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Phototrophic microendoliths bloom during coral “white syndrome”

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Abstract Following rapid lesion progression of white syndrome in tabular *Acropora* spp., the white bare skeleton gradually changes to green, a result of endolithic algal blooms (primarily *Ostreobium* spp.). Endolithic algal biomass and chlorophyll concentration were found to be an order of magnitude higher in the green zone compared with healthy appearing parts of each colony. Chl *b* to Chl *a* ratio increased from 1:1.6 in the healthy area to 1:2 and 1:3.5 in the white exposed skeleton and green zones, respectively. These observations together with pulse amplitude modulated (PAM) fluorometry suggest photoacclimation of the endoliths in the green zone. Histopathological microscopy revealed that the endolithic algal filaments penetrate the coral tissue. This study highlights the interaction of endolithic algae with both the skeleton and host tissue. This may have a critical role in the processes that accompany the post-disease state in reef-building corals.

Keywords Coral disease · Endolithic algae · *Ostreobium* · White syndrome

Introduction

The emergence and prevalence of coral diseases/syndromes in the past few decades appears to be unparal-

leled in the geological record, particularly in the Caribbean region (Harvell et al. 1999, 2001; Green and Bruckner 2000; Aronson and Precht 2001), although recent research indicates an increase in the prevalence of disease-like syndromes on the Great Barrier Reef (GBR) (Willis et al. 2004) and wider Indo-Pacific region (Sutherland et al. 2004). The most notable increase in prevalence is that of ‘white syndromes’ (WS), consisting of a group of syndromes that are characterised by a distinct line between healthy appearing coral tissue and freshly denuded coral skeleton (Bythell et al. 2004). Lesion progression rates range from 1.0 to 20 mm day⁻¹ in white band disease (Antonius 1981; Gladfelter 1982) up to 10 cm² day⁻¹ in white pox (Patterson et al. 2002).

Healthy corals live in close association with symbiotic dinoflagellates (*Symbiodinium*) and almost all coral species host a range of endolithic organisms (algae, fungi, and bacteria) (Highsmith 1981; Le Campion-Alsumard et al. 1995), primarily the siphonaceous green algae *Ostreobium* (Jeffrey 1968; Lukas 1974). All members of the holobiont consortium live in equilibrium but environmental stress can disrupt this balance, which may be critical for the health of the coral host (Rohwer et al. 2002; Golubic et al. 2005). The endolithic community lives in a sheltered environment within the coral skeleton (Shashar et al. 1997) where less than 5% of the photosynthetically active radiation (PAR) penetrates (Halldal 1968; Shibata and Haxo 1969; Schlichter et al. 1997). Low light conditions prevail because of light absorption by the unicellular endosymbiotic algae (zooxanthellae) and the inorganic skeleton itself (Kanwisher and Wainwright 1967). The rapid exposure of the skeleton of WS affected corals, as the disease progresses, leads to a dramatic increase in the light environment of photosynthetic microendolithic borers because shading by coral tissue is reduced.

During surveys of Heron Reef (Southern GBR) in the Austral summer of 2004, a form of WS characterised by a distinct line between ‘healthy’ coral tissue and exposed skeleton was recorded in the colonies of tabular *Acropora* spp. (G. Roff et al. unpublished). Following

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lesion progression, significant green pigmentation associated with phototrophic microendoliths was observed in the regions of recently denuded white skeleton. These observations suggested that the endolithic community bloomed during the progression of WS despite the fact that only a minor phototrophic endolithic community are thought to occur in tabular Acroporids. As phototrophic endoliths are significant agents of bioerosion (Tribollet et al. 2002) and may have strong interactions with coral tissue (Fine and Loya 2002), this project investigated these endolithic algal blooms relative to their role in the post-disease state of WS affected corals.

Materials and methods

The study was undertaken at Heron Reef and neighbouring Wistari Reef on the southern Great Barrier Reef (23.44°S, 151.91°E), Australia during 2004. Colonies of tabular *Acropora* spp. showing signs of WS lesion progression ($n = 9$) were monitored weekly between February and April 2004 using digital photography (Fig. 1). The images were analysed (Matrox Inspector, v2.1, Matrox Imaging, Canada) for rates of lesion progression and correlation between rate of WS progression and widths of the white or green sections.

To quantify biomass and photosynthetic pigments of endolithic algae within *Acropora* spp. skeletons, eight fragments, 5 cm in length, were sampled from each of three zones (healthy tissue, exposed white skeleton and green skeletal parts, Fig. 1) from four colonies affected by WS. Tissue and epilithic organisms were removed using an air gun and the skeleton was thoroughly brushed using a toothbrush and inspected for tissue or zooxanthellae remnants. Following Schlichter et al. (1997) skeletons were dissolved and organic residues comprising the microendolithic organisms were filtered (0.45 µm pore), dried (50°C, overnight) and weighed. Photosynthetic pigments were extracted after grinding the skeleton to a paste using a mortar and pestle, and placing the paste in chilled acetone (90% overnight). Chlorophyll *a* and *b* concentrations were quantified using the spectrophotometric method of Jeffrey and Humphrey (1975). A portable underwater pulsed amplitude modulated (PAM) fluorometer (Diving-PAM, Walz GmbH, Germany) was used to examine the photosynthetic efficiency and photoacclimation of endolithic algae inhabiting the skeletal sections visible during WS of tabular *Acropora* spp. Rapid light curves (RLCs) were performed on freshly collected fragments taken from five colonies (1–4 m in diameter) of tabular *Acropora* spp., where an actively progressing lesion of WS could be detected.

Following a 30-min dark acclimation (to obtain Y_{\max} values), the PAM main optic fibre was positioned on 12 different points (radius = 0.5 cm), three in each of four sites: (1) the surface of apparently healthy areas of colonies; (2) skeleton under (1) after removal of the coral tissue; (3)

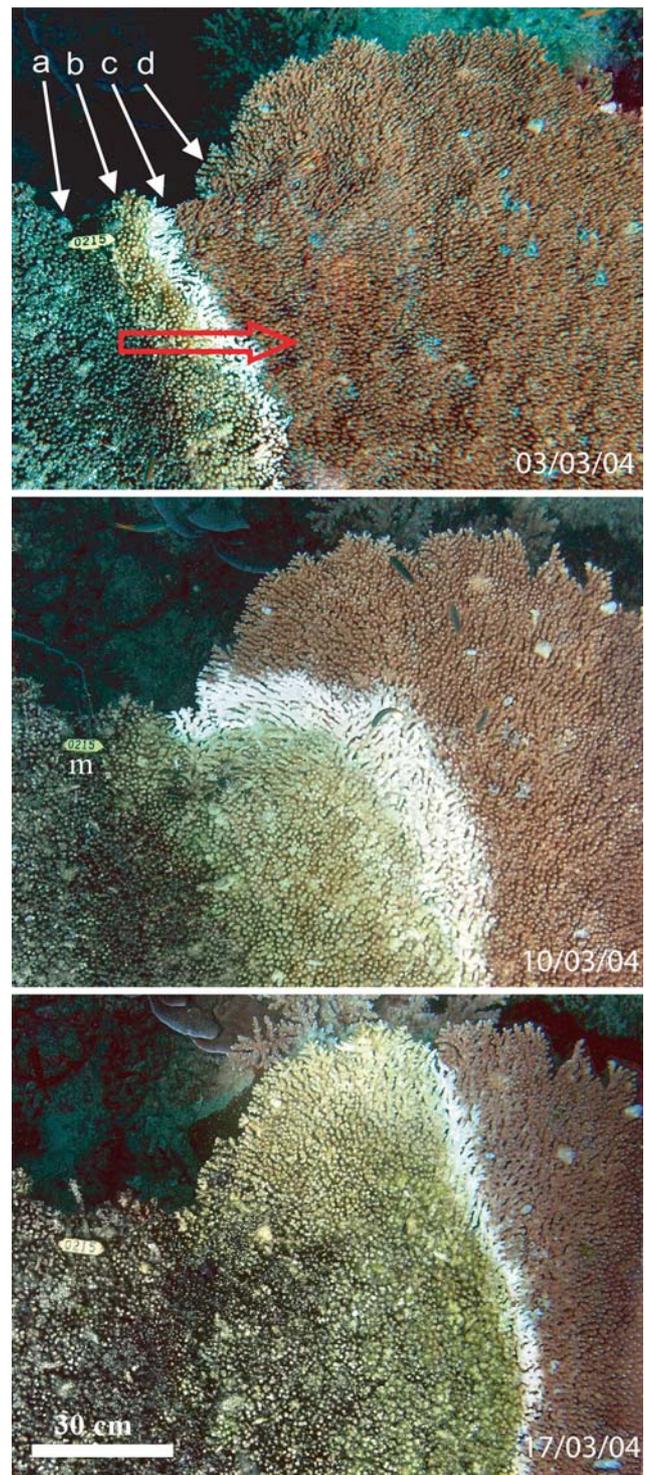


Fig. 1 Progression of white syndrome lesion over a 2-week period showing **a** old exposed regions of the colony dominated by epilithic organisms, **b** green appearance of exposed skeleton due to phototrophic endoliths blooms, **c** bare white skeleton following lesion progression, **d** apparently healthy tissue preceding the lesion border. Red arrow pointing to lesion progression direction and the colony tag (*m*) serves a marker for lesion progression rate

on white exposed skeleton after the progression of WS and (4) on exposed skeleton where the green pigments of the endoliths were visible (Fig. 1a). RLC consisted of a

series of eight irradiances (10 s each). The effective photosynthetic yield of Photosystem II (PSII) was derived from the Genty equation $Y = (F'_m - F)/F'_m$ (Genty et al. 1989). The PAM light meter was pre-calibrated against a quantum sensor (Li-Cor, USA, LI-189 light meter).

To visualise the interaction between coral tissues and endolithic communities, coral fragments ($n = 6$) from the WS lesion area were collected, fixed (4% paraformaldehyde, 8 h) and enrobed with 1.5% (w/v) agarose (Bythell et al. 2002) prior to decalcification (20% EDTA), and standard histological processing. Tissue sections (4 μm) were stained using Harris's haematoxylin and eosin and prepared for light microscopy.

Results and discussion

A cascade of changes may occur when the holobiont (Rohwer et al. 2002; Wegley et al. 2004) is under environmental stress. Little knowledge exists, however, on the changes to the microendolithic community that occur during stress and the potential implications to lesion recovery and tissue regeneration (Le Campion-Alsumard et al. 1995; Diaz-Pulido and McCook 2002). In the present study the interaction between the coral and its associated endolithic microbiota was explored in the colonies of tabular *Acropora spp.* affected by WS from the southern GBR. Significant temporal and spatial variation in lesion progression was observed during the monitoring period, with rates of tissue loss varying from 1.0 to 124.6 $\text{cm}^2 \text{day}^{-1}$, exposing bare white skeleton (Fig. 1) of $108.8 \pm 96.6 \text{ cm}^2$ (mean \pm SD) in coral colonies of 1.5–4 m in diameter, hence up to 5% coral tissue loss from a colony per day, among the highest ever recorded for white diseases (see Bythell et al. 2004). A green endolithic section, often wider than the white band ($225.0 \pm 248.6 \text{ cm}^2$, mean \pm SD) was clearly visible bordering the recently exposed skeleton (Fig. 1). The rate of progression of the WS lesion was not correlated with the width of the white or green zones. Similarly, the width of white zones was not correlated with the width of the green zones, implying a non-linear progression rate.

Biomass of endolithic flora increased dramatically with increasing distance from the WS lesion (Fig. 2a) with remarkable biomass values in the green zone, where a dense mat of *Ostreobium spp.* was observed within 3–5 days of skeleton exposure. It should be noted that since the tissue in the healthy section of the WS colony was removed prior to skeleton decalcification, possibly together with some endolithic filaments penetrating it, the biomass in the healthy section may be slightly underestimated. The rapid increase in endolithic biomass is almost certainly a consequence of the increase in light levels as the skeleton is denuded of the overlying tissues and pigments during the disease progression. This is evident, from the changes in photosynthetic pigments (Fig. 2b), specifically the increasing ratio of chlorophyll a – b with increasing distance from the intact tissue

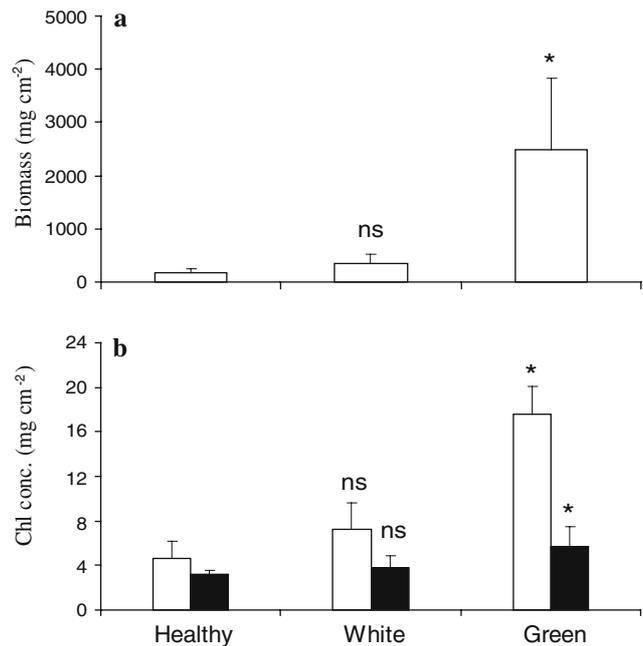


Fig. 2 Endolith biomass (a) and chlorophyll a (blank) and b (black) (b) in each of the skeletal zones (mean \pm SD) following lesion progression of white syndrome. Each bar represents 32 fragments (eight from each of four colonies). *Significantly different from healthy tissue ($P < 0.05$). ns not significantly different from healthy tissue ($P > 0.05$)

(Fig. 2b) as well as the low dark-adapted F_v/F_m values of microendoliths under intact tissue (Fig. 3a) and within the newly exposed skeleton (Fig. 3b). Higher photosynthetic efficiencies (Fig. 3c) were measured in the green zone, further demonstrating photoacclimation of PSII.

The rapid light curves performed on microendolithic algae also demonstrate that *Ostreobium spp.* can withstand dramatic changes in irradiance, from a dim light environment (less than 5% PAR, Halldal 1968) to light levels that approach full solar PAR. The ability of *Ostreobium spp.* to cope with these light changes explains the prominence of endolithic algal blooms after mass coral bleaching (Diaz-Pulido and McCook 2002; Fine and Loya 2002; Fine et al. 2004) or coral death (Le Campion-Alsumard et al. 1995). Histological sections of WS colonies showed extensive endolithic communities in exposed skeletal regions and in close proximity to (but also penetrating) the coral tissues. In addition to the dominant *Ostreobium spp.*, microendolithic algal communities appeared to be associated with some small populations of cyanobacteria. Increases in the biomass of *Ostreobium spp.* may result in an increased production of photoassimilates, which are taken up by the coral tissue, as shown by Fine and Loya (2002). Conversely, such rapid blooms of phototrophic endoliths may also result in respiration-induced dissolution of the calcium carbonate, as over 25% of the skeletal carbonate may be dissolved and removed (Le Campion-Alsumard et al. 1995). Studies have highlighted the importance of microendolithic

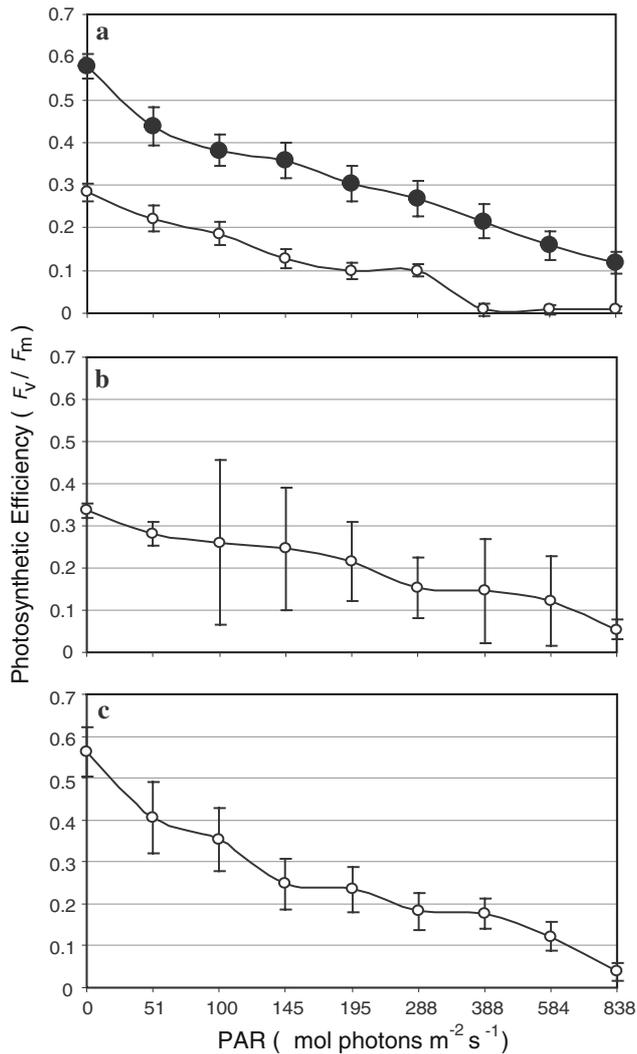


Fig. 3 Maximum F_v/F_m and rapid light curves (mean \pm SD) of endoliths in each skeletal zone, following lesion progression. **a** Dinoflagellates (filled circle) in an intact tissue and endoliths (open circle) in the skeleton under intact tissue; **b** endoliths in recently exposed white skeleton and **c** endoliths in the “green zone”. All are graphed against the photosynthetic active radiation (PAR) to which fragments were subjected

organisms in modifying the substrate, making it available for recruitment by macroborers and attractive for grazers as a source of food (Bellwood and Choat 1990; Bruggemann et al. 1996; Pari et al. 2002; Tribollet et al. 2002). These processes may determine the fate of a WS affected colony on *Acropora*-dominated reefs.

Lukas (1973) observed the endolithic communities extending up to the coral uppermost skeleton edges, but suggested these endolithic algae could penetrate the living coral tissue (Lukas 1973). Peters (1984), however, reported *Ostreobium* spp. invading the gastrodermal cells of a number of massive coral species from Puerto Rico, causing abnormalities in coral tissue (Peters 1984). In the present study, endolithic algae were found penetrating the coral tissues of tabular *Acropora* spp. Penetration may in turn affect the structure of corals

(Peters 1984) and create micro-lesions, making the coral host susceptible to infiltration by potential pathogens.

An extensive survey of coral disease is contained in the book “Coral Health and Disease” (Rosenberg and Loya 2004). Although endoliths were largely overlooked in terms of disease-related processes, nonetheless in many of the photographs in the book, green pigmentation is associated with areas of exposed skeleton after the transition from disease-like syndromes (Bythell et al. 2004; Loya 2004; Weil 2004; Willis et al. 2004). The bloom of microendolithic algae in *Siderastrea siderea* (Bythell et al. 2004, Fig. 20.1a) appears similar to the bloom observed in the present study. In other words, the microendolithic algae response may be general, but raises questions as to why endolithic algae blooms are not as common in other syndromes with a distinct and fast progressing lesion such as “Black Band Disease”. Further investigation of the patterns and function of disease-associated endolithic-microbial proliferation is likely to be of great importance in view of the enigmatic nature of many coral diseases and disease-like syndromes.

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