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# Temporal patterns in effective quantum yield of individual zooxanthellae expelled during bleaching

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## Abstract

Bleaching is a worldwide phenomenon affecting coral reefs. During elevated temperature and light conditions (bleaching), expelled zooxanthellae show distinct patterns in photosynthetic health. An innovative new device was used to collect individual expelled zooxanthellae, when a coral was exposed to bleaching conditions. This has provided new insight into the photosynthetic condition and abundance of expelled zooxanthellae. It has been assumed that expelled zooxanthellae were dead or moribund; however, we have found individual cells can have healthy effective quantum yields ( $\phi_{\text{PSII}} > 0.65$  after 8 h of bleaching conditions ( $500 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ,  $33^\circ\text{C}$ ). The population of expelled zooxanthellae from *Cyphastrea serailia* and *Pocillopora damicornis* showed distinct patterns in the frequency distribution of  $\phi_{\text{PSII}}$  over time and between locations (sun versus shade) within a colony. During the first 4 h of exposure to bleaching conditions, only 5% of expelled individual cells from *P. damicornis* were photosynthetically inactive ( $\phi_{\text{PSII}} < 0.05$ ), whereas for *C. serailia*, this was 30%. The overall photosynthetic health of expelled zooxanthellae from *C. serailia* was better than *P. damicornis* ( $0.53 \pm 0.13$  and  $0.38 \pm 0.13$  after 8 h, respectively). This was generally reflected by the in hospite measurement of the coral, yet, the in hospite cells always had a higher  $\phi_{\text{PSII}}$  than expelled cells, suggesting that host tissue provided added photoprotection for the zooxanthellae.

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**Keywords:** Chlorophyll fluorescence; Coral bleaching; Zooxanthellae; PAM

**Abbreviations:** ANOVA, analysis of variance;  $F_{\text{m}}$ , maximum fluorescence in light;  $F_{\text{t}}$ , minimum fluorescence in light; LED, light-emitting diode; PAM, pulse amplitude modulated; PAR, photosynthetically active radiation; PSII, Photosystem II; RLC, rapid light curve;  $\phi_{\text{PSII}}$ , effective quantum yield.

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## 1. Introduction

The frequency of coral bleaching events has increased over the past two decades, and this has been attributed to a number of environmental factors. Temperature-induced bleaching occurs when coral is exposed to elevated temperature, under high-light

conditions (Jones et al., 1998). The process of bleaching can involve either (or both) the expulsion of the endosymbiotic dinoflagellates (zooxanthellae) from their host tissue or a reduction in the chlorophyll content of the zooxanthellae (Jones et al., 1998). Both processes diminish the pigmentation of the coral, allowing the characteristic white colouration of the skeleton to become apparent. It was previously assumed that if the photosynthetic activity of the in hospite zooxanthellae diminished, it should then follow that the expelled zooxanthellae must be inactive or at least photosynthetically impaired as they have come from this same population (Iglesias-Prieto et al., 1992; Perez et al., 2001). Several investigations have found the expelled zooxanthellae to be viable (Glynn et al., 1985; Suharsono and Brown, 1992), and more recently, it has been shown that expelled zooxanthellae can be photosynthetically competent (Ralph et al., 2001). Bhagooli and Hidaka (2004) found that under bleaching conditions, healthy zooxanthellae were expelled while the host released a greater number of undischarged cnidae, suggesting that the host was suffering cellular damage and was therefore the more sensitive partner of the symbiosis.

Thermal tolerance of expelled zooxanthellae appears to be several degrees higher than the temperature at which bleaching occurs (Ralph et al., 2001), therefore, it seems possible that the impact of bleaching may not be exclusively linked to photosystem collapse; as has been suggested by several groups (Iglesias-Prieto et al., 1992; Warner et al., 1996; Jones et al., 1998). Given the current genetic evidence that a single colony could maintain several unique clades of zooxanthellae (Rowan and Knowlton, 1995; Rowan et al., 1997; Rowan, 1998; Ulstrup and van Oppen, 2004), it might be possible that zooxanthellae clades have a range of tolerances to bleaching stress (Rowan et al., 1997; Baker, 2001) and, therefore, the health of expelled zooxanthellae might also vary according to genotype.

The light climate impinging on a particular region of the colony is another factor which may influence the thermal tolerance of the zooxanthellae (Rowan et al., 1997). Light climate has been shown to influence the spatial heterogeneity in photosynthetic activity of zooxanthellae (Ralph et al., 1999, 2002; Hill et al., 2004b). We have also found fine-scale patterns in the photosynthetic capacity of tissues within a single

colony (Kühl et al., 1995). Furthermore, it has recently been shown that genetically distinct patterns of zooxanthellae distribution within *Acropora tenuis* and *Acropora valida* were correlated to light climate (Ulstrup and van Oppen, 2004). Therefore, microclimatic changes in light which influence zooxanthellae photosynthesis and genetic composition could equally affect bleaching tolerance within a colony.

Bleaching is not simply a stimulus→response process. Brown et al. (1995) recognised that it was necessary to understand the time course of bleaching and to correlate particular mechanisms with temporal changes in the physiological response. There are numerous regulatory processes operating at a wide range of scales, changing the overall bleaching response, which results in substantial variability in coral survival (Douglas, 2003). It is encouraging to note that not all corals bleach at the same temperature, and some species show remarkable tolerance to thermal/light stress (Marshall and Baird, 2000; Loya et al., 2001; Kayanne et al., 2002). This variability in bleaching sensitivity indicates that there are mechanisms associated with tolerant populations of zooxanthellae that we are currently not aware of. Bhagooli and Hidaka (2003) specifically addressed this issue by comparing the photosynthetic capacity of in hospite with freshly isolated zooxanthellae, where they found the photosynthetic efficiency ( $F_v/F_m$ ) of isolated zooxanthellae to be lower than in hospite. They suggested that the host coral tissue influenced the bleaching susceptibility, not just the zooxanthellae, and that zooxanthellae have different bleaching susceptibilities. These mechanisms could be linked to the cells having exceeded a physiological threshold, which initiates an alternate methods of photoprotection such as photophosphorylation (Hill et al., 2004a), or the activation of cyclic electron transport (Jones et al., 1998).

In this study, we investigate the following questions: (1) Do all expelled zooxanthellae have similar photosynthetic capacity? (2) Do zooxanthellae that are expelled early during a bleaching event have a different photosynthetic capacity than zooxanthellae expelled later on during the bleaching event? (3) Does the photosynthetic condition of the coral tissue influence the condition of the expelled zooxanthellae? To address these questions, we developed a new micro-sampler technique for collecting expelled zooxanthellae at fine

spatial scales (<2 mm). The sampler was used in conjunction with various chlorophyll fluorometers.

## 2. Materials and methods

### 2.1. Coral samples

Four shallow water (<2 m) specimens of *Pocillopora damicornis* (branching colony) and *Cyphastrea serailia* (massive colony) were collected from Heron Island Lagoon (23°31' S, 152°08' E, Great Barrier Reef, Australia) and maintained in continuously flowing seawater (26–27 °C) for 24 h under shaded conditions (<100  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) before experimentation. These two species were selected as an example of a bleaching tolerant species (*C. serailia*) and a sensitive species (*P. damicornis*). All samples for both species were the light brown colour morph.

### 2.2. Fluorescence measurement

Fluorescence measurements on single zooxanthellae cells were performed using a Microscopy-PAM (Walz, Effeltrich, Germany; settings MF=5, SI=8, SW=0.4, PG=23). Measurements of the photosynthetic capacity of the coral tissue (adjacent to each micro-sampler tip) were performed using a Diving-PAM (Walz; settings MI=8, SI=8, SW=0.8, G=2) with a 1.5 mm acrylic fibre optic. Effective quantum yield of PSII ( $\phi_{\text{PSII}}$ ) was determined according to the following expression  $(F_m' - F_t)/F_m'$  where  $F_m'$  is the light-adapted maximum fluorescence and  $F_t$  is the fluorescence before a saturating pulse (Genty et al., 1989).

### 2.3. Bleaching treatment

Bleaching was induced by exposing samples to 33 °C seawater, under moderate light conditions (500  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) (Fitt and Warner, 1995, Warner et al., 1996). Our experiment was an acute thermal/light stress exposure, which follows conditions on Heron Island where water temperature can increase by 8 °C over a single tidal cycle (Jones and Hoegh-Guldberg, 2001). Illumination was provided by a quartz halogen lamp (Sylvannia EFR 15V 150W), and irradiance was measured with a calibrated light probe (Biospherical Instruments, USA). A colony was placed in a 10-L aquarium, containing fresh aerated seawater, which was held within a 50-L controlled temperature water bath (Haake E3, West Germany). Four colonies of each species were bleached and approximately 190 individual cells per colony were measured. Temperature was maintained at  $33 \pm 0.2$  °C. The experiment commenced once the experimental aquarium reached 33 °C (usually within 2 h).

### 2.4. Microsampler

Expelled zooxanthellae were collected using a “micro-sampler” which consisted of a series of six canister filters (40 mm diameter  $\times$  160 mm, light proof) attached to a peristaltic pump (Fig. 1). Pasteur pipettes were positioned with a micromanipulator  $\sim$ 1 mm above a selected light microhabitat (sun/shade) on each coral colony. A peristaltic pump drew seawater from the Pasteur pipette tip through a custom-built canister filters (containing 5- $\mu\text{m}$  nylon filter mesh) at a rate of  $\sim$ 9 ml/min. Each Pasteur

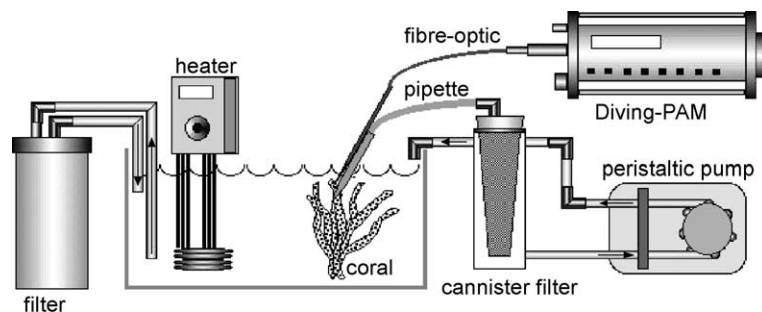


Fig. 1. Schematic of micro-sampler and experimental set up. The background sampling pipette was positioned in the corner of tank, farthest from the coral specimen.

pipette was attached to a 1.5 mm acrylic fibre optic, allowing the photosynthetic capacity to be measured (using a Diving-PAM) of the coral tissue adjacent to regions where the “microsampler” collected expelled zooxanthellae. Three “sun” (tip of upward facing region,  $500 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) and three “shade” (basal region of branch/colony,  $50\text{--}100 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) positions on each of four coral specimens

were sampled. To minimize the chance of collecting expelled zooxanthellae from other regions of the colony, the water within the exposure tank was continuously filtered using an ultra-fine filtration unit (Eheim, 2015; 500 L/h) with. An additional micro-sampler line was deployed in the corner of the aquaria to monitor the density and photosynthetic capacity of any expelled zooxanthellae from the rest of the colony

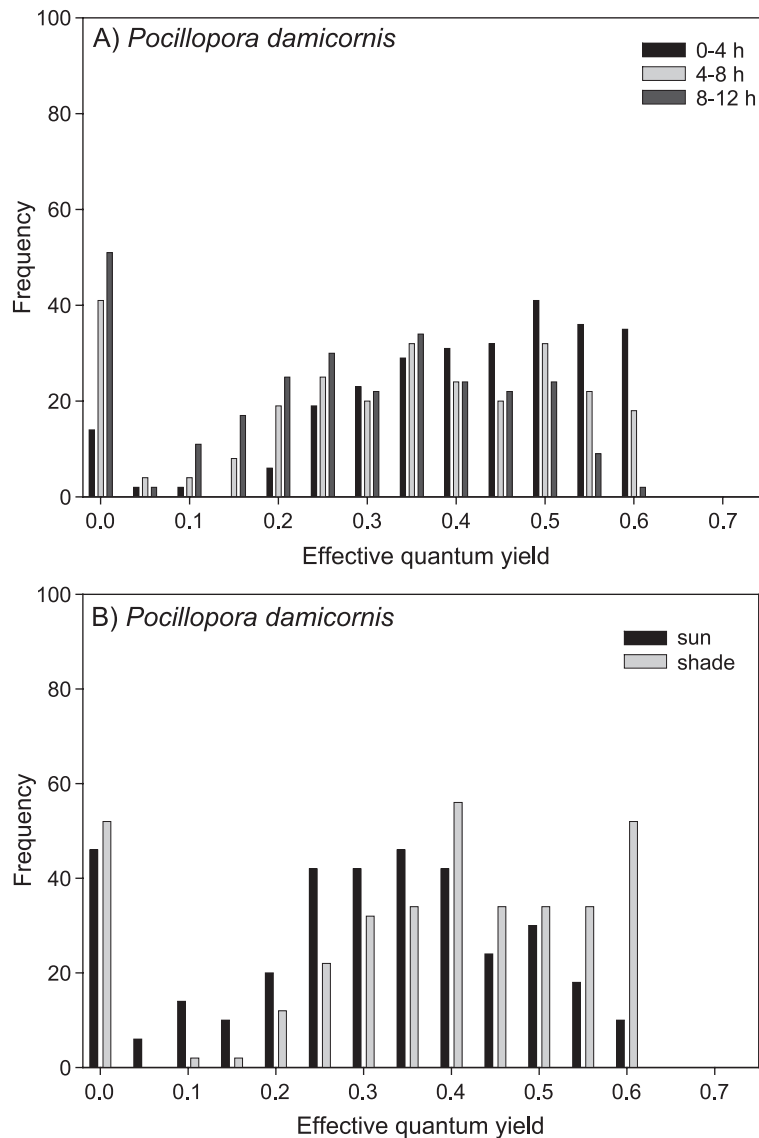


Fig. 2. (A–B) Frequency histogram of effective quantum yield measurements from expelled *P. damicornis* zooxanthellae either (A) grouped according to expulsion time 0–4, 4–8 and 8–12 h or (B) position on colony sun (tips and upper branches) or shade (lower branches). Effective quantum yield classified into 0.05 categories for a total of 760 cells.

that were not collected by the filtration system (background or supernumerate cells).

### 2.5. Zooxanthellae collection

Expelled zooxanthellae were flushed from the 5- $\mu\text{m}$  nylon mesh filter into a 15-ml centrifuge tube after 4, 8 and 12 h bleaching. Zooxanthellae were

concentrated by centrifuging the filtrate at 2000 rpm for 2 min (Beckman GP) and re-suspending the pellet into 0.5 ml of 0.45- $\mu\text{m}$ -filtered seawater. Zooxanthellae remained photosynthetically active in 0.45- $\mu\text{m}$ -filtered seawater for up to 2 days. A small aliquot of this solution was mounted on a microscopic slide for photosynthetic assessment using the Microscopy-PAM. Time between the removal of zooxanthella

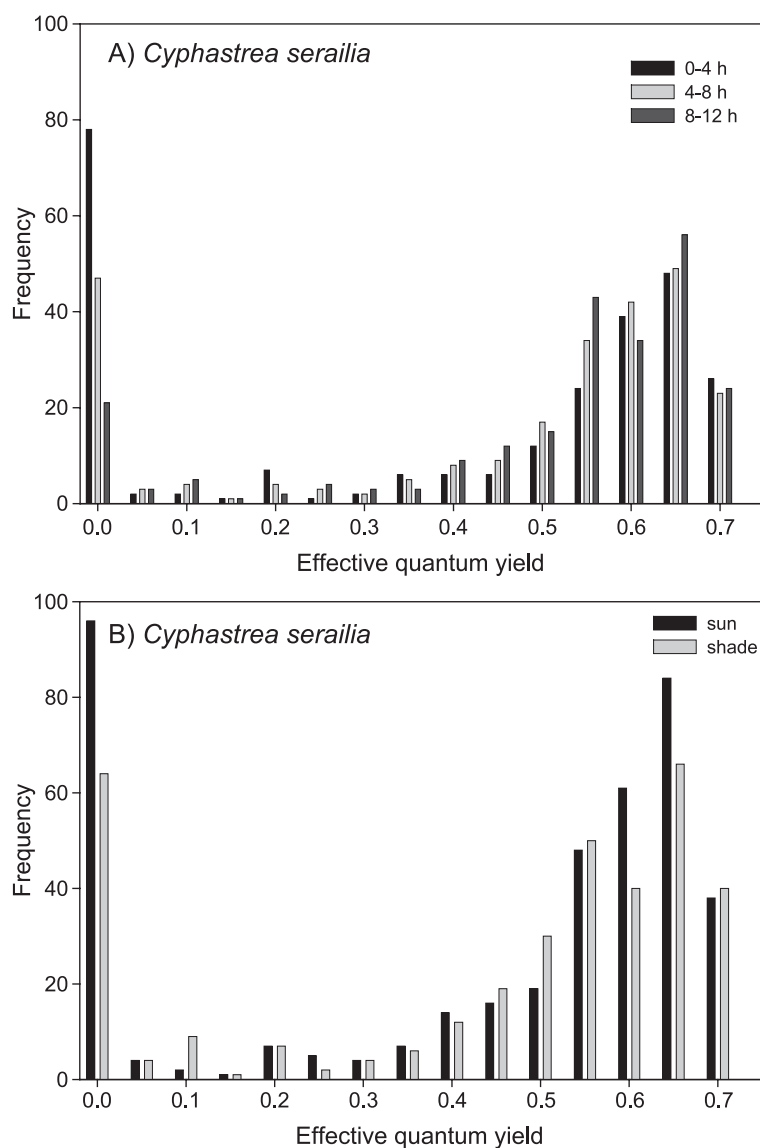


Fig. 3. (A–B) Frequency histogram of effective quantum yield measurements from expelled *C. serailia* zooxanthellae either (A) grouped according to expulsion time 0–4, 4–8 and 8–12 h or (B) position on colony sun (upper surface) or shade (lower surface). Effective quantum yield classified into 0.05 categories for a total of 766 cells.

sample from mesh filter to assessment under microscope was 10–15 min.

### 2.6. Zooxanthellae counts

The cell density for each filtered sample was determined using a Neubauer haemocytometer (Superior, Germany). Cells were counted in 25 grid cells and four replicates per sample were averaged ( $n=4$ ).

### 2.7. Statistical analysis

A Kolmogorov–Smirnov two-sample test (Systat vers. 8.0) was used to determine whether the frequency distribution of effective quantum yields for different time periods and sun versus shade populations of expelled zooxanthellae were similar. This test was sensitive to differences in location, dispersion and skewness of distribution (Sokal and Rohlf, 1995). The standard error of skewness ( $SE_{skew} = \sqrt{(6/n)}$ ) and kurtosis ( $SE_{kurt} = \sqrt{(24/n)}$ ) were also calculated to describe the shape of the frequency distributions (Ashcroft and Pereira, 2003). One-factor analysis of variance was performed on sun, shade and background cell counts to test for difference over time (not between species). A two-sample  $t$  test was used to identify differences between the effective quantum yield values of in hospite and expelled cells.

## 3. Results

Measurements of individual expelled zooxanthellae (~190 cells/colony) from four coral samples were pooled to provide frequency distributions of photosynthetic condition (Figs. 2 and 3). The effective quantum yield ( $\phi_{PSII}$ ) of expelled zooxanthellae from *P. damicornis* ( $n=760$ ) and *C. serailia* ( $n=766$ ) were monitored over 12 h of bleaching conditions (33 °C, 500  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ). When the  $\phi_{PSII}$  of these populations of expelled zooxanthellae were plotted as frequency histograms, it is apparent that the photosynthetic capacity of these cells was generally not normally distributed. Expelled zooxanthellae could be broadly classified as either photosynthetically inactive ( $\phi_{PSII} < 0.05$ ) or active (Table 1). During the first 4 h of exposure to bleaching conditions, *P. damicornis* released a large population of photosynthetically

Table 1

Kolmogorov–Smirnov two-sample test for differences in frequency distribution

	Early versus late <sup>a</sup>	Late versus middle <sup>a</sup>	Middle versus early <sup>a</sup>	Sun versus shade
<i>All cells included</i>				
<i>P. damicornis</i>	0.000*	0.002*	0.000*	0.000*
<i>C. serailia</i>	0.000*	0.108	0.074	0.476
<i>Excluding cells with <math>\phi_{PSII} &lt; 0.05</math></i>				
<i>P. damicornis</i>	0.000*	0.002*	0.000*	0.000*
<i>C. serailia</i>	0.054	0.676	0.545	0.245

<sup>a</sup> Early=0–4 h, middle=4–8 h and late=8–12 h.

\* Significant difference in populations at  $p=0.05$ .

“healthy” cells ( $\phi_{PSII} > 0.5$ ). Less than 5% of expelled cells during the first 4 h of treatment were photosynthetically inactive. Over the next 4 h, this increased to 10%, and after 8 h of bleaching, 16% of the expelled cells were inactive. Over the 12-h sampling period, 20.5% of the expelled cells had limited photosynthetic capacity ( $\phi_{PSII} \leq 0.2$ ). These are arbitrary thresholds attributed to  $\phi_{PSII}$  measurement of individual cells; however, it is expected that any cell in such poor photosynthetic condition is unlikely to survive. If this threshold was increased, the proportion of impacted zooxanthellae would be greater. The 0–4 h distribution of expelled cells was not normal ( $SE_{skew} = -3.6$ ), while the 4–8 and 8–12 h were normally distributed ( $SE_{skew} = -1.3$ ). Cells expelled during the first 4 h had a distribution skewed towards healthier cells; however, the median  $\phi_{PSII}$  decreased with exposure (0.46, 0.38 and 0.33 respectively). All three expelled zooxanthellae frequency distributions were significantly different from each other (KS two-sample test,  $p < 0.001$ ; Table 1).

When all *P. damicornis* expelled cells were grouped according to light microhabitat (sun versus shade, Fig. 2B), the expelled zooxanthellae from the high-light exposed regions (sun) had a lower median  $\phi_{PSII}$  than the shaded regions (0.32 and 0.37, respectively). A Kolmogorov–Smirnov two-sample test indicated that the distribution of  $\phi_{PSII}$  from the sun microhabitat was significantly different from the shaded regions ( $p < 0.001$ ; Table 1). Over the 12-h exposure period, the number of photosynthetically inactive cells from either sun or shaded regions of *P. damicornis* were similar.

We found a notable difference between the population distribution of expelled cells from *C. serailia* (Fig. 3A and B; Table 1) and *P. damicornis*. Firstly, with *C. serailia*, a substantial proportion of the early expelled cells were photosynthetically inactive ( $\phi_{PSII} < 0.05$ ). Almost 30% of the cells expelled in the first 4 h of bleaching conditions were inactive, by 8 h, this decreased to 19% and by 12 h, it was 9% of the population of expelled cells was inactive. It should be noted that *C. serailia* released significantly fewer cells than *P. damicornis* (Fig. 4). Given the significant propor-

tion of inactive cells, interestingly the photosynthetic capacity of the remaining expelled population was remarkably healthy (68% with  $\phi_{PSII} > 0.5$ , excluding inactive). The median  $\phi_{PSII}$  for *C. serailia* during the three sample periods (0–4, 4–8 and 8–12 h) were similar (0.57, 0.56 and 0.56, respectively). All three distributions (excluding inactive cells) were non-normal and skewed towards elevated  $\phi_{PSII}$  ( $SE_{skew} > -11.4$ ) with a positive kurtosis ( $SE_{kurt} > 8.9$ ) indicating the peak of the distribution was higher than if it was a normal distribution. Cells expelled from *C. serailia*

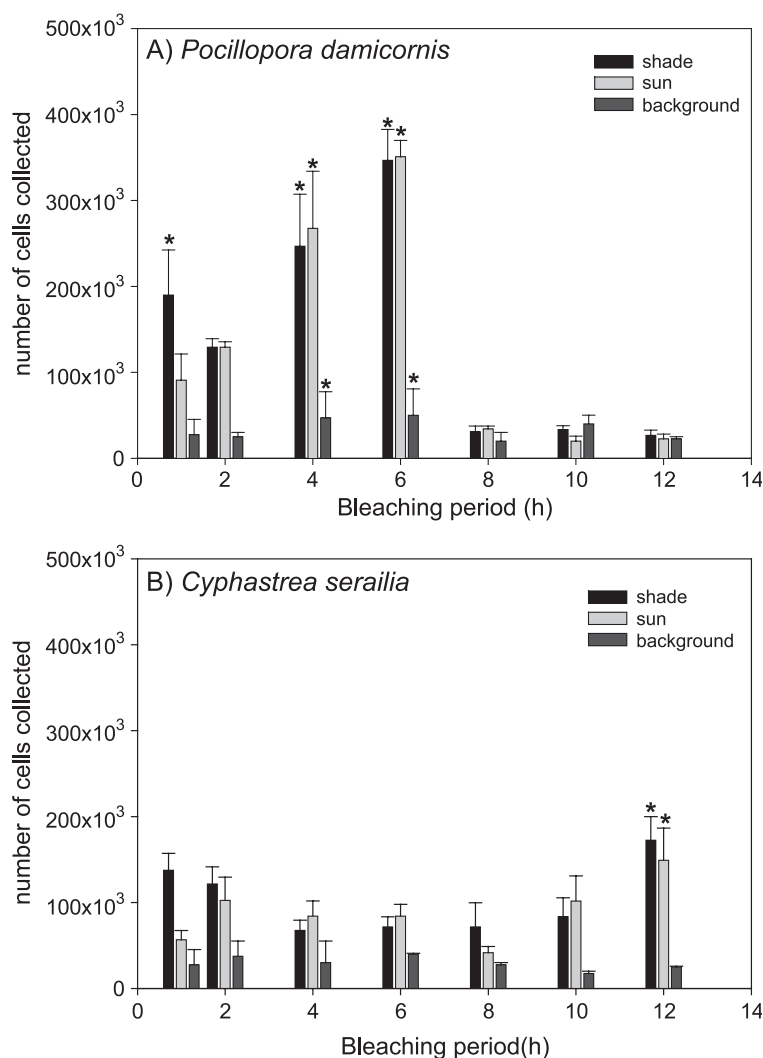


Fig. 4. (A–B) Number of zooxanthellae collected from filtration unit of micro-sampler every 2 h after the beginning of bleaching exposure for (A) *P. damicornis* and (B) *C. serailia*. ( $n=3 \pm S.E.M.$ ). \*Samples that were significantly different ( $p < 0.05$ ) across bleaching periods.



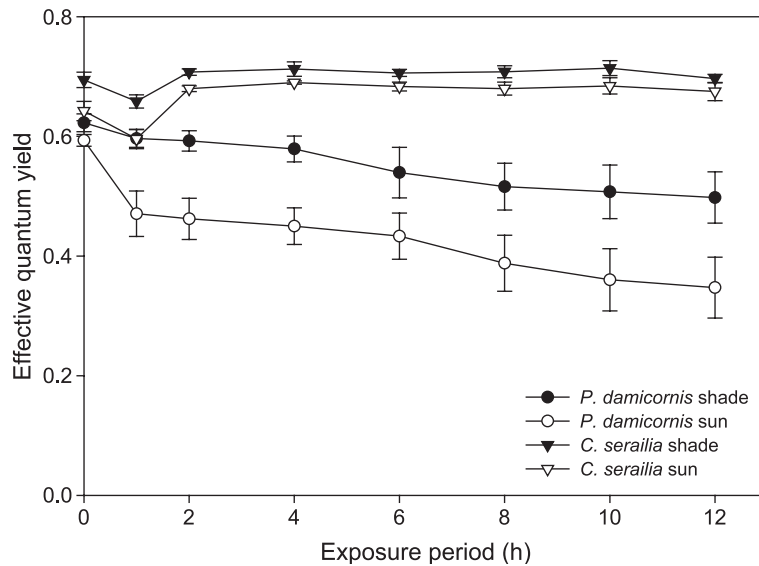


Fig. 5. Effective quantum yield of coral tissue measured after beginning of bleaching experiment. Mean and standard error of mean ( $n=4$ ) are plotted against time. Two regions are graphed; upper sun-exposed surface and lower shaded surface of *P. damicornis* and *C. serailia*.

during the three sampling period were similarly healthy (KS two-sample test; Table 1, when inactive cells excluded), yet, there were fewer inactive cells in the later sampling periods. When the entire population of expelled *C. serailia* cells were grouped according to light microhabitat (sun versus shade, Fig. 3B), there was no difference in the overall distribution (KS two-sample test; Table 1).

The number of cells collected from each microhabitat is shown in Fig. 4A–B. *P. damicornis* showed a progressively greater density of cells being collected in the micro-sampler up to 6 h, then a substantial reduction in the number of cells expelled from shaded regions of the samples (ANOVA: shade,  $p<0.001$ ; sun,  $p=0.071$ ). The number of cells not collected by the canister filter (background) in *P. damicornis* tanks increased in the 4- and 6-h samples (ANOVA,  $p=0.22$ ); however, this represented less than 15% of cells collected. This suggests that the ultrafine filter collected most floating cells and so preventing these cells from being sampled by the Pasteur pipettes located adjacent to specific sample sites. *C. serailia* released substantially fewer cells than *P. damicornis* during the 12-h collection period (Fig. 4). Cells with  $\phi_{PSII}>0.55$  were still being released from *C. serailia* samples after 24 h (data not presented). There was no significant difference in

background cell number over 12 h for *C. serailia* (ANOVA,  $p=0.500$ ).

Each of the coral specimens was monitored using Diving-PAM to assess the degree of photosynthetic damage that was evident in the regions where the micro-samplers were positioned. Both *P. damicornis* and *C. serailia* sun and shade samples showed a decline in  $\phi_{PSII}$  over the period of exposure (Fig. 5). High-light exposed (sun) regions showed a greater decline in  $\phi_{PSII}$  than shaded regions. Photosynthetic capacity ( $\phi_{PSII}$ ) of the average expelled zooxanthellae was found to be less than the corresponding in hospite

Table 2

Comparison of in hospite<sup>a</sup> and expelled<sup>b</sup> zooxanthellae effective quantum yield using a two-sample *t* test

Species	Zoox type	0–4 h	4–8 h	8–12 h
<i>P. damicornis</i>	in hospite	0.543±0.079	0.484±0.094	0.436±0.110
	expelled	0.433±0.113	0.334±0.121	0.379±0.131
	<i>p</i> -Value	0.000*	0.000*	0.026*
<i>C. serailia</i>	in hospite	0.674±0.038	0.696±0.017	0.690±0.022
	expelled	0.528±0.131	0.530±0.130	0.528±0.132
	<i>p</i> -Value	0.000*	0.000*	0.000*

Mean with standard deviation.

<sup>a</sup> Average  $\phi_{PSII}$  value of sun and shade over time period ( $n=18$ ).

<sup>b</sup> Excluded all inactive cells ( $n>80$ ).

\* Significant difference at  $p=0.05$ .



value for each of the sampling periods (Table 2). When considering the data in this table, it should be realised that the in hospite estimate of  $\phi_{\text{PSII}}$  was taken under 500  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ , while the expelled cells were exposed to room irradiance (20  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ ) on the microscope stage before measurement.

#### 4. Discussion

Bleaching causes changes in cell density and/or pigment content, which by themselves do not affect measurements of quantum yield ( $\phi_{\text{PSII}}$  or  $F_v/F_m$ ) as they only reflect the photosynthetic capacity of the zooxanthellae (Jones et al., 2000). A change in  $\phi_{\text{PSII}}$  reflects an impact on the photosynthetic condition of the zooxanthellae. Therefore, in hospite measurements may be based on a reduced population of zooxanthellae (due to partial expulsion of the population), but the in hospite photosynthetic capacity does reflect only the remaining cells. Jones et al. (2000) assumed that not all expelled zooxanthellae were equally impacted by the bleaching conditions, and that the algal population was normally distributed around a mean response. In our study, we found that the frequency distribution of individual expelled cells from *P. damicornis* was initially normally distributed, whereas *C. serailia* were always skewed to higher  $\phi_{\text{PSII}}$ .

Distinct patterns can be seen in the rate and photosynthetic condition ( $\phi_{\text{PSII}}$ ) of expelled zooxanthellae in relation to the period of exposure. Perez et al. (2001) found that aposymbiotic *Aiptasia pallida*, infected with a range of different zooxanthellae, expelled various types of zooxanthellae at different rates depending on susceptibility of the symbiont to bleaching. Our data are not in conflict with this work but does not support their conclusion that *Ai. pallida* expel symbionts when they become photosynthetically compromised and no longer autotrophic. Initially, *C. serailia* released a substantial number of inactive zooxanthellae within the first 4 h (Fig. 3A), while concurrently releasing another distinct population of healthier zooxanthellae.

Recovery of partially bleached colonies could be related to the selective removal of photo-damaged dinoflagellates (Jones et al., 2000), suggesting that only damaged (inactive) cells would be expelled. We found that a large proportion of the expelled

population of zooxanthellae for both species were photosynthetically competent and apparently autotrophic. The later sampling periods (4–8 and 8–12 h) showed fewer inactive cells, while the overall distribution of photosynthetically active cells remained constant. Given that the quantity of expelled cells from *C. serailia* was relatively consistent over the 12-h period (Fig. 4B), it could be assumed that this initial group of inactive cells ( $\phi_{\text{PSII}} < 0.05$ ) was a first flush of supernumerate cells, and the quantity of these inactive cells decreased with exposure time (Fig. 3A).

The substantial reduction in the number of inactive cells from *C. serailia* in the later sampling period could suggest that a bleaching-sensitive population of cells were impacted and expelled, leaving a more bleaching-tolerant population that were expelled at a relatively slower rate (compared to *P. damicornis*). However, these later expelled cells were essentially healthy, showing no shift in the condition of the population towards more damaged cells, as seen with the *P. damicornis* zooxanthellae. *C. serailia* has been identified as a highly resistant taxa, based on field surveys of bleaching mortality (Marshall and Baird, 2000). The pool of photosynthetically active cells expelled from *C. serailia* could provide the seed population to re-infect partially bleached colonies, once the bleaching conditions are alleviated; supporting the adaptive bleaching hypothesis (Buddemeier and Fautin, 1993). The sun and shade regions of *C. serailia* showed no obvious difference in the photosynthetic capacity of the population of expelled zooxanthellae, therefore, it is unlikely that zooxanthellae with different thermal or light tolerances were spatially distributed into specialized microhabitats within a single colony of *C. serailia*. Brown et al. (2002) found within colony differences in a similar massive species (*Goniastrea aspera*), between east and west faces of the colony; while we examined the top and side (irrespective of aspect) of the colonies which could explain the similarity of our data.

*P. damicornis* showed a more variable response with a greater proportion of inactive cells being expelled after longer exposure periods and a wider range of photosynthetic capacity over time (Fig. 2). Cells expelled during the 0–4-h period showed an overall healthy photosynthetic capacity ( $0.43 \pm 0.11$ ). Most of the population had a  $\phi_{\text{PSII}} > 0.2$  with few photosynthetically inactive cells. This variability in

photosynthetic condition of *P. damicornis* zooxanthellae could reflect the symbionts' genetic tolerance to bleaching. With increasing thermal/light stress, the population of expelled zooxanthellae showed increasing stress and substantially more inactive cells.

Interestingly, the number of cells expelled from the *P. damicornis* samples showed a distinct temporal pattern, where most of the cells were released during the first 6 h, corresponding to the population of cells with generally higher  $\phi_{\text{PSII}}$ . This implies that during the initial stages of thermal stress, large numbers of healthy cells were expelled, while the remaining in hospite cells suffer increasing photosynthetic damage, resulting in more damaged cells being released later in the exposure period. Titlyanov et al. (1996) showed that it took about 6 h for degraded zooxanthellae to migrate from tentacles and coenosarc tissue into the gastrodermal cavity for extrusion. This could suggest that large number of cells expelled during the first 6 h were responding to a host-mediated removal mechanism, whereas the later expulsion of cells was linked to the onset of bleaching conditions.

Microscale light climates have been shown to influence photosynthesis of zooxanthellae (Kühl et al., 1995; Ralph et al., 2002). Cells from sun-adapted regions of the *P. damicornis* colony generally showed a reduced  $\phi_{\text{PSII}}$ . Preferential bleaching from sun-exposed regions of corals has previously been observed by Williams and Bunkley-Williams (1990). This could also indicate photoinhibition due to the position of the host tissue, as the cells were under additional light stress in comparison to the shaded region. It is important to note that there was no difference in the number of photosynthetically inactive cells expelled from sun or shade regions.

Most research has focused on the photosynthetic capacity of the in hospite zooxanthellae, using non-destructive sampling of the quantum yield of small sections of coral (Jones et al., 1998; Ralph et al., 1999). We present here the first attempt at understanding the photosynthetic capacity of individual expelled zooxanthellae from corals exposed to bleaching conditions. Our coral colonies showed a typical decline in effective quantum yield during exposure to elevated temperature (Jones et al., 1998; Ralph et al., 2001). In hospite zooxanthellae were able to maintain a higher  $\phi_{\text{PSII}}$  than expelled zooxanthellae for both species (Table 2). Assuming that both in hospite and

expelled zooxanthellae had similar frequency distributions of  $\phi_{\text{PSII}}$ , this would suggest that the host tissue influences the condition of cells remaining within the colony. Bhagooli and Hidaka (2003) found a similar result with in hospite and freshly isolated zooxanthellae, and concluded that host tissue was affording in hospite cells, such as additional photoprotection (Salih et al., 2000). Further experiments by Bhagooli and Hidaka (2004) suggest that bleaching conditions resulted in a primary impact on the host (increased undischarged cnidae release), which lead to the non-selective expulsion of healthy zooxanthellae. This finding confirms our earlier results of photosynthetically competent expelled zooxanthellae (Ralph et al., 2001), however, given the distinct patterns of  $\phi_{\text{PSII}}$ , we would suggest that the expulsion was controlled by a presently unknown selective factor. The capacity of zooxanthellae to survive bleaching lends further evidence to the hypothesis that bleaching is linked to a dysfunction between the host–algae symbiosis, not just the photosynthetic processes of the zooxanthellae.

Differences between in hospite and expelled zooxanthellae suggest that host-mediated protection could be important. The mean  $\phi_{\text{PSII}}$  of expelled zooxanthellae was up to 31% lower than in hospite  $\phi_{\text{PSII}}$  from the same region (Table 2). The expelled cells were collected from a darkened canister and only exposed to room light during measurement, therefore, they had a greater opportunity for recovery/repair than the in hospite cells. Given this difference in light treatment, it would imply that there could be additional factors influencing the condition of expelled zooxanthellae. It is clear that expelled cells are photosynthetically active, but if they were from the same population as that remaining inside the colony, the  $\phi_{\text{PSII}}$  would be the same, but this is clearly not observed. This could be explained by differential expulsion of zooxanthellae residing in polyp regions, as opposed to the more sun-exposed coenosarc tissues. Zooxanthellae within polyps could also be experiencing additional stress such as gas exchange problems during the bleaching conditions (Kühl et al., 1995; Patterson, 1995).

The presence of photosynthetically active cells within the population of expelled cells raises several questions linked to recovery and genetics. Firstly, the “adaptive bleaching hypothesis” suggests that corals selectively lose the more vulnerable symbiont pop-

ulations and possibly select new and more competent populations of zooxanthellae from the water column (Buddemeier and Fautin, 1993). Our results lend support to this theory by demonstrating that corals can expel both healthy and photosynthetically compromised zooxanthellae. It is possible that some expelled zooxanthellae can survive a bleaching event, providing the pool of cells, which given the correct conditions may be able to re-infect previously bleached corals.

On the issue of how genetics influences the condition of expelled zooxanthellae, it was clear that both coral species showed evidence that the expelled zooxanthellae had different levels of tolerance to bleaching conditions. Molecular analysis of zooxanthellae has found up to five distinct clades of zooxanthellae, which may be linked to photophysiological traits (Rowan and Knowlton, 1995; Rowan et al., 1997). If it was found that a coral possessed multiple clades (or strains) of zooxanthellae, this could partially explain the variation in photosynthetic health of the expelled cells. Distinct populations of expelled zooxanthellae (Figs. 2 and 3) could suggest that these species of coral contained mixed populations of zooxanthellae with different degrees of bleaching tolerance (Perez et al., 2001); however, this speculation requires further detailed investigation.

In conclusion, we show that when subjected to bleaching conditions, two species of coral expelled photosynthetically active zooxanthellae. A wide frequency distribution of  $\phi_{\text{PSII}}$  corresponded to the individual cells collected. This provides new insight into the photosynthetic health at the organism level (individual cells), not a bulk measurement of a population of zooxanthellae. Surprisingly, cells with  $\phi_{\text{PSII}} > 0.5$  were common even after 12 h of bleaching. Both *P. damicornis* and *C. serailia* expelled photosynthetically active cells and showed temporal and spatial patterns in the health of expelled zooxanthellae. In hospite measurements of zooxanthellae were higher than expelled; suggesting additional photoprotection of zooxanthellae occurs within the host tissue.

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