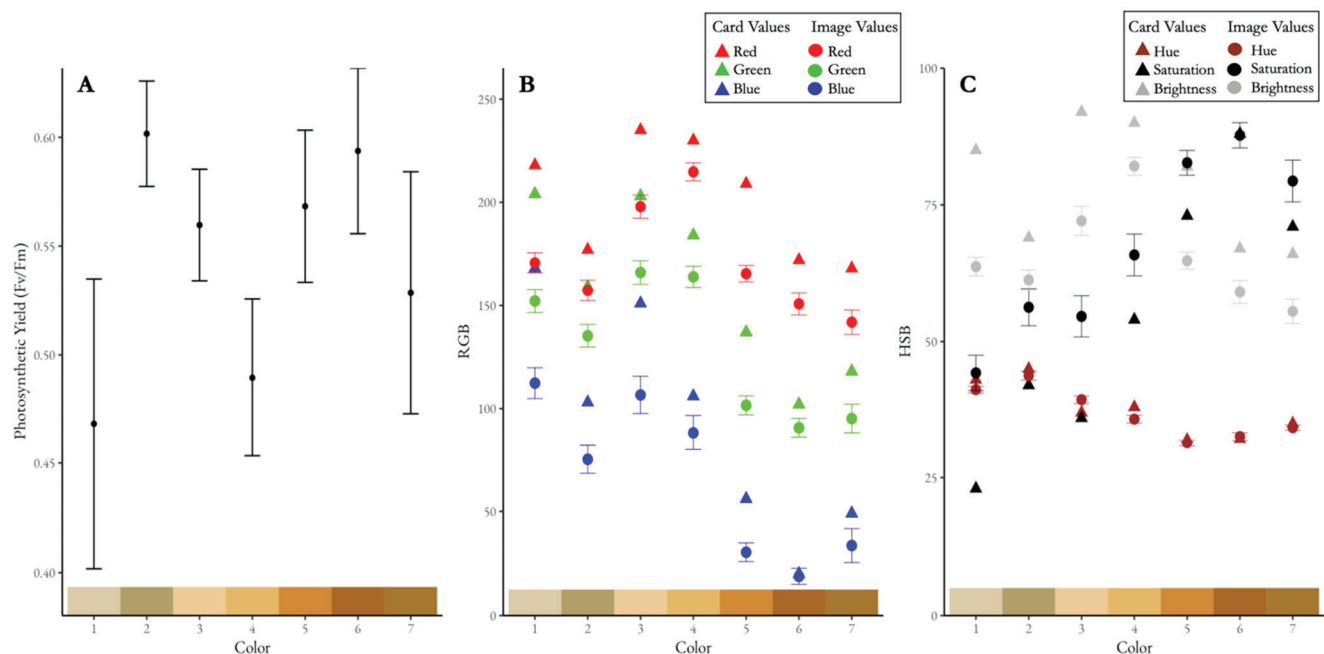


Figure 4. The seven colors (1 (n=6), 2 (n=6), 3 (n=5), 4 (n=5), 5 (n=8), 6 (n=6), 7 (n=5)) (n=number of individuals) of the final color card correlated to the corresponding coral's photosynthetic yield (A), red, green, blues (RGB) values (B), and hue, saturation, and brightness (HSB) values (C). The triangles represent the actual RGB and HSB values, and the circles represent the mean coral RGB and HSB values with standard error (SE) depicted with error bars.

Qualitative Validation

Four successive surveys were developed to validate the colors for the color card and quantify observer variation. A photograph of the color card was placed alongside photographs of each coral, and the observer independently determined the best representative color for each coral. Observers for each survey ranged from ages 18 to 64, representing diverse education and coral experience levels. Survey one had 48 participants observe between three to five colors specific to each species (n=3) at each time point (Table 1). The results of this survey narrowed down which colors were well-represented the most for each species. Results showed >50% observer agreement on a representative color for 23 of 31 corals. These results were used to develop a 10-color-swatch for the second survey. There was >50% agreement on a single color for 64.5% of fragments. Five fragments had a close split between two colors, and the other six showed no agreement, leading to a reassessment of colors. For survey three, two colors were removed because these colors were shown not to be well represented by the corals in survey 2. The colors were re-oriented to maximize differences in pigmentation between colors for ease of use. If colors 1 and 3 were often selected together for a fragment, they were placed next to each other to show the differences in pigmentation. This survey showed general agreement between over 100 participants, with a few exceptions. Survey two showed 83.9% of corals received >40% agreement, and 64.5% received >50% agreement for a single color. Survey three showed 87.1% of corals received >40% agreement, and 54.8% received >50% agreement from all observers. Therefore, it was observed that removing two colors from survey three did not change the agreement between observers and made the final card more accurate. It is assumed that for some species like *S. radians* and *A. palmata*, high observer variation was caused by a difference in the coloration between the coenosarc and polyps. For those two species, differences in color across the individual caused observer variations in the surveys. However, other species like *A. cervicornis* and *Porites* spp. did not have observer variation (>50% agreement), as the individuals had uniform coloration. The final 7-color card was created from the results of surveys two and three. Survey four tested the final color card, but only the 14 corals that showed the most observer variation were used. Survey four justified 85.7% of corals received >40% agreement, and 57.1% received >50% agreement. This final survey was conducted to clarify any discrepancies in color agreement. The corals that already had >50% agreement were omitted. The results of each survey showed that agreement among observers stayed the same as colors were removed.

DISCUSSION

This study successfully created a 7-color card for five key Caribbean coral species. As evidenced by the data in this study, there was a visual decline in photosynthetic yield (F_v/F_m), which correlated with a notable color change. Lighter colors primarily represented lower F_v/F_m ; however, some colors, like 1 and 7, represented more extensive F_v/F_m ranges because of numerous species matching with that color (Fig. 4A). The colors on the card also successfully matched the RGB (Fig. 4B) and HSB (Fig. 4C)

values of the coral photos. These correlations between photosynthetic efficiency and color scales help to validate the method used to develop the color card.

With further development, this card could be used for coral health monitoring surveys in the Caribbean, from large-scale remote sensing²³ to smaller-scale citizen science projects.⁵ During this study, we noted some limitations and biases with the experimental method; one such limitation was having too few replicates in our samples. To overcome this, future studies should have a larger sample size with more replicates to avoid the discrepancies seen in this study. The number of corals indicated by our card was limited by the number of coral species available at the Texas State Aquarium (TSA). Another limitation is the subjectivity of color. Color as an indicator of health is subjective as people perceive color differently, which should be considered when using the card for data collection.

The color card should not be used as a stand-alone tool but in collaboration with other health assessment techniques. Nevertheless, the Caribbean color card developed in this study currently only represents five species, and more species should be added to reflect Caribbean coral reef assemblages. Similar to the Hawaiian Ko'a card, a key should be included to decipher the different morphologies among species. A key could sufficiently help users determine the meaning of the color based on the species since one color may represent the health of various species differently. For example, high variation was observed in color 1 because unhealthy *Porites* spp. and healthy *S. radians* represent this color well, and having a morphology key would clarify which species the color is associated with. Additionally, this study could not perform any experimental tests for chlorophyll or symbiont extractions because of the Association of Zoos and Aquariums (AZA) animal wellness expectations to maintain coral health, limiting the extent of the study. Since experimental data could not be collected, the card does not show the stages of bleaching. However, studies have shown that PAM fluorometry measurements capture damage to photosystem II and the underlying physiological collapse of the symbiont regardless of bleaching.¹⁷ Regarding the PAM measurements, two rapid light curves were conducted for each coral fragment and averaged to extrapolate the health of the coral from a point-based measurement. There may be some limitations and biases in two points reflecting the individual's overall health. Subsequent studies should include more experimental data to better understand physiology and how environmental changes affect coral health in aquaria and the field. Aside from coral reef health assessments in the field, color cards could also be used in the zoo and aquaria trade. For example, the AZA carefully evaluates zoos and aquariums to ensure they meet animal welfare, care, and management standards.²⁴ Animal care is defined as excellent animal husbandry procedures that ensure excellent animal health and welfare.²⁴ The color card could supplement existing or new AZA-accredited zoos and aquariums standard practices by implementing a way to diagnose, manage, and track coral health in captivity. The card could also be used as an interactive educational tool for public engagement to illustrate the effects of coral bleaching and how climate change affects marine ecosystems. Existing education tools and resources have been designed to cover a wide range of topics related to coral reefs, such as the many lesson plans available for K-12 classrooms.²⁵⁻²⁷ But there has been an effort to make coral educational tools more interactive and hands-on through 3D Coral Polyp Models and art projects.²⁵⁻²⁸ Therefore, implementing this color card in aquariums and zoos could feasibly provide an engaging and educational coral exhibit for the general public.

CONCLUSION

Understanding coral health is critical for the success of coral reefs under our changing climate. Coral reefs are one of the most biodiverse ecosystems on Earth, supporting roughly 25% of all marine life and billions of humans through food, income, and coastal protection. However, corals are in decline due to human-induced environmental change. Therefore, it is essential to monitor coral health to conserve a valuable marine ecosystem and protect marine biodiversity. Coral reefs in the Caribbean face the synergistic threat of climate change and disease (e.g., stony coral tissue loss disease, white band disease).²⁹⁻³¹ The 7-color card created in this study provides an objective tool to monitor and assess the coral health of 5 key coral species in this region non-invasively. This tool was created by correlating changes in photosynthetic efficiency (F_v/F_m) to changes in coral color. A color card, such as the one created in this study, can be an effective way to implement a visual assessment of the health of corals in captivity. Developing an objective, science-based tool also helps support local and global efforts to monitor the effects of climate change and disease on coral reefs. Individual color cards should be developed as place-based tools to support global efforts since coral species and *Symbiodinium* clades differ between regions.

The next steps for this study are to assess more healthy corals to represent species diversity in the Caribbean and more non-healthy corals to expand the color card to monitor bleaching events. The best way to do this would be through experimental data in the lab that would represent a greater variation of health for the species on the color card. Ultimately, more corals must be assessed to fully understand the Caribbean species' coral color health metric. The color health metric is species-specific, so a morphological key must be added to determine what a given color means for each species. For the card to be used as a monitoring tool in the field, field studies would have to be conducted to validate that the data represents corals in their natural environment.

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REFERENCES

1. Knowlton, N., Brainard, R.E., Fisher, R., Moews, M., Plaisance, L., and Cailey M.J. (2010) Coral reef biodiversity, in: *Life in the World's Oceans: Diversity, Distribution, and Abundance* (McIntyre, A., Ed.) 65–76, Blackwell Publishing Ltd.
2. Castro, P., and Huber, M.E. (2010) Marine Biology, P. Castro and M. E. Huber, Marine Biology (8th eds). McGraw Hill Comp., NY, 2010.
3. Bellwood, D.R., Hughes, T.P., Folke, C., and Nystrom, M. (2004) Confronting the coral reef crisis. *Nature* 429, 827–833. <https://doi.org/10.1038/nature02691>
4. Harris, D.L., Rovere, A., Casella, E., Power, H., Canavesio, R., Collin, A., Pomeroy, A., Webster, J.M., and Parravicini, V. (2018) Coral reef structural complexity provides important coastal protection from waves under rising sea levels. *Sci Adv* 4(2). <https://doi.org/10.1126/sciadv.aao4350>. NOAA, Coral reef ecosystems. National oceanic and atmospheric administration, <https://www.noaa.gov/education/resource-collections/marine-life/coral-reef-ecosystems> (accessed Dec 2022)
5. Bahr, K., Severino S., Tsang, A., Han, J., Dona, A., Stender, Y., Weible, R., Graham, A., McGowan, A.E., and Rodgers, K.S. (2020) The Hawaiian Ko'a Card: coral health and bleaching assessment color reference card for Hawaiian corals. *SN Appl. Sci.* 2, 1–15. <https://doi.org/10.1007/s42452-020-03487-3>
6. Muscatine, L., and Porter, J.W. (1977) Reef corals: mutualistic symbioses adapted to nutrient-poor environments. *Bioscience*, 27(7), 454–460. <https://doi.org/10.2307/1297526>
7. Polovina, J.J. (1984) Model of a coral reef ecosystem. *Coral Reefs* 3, 1–11. <https://doi.org/10.1007/BF00306135>
8. Jokiel, P.L. (2004) Temperature stress and coral bleaching, in: *Coral Health and Disease* (Rosenberg, E. and Loya, Y., Ed.) 401–425, Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-662-06414-6_23
9. Allemand, D., and Furla, P. (2018) How does an animal behave like a plant? Physiological and molecular adaptations of zooxanthellae and their hosts to symbiosis. *C. R. Biol* 341, 276–280. <https://doi.org/10.1016/j.crv.2018.03.007>
10. Hughes, T.P., Baird, A.H., Bellwood, D.R., Card, M., Connolly, S.R., Folke, C., Grosberg, R., Hoegh-Guldberg, O., Jackson, J.B.C., Kleypas, J., Lough, J.M., Marshall, P., Nyström, M., Palumbi, S.R., Pandolfi, J.M., Rosen, B., and Roughgarden, J. (2003) Climate change, human impacts, and the resilience of coral reefs. *Science* 301, 929–933. <https://doi.org/10.1126/science.1085046>
11. Baird, A.H., Bhagooli, R., Ralph, P.J., and Takahashi, S. (2009) Coral bleaching: the role of the host. *Trends in Ecology and Evolution*. *Cell Press* 24(1), 16–20. <https://doi.org/10.1016/j.tree.2008.09.005>
12. Glynn, P.W. (1996) Coral reef bleaching: facts, hypotheses, implications. *GCB* 2(6), 495–509. <https://doi.org/10.1111/j.1365-2486.1996.tb00063.x>
13. Siebeck UE, Logan D, and Marshall NJ. (2010) CoralWatch – a flexible coral bleaching monitoring tool for you and your group. Coral Reefs. Proceedings of the 11th International Coral Reef Symposium; 7–11 Jul 2008; Ft Lauderdale, FL. Dania Beach, FL: National Coral Reef Institute.
14. Warner, M.E., Lesser, M.P., and Ralph, P.J. (2010) Chlorophyll fluorescence in reef building corals, in: *Chlorophyll a Fluorescence in Aquatic Sciences: Methods and Applications. Developments in Applied Phycology, vol 4.* (Suggett, D., Prášil, O., Borowitzka, M., Ed.). 209–222, Springer, Dordrecht. https://doi.org/10.1007/978-90-481-9268-7_10
15. Ralph, P.J., Schreiber, U., Gademann, R., Kuhl, M., and Larkum, A. (2006) Coral photobiology studied with a new imaging pulse amplitude modulated fluorometer. *Phycological Society of America* 41(2), 335–342. <https://doi.org/10.1111/j.1529-8817.2005.04034.x>
16. Warner, M.E., Fritt, W.K., Schmidt, G.W. (1999) Damage to photosystem II in symbiotic dinoflagellates: a determinant of coral bleaching. *PNAS* 96(14), 8007–8012. <https://doi.org/10.1073/pnas.96.14.8007>
17. Warner, M.E., Berry-Lowe, S. (2006) Differential xanthophyll cycling and photochemical activity in symbiotic dinoflagellates in multiple locations of three species of Caribbean coral. *Journal of experimental marine biology and ecology* 339(1), 86–95. <https://doi.org/10.1016/j.jembe.2006.07.011>
18. Dellisanti, W., Chung, J.T.H., Chow, C.F.Y., Wu, J., Wells, M.L., Chan, L.L. (2021) Experimental techniques to assess coral physiology in situ under global and local stressors: current approaches and novel insights. *Front Physiol* 594(12). <https://doi.org/10.3389/fphys.2021.656562>
19. Ralph, P.J. and Gademann, R. (2005) Rapid light curves: a powerful tool to assess photosynthetic activity. *Aquatic botany* 82(3), 222–237. <https://doi.org/10.1016/j.aquabot.2005.02.006>

20. Idris, Putri A.R., Adiwijaya, C., Gilang, M., Santoso, P., Prabowo, B., Muhammad, F., Andriyani, W., Lestari, D.F., Setyaningsih, W.A. and Zamani, N.P. (2020) Assessment of coral reefs health in nature recreation park (TWA = taman wisata alam) Sangiang Island, Banten. *IOP Conf Ser: Earth Environ Sci* 429: 012020. <https://doi.org/10.1088/1755-1315/429/1/012020>
21. Miller, I., and Müller, R. (1999) Validity and reproducibility of benthic cover estimates made during broadscale surveys of coral reefs by manta tow. *Coral Reefs* 18, 353–356. <https://doi.org/10.1007/s003380050212>
22. Kautsky, H., and Hirsch, A. (1931) Neue Versuche zur Kohlensäureassimilation. *Naturwissenschaften*, 19, 964. <https://doi.org/10.1007/BF01516164>
23. Leiper, I.A., Siebeck, U.E., Marshall, N.J., Phinn, S.R. (2009) Coral health monitoring: linking coral color and remote sensing techniques. *Canadian Journal of Remote Sensing* 35(3), 276-286. <https://doi.org/10.5589/m09-016>
24. AZA, Animal management. Association of Zoos & Aquariums, <https://www.aza.org/animal-management> (accessed Dec 2022)
25. Living Oceans Foundation, Coral reef ecology curriculum. Khaled bin Sultan Living Oceans Foundation Coral Reef Ecology Curriculum, <https://livingoceansfoundation.org> (accessed Dec 2022)
26. NOAA, Background information. Coral reef information system, <https://www.coris.noaa.gov/activities/oa/welcome.html> (accessed Dec 2022)
27. National Geographic, Coral reefs, <https://education.nationalgeographic.org/resource/coral-reefs> (accessed Dec 2022)
28. NOAA, 3D printed model brings coral education to life. Coral reef conservation program, <https://coralreef.noaa.gov/education/polypmodel.html> (accessed Dec 2022)
29. Aronson, R.B., and Precht, W.F. (2001) White-band disease and the changing face of Caribbean coral reefs. *Hydrobiologia* 460, 25–38. <https://doi.org/10.1023/A:1013103928980>
30. Precht, W.F., Gintert, B.E., Robbart, M.L., Fura, R., van Woessik, R. (2016) Unprecedented disease-related coral mortality in Southeastern Florida. *Sci Rep* 6(31374). <https://doi.org/10.1038/srep31374>
31. AGRRA, Coral disease outbreak. Atlantic and Gulf Rapid Reef Assessment, <https://www.agrra.org/coral-disease-outbreak/> (accessed Dec 2022)

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PRESS SUMMARY

Coral reefs are one of the most biodiverse and productive ecosystems on Earth, and color has been shown as an indicator of health in Australian and Hawaiian reef systems. But there is no standardized method to quantify coral health for Caribbean corals. Therefore, a health assessment card using coral color to non-invasively monitor coral health was developed for five species of Caribbean corals. Nondestructive measurements of “health” were correlated with coral color pigmentation to develop a rapid, non-invasive tool for coral health monitoring. The results of this work successfully corresponded photosynthetic efficiency to coral color for five species of dominant Caribbean corals to develop a Coral Health Assessment Card for Caribbean reefs. By implementing a standardized assessment of coral health, long-term and widespread monitoring projects can be implemented to track overall Caribbean reef health. Consequently, the results of this work support global efforts to conserve marine ecosystems and protect biodiversity.