Zooxanthellae Harvested by Ciliates Associated with Brown Band Syndrome of Corals Remain Photosynthetically Competent

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Brown band syndrome is a new coral affliction characterized by a local accumulation of yet-unidentified ciliates migrating as a band along the branches of coral colonies. In the current study, morphologically intact zooxanthellae (=Symbiodinium) were observed in great numbers inside the ciliates (≥50 dinoflagellates per ciliate). Microscale oxygen measurements and variable chlorophyll a fluorescence analysis along with microscopic observations demonstrated that zooxanthellae within the ciliates are photosynthetically competent and do not become compromised during the progression of the brown band zone. Zooxanthellae showed similar trends in light acclimation in a comparison of rapid light curve and steady-state light curve measures of variable chlorophyll a fluorescence. Extended light exposure of steady-state light curves resulted in higher quantum yields of photosystem II. The brown band tissue exhibited higher photosynthetically active radiation absorbity, indicating more efficient light absorption due to a higher density of zooxanthellae in the ciliate-dominated zone. This caused relatively higher gross photosynthesis rates in the zone with zooxanthella-containing ciliates compared to healthy coral tissue. The observation of photosynthetically active intracellular zooxanthellae in the ciliates suggests that the latter can benefit from photosynthates produced by ingested zooxanthellae and from photosynthetic oxygen production that alleviates diffusion limitation of oxic respiration in the densely populated brown band tissue. It remains to be shown whether the zooxanthellae form a stable symbiotic association with the ciliate or are engulfed incidentally during grazing on coral tissue and then maintained as active inside the ciliate for a period before being digested and replaced by new zooxanthellae.

A recently identified coral disease, brown band syndrome (BrB), was described for the first time in three coral families (Acroporidae, Pocilloporidae, and Faviidae) in the northern and southern sectors of the Great Barrier Reef (GBR), Australia (55). Macroscopic symptoms of the syndrome manifest as a brown zone on the coral preceded by healthy tissue and followed by exposed white skeleton (see Fig. 1A). Sometimes, a white zone is observed between the brown band and healthy tissue which may comprise bleached tissue and/or denuded skeleton (55). Microscopic investigations of the brown zone revealed the massive presence of an unknown protozoan ciliate which accumulates zooxanthellae intracellularly, resulting in the characteristic brown coloring of the observed syndrome. The ciliate band has been observed to migrate along the length of branching corals from base to tip at a rapid rate (>5 cm per day [B. Willis, personal communication]). The ciliates appear to ingest coral tissue at the lesion interface, accumulating the symbiotic dinoflagellates, known as zooxanthellae (=Symbiodinium), from the coral endoderm. However, it is unknown whether the zooxanthellae remain photosynthetically active inside the ciliates.

In this study, we used variable chlorophyll a fluorescence analysis with Mini- and Imaging-PAM (pulse-amplitude-modulation) instruments (22, 23, 37) and the saturation-pulse technique (reviewed in reference 45) to obtain measures of maximum and effective quantum yields of photosystem II (PSII) and derived measures of photosynthetic electron transport (24, 36). These measurements were combined with oxygen microelectrodes (25, 53) to assess the photosynthetic competency of zooxanthellae of corals affected with BrB. The combination of their fast response times, small tip diameters, and extremely small oxygen consumption means that O2 microsensors are able to measure rapid changes in oxygen depletion as well as microprofiling of the diffusion boundary layer (DBL) (required to measure net photosynthesis and dark-respiration rates) at a spatial resolution of better than 100 μm and with response times of <0.2 to 0.5 s (26, 40, 53).

Only a limited number of studies have examined coral-protozoan associations (1, 2, 7, 29, 30, 47, 56). In this study, we compare the photosynthetic performances of zooxanthellae in healthy tissue preceding the brown band zone and within ciliates accumulating in the brown band zone of affected Acropora muricata corals. Direct microscopic and histological observations combined with photophysiological responses were analyzed to determine the competency of internalized zooxanthellae. Our results show that the dynamics of photosynthesis regulation of visually healthy zooxanthellae accumulated in the brown band zone were greatly altered (as shown by O2-derived measures) from that of zooxanthellae in the healthy tissue. Nevertheless, the capacity for photosynthesis appeared unchanged (as shown by variable chlorophyll a fluorescence-derived measures).
**MATERIALS AND METHODS**

**Sampling.** Specimens of the branching coral *Acropora muricata* exhibiting symptoms of BrB were collected in November 2005 from an exposed reef flat at Davies Reef (18°49.86′S, 147°38.2′E) on the GBR and brought to the facilities of the Australian Institute of Marine Science in Townsville for further analysis.

**Histological and microscopic examination.** For histological examination, the brown band mass including ciliates and coral tissue was scraped from a coral branch and concentrated by centrifugation (1,000 × g) with excess liquid removed. Samples were fixed in Davidson’s fixative (21) for 24 h prior to processing, which was done using a Leica ENV 165B (Leica Microsystems, Germany) (Sigma-Aldrich). The height (h) and radius (r) of the cylinder-shaped and fingertip-sized coral pieces, from which tissue was extracted, were measured to the nearest mm using calipers and applied to the formula for the volume of a cylinder: $V = \pi r^2 h$.

**PAR Abs and chlorophyll a determinations.** The Imaging-PAM estimates photosynthetically active radiation (PAR) absorbance (Abs), i.e., the fraction of incident PAR that is absorbed, from the ratio of reflectance of nonabsorbed near-infrared light (780 nm) (NIR) from photosynthetically active radiation (PAR) absorptivity (Abs), i.e., the fraction of light absorbed by a pigment preparation after passing through one millimeter of the preparation. The light source (Schott 2500) was calibrated against a light sensor (LI-192SB; LI-COR). The oxygen flux (dark-respiration rate) was calculated from the oxygen concentration profile as

$$J = \frac{dC}{dz}$$

where $dC/dz$ is the light-dark shift of oxygen, and $C$ is the oxygen content (tabulated value of 204.3 mmol O$_2$ liter$^{-1}$ at 27°C and salinity of 35 ppt; Unisense). The oxygen flux (dark-respiration rate) was calculated from the oxygen concentration profile as

$$J = \frac{dC}{dz}$$

where $dC/dz$ is the light-dark shift of oxygen, and $C$ is the oxygen content.

**Statistical analysis.** One-way analysis of variance tests (SPSS v11.0.0) were used to determine if significant differences in the following parameters were present between healthy and brown band tissue. $F_{tc}$, $\Delta F/\Delta Fm$, $Q_{o}$, and $\alpha$ (quantitative parameters of RLCs and steady-state light curves (fluorescence and $O_2$ derived), PAR absorptivity, and chlorophyll a content). Where the assumptions of normality and equal variance failed ($P < 0.05$), data were transformed using natural log; this applied only to $\alpha$ and $Q_{o}$ for RLCs and SSSLs. Transformed data successfully met the assumptions of normality and equal variance. Post hoc comparisons were performed on quantitative parameters of RLCs and fluorescence derived steady-state light curves using the Tukey-Kramer honestly significant difference test.

**RESULTS**

Field and microscopic observations of BrB. BrB exhibits distinctive macroscopic symptoms in the field (Fig. 1A), which
are derived from ciliates gliding over the exterior of the coral samples and into the coelenteron and cavities of the coral polyps. Light microscopic analysis of the ciliates demonstrated an accumulation of intact zooxanthellae within the ciliates (Fig. 1B). The ingested zooxanthellae maintained a strong localized chlorophyll autofluorescence, indicating intact chloroplasts and a potential capacity for photosynthesis (Fig. 1C). In addition, histological cross sections of the sampled brown band mass demonstrated that the ciliates contained intact zooxanthellae that were not surrounded by food vacuoles (Fig. 1D).

**PAR absorptivity and chlorophyll a determinations.** Variable chlorophyll a fluorescence imaging showed high fluorescence along with high PAR absorptivity in the brown band tissue (Fig. 2A and C), and active photosynthesis in the brown band tissue was evident by a maximum quantum yield ($F_v/F_m$) comparable to or slightly higher than that in the healthy tissue (Fig. 2B).

Measurements of PAR absorptivity were significantly lower ($P = 0.001$) in the healthy tissue (0.304 ± 0.02) than in the brown band tissue (0.592 ± 0.03) (Fig. 3). However, chlorophyll a content in the healthy tissue (2.22 ± 1.79 μg/cm² surface area) was not significantly different ($P = 0.904$) from the content found in brown band tissue (1.92 ± 1.55 μg cm² sur-
face area). This indicates that pigments were distributed differently between the healthy tissue and the brown band.

**Combined measures of variable chlorophyll a fluorescence and O₂ productivity.** Maximum quantum yield ($F_{v}/F_{m}$) and effective quantum yield ($\Delta F/F_{m}'$) as well as $Q_{m}$ of the fluorescence-derived dark-acclimated and light-acclimated RLCs are shown in Fig. 4. $F_{v}/F_{m}$ was unchanged ($P = 0.579$) between healthy (0.572 ± 0.032) and brown band (0.601 ± 0.037) tissue. $\Delta F/F_{m}'$ of healthy tissue (0.069 ± 0.006) was significantly lower ($P = 0.022$) than that of brown band tissue (0.109 ± 0.012). This relationship was reflected in subsequent calculations of the maximum excitation pressure over PSII ($Q_{m}$), which was higher (0.879 ± 0.013) in healthy tissue than in brown band tissue (0.813 ± 0.032), although the difference was not significant ($P = 0.102$) (Fig. 4).

The quantitative parameters of RLCs ($rETR_{\text{max}}$, $\alpha$, and $E_{c}$) measured initially in the dark and again after increasing light exposure for 80 min were statistically compared to $rETR_{\text{max}}$, $\alpha$, and $E_{c}$ calculated for fluorescence-derived SSLCs (Table 1). $rETR_{\text{max}}$, calculated for light- and dark-acclimated RLCs of healthy and brown band tissue showed similar values. However, the RLC performed on dark-acclimated brown band tissue also shared similar values with RLCs calculated for SSLCs of healthy tissue which were significantly different ($P = 0.003$). The $rETR_{\text{max}}$ calculated for SSLCs of brown band tissue was similar to values collected for all other groups of tissue and light curve types (Table 1). The slope of the $rETR$ versus irradiance curves, $\alpha$, showed similar values between healthy and brown band tissue within all three groups of light curves (Table 1). However, RLCs performed in the dark showed a significantly lower ($P = 0.011$) $\alpha$ than those calculated for SSLCs. $\alpha$ values calculated for RLCs performed in the light were similar to those for both dark-acclimated RLCs and SSLCs. There was no significant difference among groups of light curves and tissue types in minimum saturating irradiance, $E_{c}$ ($P = 0.571$) (Table 1; Fig. 5).

Healthy and brown band tissues exhibited widely different O₂ concentration levels over the entire irradiance range (Fig. 6). At the highest irradiance level (845 μmol photons m⁻² s⁻¹), the O₂ concentration of healthy tissue was 223 ± 4 μmol liter⁻¹ whereas the O₂ concentration of the brown band tissue was only 63 ± 22 μmol liter⁻¹. This amounts to ~30% of saturation O₂ concentration. In the dark, the O₂ concentration of the healthy tissue was 60 ± 28 μmol liter⁻¹ but virtually 0 (3 ± 3 μmol liter⁻¹) for brown band tissue due to high respiration activity coupled with O₂ transfer limitation imposed by the DBL (Fig. 6). The brown band tissue was almost completely hypoxic at irradiances below 60 μmol photons m⁻² s⁻¹ and never reached the compensation irradiance ($E_{c}$), i.e., the irradiance above which the tissue exhibits net oxygen production. The healthy tissue reached compensation irradiance at ~230 μmol photons m⁻² s⁻¹.

Microsensor measurements generally showed a thin DBL ranging from 100 to 200 μm and strong oxygen gradients across the DBL. Motility of the ciliates caused fluctuating oxygen signals, and this resulted in relative high variability in our flux

![](Image)

**FIG. 4.** Dark-acclimated maximum quantum yield ($F_{v}/F_{m}$) (black bars), high-light-acclimated effective quantum yield ($\Delta F/F_{m}'$) at 845 μmol photons m⁻² s⁻¹ (gray bars), and maximum excitation pressure over PSII ($Q_{m}$) at 845 μmol photons m⁻² s⁻¹ (○) (n = 4) of healthy coral tissue and brown band tissue (BrB) of Acropora muricata.

**FIG. 5.** $rETR$ of dark-acclimated RLCs and SSLCs of healthy coral tissue (○) and brown band tissue (●) of Acropora muricata. Light-acclimated RLCs are not shown. Average values (n = 4) ± standard errors are given. The fitted $rETR$ curves of RLCs and SSLCs are superimposed with a broken and a solid line, respectively. a.u., arbitrary units.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>RLC (0) Healthy</th>
<th>RLC (0) BrB</th>
<th>SSLC Healthy</th>
<th>SSLC BrB</th>
<th>RLC (845) Healthy</th>
<th>RLC (845) BrB</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>$rETR_{\text{max}}$</td>
<td>83 ± 11 A</td>
<td>108 ± 4 A,B</td>
<td>172 ± 36 B</td>
<td>131 ± 15 A,B</td>
<td>71 ± 8 A</td>
<td>94 ± 22 A</td>
<td>0.003</td>
</tr>
<tr>
<td>$\alpha$</td>
<td>0.39 ± 0.09 A</td>
<td>0.46 ± 0.05 A</td>
<td>0.69 ± 0.11 B</td>
<td>0.74 ± 0.06 B</td>
<td>0.49 ± 0.06 A,B</td>
<td>0.49 ± 0.05 A,B</td>
<td>0.011</td>
</tr>
<tr>
<td>$E_{c}$</td>
<td>258 ± 79 A</td>
<td>243 ± 18 A</td>
<td>288 ± 85 A</td>
<td>179 ± 19 A</td>
<td>163 ± 45 A</td>
<td>196 ± 48 A</td>
<td>NS</td>
</tr>
</tbody>
</table>

*a $rETR_{\text{max}}$ is in arbitrary units; $\alpha$ and $E_{c}$ are in μmol photons m⁻² s⁻¹. Numbers in parentheses after RLC are the irradiances applied (μmol photons m⁻² s⁻¹). Average values (n = 4) ± standard errors for healthy and BrB tissue are given. Values with different superscript capital letters were significantly different. NS, not significant.*
calculations. Oxygen respiration rates in the dark were significantly different \((P = 0.011)\) between healthy tissue \((0.22 \pm 0.04 \text{ nmol O}_2 \text{ cm}^{-2} \text{ s}^{-1})\) and brown band tissue \((0.48 \pm 0.05 \text{ nmol O}_2 \text{ cm}^{-2} \text{ s}^{-1})\) (Fig. 7). The compensation irradiance, \(E_{c}\), i.e., the irradiance above which the tissue exhibits net oxygen production, was reached between 130 and 247 \text{ pmol photons m}^{-2} \text{ s}^{-1}\) in healthy tissue. However, maximum net production recorded beyond the compensation irradiance was low and highly variable \((0.15 \pm 0.09 \text{ nmol O}_2 \text{ cm}^{-2} \text{ s}^{-1})\). The brown band tissue exhibited net oxygen consumption at all irradiances, but consumption rates decreased with increasing irradiance, stabilizing around the \(E_{c}\) determined for the healthy tissue (Fig. 7).

Although no net oxygen production was measured in the brown band tissue due to intense respiration, there was substantial gross photosynthesis (Fig. 8). There was no significant difference between quantitative parameters \((P_{g,\text{max}}, \alpha, E_k)\) calculated from fitted gross photosynthesis rate and those calculated from irradiance curves (Table 2). Brown band tissue showed higher \(P_{g,\text{max}}\) than healthy tissue (Fig. 8), although the difference was not significant \((P = 0.170)\) (Table 2). There was no significant difference between healthy and brown band tissue in \(\alpha (P = 0.138)\) or \(E_k (P = 0.517)\) (Table 1).

**DISCUSSION**

Intact zooxanthellae in ciliates associated with BrB. Reports concerning diseases of coral reef organisms have increased substantially over the last 2 decades (19, 28, 54). Coral diseases appearing with progressively greater frequency and wider distributions have been shown to alter total coral abundance and species diversity (20, 31, 44). A wide range of microorganisms including fungi, bacteria, cyanobacteria, and protozoans have been demonstrated to associate with both healthy and diseased corals (6, 15, 29, 30, 34, 41, 42, 43). BrB was recently described as a newly identified coral disease on the GBR. The distinctive macroscopic field symptom of BrB is the formation of a brown zone on the coral harboring high densities of motile yet unidentified protozoan ciliates (55). Although the prevalence of BrB on three reefs in the northern GBR was quite low, i.e., <1% of total number of coral colonies surveyed, corresponding to 12 to 24 cases per investigated reef (55), the high spreading rates and efficient transmission of BrB may cause significant damage to coral reef assemblages and can

**TABLE 2.** Quantitative parameters derived from fitted gross photosynthesis rate versus irradiance curves

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Healthy</th>
<th>BrB</th>
</tr>
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<tbody>
<tr>
<td>(P_{g,\text{max}})</td>
<td>(5.37 \pm 1.06)</td>
<td>(11.09 \pm 3.11)</td>
</tr>
<tr>
<td>(\alpha)</td>
<td>(0.024 \pm 0.007)</td>
<td>(0.065 \pm 0.02)</td>
</tr>
<tr>
<td>(E_k)</td>
<td>(302 \pm 120)</td>
<td>(204 \pm 66)</td>
</tr>
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\(a P_{g,\text{max}}\) is given in nmol \text{ O}_2 \text{ cm}^{-2} \text{ s}^{-1}; \alpha and \(E_k\) are given in \text{ pmol photons m}^{-2} \text{ s}^{-1}\). Average values \((n = 3)\) ± standard errors for healthy and brown band tissue (BrB) are given. NS, nonsignificant.
thus have large ecological impacts (B. Willis, personal communication).

Although other organisms such as bacteria and diatoms are also associated with the brown band zone of affected corals, the ciliates and ingested zooxanthellae account for the largest biomass. Light microscopy of the ciliates in the brown band tissue showed intact internal zooxanthellae with strong chlorophyll autofluorescence (Fig. 1B and C), lending support to the notion of photosynthetic competency of the zooxanthellae. The degeneration of symbiotic zooxanthellae in symbio is a routine process as cells divide and senesce (13, 14). Morphological changes associated with degeneration are characterized by vacuolization, disorganization of thylakoids, and enlargement of the accumulation body (5, 9, 13). Increased vacuolization in corals has previously been observed to result in loss of symbiont autofluorescence under green excitation light (13), but this was not observed in the grazed zooxanthellae investigated in this study. In addition, histological sections of ciliates also showed intact zooxanthellae within the cell cytoplasm (Fig. ID) and without surrounding food vacuoles, which are otherwise indicative of the degradation of ingested food (18, 38). Our microscopic observations thus clearly showed that in BrB the ingested zooxanthellae are not degraded rapidly and remain intact within the ciliates.

Photophysiology of ingested zooxanthellae. Based on a novel methodological approach we showed that the zooxanthellae within the ciliates were photosynthetically competent. While microsensors for oxygen and variable chlorophyll a fluorescence have previously been employed separately to investigate the heterogeneity of zooxanthellar photosynthesis in hospite (25, 36), this study provides the first combined measurements of PSII quantum yields and derived rETRs together with measurements of steady-state O2 concentration, rates of O2 flux, and gross photosynthesis in diseased coral tissues. Our comparison between RLCs and SSLCs obtained from diseased corals is also novel. Our results show that SSLCs are different from RLCs, which delay only 10 to 20 s between each irradiance level. It is evident that if zooxanthellae are allowed to acclimatize for an extended period of time to each irradiance level, their photosynthetic performance yields higher rETR values. Thus, fluorescence-derived light curves obtained using PAM instruments are dependent on previous light exposure (see also reference 27).

Photopigment and optical properties. Although the chlorophyll a content was similar across the disease lesion, the PAR absorptivity was significantly higher in the brown band zone (Fig. 2B and 3), suggesting that the light climate available to the zooxanthellae in this region was different from that of zooxanthellae associated with the healthy tissue. The higher absorptivity in the brown band indicates a more densely pigmented surface layer due to the high density of zooxanthellae-containing ciliates in this zone. In healthy tissue, the pigments are distributed differently and primarily in the coral endoderm below the surface. The different tissue composition of the healthy and brown band zones invariably results in differing spectral properties and intensities of the light reaching the zooxanthellae in the two zones (10, 25). The paler tissue coloration in the healthy tissue (Fig. 1A) thus indicates a higher contribution of diffuse scattered light from the skeleton to the ambient light field of the zooxanthellae compared to the brown band tissue, and this will influence light-dependent measures of photosynthesis activity such as the effective quantum yield (ΔF/Fm′) of PSII.

Variable chlorophyll a fluorescence analysis. There was no significant difference in Fv/Fm between healthy and brown band tissue, indicating that PSII activity was not inhibited as a consequence of ingestion by ciliates (Fig. 2 and 4). Actually, the efficiency of light utilization, characterized by the effective PSII quantum yield, ΔF/Fm′, apparently increased in zooxanthellae associating with ciliates.

Interestingly, the healthy tissue exhibited slightly lower rETR values throughout the irradiance range in dark-acclimated RLCs than did brown band tissue (Fig. 5), and this was mirrored in the gross photosynthesis irradiance curves (Fig. 8). Although the quantitative parameters of the RLCs are not significantly different (Tables 1 and 2), it is notable that the trend is reproduced across different techniques.

No photoinhibition, i.e., a decline in quantum yields or O2 production measures at high irradiances, was observed except in the fluorescence-derived SSLC measures of healthy tissue (Fig. 5). The lack of significant differences in other fluorescence-derived light curves suggests that the photoinhibition observed is a reflection of a decrease in PAR absorptivity observed in healthy tissue that may cause higher scattering and light exposure (Fig. 3) (10).

Oxygen dynamics. The O2 dynamics measured at the tissue surface showed a typical pattern of increasing O2 levels, which saturated and approached a maximum value asymptotically with increasing irradiance for healthy tissue (Fig. 6). From such curves, the compensation irradiance, Ec, can be estimated as the irradiance at which there is no oxygen gradient across the DBL between the tissue surface and the surrounding water. At this irradiance there is no net exchange as oxygen consumption and production balance; above Ec the system thus switches from being net heterotrophic to becoming autotrophic. In healthy tissue this occurred at a higher irradiance (~230 μmol photons m−2 s−1) than that previously reported for corals (25, 53), reflecting the fact that the corals were high light adapted (the Acropora muricata branches were from the reef flat) and thus exhibited a high respiration rate. In contrast, the brown band tissue demonstrated much lower O2 concentration at the tissue surface, and oxygen levels at the highest irradiance reached only ~25% of the O2 concentration in the surrounding water due to high respiration of the ciliates and other constituents of the brown band tissue. In darkness and at the lowest irradiances, the brown band tissue was almost anoxic at the surface, indicating diffusion limitation of the oxygen supply across the DBL (Fig. 6). This limitation was clearly alleviated by the photosynthetic activity of the zooxanthellae at higher irradiance. Oxygen flux calculations for brown band tissue showed a net oxygen uptake, indicating that respiration was greater than gross photosynthesis at all experimental irradiances (Fig. 7).

Oxygen levels at the brown tissue surface were fluctuating due to the presence of highly motile and dense ciliate populations, and this caused high variability in our oxygen profiles. It is also possible that boundary layer compression due to the microsensor (see reference 16) affected our oxygen microprofiles and the derived oxygen fluxes. Finally, high densities of ciliates have previously been shown to affect the local transport
coefficient of oxygen (17), and if such enhancement was present in the brown band tissue it would affect our flux calculations. Advection induced by the ciliates could alleviate diffusion limitation significantly, as has been shown for large motile bacteria (12, 52). Enhancement of transport was shown to depend on both size and cell density of ciliates. For large filter-feeding ciliates such as Euplotes sp., it was shown that at cell densities above $10^7$ cm$^{-2}$ oxygen transport was significantly enhanced over the oxygen supply by diffusion alone (17). A zooxanthella-containing Euplotes uncinatus was recently described by Lobban et al. (30). A quantification of the ciliate density along with more detailed microsensor measurements and imaging of ciliate behavior would provide more insights into the mechanisms that affect oxygen fluxes in BrB, and our oxygen turnover rates reported here should thus be regarded as rough estimates. Nevertheless, the data clearly support our other data showing that zooxanthellae in the ciliates exhibit active photosynthesis.

**Photosynthesis in healthy versus brown band tissue.** The A. muricata branches studied in this sample were collected from a reef flat exposed to high irradiance, and as such the healthy tissue was visibly pale, indicative of a relatively low density of zooxanthellae in the coral tissue (Fig. 1A). The low net photosynthesis rates observed suggest that these high-light-exposed corals acquire necessary photosynthates from reduced zooxanthellar densities and thereby also reduce the risk of exposure to excess production of reactive oxygen species (32). In addition, the causative agent of BrB syndrome has not been determined, and it is unknown if the net photosynthesis production of healthy tissue preceding the brown band zone is compromised by a bacterial or viral pathogen. Pantos et al. (33) demonstrated that the bacterial community of the whole coral colony is affected even when just a small part of the colony shows signs of disease.

Results of gross photosynthesis measures (Fig. 8) confirm that the brown band tissue is indeed highly dynamic. The high variation observed in measurements, indicated by the large standard error, reflects the motility of the layer, which causes signal fluctuations even at steady state. The observed variation may also be due to variable health of the zooxanthellae, though this is unlikely since the PAM data do not support the assertion of compromised zooxanthellar health. Gross photosynthesis rates were higher, although not statistically significant, in the brown band tissue than in healthy tissue. Although the chlorophyll a contents of healthy tissue and brown band tissue were similar, the local density of zooxanthellae in the ciliates was enhanced, as seen in the higher absorptivity in the brown band tissue. Gross photosynthesis rate measurements with oxygen microsensors are influenced by the local density of active zooxanthellae around the tiny measuring tip, and this, along with a more efficient light capture, can explain the increased productivity in the brown band zone compared to healthy tissue. However, measurements of oxygen levels and fluxes showed that most of this productivity is channeled into respiration in the highly motile ciliate band.

**Ciliates with chloroplasts or photosymbionts.** Protozoa harboring photosymbionts are abundant in aquatic ecosystems (4, 11, 51) with mixotrophic (chloroplast-sequestering) oligochrich ciliates obtaining fixed carbon from photosynthesis as well as through ingestion of particulate matter (phagotrophy) (8). Species of oligochrichs in the genera Tontonia, Laboea, and Strombidium have been shown to enslave chloroplasts from ingested flagellates, and such kleptochloroplasts remain functional for periods ranging from hours to days fixing carbon that is metabolized by the ciliate (48, 49). However, kleptochloroplasts do not reproduce inside the ciliate and must therefore be constantly replaced through feeding. Such mixotrophic marine oligochrichs use photosynthesis primarily to cover respiratory demands and to increase their growth efficiency, but photosynthesis may also alleviate starvation when food is scarce (8, 50). To confirm such a survival strategy for brown band ciliates, life history studies of the ciliates are required.

Recently, the marine ciliates Maristentor dinoferos (Heterotrichacea) and Euplotes uncinatus have been demonstrated to form associations with symbiotic zooxanthellae (29, 30). In the case of Maristentor dinoferos, approximately 50 to 800 dinoflagellates in the genus Symbiodinium clade C lineage have been observed within single ciliates (29). It is speculated that such ciliate-zooxanthella associations allow nutrients to pass from the ciliate to the dinoflagellate and photosynthates from dinoflagellate to the heterotrophic ciliate (30). Sommaruga et al. (47) detected UV-absorbing mycosporine-like amino acids in Maristentor dinoferos and proposed an additional mutu- alistic benefit of intracellular zooxanthellae, which in some cases are known to produce mycosporine-like amino acids (46). Whether similar UV protection occurs for ciliates in the brown band tissue requires a more detailed analysis of their pigmentation.

**Conclusion.** Our results show that zooxanthellae ingested by ciliates associated with BrB are photosynthetically competent and do not become compromised during the progression of the ciliate band. Ciliates feeding on coral tissue within the brown band tissue can thus gain additional energy from photosynthates and possibly alleviate oxygen limitation due to the photosynthesis of ingested zooxanthellae. Whether this association is a stable symbiosis or whether the zooxanthellae undergo a turnover and slow degradation in the ciliate, which requires constant uptake of new photosynthetically competent zooxanthellae, awaits further investigation.

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