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A photographic method for analyzing areal chlorophyll and bleaching status of the coral *Stylophora pistillata*

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Abstract. With the increasing frequency and severity of coral bleaching events, this phenomenon has been documented and studied by scientists around the world. Methods to quantify bleaching status are based on sacrificing the samples being tested or using expensive equipment (like the diving PAM). In recent years, the performance of digital cameras in image processing has improved and they have become more affordable, thus, we used these to develop a new, economical and nondestructive way to monitor bleaching and recovery processes in coral tissues, to be used by conservationists and researchers. In the present study, we report a reliable bioassay for computerized quantification of areal chlorophyll density in corals. We used a regular digital camera and standard computer software, both inexpensive and easily available. The proposed bioassay could be used on any coral to rapidly and economically gather data on coral bleaching and disseminate them.

Key words: Coral, Bleaching, photography, chlorophyll.

Introduction

Corals and their colors

Corals and coral reef ecosystems depend on the unique symbiosis between the host animal, the coral, and single-cell algae, the zooxanthellae (Brandt 1881). These live within the coral cells, in specialized vacuoles, the symbiosomes (Trench 1971, 1974; Gates 1992).

Besides some effect of the animal's water-soluble pigments (Shibata 1969), the color of corals depends primarily on the areal concentration of the photosynthetic pigments of the zooxanthellae. Thus, it reflects the product of the density of the symbionts and their cellular pigment content, with chlorophylls being the most prominent.

Coral bleaching

In well-publicized bleaching events, corals have turned white, usually due to loss of zooxanthellae (Glynn 1996), but occasionally as the result of a decrease in algal pigmentation (paling). These bleaching episodes are triggered by a sustained increase of water temperature, occasionally acting synergistically with high light, UV radiation, eutrophication, and disease (Glynn 1990, 1993; Dubinsky & Stambler, 1996), and result in widespread colony mortality and reef destruction.

The documentation of bleaching

In order to document and monitor bleaching events around in real time, it is of great importance and urgency to develop reproducible, rapid, reliable, inexpensive, and nondestructive ways to quantify coral pigmentation. Much of the early and current reporting of bleaching events is based on diver information and qualitative descriptions (Wilkinson and Buddemeier 1994). These are insufficient and there is a need for quantifiable methods for revealing the onset of bleaching in corals, and document instances in which recovery takes place.

Past attempts to achieve this goal were difficult and tedious. Thieberger et al. (1995) used 35 mm color slides taken under standard conditions, which were then scanned in a specially designed adapter in a spectrophotometer. Several subsequent efforts at different spatial scales and resolutions were tried in order to document the status of entire reefs, usually based on expensive, custom-made, hyperspectral scanners (Elvidge 2003). However, these were inadequate for determining the advent of bleaching on a single-colony level. The use of a digital camera to determine bleaching status in corals using a color reference card has been reported recently (Siebeck at al 2006) however the present method is more convenient and precise.

Most reefs are found in remote areas and in developing countries, far from well-equipped, academic research centers. Therefore, what is needed is a means to monitor coral-bleaching events and their aftermath using straightforward, standardized procedures. Ideally, such a system – based on off-the-shelf digital underwater cameras – should be designed to be used rapidly and economically by field technicians and reserve operating personnel at hundreds of locations. The resulting digital information can then be easily communicated worldwide.

Materials and Methods

Coral fragments

Three colonies of *Stylophora pistillata* were gathered from the reef near the Interuniversity Institute, Eilat, Israel, in the Gulf of Eilat (Aqaba). Corals of 15-cm diameter were collected from artificial objects at a depth of 2.5-3.5 m. The corals were allowed to acclimate for one month in our main aquarium system under 500µ mole quanta m^{-2}. Then the corals were divided into 3-cm-long fragments and glued with Super Glue Gel (Scotch, 3M) to plastic tips (Fig. 1).

Figure 1: *Stylophora pistillata* fragments during acclimation in our main aquarium system

The Fragments had divided between controls remaining in the light and the bleaching ones in the dark for 44 days. Every two weeks 3 fragments were photographed and extracted, and from these data the calibration curve (Fig. 4) and figure 5 were obtained.

Digital photographs

Coral fragments were taken from the aquaria in a seawater-filled beaker, photographed, and immediately returned to the water to minimize air exposure, or they were processed for analyses as described below.

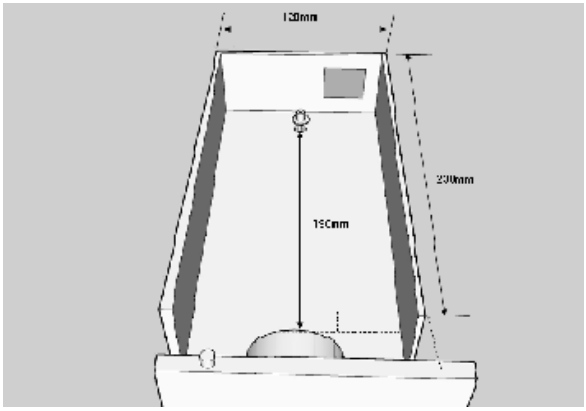

Figure 2: Camera stand sketch. The coral fragment was placed in the center of the image at 190 mm from the camera lens, with the gray plate at its right.

Digital images were taken with an Olympus 750 camera with 4 M pixel resolution. To be able to compare images, camera-to-coral distance, illumination, and state of extension of the polyp tentacles were kept constant. A gray 30□40-mm PVC plate was included as a reference (red=200, green=200, and blue=200 as a standard color reference card, see below for details) (Fig. 2) in each photograph.

We used a plastic stand to position the fragments and camera at a fixed distance and angle (Fig. 2), to the right side of the fragment position we attached the PVC plate. The distance between the camera lens and the fragment's nearest surface was 190 mm and the fragment was orientated to be at the center of the image. All the photographs were taken using the camera's internal flash. To keep the polyp tentacles fully retracted, we photographed the fragments out of the water. For an in-water procedure, one can disturb the water near the coral (by hand or pump) and cause the tentacles to retract. The gray PVC sheet was attached at the same distance from the camera as the fragment, in order to eliminate any differences in lighting since we regard its color as the standard for calculations. All pictures were taken with a shutter speed of 1/60s and aperture of f=1.8.

Picture analysis

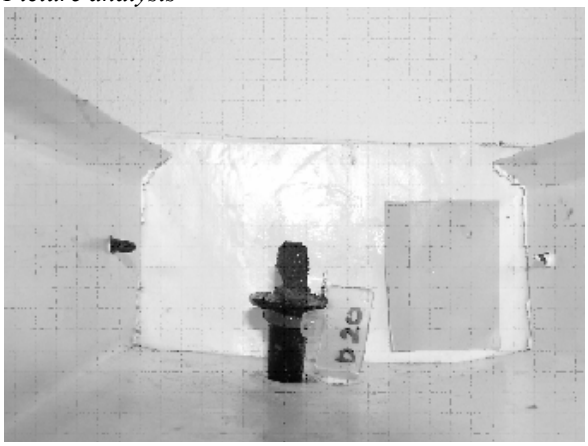

Figure 3: *A sample of the image taken by the camera. The coral fragment is in on the plastic stand with the grey reference plate to its right*.

Analysis of the digital images (Fig. 3) was done using Paint Shop Pro (Corel Corporation, Ottawa, Ontario, Canada). All images were cropped to a square of 100*100 pixels, covering an area of 7.5 by 7.5 mm live coral tissue. Likewise, 100*100 pixels of the gray sheet image were cut from the picture. For each such square, a wavelength histogram graph (including only the green channel) was produced by the appropriate Paint Shop option, and the average value of the 10,000 pixels (100*100) was calculated. This value was found to be between 0 for black and 255 for white paper.

The sample size was chosen as to include the largest possible homogenous fragment area. In the branched *Stylophora pistillata*, the average width of

the subcylindrical branches was found to be ~10 mm. The chosen size allowed us to photograph a nearly flat surface perpendicular to the camera lens. In preliminary experiments, we found that correlation improved as we increased the sample area to the maximum of 7.5 mm square.

For standardizing images and results, all readings were corrected for the gray standard as seen in Eq. 1. In this way, the lighter the coral is, the higher the resulting value.

Eq. 1 P = 255 - [Gray sheet average] + [Coral tissue average]

Thus, low P values correspond to a darker image and higher chlorophyll concentrations, the P value ranged between 100 (high) to 250 (low).

Pigment extraction and quantification

In order to calibrate the data obtained from the photographs, we used the standard methods for quantifying chlorophyll in *S. pistillata* and other corals (Dubinsky et al. 1990; Titlyanov et al. 2000; Nordemar et al. 2003). The procedure requires sacrificing the sample using the Water-Pik method (Joahnnes and Wiebe 1970; Falkowski and Dubinsky 1981; Hoegh-Guldberg and Smith 1989; Edmunds and Gates 2002) to remove the animal tissue with the zooxanthellae from the skeleton. The resulting homogenate was filtered through a 25-mm Ø glass fiber paper filter (Whatman GFC). The zooxanthellae collected on the filter were extracted in 10 ml 90% acetone/water by grinding the filter with a glass/PTF homogenizer. The resulting slurry was filtered again through the same type filter. Chlorophyll concentration was quantified spectrophotometrically with a Varian DMS 100S, according to the Jeffrey and Humprey (1975) equations from the ODs 665nm and 755nm, and normalized to sample area.

Fragment area was determined using the aluminum foil method (Falkowski and Dubinsky 1981; Hoegh-Guldberg and Smith 1989; Edmunds and Gates 2002).

Results & Discussion

Comparison the extracted chlorophyll measurements and our estimates based on analysis of the photographs of the same fragments, resulted in a logarithmic trend line (Fig. 4, Eq. 2)

Eq. 2 y = -11.41n(x) + 62.274, R2= 0.919

This proves that estimating areal chlorophyll density can be done by photographing and analyzing a digital image of a coral fragment or colony. The S. pistillata control fragments had a concentration of 7.2±0.9 chlorophyll a mg cm-2 as determined by the standard method, and 6.8±0.6 mg cm-2 estimated by our photographic method.

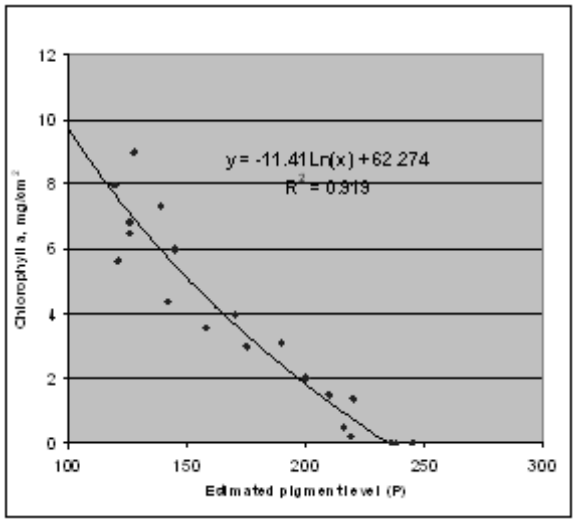

Figure 4: *The relation of estimated pigment level (P) to measured chlorophyll density. Data points are from different times during the bleaching and recovery processes*.

The usefulness of our method was further demonstrated by the results from monitoring the time course of the experimentally induced bleaching of *S. pistillata* fragments in the dark as described in the Methods section. A steady decline in chlorophyll density is seen until the 44th day, when the corals were completely bleached (Fig. 5).

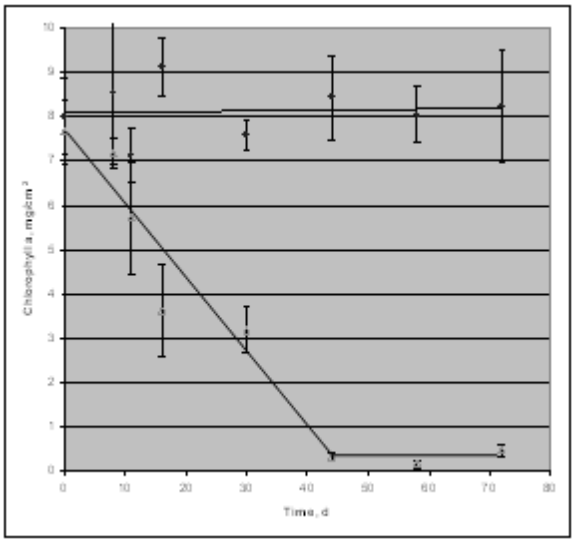

Figure 5: *Photographically estimated chlorophyll density of bleached fragments △ vs. control ● (n=5). The bleaching was complete by day 44 (as seen in the graph)*..

A calibration curve should be determined for each coral species, since the distribution of the zooxanthellae between polyp body and tentacles, the depth of algae within the calyx, and the surface rugosity, are all species-specific properties that affect

the relationship between the photographically estimated chlorophyll and its true areal concentration. Put in a different way, the color of a zooxanthellate coral, as it appears to the eye or camera, depends not only on the pigment concentration but also on its distribution over the coral surface. If the conditions under which the image is taken (distance, light, exposure, background, and camera) are changed, a new calibration curve should be generated. It is also important to take all such photographs with the tentacles in the same stage, either withdrawn or expanded, since tentacle status considerably affects the optical properties of corals (Levy et al. 2003).

The advantages of the method are obvious: once calibration is established, the tedious and error-prone tissue homogenization, pigment extraction, and area determination procedures are no longer required. Furthermore and most important, our method is totally nondestructive, rapid, inexpensive, readily computerizable and transmissible.

We believe that this bioassay can be further developed for use even on the reef scale, as long as photographic activity is done under similar conditions regarding distance and angle of the camera in relation to the reef, solar elevation, cloud cover, wave height, tidal state, etc.

By using this bioassay, one can gather information on reef status by relatively simple and inexpensive tools. We hope that this will encourage scientists and government officials to support the creation of an extensive worldwide network monitoring the health of reefs in real time, and including most remote and poorly explored reef areas.

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