Marine Pollution Bulletin xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Effect of low concentrations of Irgarol 1051 on RGB (R, red; G, green; B, blue) colour values of the hard-coral *Acropora tenuis*

Keita Hirayama[a], Kotaro Takayama[a,b], Shinsuke Haruta[a,b], Hiroshi Ishibashi[a,b], Ichiro Takeuchi[a,b,*]

[a] Graduate School of Agriculture, Ehime University, 3-5-7 Tarumi, Matsuyama, Ehime 790-8566, Japan
[b] Center of Advanced Technology for the Environment, Graduate School of Agriculture, Ehime University, 3-5-7 Tarumi, Matsuyama, Ehime 790-8566, Japan

ARTICLE INFO

Keywords:
Acropora
Coral
Coral bleaching
Environmentally relevant low concentration
Irgarol 1051
RGB values

ABSTRACT

Colour change in *Acropora tenuis*, a representative species of Indo-Pacific hard coral, in response to low concentrations of Irgarol 1051 was examined in the laboratory. Branches of *A. tenuis* were exposed to 0, 1, and 10 μg Irgarol 1051/L for 14 days, and photographed daily using digital camera. These Irgarol 1051 concentrations were similar to those recorded at a number of sea ports. Red, green and blue (RGB) coral colour values were quantified from the photographs, with black represented by R = G = B = 0 and white as R = G = B = 255. Exposure to Irgarol 1051 caused RGB values to increase, moving towards the 'white' end of the spectrum as Irgarol 1051 concentration increased. These results suggest that the ambient levels of Irgarol 1051 recorded from port environments could be implicated in coral bleaching, if concentrations over nearby reef ecosystems are similar.

1. Introduction

Coral reefs, sometimes known as "the rain forests of the sea" are one of the most biologically rich and productive ecosystems in the world, providing important sources of food and income for humans, serving as nursery areas for commercially important fish species, attracting divers and snorkelers from around the world, even generating the sand on tourist beaches, and protecting shorelines from the ravages of storms (Burke et al., 2011). However, approximately 60% of coral reefs are immediately and directly threatened by one or more anthropogenic impacts, including overfishing and destructive fishing, coastal development, watershed-based pollution, and marine-based pollution and damage (Burke et al., 2011). Moreover, unless thermal thresholds change, coral reefs are expected to experience an increase in frequency and severity of mass coral bleaching, disease and mortality as ocean acidification and temperatures increase (Hoegh-Guldberg et al., 2007). Hoegh-Guldberg et al. (2015) reported that at least 50% of reef-building corals on tropical reefs in Southeast Asia, Australia, the Western Pacific, Indian Ocean and the Caribbean have been lost from reefs over the last 30 years. They also estimate that, at current rates of ocean temperature rise, coral reefs will disappear altogether by 2050, resulting in the loss of the world's most biologically diverse marine ecosystem. Recognized as an impact of extreme concern since the early 1980s, the most recent mass bleaching event occurred in March 2016, with extensive coral bleaching reported widely from the Great Barrier Reef along the eastern coast of Australia (Normile, 2016). The Ministry of the Environment, Japan (2017) reported that 70.1% of coral reef in Sekisei Lagoon, Japan's largest coral reef located at the southern tip of the Japanese Archipelago, were dead after the mass bleaching during 2016 summer. Normile (2016) reports that some coral reef experts suggested that "the world is on course to lose coral reefs entirely by 2040", bringing forward by ten years an assessment made only the previous year (Hoegh-Guldberg in Normile, 2016).

Contamination of anthropogenic chemicals including herbicides has recently been reported in several studies of watershed and marine pollution (see Jones and Kerswell, 2003; Jones, 2005; Shaw et al., 2008). Approximately half of the commercially available herbicides, including Irgarol 1051 (hereafter, Irgarol), diuron and ametryn, work through chloroplast electron transport chain inhibition (see Jones and Kerswell, 2003). Irgarol and diuron are also being used in a new generation of antifouling paints that provide alternatives for tributyltin (TBT) containing paints, the use of which were globally banned in 2008. Jones and Kerswell (2003) reported that, of 8 species of herbicides, Irgarol showed the lowest median effective concentration [EC_{50}] required to reduce the maximum effective quantum yield ($\Delta F/Fm'$) of *Seriatopora hystrix* of 0.7 μg/L. This concentration of Irgarol is close to those recently recorded from the coastal waters of tropical, subtropical and temperate regions (see Basheer et al., 2002; Bowman et al., 2003;

* Corresponding author at: Graduate School of Agriculture, Ehime University, 3-5-7 Tarumi, Matsuyama, Ehime 790-8566, Japan.
E-mail address: takeuchi@agr.ehime-u.ac.jp (I. Takeuchi).

http://dx.doi.org/10.1016/j.marpolbul.2017.05.027
Received 7 December 2016; Received in revised form 7 May 2017; Accepted 11 May 2017
0025-326X/

Lam et al., 2005; Carbery et al., 2006; Balakrishnan et al., 2012; Ali et al., 2013).

Waterproof reference cards (the 'Coral Colour Reference Card', CCRC) have been developed to code the visible colour of coral in the field to record changes in bleaching state, providing an inexpensive, rapid and non-invasive means of assessing coral bleaching in the field (Siebeck et al., 2006). This method was used to assess the extent of bleaching of the zoantharian coral *Palythoa tuberculosa* along the coast of Okinawa Island, Japan (Hibino et al., 2013; Parkinson et al., 2016). The use of still photography for documenting coral colour has also been applied to assess coral bleaching in the field (Winters et al., 2009). Such techniques follow on from those used by commercial greenhouses, where high-resolution colour still photography is established as one of the most effective techniques for monitoring living plant growth (see Omasa et al., 1987; Takayama and Nishina, 2009). The still imaging method scores coral colour by its RGB values, where R, G and B component values range from 0 (darkest) to 255 (brightest); "black" is represented by $R = G = B = 0$, and "white" by $R = G = B = 255$. Scoring colour using RGB values is now one of the most commonly used methods in visual image processing hardware (e.g. LCD displays for PCs, computers and digital cameras) and software (e.g. the Photoshop and Illustrator series by Adobe Systems Incorporated, San Jose, CA, USA). Winters et al. (2009) proposed two methods for controlling for variations in natural lighting conditions. The first was the use of a custom-made opaque funnel with a white Light Emitting Diode (LED) as the standard light source. The other method calibrated natural irradiances using a Kodak grey scale irradiance reference. Both of these methods require specialist equipment, either making a bespoke light case or an underwater housing for the digital camera. Both methods also involve on-camera image processing, which is a non-linear system of equations that are specific to the camera brand and model, which alter the colour, contrast, and white balance of images (Akkaynak et al., 2014). The image formatting settings were not reported in Winters et al. (2009) for either method.

Here we develop a digital colour imaging system for corals in the laboratory based on NEF (RAW) files using a commercially available digital single-lens reflex camera and wireless speed light system. Baseline results for this more readily available, off the shelf, system are reported. We also conducted experiments to monitor the RGB colour response of the coral *Acropora tenuis* to the presence of concentrations of Irgarol similar to those reported to occur around a number of port locations. *Acropora* is the dominant genus of hard hermatypic corals in the seas off southern Japan (Veron, 1992).

2. Materials and methods

2.1. Digital camera settings

A colony of *Acropora tenuis* was aquacultured by Uminotane Inc. (Yomitan, Okinawa, Japan) and transported to the laboratory at Matsuyama, Ehime Pref. using a door-to-door parcel delivery service (Yamato Transport Co., Ltd., Chuo-ku, Tokyo, Japan). Corals were acclimated in 36 L aquaria (26 °C) for two weeks. Apical branches were cut from the colony in ~1 cm lengths, and secondarily acclimated for an additional two days in the same aquarium under the same conditions. Aquaria were filled with an artificial seawater (34 psu) containing LIVEsea salt (Delphis Inc., Itami, Hyogo, Japan), and illuminated with an LED light source (PowerShot; Kotobuki Co., Ltd., Matsubara, Osaka, Japan).

Prior to the bleaching treatment, the cut apical branches ($n = 8$) were transferred into a Petri dish, and photographed with a digital colour imaging system. The system comprised a commercial grade digital single-lens reflex camera, Nikon df (Nikon Corporation, Chiyoda-ku, Tokyo, Japan) with lens, AF-S Nikkor 50 mm (Nikon Co.) and wireless speed light system, wireless speed commander, SU-800 (Nikon Co.) with wireless remote speed light, SB-R200 (Nikon Co.) and extreme close-up positioning adapter, SW-11 (Nikon Co.) (Fig. 1). This digital camera processes images using a non-linear system of equations that are unique to the camera company and model, which alter the colour, contrast, and white balance of images (Akkaynak et al., 2014). Photographed images were transformed to a nonlinear RGB colour model and compressed into a NEF (RAW) file format. The photographic system was operated manually using a light sensitivity of ISO 100, a shutter speed of 1/125 s and a manual flash mode. Each apical branch was photographed with a range of apertures from f5 to f16 (Table 1). A colour plate (Kodak Colour Control Patches; Eastman Kodak Company, Rochester, NY, USA) was included in the photograph to allow calibration for any miniscule colour differences in corals in the before and after bleaching treatment images. The NEF (RAW) files were then subject to RGB value analysis.

Fig. 1. Digital imaging system for recording the colour of the hard coral *Acropora tenuis*; digital single-lens reflex camera, Nikon df (Nikon Corporation, Chiyoda-ku, Tokyo, Japan); lens, AF-S Nikkor 50 mm (Nikon Co.); wireless speed light system, wireless speed commander, SU-800 (Nikon Co.) and wireless remote speed light, SB-R200 (Nikon Co.) covered with extreme close-up positioning adapter, SW-11 (Nikon Co.).

Corals were then bleached for 48 h using a commercial home kitchen bleaching agent, Uzushio Kitchen-Bleach (Sankyo Co. Ltd., Kumamoto, Japan). The major components of this bleaching solution are sodium hypochlorite, alkyl amine oxides (surfactant) and an alkali agent. Following the bleaching treatment, corals were photographed following the method outlined above for the pre-bleaching treatment.

The photographic images captured using this system were then converted into RGB values using Adobe Photoshop CC (Adobe Systems Incorporated, San Jose, CA, USA) based on NEF (RAW) files. The image

Table 1
Main settings for recording hard coral colour using a digital single-lens reflex camera, Nikon df, and related wireless light system (Nikon Co. Ltd.).

Setting	
ISO sensitivity	ISO 100
Shutter speed	1/125 s
Aperture	f5, f6.3, f7.1, f8, f9, f10, f11, f13, f14, f16
White balance	Flash (colour temperature 5400 K)
Image quality	NEF(RAW) + JPEG fine[a] (NEF(RAW); uncompressed 14-bit and JPEG image at compression ratio of 1:4)
Image size	Large 4928 × 3280 pixels
Focus mode	Manual
Picture control	Standard
Manual flash output level	M1/1
Distance from lens to object	33 cm

[a] NEF(RAW) is used for R, G, B analysis in the present study.