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Field-based remote sensing of intertidal epilithic chlorophyll: Techniques using specialized and conventional digital cameras

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ABSTRACT

Information on the amount of chlorophyll (as an index of micro-algal abundance) on rock surfaces is essential for many reasons, including studies of grazing and its role in structuring intertidal assemblages. Many methods are destructive, error-prone and expensive. Remote sensing allows non-destructive, inexpensive and quantitative measurements to be made of chlorophyll *in situ*. One specialized and two inexpensive commercially-available digital cameras (Fuji IS1 and Sony DSC-V1) are evaluated for estimating amounts of chlorophyll on rock surfaces. To compare measurements from different images, they are calibrated, using reflectance standards of different brightness. To test the calibration, images of a natural rock platform were acquired under variable solar illumination and camera-exposure times. Analyses before and after calibration showed that the method was effective.

A range of quantities of micro-algae was grown on sandstone disks in an aquarium over different intervals of time. Red and NIR reflectance images were obtained from the cores. For each core, the amount of chlorophyll was determined spectrophotometrically and estimated from the images using the Ratio Vegetation Index (RVI) and Normalised Difference Vegetation Index (NDVI). Each of these was linearly related to the measured chlorophyll, with r^2 ranging from 0.78 to 0.9. These techniques can be applied to the study of intertidal and freshwater benthic habitats.

1. Introduction

Herbivory and competition by intertidal gastropods are important processes influencing intertidal assemblages (Lubchenco and Gaines, 1981; Hawkins and Hartnoll, 1983). The primary food of many gastropods are micro-algae which coat the surface of rocks in intertidal areas (Underwood, 1984a, 1992). These biofilms comprise assemblages of unicellular algae, including diatoms and cyanobacteria and the spores and sporelings of macro-algae. Measures of micro-algal food resources are essential for the studies of competition within and between species, particularly where interference and exploitative interactions need to be separated (Underwood, 1984b; Marshall and Keough, 1994; Boaventura et al., 2003). Abundance of micro-algae is most commonly determined indirectly by removing the biofilm from the rock surface, extracting the chlorophyll and quantifying amounts of absorption by spectrometry or HPLC. Removing biofilm is usually done by scraping, brushing or removing chips of rock with intact biofilm; it is error-prone, laborious and damaging to the habitat.

Recent advances in field-based remote sensing have significantly improved our ability to measure amounts of chlorophyll (as an index of biomass) in biofilms *in situ*. Field-based remote sensing was used to produce the first quantitatively-derived images of biofilms in soft sediments (Murphy et al., 2004) and on rocky substrata (Murphy et al., 2006). Such image-based approaches have advantages over conventional methods: acquisition of images is quick and non-destructive (enabling repeated observations to be made of the same area without interfering with any experiment) and large areas can be sampled in a single image. Data can be assembled into any hierarchy of spatial scales within the boundary of an image and the relative locations of samples within a plot are implicitly stored within the image.

Most published studies employing field-based imagery concerning epilithic chlorophyll (e.g. Murphy et al., 2004, 2006, 2008; Murphy and Underwood, 2006; Underwood and Murphy, 2008) have used a digital colour-infrared (CIR) camera which records reflected light at green (525–575 nm), red (645–689 nm) and Near-InfraRed (NIR; 758–833 nm) wavelengths. Quantitative measurements of chlorophyll can be made using reflectance at red and NIR wavelengths because, with increasing amounts of chlorophyll, there is increasing absorption at red relative to NIR wavelengths. Reflectance at NIR wavelengths is dominated by scattering of light by algal cells and

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variations in brightness of the rock. Amounts of chlorophyll are estimated using chlorophyll (vegetation) indices that describe the amount of absorption by chlorophyll at red wavelengths, relative to NIR reflectance.

Digital CIR cameras used for quantitative imaging separately measure green, red and NIR light, thus a separate CCD array is used to record reflectance at each band. Cameras with more than one CCD array are expensive and few commercially-produced models are available. Murphy et al. (2004) and in subsequent studies used a 3-CCD array CIR camera originally developed for NASA for other applications. The camera is large, requires a frame-grabber (National Instruments PCI-1428) and separate 12-volt batteries are required for each. These, together with the large metal stand required to mount the camera normal to the substratum, make the imaging system cumbersome in the field. Cheaper and smaller systems which could easily be moved around the field would greatly reduce the logistical constraints. Use of a single CCD array camera to measure both red and NIR light would greatly reduce the cost of equipment. NIR light, in the context of conventional photography, causes images to have a “washed out” appearance, degrades the quality of colour and can affect the focusing of the camera. Digital cameras therefore have an NIR blocking filter to prevent entry of NIR light; this filter is integrated into the machinery of the camera. Conventional cameras can be modified by removing the NIR blocking filter to permit NIR photographs to be taken. This is time-consuming, requires delicate instruments, nullifies any warranty and the procedure is different for each make or model of camera.

Digital cameras have been used to provide quantitative information on biogeochemical variables in relation to forest canopies (e.g. Dean et al., 2000) and bodies of water (e.g. Goddijn and White, 2006). This paper describes two different approaches to acquiring images at NIR and red wavelengths using a camera with a single CCD, to quantify amounts of chlorophyll on rock surfaces. The first uses a commercially-available single CCD array camera, the Fuji IS1, to acquire NIR and red images using different blocking filters attached to the outside of the lens. The second uses a conventional camera with a night-shot facility, the Sony DSC-V1. In night-shot mode, the integrated NIR blocking filter is automatically removed from the field of view of the camera and the CCD array is able to record NIR light. These approaches do not require any modifications to the camera and use standard, commercially-available filters. Here, results from these cameras are compared with those from the 3-CCD array camera used by Murphy et al. (2004), using two chlorophyll (vegetation) indices: the Ratio Vegetation Index (RVI; Jordan, 1969) and the Normalised Difference Vegetation Index (NDVI; Rouse et al., 1973). Vegetation indices have been used for many years to quantify green biomass in terrestrial environments from satellites and aircraft. The central hypothesis tested in this paper is that single CCD array cameras are similarly effective in quantifying epilithic chlorophyll as is a 3-CCD array camera.

2. Materials and procedures

2.1. Growth of micro-algal samples

Epilithic micro-algae were grown in a recirculating seawater aquarium on small (12 mm diameter) sandstone cores. Sandstone cores of different colours and brightness were selected to represent the approximate variations observed on the natural rock platforms around Sydney. Colour variations ranged from light cream to orange-red. To ensure an adequate range of amounts of chlorophyll, cores were placed into the aquarium at nine intervals, each separated by 1 week (216 cores in total). At each time, 24 cores were placed into foam mountings so that only their top surfaces were exposed to water and light. The foam rubber prevented growth of micro-algae on the sides and bottom of the cores, but, to ensure that these areas were completely free of algae, they were tightly covered by plumbers' tape. The tape was removed prior to extraction of chlorophyll. Cores were illuminated with standard aquarium lights for 6 h/day.

2.2. Camera specifications and methods

Cameras with multiple (usually 3) CCD arrays use separate CCD arrays to record each colour (range of wavelengths or bands), using an internal filter which admits only light of the required range of wavelengths. The images are spatially-registered because each pixel in each band measures exactly the same area of ground. Cameras with a single CCD array typically record colour images using a Bayer-pattern filter, where individual elements of the array are assigned to admit red, green or blue light. The Bayer-pattern filter still admits NIR light, so an additional blocking filter is used to remove most, but not all, NIR light. Because the eye has greater resolving power at green wavelengths, there are more green elements in the Bayer-pattern array (Bayer, 1976). Full-resolution images are reconstructed using internal software, by interpolating between array elements measuring the same colour. The software then constructs an image with three layers — red, green and blue. A particular colour can be extracted from the three-layered colour image. To acquire NIR images, it is necessary to use an externally attached filter to block out visible and ultra-violet (UV) light. Although this filter blocks out all visible and UV light, the Bayer pattern on the CCD array still considers the incoming light as a three band ‘colour’ image and constructs a three-layered image, accordingly. Thus, like a colour image, a NIR image is stored by the camera as a single image with three layers of different brightness. All of these layers are a measure of infrared light (the image pixel values are tightly correlated), but the brightness of each layer is different because it has passed through a differed coloured element of the Bayer filter. We use the middle layer (i.e. the layer which would normally be encoded as green) as the NIR image because there are more green elements in the Bayer-pattern filter. Thus, to measure amounts of chlorophyll with a single CCD array camera, two separate images must be acquired (red and NIR). Unlike images from a multiple CCD array camera, these images will not be exactly registered, because changing the filter can move the camera, causing mis-registration by a few pixels. Generally, multiple and single CCD array cameras store data at 8-bit or, less commonly, 10-bit resolution per band (i.e. the range of integer values in each band is between 0 and 255 or 0 and 1023, respectively).

A few digital cameras with a single CCD array use a Bayer-pattern filter to produce a 3-layered image comprising green, red and NIR layers which are analogous to the red, green and blue layers produced by conventional digital cameras. These cameras, sometimes termed NDVI cameras, are considerably more expensive than cameras that use external changeable filters, but have the advantage of producing images which are spatially-registered and they often come with software to derive chlorophyll indices. Although such cameras could be used to measure epilithic chlorophyll, they were not used in this study because we wished to evaluate the least expensive, most readily-available methods.

The Redlake 3-CCD array CIR camera simultaneously acquires images of reflected light at green, red and NIR wavelengths (bands), which are 1392×1039 pixels in size and in TIFF format. The camera was fitted with a Sigma 14 mm f2.8 ES/HSM super-wide angle lens and mounted 1 m above and nadir to the surface being imaged. This configuration yielded an image of $490\ mm \times 370\ mm$ of surface, each pixel imaging an area of ground ~$0.12\ mm^2$. The brightest objects in the image were the sandstone cores with small amounts of or no chlorophyll and the 30% reflectance standard. To ensure the greatest signal-to-noise ratio, the brightness of the image was adjusted to produce the maximum image brightness (large pixel values) without pixels over the cores or reflectance standards becoming saturated (i.e. few or no pixels in the image had a value of 255). This was done by