



## Photographic assessment of coral chlorophyll contents: Implications for ecophysiological studies and coral monitoring

G. Winters<sup>a,b,\*</sup>, R. Holzman<sup>b,c,1</sup>, A. Blekhan<sup>d</sup>, S. Beer<sup>a</sup>, Y. Loya<sup>e</sup>

<sup>a</sup> Department of Plant Sciences, Tel Aviv University, Tel Aviv 69978, Israel

<sup>b</sup> The Inter University Institute for Marine Sciences at Eilat, Eilat P.O.B. 469, Israel

<sup>c</sup> Department of Evolution, Systematics and Ecology, the Hebrew University of Jerusalem, Israel

<sup>d</sup> Department of Electrical Engineering, Technion, Israel Institute of Technology, Haifa 32000, Israel

<sup>e</sup> Department of Zoology and the Porter School for Environmental Studies, Tel Aviv University, Tel Aviv 69978, Israel

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### ABSTRACT

Assessing environmental impacts on coral reef communities has become a growing discipline. As most corals grow relatively slowly, the common method of monitoring changes in coral communities may limit our ability to identify stressors and stress responses. Since chlorophyll density (amount of chlorophyll  $a + c_2$  per unit of coral surface area) in corals may correlate with coral color, the latter has been suggested as an indicator of the natural seasonal changes in undisturbed (“healthy”) corals, as well as an indicator of environmentally-induced stresses in corals, including those related to bleaching. However, the color of underwater objects as perceived through the naked eye or a camera is affected by attenuation of irradiance with depth, changes in spectral properties of underwater light, turbidity and dissolved materials in the water, among other factors. Thus, methodological issues have so far held back the use of color as a quantitative indicator for chlorophyll content. Presented here are two accurate and easy to use methods for quantitative measurements of chlorophyll density from digital photographs of corals. In these methods, the intensities of the red, green and blue channels in digital photographs were compared to measured chlorophyll densities in *Stylophora pistillata*. Variations in external light, known to bias the true color in underwater images, were eliminated either by photographing corals through a specially built funnel with an internal light source or by mathematically normalizing color channels to a gray reference scale. In both methods, chlorophyll density was highly correlated with the intensity of the red channel, despite large variations in lighting conditions during the photography.

These photographic methods enabled the estimation of natural spatial and temporal changes of the chlorophyll density of corals. By predicting chlorophyll density in corals at very low costs, both methods presented here could facilitate the study of large-scale physiological changes in corals.

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### 1. Introduction

Over the last few decades, coral reefs worldwide have suffered declines in abundance, diversity and habitat structure (Hughes, 1994; Gardner et al., 2003). Among other things, these declines entail severe economic consequences (Costanza et al., 1997; Donner and Potere, 2007). Such long-term declines have been attributed to overfishing and pollution (Hughes, 1994; Jackson et al., 2001), land-use practices (Richmond, 1993) and the growing threat of global warming (Hoegh-Guldberg, 1999) (reviewed by Hughes et al., 2003). Indeed, much

effort is invested in monitoring coral reefs around the world (Porter and Ouida, 1992; Bythell et al., 1993; Hodgson, 1999; Dustan et al., 2000) in an attempt to identify causes and effects of potential coral stressors, thereby improving the management and conservation of reefs. Coral monitoring is mostly done by following changes in coral community structure (coral cover, density and species diversity) with time, usually in the scale of years (Connell et al., 1997; Loya, 2004), by means of belt/line transects and/or photographing quadrants on regular intervals. However, due to the relatively slow growth rates of corals, there is a large time lag between the temporal scale of disturbance events (e.g. pollution discharges) and the community response (i.e., a measurable change in the community structure). This large time lag (sometimes in the order of years) hinders the efforts to identify the causes of community dynamics. Moreover, the observed changes in community structure might be identified too late to allow an opportunity for effective human intervention. Indeed, both Brown (1988) and Risk (1999) have emphasized the importance of

\* Corresponding author. Tel.: +49 251 83 21041; fax: +49 251 83 24668.

E-mail address: [wintersg@uni-muenster.de](mailto:wintersg@uni-muenster.de) (G. Winters).

<sup>1</sup> Current address: Section of Evolution and Ecology, University of California in Davis, One Shields Ave, Davis, CA, 95616, USA.

<sup>2</sup> Institute for Evolution and Biodiversity, Westfälische Wilhelms-Universität, Hüfferstrasse 1, D48149 Münster, Germany.

developing techniques that enable detection of early stages of coral stress.

Preceding changes in coral community patterns are physiological changes on the organismal level that occur at a much more rapid rate of hours to weeks. These physiological changes, e.g. changes in zooxanthella density and chlorophyll content, are considered reliable indicators not only of seasonal changes (Fitt et al., 2000; Winters et al., 2009), but also of stress due to increased temperature (Glynn and D'Croz, 1990; Jones, 1997a,b; Warner et al., 2002), changes in salinity (Hoegh-Guldberg and Smith, 1989) and pollutants (e.g. cyanide; Jones et al., 1999). However, measurements of zooxanthella biomass parameters involve the collection of corals and the removal of coral tissue (thereby sacrificing or severely injuring the coral) followed by relatively time consuming laboratory protocols, thus limiting the temporal and spatial scales over which such studies can take place. Concomitantly, studies addressing the physiological response of corals to environmental changes are usually restricted to short-term effects and are performed at the laboratory or a limited number of sites (Hoegh-Guldberg and Smith, 1989; Dubinsky et al., 1990; Glynn and D'Croz, 1990; Jones, 1997a). In addition, the intrusive nature of such procedures might limit research to certain coral species (abundant/fast growing) or to certain reef sites (those that are not within marine protected areas), and to specific laboratories (equipped with centrifuges, spectrophotometers, microscopes etc.).

Since both zooxanthella density and chlorophyll content contribute to the overall chlorophyll density (amount of chlorophyll  $a + c_2$  per unit of coral surface area), coral color, which is proportional to chlorophyll density, has been suggested as an indicator of environmentally-induced stress in corals, including that related to bleaching (Thieberger et al., 1995; Edmunds et al., 2003; Siebeck et al., 2006; Table 1). However, using underwater images to quantify coral color involves several possible biases arising from variations in ambient (above-waters) illumination, the attenuation of irradiance and changes in the color spectrum with depth, difficulties in comparing photographs taken under different lighting conditions and the question of how to “score” color, all of which have limited this approach. Trying to restrict the local light field, both Thieberger et al. (1995) and Edmunds et al. (2003) used powerful strobes to override the effect of sunlight, while Maguire et al. (2003) shaded the photographed corals with black plastic sheets. Some of the distortion of coral color found in photographs taken underwater, caused by changes in external illumination, increased depth and processing conditions of the slides, can be corrected by the use of color and gray scale reference cards (Thieberger et al., 1995; Edmunds et al., 2003; Maguire et al., 2003). However, color calibration in most of these studies was done subjectively (Edmunds et al., 2003; Siebeck et al., 2006).

In trying to “score” coral color, several studies have related corals to different color categories (Thieberger et al., 1995; Edmunds et al., 2003; Siebeck et al., 2006) ranging from healthy coral color to completely bleached (white). However, the resolution of such methods depends largely on how many color ranks are to be used (e.g., Edmunds et al., 2003 whom divided all coral colors into only 4 categories). More importantly, since color vision is constructed in the brain based on the signals it receives from photoreceptors (cones) sensitive to different parts of the color spectrum, the perception of color in humans is variable (Neitz and Jacobs, 1986). Hence, scoring color using color categories (or color cards) is subjective, and introduces inter-observer variability (Thieberger et al., 1995; Siebeck et al., 2006; see further details in Table 1).

The objective of this study was to develop an accurate, inexpensive and easy-to-use photographic method for quantitative estimates of chlorophyll density using coral color. Effects of variations in external light were normalized by either photographing the corals in situ through a specially built funnel with an internal light source or by using a gray-scale as a color reference and then mathematically

normalizing the color intensity values of the gray scale to values obtained in a reference image. These techniques were then used to evaluate the combined effects of natural spatial and temporal changes of the chlorophyll density of corals.

## 2. Materials and methods

### 2.1. Study subject and site

The branching coral *Stylophora pistillata* was chosen as a model coral for this study. It is one of the most dominant coral species of the Red Sea (Loya, 1972), and has been the subject of numerous studies on coral growth, reproduction (Loya, 1976), photophysiology (Falkowski and Dubinsky, 1981) and photosynthesis (Winters et al., 2003). The present work was done on specimens growing in the reefs adjacent to the Inter-University Institute (IUI) in Eilat, Israel (Gulf of Aqaba, Northern Red Sea, 29°30'N, 34°55'E) at depths of 5–20 m, between September 2004 and December 2005.

*S. pistillata* growing in this area and in these depths is also known to have no endolithic algae.

### 2.2. Underwater control of spectral properties (the “hardware method”)

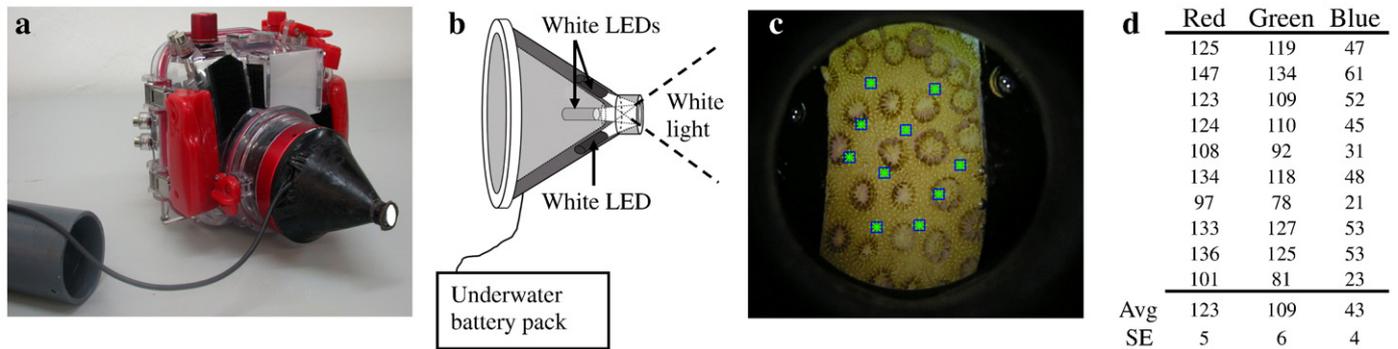
To exclude the variability associated with the ambient light, corals were photographed in a super-macro mode through an opaque black plastic funnel (5 cm long, 16 mm diameter at its narrow end; Fig. 1). Illumination was provided by three white 5 mm light-emitting-diodes (LEDs; OSPW5121A-PQ; 3.5 V, 5800 milicandles each, 20°, spectral peak at 459 nm [see electronic supplementary material (Appendix A) for emission spectrum], OptoSupply, Hong Kong, China), with a cumulative intensity of 21  $\mu\text{mol photons m}^{-2}\text{s}^{-1}$  (measured at the tip of the funnel using a LI-190 2 $\pi$  quantum sensor and a LI-1400 data logger; LI-COR Biosciences, USA). A 3.6 V battery (Panasonic HHR-P401, 1150 mA) in a custom-made underwater housing provided power for the LEDs. Estimated costs for funnel and battery pack are 150\$. The funnel was connected to an Olympus C-5060 digital camera in a P-20 Olympus underwater housing (Olympus, Japan) at its wide end, while a layer of 1 mm thick black rubber foam at its narrow end (Fig. 1a,b) prevented both damage to the corals during photography and penetration of light from the surrounding environment.

Colonies of *S. pistillata* growing at 5, 10 and 20 m (6–9 colonies per depth; total  $n = 21$ ) were used to test the relationship between coral color and chlorophyll density. Corals were chosen randomly by swimming for 10s at different directions within the same depth ( $\pm 1$  m), and then choosing the nearest coral to the point reached. Up to 10 successive photographs were taken underwater (by SCUBA divers) from different south-facing branches of each of the 21 corals (each photograph covering a total area of 27 mm<sup>2</sup>; Fig. 1c). Camera settings for all photographs taken in this protocol were pre-fixed and included aperture speed of 1/60 s, ISO = 100 (film speed),  $F = 3.2$  (the aperture size), lens focal length = 11.5 mm, super macro mode, flash off and spot metering. Since the funnel was always placed physically on the corals' flat surface, the target distance was constant. Following the coral photography, a coral fragment (usually a 3–4 cm long branch) was removed from one of the areas that had just been photographed, and placed in a tagged Ziploc bag filled with ambient seawater. Fragments within the Ziploc bags were kept in shaded collection bags, brought to the lab and frozen (without the seawater) at  $-70$  °C within 1 h for later analysis (see below).

Color analysis in this part of the study was based on the red, green and blue (hereafter RGB) color model, an additive model in which red, green, and blue are combined in various ways to reproduce other colors. This model is by far the most commonly used color model in visual image processing hardware (e.g. consumer grade digital cameras and computer displays) and software (e.g. ImageJ, Photoshop). Thus, the quantitative analysis of coral color described here

**Table 1**  
Comparison with other studies.

Reference	Photographic setting, equipment used	Standard used	How was color measured	Scoring of coral color	correlation/validation technique	Remarks
Thieberger et al. (1995)	Photographs were taken underwater using an SLR camera equipped with a strobe	One gray was used as the color standard	Corals were photographed on a slide film. The absorption of slides was read at 439 nm with a spectrophotometer	Optical density (OD) measurements were used to create a black and white chart of increasingly darker colonies. Chart was then used for in situ visual estimation of chlorophyll a	A significant correlation was found for 3 species between the OD of the slides and areal chlorophyll a content (curved, non linear regression)	Thieberger et al. subjectively selected the corals to be photographed – only even surface colonies were photographed. The chart created in this method was not actually tested. The authors suggest that such a chart could cause subjective color scoring
Maguire et al. (2003)	Photographs were taken underwater using an SLR camera equipped with a strobe. The natural irradiance was controlled by holding a 2 × 2 m black plastic sheet above the coral being photographed	Used the Kodak gray scale, which included 20 gray intensities	Corals were photographed on a slide film. After digitalizing slides by scanning them, the brightness of Red, Green and Blue was measured using Photoshop's histogram function	Scoring was based on the 0–255 scale	A significant correlation was found between chlorophyll a concentration and the brightness in each one of the RGB channels. Spearman rank order correlation coefficients (rs); rs = 0.929 for Red, 0.904 for Green and 0.929 for Blue – $P < 0.05$ for all channels	Maguire et al. subjectively selected the areas in the photographs that were going to be measured – only areas that were even in color and brightness) were chosen
Edmunds et al. (2003)	Photographs were taken underwater using an SLR camera equipped with a dual strobe	Used color standard bands of Red, Green, and Blue (RGB)	Corals were photographed on a slide film and were digitized by scanning. The brightness of Red, Green and Blue was measured using Photoshop's histogram function	The adjusted RGB values were collapsed into a single measure (PCA). Color was assigned to a subjective rank on an ordinal scale (of 1–4)	A weak negative correlation ( $r = -0.447$ ) was found between color ranking and zooxanthellae density	Edmunds et al. adjusted for differences in the exposure manually. They also used subjective color ranking
Siebeck et al. (2006)	For the initial work of developing the actual color chart, photographs were taken out of water in the laboratory using a digital camera and under the same controlled light conditions. Each coral was photographed under three different exposures	Five colored areas of Black, White, Red, Green and Blue	For the initial work of developing the color chart, Photoshop's histogram function was used to measure hue (reflected color), saturation (proportion of gray in the hue), and brightness (relative lightness and darkness) (on a 0–255 scale) of the lightest part of bleached coral (controlled bleaching experiment) and the brightest and darkest (photographic field survey) part of the corals	For developing the color chart, coral colors were grouped into 4 hue categories and 6 categories of brightness and saturation (hence, in total 24 hue, saturation and brightness combinations). Color scoring (0–6) was based on differences of brightness and saturation	For several species a significant positive relationship was found between color score and symbiont density ( $r^2 = 0.63$ ) and between color score and chlorophyll a ( $r^2 = 0.36$ ). Results for <i>A. aspera</i> from a laboratory bleaching experiment showed an improved correlation between symbiont density and color score ( $r^2 = 0.93$ )	The initial work included subjective screening of images based on color standards. The method was not tested underwater. Indeed, the authors suggest that the method is applicable only in shallow waters (< 5 m). The actual scoring of color was subjective. The experimental calibration artificially excluded intermediate colors, although these would be found in normal use. Method provides a rapid estimation of coral color
“Hardware method” – this study	Photographs were taken underwater using a digital camera. The corals were photographed through a specially built opaque funnel with an internal light source that excluded outside irradiance	No color standard was used	All images taken were already in digital format. The intensity of Red, Green, and Blue was measured using quadrates randomly placed on the coral surface. Quadrates from the same colony/ fragment were pooled and averaged for a single value for each of the R, G, and B channels	Scoring was based on the 0–255 scale. Although rapid, scoring of color requires computer time	A significant negative relationship (linear regression, $r^2 = 0.82$ , $P < 0.01$ ) between the intensity within the red channel and chlorophyll density (Fig. 3c)	The method is not limited by depth. Changes in LED intensity should be controlled, measured and/or accounted for. The surface area covered by each photograph is relatively small. We recommend taking at least 10 photographs to cover a 3–4 cm coral fragment. Although rapid, scoring of color requires computer time
“Software method” – this study	Photographs were taken underwater using a digital camera, under natural irradiances and with a Kodak gray scale as reference	Used the Kodak gray scale, which included 20 gray intensities		Scoring was based on the 0–255 scale. Although rapid, scoring of color requires computer time	A significant negative relationship (linear regression, $r^2 = 0.71$ , $P < 0.01$ ) between the intensity within the red channel and chlorophyll density (Fig. 4d)	The method is not limited by depth. Coral color was normalized mathematically before intensity of color was measured (objective normalization), involving a pre color-scoring process. The surface area covered by each photograph is relatively large



**Fig. 1.** Setup for underwater photographing of the coral *Stylophora pistillata* at different depths and otherwise varying conditions of irradiance (“hardware method”). (a) A custom-made opaque plastic funnel was connected to an underwater digital system (Olympus digital camera C-5060 in P-20 housing) at its wide end. (b) The black plastic funnel (5 cm long, 16 mm in diameter at its narrow end) was equipped with three white LEDs providing a fixed illumination intensity and color for each photograph. A ring of black foam was fitted at the narrow end of the funnel to reduce light penetration and prevent damage to the corals during photography. Power to the LEDs was provided by an underwater 3.6 V battery pack. Image analysis of these photographs (c) was based on measurements of color intensity within 10 different quadrates (of  $25 \times 25$  pixels each) placed randomly on the coral within the image. For each quadrate, color intensity for each of the RGB channels was measured in the scale of 0 (darkest) – 255 (brightest) (d).

was based on measurements of intensities with each one of the RGB channels ranging from 0 (for the darkest) to 255 (for the brightest) in each channel. For each photograph the color intensities were sampled in 10 randomly placed quadrates of  $25 \times 25$  pixels each, corresponding to an overall area of  $1.12 \text{ mm}^2$  (Fig. 1c). Intensity measurements were made using a custom-made macro (“AnalyzeIntensity” utility; see electronic supplementary material (Appendix A)) in MATLAB (Ver 6.5, MathWorks Inc, MA, USA), which was based on the image analysis toolbox within this software. For each coral, color intensities from all the images were averaged (separately for each channel) and used as a single output (see the average example calculated in Fig. 1d) which was compared to the coral’s chlorophyll density (see below).

The use of the “hardware method” depends both on the ability of the funnel to exclude external illumination and on the stability and consistency of the illumination within the black funnel. The ability of the funnel to exclude the outside light was tested by photographing a standard gray under different external light conditions including 1, 5, and  $57 \mu\text{mol photons m}^{-2}\text{s}^{-1}$  of artificial light provided by fluorescent neon lamps, and 110 and  $1000 \mu\text{mol photons m}^{-2}\text{s}^{-1}$  from natural irradiance. The standard gray was photographed five times under each of these irradiances. Irradiance was measured using a LI-190  $2\pi$  quantum sensor connected to a LI-250A light meter (LI-COR Biosciences, USA). Using the “AnalyzeIntensity” utility (mentioned above) we measured the intensity of color within each of the RGB channels in ten randomly placed quadrates on each of the 25 photographs taken. We found no significant differences between the red values from photographs taken under different lighting conditions (repeated measures ANOVA,  $F_{4, 196} = 1.5486$ ,  $P > 0.18$ ). Similar results were found for values of green and blue ( $F_{4, 196} = 1.56$ ,  $P > 0.18$  and  $F_{4, 196} = 1.40$ ,  $P > 0.23$ , respectively). Photographs taken under different lighting conditions but with the LEDs turned off, always resulted in complete darkness (intensity values of 1,1,1 in the RGB channels, respectively) regardless of external light conditions. Results from both of these tests confirmed the ability of the funnel to completely exclude outside irradiance.

The stability of the LEDs’ light source over time was also tested by photographing a standard gray while leaving the LEDs on (in order to consume the battery level) for a period of 90 min. Photographs of the gray standard together with measurements of PAR (using a LI-1400 data logger with a LI-190 quantum sensor; LI-COR Biosciences, Lincoln, USA) were taken every 10 min. This examination revealed no significant changes in both the LED intensity (linear regression,  $F_{1, 8} = 0.3673$ ,  $P > 0.5$ ;  $CV = 2.6\%$ ) and in the intensity of the red channel of the gray being photographed over a 90 min period (repeated measures ANOVA,  $F_{8, 72} = 0.22934$ ,  $P > 0.05$ ). However,

these tests were done for a fairly new battery. Users should be aware of these issues and either charge/change the batteries used and measure the light intensity within the funnel regularly.

The consistency of the light source within the measurement area was also tested. Users should note that LED outputs tend to be uneven with an obvious hot spot in the center. We were technically unable to place diffusers on the LEDs themselves. However, we found that the LED’s uneven output could be solved in the color intensity measurement stage by placing the quadrates randomly to cover the entire photograph (as shown in Fig. 1). Use of randomly placed quadrates was actually tested by analyzing the spatial variance in 5 images of uniform gray paper. Measurements of red channel intensity were taken by placing 10 randomly placed quadrates making sure that the entire photograph was being covered. This analysis found no significant differences between the red values taken from the different images (one way ANOVA,  $F_{4, 45} = 0.1245$ ,  $P > 0.5$ ).

### 2.3. Post-hoc normalization of image colors (the “software method”)

Correcting for the effects of variations in light intensity and spectrum was also done post-hoc using a normalization procedure involving the use of a predefined gray scale. Colonies of *S. pistillata* ( $n = 14$ ), collected from 5 m depth, were broken into ca. 60 fragments (3–5 cm long each). The fragments were mounted on plastic holders using underwater epoxy glue (AquaMend, USA) and placed on an underwater table at 5 m depth. Following a 3 month period during which fragments were allowed to recover from this fragmentation process, the fragments were divided into two sets with repeated photographs taken from one set ( $n = 8$  colonies) and fragments taken for chlorophyll analysis from the second set ( $n = 6$  colonies).

Once a month (September 2004–August 2005;  $n = 12$  months) a fragment from each original colony was taken for chlorophyll analysis (as described below;  $n = 6$  different colonies each month). Concurrently, fragments from the larger set ( $n = 8$  colonies) were photographed each month underwater using a Nikon 995 Coolpix in an Ikelite (USA) housing placed on a custom-made tripod to ensure fixed target distance (1.2 m), angle ( $90^\circ$ ) and relative height. White balancing was done prior to each dive (from a fixed distance) using the same camera settings against the white board onto which the Kodak gray scale was glued. Photographs were taken in manual mode so that the gain and white balance settings remained constant. Camera settings for all the photographs taken each month using this protocol included the following: flash was turned off, ISO = 100,  $F = 3.6$ , aperture speed of  $1/60 \text{ s}$  and full frame metering were used. Each fragment was photographed in situ along with a Kodak gray

scale (Kodak color separation guide and gray scale Q-13, Kodak, USA) and a measuring tape (Fig. 2a). The Kodak gray scale is a quality-control device of stepped neutral values to help the photographer compare photographs taken under different photographic conditions. The scale is composed of 20 gray squares representing 20 density steps, with image intensity values declining linearly from 255 (white; gray # 1) to 0 (black; gray # 20).

Normalization of image color was performed using a custom-made macro in MATLAB (“CalibrateImageA” utility 1.0; see Appendix A), which adjusted the RGB components in the images to an a priori known RGB profile of the Kodak gray scale. The principle of this normalization procedure relies on the fact that under perfect conditions (illumination and camera), for each gray within this scale the intensity of each of the three RGB color channels is equal to one another ( $I_{(Rj)} = I_{(Gj)} = I_{(Bj)}$ , where  $I_{(Rj)}$ ,  $I_{(Gj)}$ ,  $I_{(Bj)}$  are the intensities of the each color (R,G,B) for pixels located within the jth gray). Since the decrease in the intensity of the grays on this scale is linear, the intensity of the red channel (for example) as a function of gray number can be described as:

$$I_{(Rj)} = a_{(R)} \times j + b_{(R)} \quad (1)$$

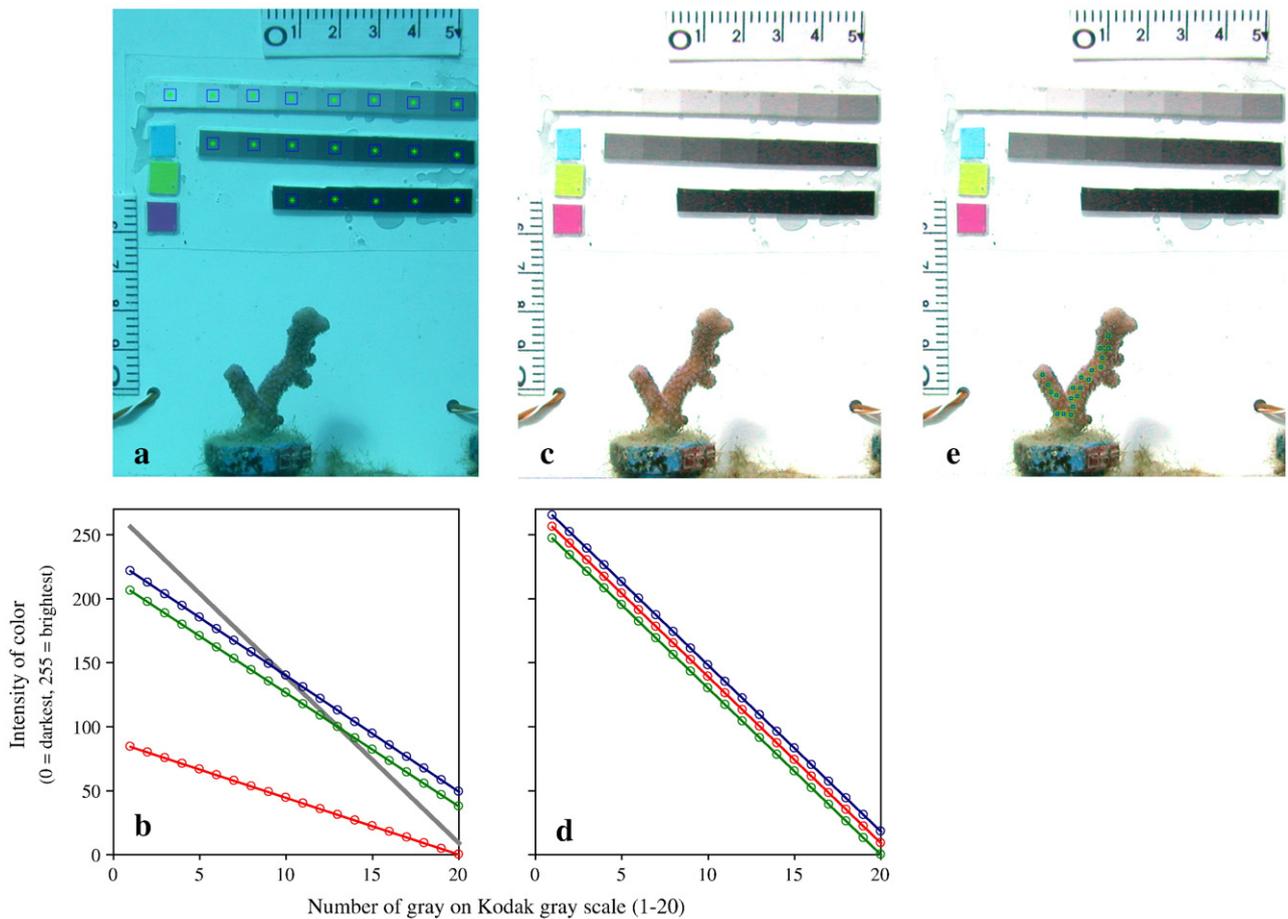
where  $a_{(R)}$  and  $b_{(R)}$  are slope and intercept coefficients for the red channel. Given that 20 intensities cover the range of 0–255, and if the intensity of a complete black ( $j = 20$ ) is zero (assuming perfect conditions), it follows that  $a_{(R)} = a_{(G)} = a_{(B)} = -(255/20)$ , and  $b_{(R)} = b_{(G)} = b_{(B)} = (255 - a)$ . In

reality, these values could be slightly different (for example, black is rarely 0 due to electric noise), but they can be empirically determined for a calibration image taken under “close to perfect” conditions (the photographs of the Kodak gray scale used for the calibration were taken outside of the water under natural midday sunlight at  $\sim 1500 \mu\text{mol photons m}^2\text{s}^{-1}$ ).

When taking images in realistic underwater scenarios, colors change due to differential absorption of by water across the spectrum, as well variations in illumination conditions. Consequently,  $a_{(R)}^* \neq a_{(G)}^* \neq a_{(B)}^* \neq -(255/20)$  and  $b_{(R)}^* \neq b_{(G)}^* \neq b_{(B)}^* \neq (255 - a)$  [\* indicates coefficients in the underwater image; notice the difference in attenuation of the R channel compared to G and B in Fig. 2b]. However, the intensity within each channel is still linearly correlated with gray number following the Eq. (1). Between different underwater images,  $a_{(R)}^*$  and  $b_{(R)}^*$  are expected to vary due to varying illumination, reflectance and absorption conditions, producing the variation in color observed in unprocessed images. Since the original values of  $a$  and  $b$  are known, it is possible to calculate the corrected intensity of each channel for each pixel through linear transformation so that:

$$I_{corrected(Rxy)} = [(a_{(R)} / a_{(R)}^*) \times (I_{measured(Rxy)} - b_{(R)}^*)] + b_{(R)} \quad (2)$$

where  $I_{corrected(Rxy)}$  is the corrected (reconstructed) intensity value of the red channel in pixel  $x, y$ , and  $I_{measured(Rxy)}$  is that value in the



**Fig. 2.** Normalization and sampling of coral color using the “software method”. (a) original image, (b) measured intensity values of the gray reference card for the three color channels (Red, Green, Blue; measured under the Kodak gray scale quadrates). The gray line represents the expected intensity of the three channels under perfect conditions. (c) Normalized image. The intensity value in each color channel was linearly transformed in each pixel based on the deviation in each color channel from the expected slope [gray line in panel b]. (d) Following normalization, re-measured intensity values of the gray reference card for the three color channels (Red, Green, Blue; measured under the Kodak gray scale quadrates) coincide with the reference line. Data in (d) were vertically shifted for visualization purposes; measured values of the three channels were identical. (e) Measurement of color intensity using 20 quadrates of  $15 \times 15$  pixels randomly placed at different parts of the normalized coral photograph.

original underwater image,  $a$  and  $b$  are the coefficients measured for the calibration image and  $a_{(R)}^*$  and  $b_{(R)}^*$  are empirically measured coefficients describing the intensity of the red channel in the underwater image as a function of gray number. Note, that  $a_{(R)}^*$  and  $b_{(R)}^*$  are image-specific and have to be measured for each image independently. A similar process is then made for the G and B color channels to produce a reconstructed image (Fig. 2c), in which the colors in each image are normalized, so that the intensities of the three colors measured for the gray scale would fit their real values (Fig. 2d). It should be noted that this normalizing process (described above) is analogous to the techniques of histogram equalization and gamma correction (Gonzalez et al., 2004).

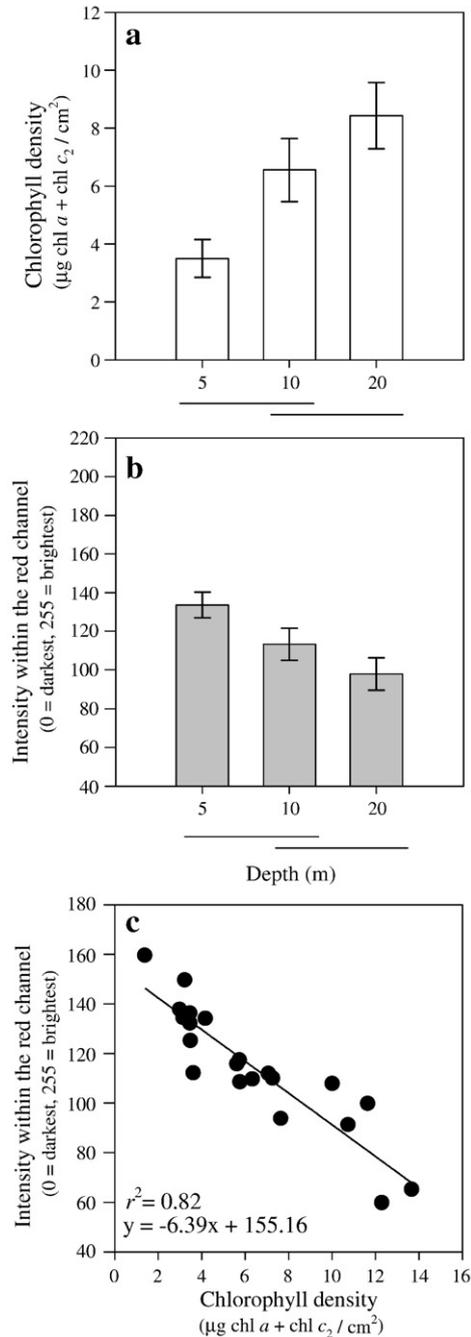
Following normalization (Fig. 3c,d), 20 quadrates of  $15 \times 15$  pixels (each one equivalent to  $6.5 \text{ mm}^2$ ) were randomly placed at different parts of the normalized coral photograph (Fig. 2e) and color intensity was measured for the R, G, and B channels using the “AnalyzeIntensity” macro described above. Using this macro, RGB values of gray # 1, 4, 8, 12, 16 and 20 were also measured monthly to account for the possible change in the gray scale plate, or in the camera, with time. Each month (September 2004–August 2005;  $n = 12$  months) six photos were taken from each one of these grays ( $n = 6$  for each of the 6 different grays). Color intensity within these photographs was taken using one  $15 \times 15$  quadrate placed on the particular gray being measured in each photograph.

#### 2.4. Measurement of chlorophyll density

Chlorophyll density (amount of chlorophyll  $a + c_2$  per unit of coral surface area) was measured following Fitt et al. (2000). Briefly, coral tissue was removed from the skeleton using an air brush (VL, Paasche, USA) filled with  $0.2 \mu\text{m}$  membrane filtered seawater (FSW). The resulting slurry was centrifuged (5 min at  $2300g$ ), the zooxanthellae containing pellet was resuspended in 10 ml FSW, homogenized and centrifuged again (as above). The pellet was resuspended in 1 ml FSW for cell counts and chlorophyll analysis. For chlorophyll analysis, algal pellets were resuspended in 90% acetone and stored in darkness overnight (14 h) at  $4^\circ\text{C}$ . After centrifugation, the chlorophyll  $a$  and chlorophyll  $c_2$  contents were determined spectrophotometrically according to absorptions at 664 and 630 nm, respectively (Jeffrey and Humphrey, 1975). Coral surface area was determined using melted wax maintained at  $65^\circ\text{C}$  in a water bath (Stimson and Kinzie, 1991). Fragments were dried and weighed, then dipped in melted wax for a standard period of 5 s, taken out, shaken slightly 6 times (see Hoegh-Guldberg, 1988) and after cooling for 20 min at  $20^\circ\text{C}$  were weighed again. For improving the accuracy of measuring surface area using the wax method, the wax was removed from the coral skeleton by multiple washes in boiling fresh water, followed by drying the fragments in air for 2 h, weighing the dried fragments before and after dipping them again in boiling wax. This process was repeated so that in total the wax weight of each fragment was measured 3 times. Wax weight was transformed to surface area using a calibration curve ( $r^2 = 0.92$ ) based on 7 different standardized cubes produced from sandpapering *S. pistillata* coral skeletons to known surface areas. For each coral skeleton measured (sample and calibration cubes) the three weights were averaged.

#### 2.5. Comparing the “hardware” and “software” methods

The “hardware” and “software” methods were compared by photographing 12 coral fragments through the opaque funnel (“hardware” method; setting mentioned above) followed immediately by photographing the same corals with a gray scale beside the fragments (“software” method; setting mentioned above). For this test, *S. pistillata* colonies ( $n = 4$ ) were collected from 2 m and brought to the lab where each colony was broken into 3 fragments. Each one of the three fragments was fixed in an upright position (as mentioned above) and



**Fig. 3.** Measurements of (a) chlorophyll density and (b) intensity of the red channel for *Stylophora pistillata* colonies (6–9 colonies at each depth; total  $n = 21$ ,  $\pm$  SE) growing at 5, 10 and 20 m depth. Color intensity, measured using the “hardware method” (Fig. 1) was significantly correlated with chlorophyll density (c). Values represent averages  $\pm$  SE of 10 randomly placed quadrates of  $25 \times 25$  pixels each distributed on 4–10 photographs of the same colony. Horizontal lines indicate results that are not significantly different (ANOVA followed by Tukey HSD post-hoc test;  $P > 0.05$ ).

allowed to photoacclimate in outdoor aquariums exposed to 90, 70 and 50% of surface irradiance using different black plastic shading nets, with light intensity measured using the LI-190 quantum sensor connected to a LI-1400 data logger (LI-COR Biosciences, USA). The 12 fragments were allowed to photoacclimate this way for 3 months before photographing them. Growth in these different light regimes resulted in variability in coral colors between the 12 fragments. All photographs were taken under natural irradiance and underwater, using the aforementioned Olympus C-5060. For each method, the intensity of the color red was

measured using 10 quadrates (of 15 × 15 pixels each) randomly placed on the coral surface photograph (as mentioned above).

## 2.6. Statistical analyses

In using either the “hardware” or “software” methods to control for image illumination, the basic “sampling unit” was the single coral colony or fragment, respectively. In both approaches, quadrates from the same colony or fragment were pooled and averaged for a single value for each of the R, G, and B channels.

The effect of collection depth on chlorophyll density was tested using one-way ANOVA with depth (3 levels) as categorical predictor (factor) and chlorophyll density measured per coral ( $n = 6$ –9 of each depth) as dependent factor. A similar analysis was made for the effect of depth on intensity of coral color. ANOVAs were performed only after verifying homogeneity of variances and testing for normality on the standardized residuals. Following significant ANOVA results, differences between the treatments were tested using Tukey HSD post-hoc tests.

For the work on temporal changes in coral color, each fragment was measured repeatedly each month ( $n = 12$ ) thus repeated measures ANOVA was used to test the effect of month (12 levels) on coral color ( $n = 8$  fragments). Since the same coral colonies were sampled every month, a similar analysis was made to test the effect of month on chlorophyll density. Data on the intensity of the red channel throughout the year was log transformed to comply with the sphericity assumption. The consistency of the procedure and equipment used throughout the year in the “software method” was tested with the null hypothesis that the slope and intercept of the relationship between gray # (on the Kodak gray card) and the measured value of red channel for that gray were not significantly different between sampling months or individual corals. For each month, the intensity of the red values (ranging from 0 = darkest, to 255 = brightest) was measured for grays # 1, 4, 8, 12, 16 and 20. These measurements were tested using a two-way ANCOVA with gray # as continuous predictor (6 grays), months and coral ID as categorical predictors with the measured gray intensity ( $n = 6$  measurements per gray per month; each taken concurrently with a different coral) as the dependent factor.

Forward stepwise multiple linear regression was used to examine the relationship between each of the R, G and B channels (as dependent variables) and chlorophyll density.

## 3. Results

### 3.1. Assessment of the “hardware method”

To assess the correlation between coral color and chlorophyll content, we used the naturally existing spatial variation in coral color found in corals growing along a depth gradient. For this, *S. pistillata* colonies naturally growing at 5–20 m were photographed and collected for measurements of their chlorophyll density. Deeper colonies were expected to have higher densities of chlorophyll and thus be darker (Falkowski and Dubinsky, 1981). Indeed, darkening of *S. pistillata* colonies with increased depth was evident both from the significant increase in chlorophyll density (Fig. 3a; ANOVA  $F_{2,18} = 5.64$ ,  $P < 0.05$ ) and the parallel significant decrease in the intensity of the red channel (Fig. 3b; ANOVA  $F_{2,18} = 4.15$ ,  $P < 0.05$ ) of deeper colonies compared to their shallower counterparts. Although not shown, similar decreases in the intensity of color for deeper colonies were also found for the blue and green channels.

For *S. pistillata* colonies growing along this 5–20 m depth gradient, a significant negative relationship was found between the chlorophyll density measured for each one of the corals collected and the intensity within the red channel measured from photographs taken using the “hardware method” (linear regression,  $F_{1,20} = 89.36$ ,  $P < 0.0001$ ,

$r^2 = 0.82$ ; Fig. 3c). Similar relationships were found for the blue and green channels ( $F_{1,20} = 24.96$ ,  $P < 0.0001$ ,  $r^2 = 0.56$  and  $F_{1,20} = 91.06$ ,  $P < 0.0001$ ,  $r^2 = 0.82$ , respectively). There was no significant effect of sampling depth on the slopes of the regression between the intensity of the red channel and chlorophyll density (ANCOVA  $P > 0.05$ ).

### 3.2. Assessment of the “software method”

To assess the “software method”, we followed the natural temporal variation of both color and chlorophyll density of *S. pistillata* colonies growing at 5 m. Continuous changes in coral color were evident from photographs taken in situ under natural illumination throughout the year, normalized using the Kodak gray scale in the “software method” (as depicted in Fig. 2) and sampled for color intensity within the red channel (Fig. 4a). Significant changes over time were found for the intensity of the red channel (repeated measures ANOVA on ln transformed data,  $F_{11,77} = 31.06$ ,  $P < 0.001$  followed by Tukey HSD post-hoc test,  $P < 0.05$ ; Fig. 4a) and for the green and blue channels (data not shown). Starting with relatively bright colonies (September 2004, Fig. 4a), branches become darker with the onset of winter (December 2004–January 2005), seem to be the darkest (i.e., lowest intensity within the red channel) during early spring (February–March 2005) and then brighten again with the onset of summer with the brightest colonies being photographed during June–July 2005.

Throughout the year, no significant changes were noted in the intensity of the red channel within the six grays measured, our control for temporal changes in the camera, gray scale, or both (Fig. 4b; for clarity only grays # 4, 8 and 12 are shown). The correlation between the gray # and the measured value was strong and significant ( $r^2 = 0.94$ , ANCOVA,  $F_{1,414} = 6664$ ,  $P < 0.001$ ) indicating a lack of changes in the camera and gray scale over time.

The significant seasonal changes in chlorophyll densities (repeated measures ANOVA,  $F_{11,33} = 10.2$ ,  $P < 0.01$ ; followed by Tukey HSD post-hoc test,  $P < 0.05$ ; Fig. 4c) mirrored those of the intensity of the red channel (Fig. 4a). In accordance, chlorophyll densities during the winter months (November 2004–January 2005) were 3 times higher than when measured during the summer months (June–August 2005; Fig. 4c).

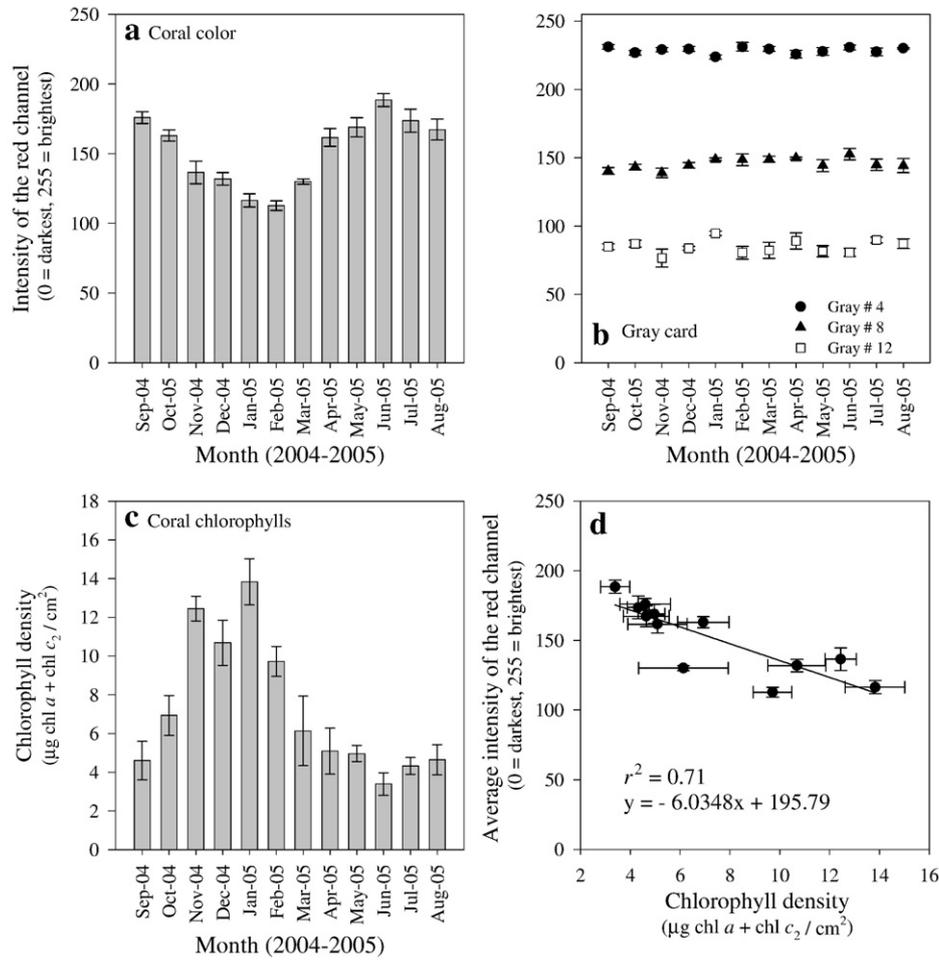
Similar to the “hardware method”, there was a significant negative relationship between the intensity of the red channel measured from photographs and the chlorophyll density (linear regression on monthly averaged red intensities and chlorophyll density values,  $F_{1,11} = 24.45$ ,  $P < 0.01$ ,  $r^2 = 0.71$ ; Fig. 4d). Similar, however weaker relationships were also found for the blue and green channels (linear regression,  $F_{1,11} = 8.86$ ,  $P < 0.05$ ,  $r^2 = 0.46$  and  $F_{1,11} = 12.10$ ,  $P < 0.01$ ,  $r^2 = 0.54$ , respectively).

Interestingly, although many studies report a number of symbiotic dinoflagellates (e.g., Fagoonee et al. 1999; Warner et al. 2002) rather than chlorophyll density, the above relationship between intensity of the red channel and chlorophyll density ( $r^2 = 0.71$ ) was higher than with zooxanthella density (linear regression,  $F_{1,11} = 16.87$ ,  $P < 0.01$ ,  $r^2 = 0.62$ ; see Fig. 2b in Winters et al., 2009).

### 3.3. Comparison of methods

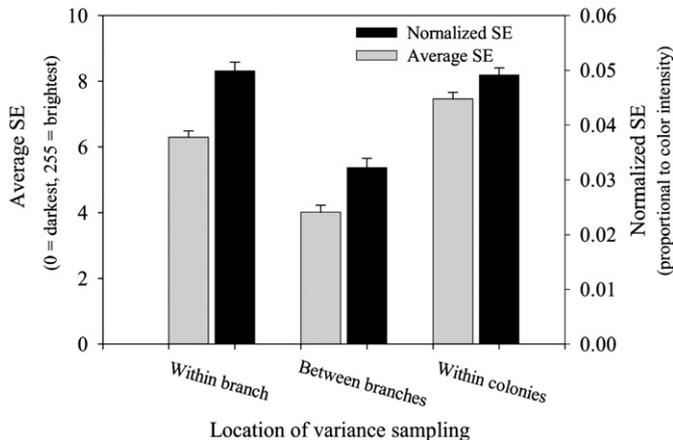
For comparing the methods, photographs of the same coral fragments were taken using both methods. In doing so, a significant positive relationship was found between the intensity of the red channel measured using the “software” and “hardware” methods (linear regression,  $F_{1,11} = 55.47$ ,  $P < 0.01$ ,  $r^2 = 0.847$ ), thus indicating that the methods were tightly correlated.

Variation in the intensity of red channel values within photographs taken of the same branch (Fig. 5, “within branch” and “within colony”) was low in both the “hardware” and “software” methods, with values of 5 and 4.8% of the mean red intensity, respectively.



**Fig. 4.** (a) Changes in intensity of color within the red channel in images of *Stylophora pistillata* colonies growing at 5 m, photographed throughout the year (September 2004–August 2005) in situ, under natural illumination. Images were normalized using the “software method” (Fig. 2). Color intensity was measured using 20 randomly placed quadrates of  $15 \times 15$  pixels each on each image. Values represent averages  $\pm$  SE of 8 different colonies photographed each month. (b) Changes in intensity within the red channel for gray # 4, 8 and 12 for images of Kodak gray scale, measured underwater throughout the year (one quadrate per each gray scale, 6 photographs of each gray every month,  $n = 6$ ,  $\pm$  SE); Although not shown, similar analyses were performed also on grays # 1, 16 and 20, indicating similar uniformity. (c) Monthly changes in chlorophyll density of adjacent colonies ( $n = 6$ ,  $\pm$  SE). (d) Linear regression between intensity of the color within the red channel (Fig. 4a) and chlorophyll density (Fig. 4c) for *Stylophora pistillata* colonies growing at a depth of 5 m.

Variation between different photographs taken of different locations in the same coral (“between branches”) was even lower ( $\sim 3\%$  of the mean red value), indicating low variability and high consistency of both methods.



**Fig. 5.** Variation in intensity of the red channel within branches, between branches and within colonies (Average SE, Left Y-axis) and in proportion to the color intensity measured (Normalized SE, right Y-axis).

**4. Discussion**

Assessing environmental impacts on coral reef communities has become a growing discipline (Porter and Ouida, 1992; Bythell et al., 1993; Hodgson, 1999; Ben-Tzvi et al., 2004; Loya, 2004). However, since most corals grow relatively slowly, relying only on coral community parameters in such studies limits the understanding of coral community dynamics and identification of stress responses but could also have important management implications. For example, in the case of some sewage discharges that caused increases in algal populations, it has been estimated that it would take a year or more for such discharges to have a measurable impact on community-level indices at the sewage discharge site (Pastorok and Bilyard, 1985). From a management perspective, such a time lag between the disturbance (e.g. this sewage discharge) and the response (i.e., a change in the community structure) could be crucial. Furthermore, the slow growth rate of corals also limits the use of community structure indices in controlled experiments in the laboratory (Brown, 1988). Hence, relying on community structure indices in order to assess possible additive, antagonistic and/or synergistic affects of different environmental stressors within the coral reef ecosystem is problematic (Brown, 1988).

Measurements of physiological parameters, specifically those related to the coral's *in hospite* zooxanthellae, have the advantage of

responding relatively fast (within hours or less) to changes in a suite of environmental conditions. Indeed, several studies have followed stress related changes in a suite of zooxanthella-related parameters such as zooxanthella density, chlorophyll content (Hoegh-Guldberg and Smith, 1989; Jones, 1997a,b) and the maximal quantum yield of photosystem II,  $F_v/F_m$  (Warner et al., 1999; Jones et al., 1999). Since there is also a need to establish what is 'normal' in order to be able to identify what is 'outside of the normal', studies have also followed the natural seasonal variations in these zooxanthella-related parameters (Fagoonee et al., 1999; Fitt et al., 2000; Warner et al., 2002; Winters et al., 2006, 2009). However, measuring zooxanthella density and chlorophyll content usually involves sacrificing the coral. While the development of underwater pulse amplitude modulated (PAM) fluorometers (e.g. the Diving-PAM; Walz, Germany) offers non-intrusive in situ measurements of photosynthetic parameters and the effects of stressors on those parameters (Jones et al., 1999; Winters et al., 2003, 2006; Okamoto et al., 2005), their high price (~\$15,000) will probably limit their worldwide use. The fast repetition rate fluorometers (FRRF), also recently used in some coral studies (Lesser and Gorbunov, 2001), were custom-made instruments, and will hence probably be even more expensive.

Measurements of changes in coral color offer not only the ability to measure early stages of coral stress, but also the possibility of combining field and laboratory studies. An accurate and cheap method of estimating coral chlorophyll (as presented here) enables the scaling up of field studies on both temporal and geographic scales. While several studies have used coral color as an indicator of coral chlorophyll contents (Thieberger et al., 1995; Edmunds et al., 2003; Siebeck et al., 2006; See Table 1), they have chosen to create underwater charts to be used for visually estimating chlorophyll densities. Although color charts provide rapid estimation of coral color and enable data to be collected by non-experts, the main disadvantages of this method are the discontinuity of color differences in the chart and the variability between observers (Thieberger et al., 1995; Siebeck et al., 2006). Furthermore, some studies (Table 1) have included subjective decisions in their analysis process, such as which area of the coral could be most effectively analyzed (Maguire et al., 2003) or photographed (Thieberger et al., 1995). Manual adjustments to exposure of photographs (Edmunds et al., 2003) also allows for a subjective influence on coral color. In contrast, the methods demonstrated here measure color on a continuous scale (ranging from 0 to 255) and, thus, can assess subtle differences in color between images taken from different corals or at different times. Adjustments of RGB components for corals photographed under natural irradiance (the normalizing process of the "software method") allow for an objective way of coral color correction. Likewise, the method used here for color "scoring" is much more objective than scoring coral color based on color charts (Thieberger et al., 1995; Edmunds et al., 2003; Siebeck et al., 2006), which can result in large inter-observer variation (Siebeck et al., 2006). Compared with destructive techniques photographic methods allow for repetitive measurements and following up on long term processes. Additionally, digital photographs can be easily stored and used for later reference. Indeed, the temporal variations in chlorophyll and coral color demonstrated here for healthy *S. pistillata* growing at 5 m (see also Winters et al. 2006, 2009) could be used as a base line helping to follow changes in coral health in the case of a natural or anthropogenically caused stress.

The work of Warner et al. (2002) on *Montastraea annularis* confirms the importance of establishing these baselines. Warner et al. (2002) measured zooxanthella densities for *Montastraea annularis* for over 5 years, finding cell densities of  $\sim 2.5 \times 10^6$  cells  $\text{cm}^{-2}$  in the winter (100%), with a loss of 40% of cells during "normal" summers ( $\sim 1.5 \times 10^6$  cells  $\text{cm}^{-2}$ ), compared to a loss of 80% of cells during the 1998 summer bleaching event ( $\sim 0.5 \times 10^6$  cells  $\text{cm}^{-2}$ ). Thus, it seems that mass bleaching events reflect a secondary decrease in

cell numbers beyond those experienced during "normal" summers. Compared with winter zooxanthella densities reported for *S. pistillata* in Eilat ( $1.45 \times 10^6$  cells  $\text{cm}^{-2}$ ; Winters et al., 2009), *S. pistillata* during the summer ( $0.85 \times 10^6$  cells  $\text{cm}^{-2}$ ) experiences a loss of  $\sim 40\%$  of its symbionts, in what seems at least visually to be another "normal" year (Winters et al., 2009). In our study, the natural fluctuations in chlorophyll densities (the software method Fig. 4c) ranged from 4 to  $14 \mu\text{g cm}^{-2}$ , with darkest corals having red intensity of  $\sim 110$  and brightest colonies red intensity of  $\sim 180$  (both on the scale of 0–255; Fig. 4a). Hence, there is still a wide enough range for assessing both darker corals (in the range of 0–110) and, during years which might be outside of the norm, brighter/bleached corals (in the range of 180–255). Similarly, in the hardware method (Fig. 3c) there is still enough range to photograph both deeper/darker corals (in the range of 0–60) and shallower/brighter corals (in the range of 180–255).

In principle, our color intensity measurements in each of the RGB channels are simplified spectral analyses that measure photon fluxes at three broad (on the order of 100-nm-wide) wavebands. Laboratory-based spectrophotometers record photon fluxes at hundreds of wavebands, each 1-nm-wide and across a much wider range (typically 200–1000 nm). Spectral analysis of corals is not a new field, and has been an area of active research since at least the late 1990s. In most of those studies, the reflected and incident fluxes were measured in order to calculate spectral reflectance and to correlate it with pigment content (Myers et al., 1999; Joyce and Phinn, 2003; Hochberg et al., 2006). Indeed, in some of those studies, the prediction of chlorophyll content and spectral reflectance in the red was much better than our intensity of the red-chlorophyll density relationship ( $r^2 = 0.7\text{--}0.82$ ; Figs. 3c and 4d) (Hochberg et al., 2006). The fact that several studies applying spectral analysis report on an absorption feature at around 675 (Joyce and Phinn, 2003; Stambler and Dubinsky, 2005; Hochberg et al., 2006) reconfirms our choice of the Red channel as best predictor of chlorophyll density. Although Hochberg et al.'s (2006) prediction of chlorophyll content from spectral reflectance in the red ( $r^2 = 0.997$  for chl a and  $r^2 = 0.941$  for chl  $c_2$ ) was much better than our intensity of red-chlorophyll density relationship ( $r^2 = 0.7\text{--}0.82$ ; Figs. 3c and 4d), our results are much better than those reported by Joyce and Phinn (2003;  $r^2 = 0.09$ ) using an underwater spectrometer and the relationship between absorbed ( $= 1 - \text{reflected}$ ) light and chlorophyll concentrations ( $r^2 = 0.57$ ) reported by Stambler and Dubinsky (2005) using the integrating sphere.

It should be noted that although some studies have measured spectral reflectance in situ (Joyce and Phinn, 2003; Hochberg et al., 2006) the price of such hand held in situ spectral reflectance systems ( $\sim$ US\$ 5000; Hochberg et al., 2006) is still much higher than digital cameras and camera housings, meaning that their immediate application to large-scale coral monitoring would probably be limited. However, spectral reflectance holds great potential for remote sensing platforms in the near future. The aim of such systems is not only to be able to discriminate between coral, algae and possibly other primary producers (Andréfouët et al., 2003, 2004), but more importantly, between healthy and bleached coral (Myers et al., 1999). While airborne systems would allow a dramatic increase in the scale of such monitoring, they would probably be used to monitor coral patches and not individual corals (Andréfouët et al., 2002).

Presented here are two different methods for accurate analysis of color intensity within the RGB color model. The two methods can be used in a variety of studies where color has to be quantitatively determined under varying external illumination that might affect the observed color. Although rapid, in both methods the scoring of color requires computer time. The "hardware method" requires contact with the photographed object (the funnel is placed physically on the coral's flat surface) and involves the costs ( $\sim$ 150\$) and time (2–3 days) of building an appropriate funnel and battery housing. Although the opening of the funnel we used was very small (16 mm diameter at its narrow end; Fig. 1a,b), it is possible to adjust the

diameter to fit corals with steeper curvature. The “software method” can be applied without contact with the focal object, as long as the gray scale plate is in the image. It involves buying (30\$) and properly laminating the Kodak gray scale and also requires some post-hoc normalization of image color before the color scoring stage. The two methods are highly comparable ( $r^2 = 0.84$ , mentioned above). Note, however, that for logistic reasons our initial test of the two methods was made with different cameras for each experiment. The two different cameras have different filter arrays and probably different image acquisition and processing procedures; this could be the reason for the slight different equation found for the intensity of red-chlorophyll density relationship between the two methods despite being performed on the same species in the same region. Therefore following organism’s color throughout the year or at a large geographic scale should be done using the same camera type, settings and orientation over time (the specific image acquisition and processing procedure of each camera is assumed to be consistent with respect to itself) or after cross-calibrations between different cameras. While both methods presented here could potentially work for all massive coral species (albeit in the hardware method, some changes might be needed to the aperture of the funnel, mentioned above), users should note that the method cannot distinguish the source of pigments. Thus, large concentrations of endolithic algae and/or large amounts of host pigments can reduce the accuracy of chlorophyll density predictions.

When preparing calibration curves between the red intensity and physiological parameters, we expect a better fit with chlorophyll density compared to zooxanthella density; While zooxanthella density is often used to reflect the physiological state of corals (Hoegh-Guldberg and Smith, 1989; Jones et al., 1999), chlorophyll density might be a more relevant parameter for coral color as it includes the density of symbiotic cells and the chlorophyll content within each cell, both of which would affect the coral’s light capturing abilities and hence, coral color.

The fact that intensity of Red was closer related to chlorophyll density in the “hardware method” compared with the “software method” is probably related to the fact that the latter involves a much larger photograph which covers much more surface area thus allowing for more variation. This is in addition to the fact that in the “software method” the branches that were photographed were not the same ones that were sacrificed and used for the chlorophyll measurements.

By predicting chlorophyll density in corals at very low costs, with relatively high accuracy ( $r^2 > 0.7$ ), we believe that both methods presented here will facilitate the study of large-scale physiological changes in corals. Users should note that to start using these methods it would be necessary to create camera- and region-specific color calibration curve for each coral species used. Indeed, different coral species might have different relationships between red and chlorophyll (e.g. due to different amounts of host pigments, or due to the presence of endolithic algae which also contain chlorophyll; discussed above). However, once color calibration curves are prepared, photographs of a specific coral species taken by local divers over time (using the same gray scale, camera and camera settings throughout the monitoring period) could be included in the analysis and serve as a baseline for further analysis. Significant deviations from this established coral color baseline could indicate early stages of coral stress.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jembe.2009.09.004.

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