

# Feeding sustains photosynthetic quantum yield of a scleractinian coral during thermal stress

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**Abstract** Thermal resistance of the coral–zooxanthellae symbiosis has been associated with chronic photoinhibition, increased antioxidant activity and protein repair involving high demands of nitrogen and energy. While the relative importance of heterotrophy as a source of nutrients and energy for cnidarian hosts, and as a means of nitrogen acquisition for their zooxanthellae, is well documented, the effect of feeding on the thermal sensitivity of the symbiotic association has been so far overlooked. Here we examine the effect of zooplankton feeding versus starvation on the bleaching susceptibility and photosynthetic activity of photosystem II (PSII) of zooxanthellae in the scleractinian coral *Stylophora pistillata* in response to thermal stress (daily temperature rises of 2–3°C) over 10 days, employing pulse-amplitude-modulated chlorophyll fluorometry. Fed and starved corals displayed a decrease in daily maximum potential quantum yield ( $F_v/F_m$ ) of PSII, effective quantum yield ( $\Delta F/F_m'$ ) and relative electron transport rates over the course of 10 days. However after 10 days of exposure to elevated temperature,  $F_v/F_m$  of fed corals was still 50–70% higher than  $F_v/F_m$  of starved corals. Starved corals showed strong signs of chronic photoinhibition, which was reflected in a significant decline in nocturnal recovery rates of PSII relative to fed corals. This was paralleled by the progressive inability to dissipate excess excitation energy via non-photochemical quenching (NPQ). After 10 days, NPQ of starved corals had decreased by about 80% relative to fed corals. Feeding treatment had no significant effect on chlorophyll *a* and  $c_2$  concentrations and zooxanthellae densities,

but the mitotic indices were significantly lower in starved than in fed corals. Collectively the results indicate that exogenous food may reduce the photophysiological damage of zooxanthellae that typically leads to bleaching and could therefore play an important role in mediating the thermal resistance of some corals.

**Keywords** Bleaching · Heterotrophy · Photosystem II · *Stylophora pistillata* · Zooxanthellae

## Introduction

Coral bleaching, the dissociation of corals and their zooxanthellae (endosymbiotic dinoflagellates of the genus *Symbiodinium*) in response to thermal stress, is a global threat to coral reefs (Hoegh-Guldberg 1999). Coral responses to elevated temperatures are, however, not uniform but often display high variation in bleaching severity, differential mortality and subsequent recovery, not only between different species but also among and within conspecific colonies in the same reefal habitat (e.g. Brown and Suharsono 1990; Marshall and Baird 2000). Recent advances in chlorophyll fluorescence and molecular techniques have led to several lines of evidence which implicate coral bleaching with chronic photoinhibition of photosynthesis and the subsequent increase in reactive oxygen species within both the zooxanthellae and coral host (for review, see Smith et al. 2005). Both symbiotic partners, however, feature elaborate protective mechanisms against oxidative cellular damage, including antioxidant enzymes, heat shock proteins (e.g. Downs et al. 2002; Richier et al. 2005) and certain mycosporine-like amino acids (Yakovleva et al. 2004). In addition, zooxanthellae are able to regulate excess excitation energy via photoprotective

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non-photochemical quenching (NPQ) processes, associated with xanthophyll cycle-dependent thermal energy dissipation of excess light via the de-epoxidation of the xanthophyll carotenoid diadinoxanthin to diatoxanthin (Ambarsari et al. 1997; Brown et al. 1999). The evidence derived from studies by Warner et al. (1996, 1999) and Hill et al. (2005) strongly suggest that those species more capable of engaging in NPQ processes are less susceptible to thermal bleaching. The work by Warner et al. (1999) and Takahashi et al. (2004) indicate that bleaching susceptibility is determined by the rate of protein turnover of the photosystem II (PSII) D1 protein and efficiency of the photosynthesis repair machinery. Conversely, thermally sensitive species have been shown to exhibit higher levels of antioxidant enzyme activities than thermally tolerant species (Yakovleva et al. 2004). Thus maintaining cellular homeostasis during thermal stress can incur high metabolic costs and divert resources away from growth and reproduction (Hawkins 1991). Recent findings by Grottoli et al. (2006) indicated that coral species that were capable of increasing their heterotrophic carbon input recovered faster from bleaching than species that remained largely dependent on photosynthate translocates. In healthy corals, photosynthetically fixed carbon is translocated from the zooxanthellae to the coral host, covering up to 100% of the host's daily energy requirements (e.g. Falkowski et al. 1984), while substantial amounts of the host's nitrogen are derived from heterotrophic sources (Cook et al. 1994; Anthony and Fabricius 2000; Fitt and Cook 2001). It is well established that under steady-state conditions, food availability tends to increase protein levels of host tissue, and chlorophyll concentrations of zooxanthellae (Titlyanov et al. 2001; Ferrier-Pages et al. 2003; Houlbrequé et al. 2003, 2004). Furthermore, Piniak et al. (2003) have demonstrated that zooxanthellae can take up zooplankton nitrogen digested within the coelenteron directly, which underscores the importance of a heterotrophic diet as a direct source of nitrogen to the endosymbiont. The heterotrophic abilities of corals can vary considerably between reefal habitats due to factors including light (Ferrier-Pages 1998; Titlyanov et al. 2000), water temperature (Palardy et al. 2005), ambient zooplankton abundance (Ferrier-Pages et al. 2003; Palardy et al. 2006) and concentrations of suspended particulate matter (Anthony and Fabricius 2000). Furthermore, factors such as water flow (Sebens and Johnson 1991; Sebens et al. 1998, 2003) and escape behaviour of zooplankters (Sebens et al. 1996) may create high levels of small-scale variability of available food among individual colonies, which may contribute to the often observed conspecific variation in bleaching susceptibility and severity. Feeding may thus present an important, yet so far overlooked, factor that contributes to differential bleaching susceptibilities not only between but also within species.

The rationale of this study was to test the prediction that exogenous food increases the thermotolerance of zooxanthellae photosynthesis through the provision of additional resources required for metabolic processes involved in protein synthesis or repair and the photoprotective mechanisms of the holobiont (coral-symbiont association). The effect of zooplankton feeding on the photosynthetic activity of PSII of zooxanthellae in the coral *Stylophora pistillata*, a thermally sensitive species (Yakovleva et al. 2004), in response to thermal stress (daily temperature rises of 2–3°C) over a period of 10 days was investigated experimentally employing pulse-amplitude-modulated (PAM) fluorometry. Specifically the following hypotheses were tested:

1. PSII of zooxanthellae in fed corals will maintain higher photosynthetic efficiency during prolonged exposure to elevated temperatures than PSII of zooxanthellae in starved corals.
2. Zooxanthellae in fed corals will retain higher levels of NPQ during prolonged exposure to elevated temperatures than zooxanthellae in starved corals.
3. Fed corals will have higher zooxanthellae densities, higher mitotic indices and higher chlorophyll *a* and *c*<sub>2</sub> concentrations per algal cell than zooxanthellae in starved corals following exposure to thermal stress.

## Materials and methods

This study was carried out at Hasanuddin University Marine Field Station on Barang Lompo Island (05°03' S, 119°19' E; Spermonde Archipelago, southwest Sulawesi) during the dry season in July–August 2005. Feeding experiments using the scleractinian coral *S. pistillata* were conducted outdoors under natural sunlight. For logistical reasons experiments were run in three consecutive, independent repeat trials. Each trial was run for 10 days. Coral samples were collected on the fringing reef around Barang Lompo at a depth of 3 m. For each trial two spatially distant (>30 m) parent colonies (brown colour morphs), assumed to be genetically distinct, were randomly selected. In order to minimize a bias effect of feeding treatment on the photophysiological responses due to genotype differences as the number of replicates in each trial was small ( $n = 2$ , one per colony), we used fragments from the same parent colony for each treatment. From each of the two colonies, three terminal branches (surface area approximately 25–30 cm<sup>2</sup>) were cut, placed into dark containers and transported to the field station within 30 min of collection. One fragment of each colony was frozen immediately at –20°C and used as the reference coral in later tissue analysis.

## Coral maintenance and experimental design

The four remaining fragments were glued to PVC nuts using non-toxic, two-component underwater epoxy, and mounted on PVC screws attached to perspex blocks. Each individual fragment was placed into one of four transparent 14-l plastic tanks. The tanks were supplied independently with oligotrophic ( $<0.3 \mu\text{M}$  total inorganic nitrogen), filtered ( $>0.5 \mu\text{m}$ ) seawater ( $0.45 \text{ l min}^{-1}$ ), and aerated continuously. Acrylic covers reduced contamination with airborne particulates and ultraviolet radiation. Each tank was covered with black plastic netting (1 mm mesh) to attain an irradiance regime similar to in situ conditions at 3 m depth (maximum  $500\text{--}600 \mu\text{mol m}^{-2} \text{ s}^{-1}$  under cloudless conditions). Seawater was pumped from a depth of 30 m into an intermediate reservoir, from where it was subsequently pumped into the experimental tanks. The water temperature in the reservoir and tanks increased from  $28\text{--}29^\circ\text{C}$  (temperature in situ) just after sunrise to  $32.5 \pm 0.05^\circ\text{C}$  ( $n = 120$ ; mean  $\pm$  SE) at 1200 hours following the solar zenith [maximum photosynthetically active radiation (PAR)  $572 \pm 5 \mu\text{mol m}^{-2} \text{ s}^{-1}$ ;  $n = 120$ ; mean  $\pm$  SE] and decreased slowly with decreasing irradiance back to in situ temperatures at  $\sim 1900$  hours. Water temperature and light in each tank were recorded hourly between 0500 and 2100 h and 0600–1800 hours, respectively. Temperature was measured using digital thermometers (accuracy  $\pm 0.1^\circ\text{C}$ ) and light levels (PAR) were measured with the fibre quantum sensor of the Diving-PAM fluorometer (Diving-PAM, Walz, Effeltrich, Germany;  $\mu\text{mol quanta m}^{-2} \text{ s}^{-1}$ , calibrated against a LI-192SA quantum sensor). Salinity was recorded daily and tanks were cleaned several times per week to prevent algal growth.

## Feeding experiments

Corals were acclimated to experimental conditions for 2 days in unfiltered seawater at subdued light ( $\sim 400 \mu\text{mol m}^{-2} \text{ s}^{-1}$ ) with maximum daily temperatures  $<30^\circ\text{C}$ . No changes in mucus production and tissue coloration were noticed and all corals displayed full polyp extension at night. Water supply was switched to filtered seawater 12 h prior to the start of the experiment, allowing sufficient time for food digestion (Rossi et al. 2004). One fragment of each colony was assigned to one of the following treatments: (1) starvation—corals were kept in filtered seawater, deprived of organic particles  $>0.5 \mu\text{m}$ ; (2) feeding—corals were provided daily with freshly hatched *Artemia salina* nauplii ( $1,070 \pm 110$  individuals  $\text{l}^{-1}$ ), and were allowed to feed for 3 h. During each feeding bout the water flow was discontinued in all tanks, while aeration was maintained, to keep the water well oxygenated and zooplankton in circulation (Sebens and Johnson 1991). At the

end of each feeding period, the tanks of both fed and starved corals were rinsed and the water flow continued. At the end of each trial corals were frozen at  $-20^\circ\text{C}$  pending tissue analyses.

## Fluorescence measurements

Photophysiological responses of zooxanthellae were assessed by chlorophyll fluorescence analyses using a pulse amplitude-modulated fluorometer (Diving-PAM) inside the tanks. All corals were dark adapted prior to each measurement. Hoegh-Guldberg and Jones (1999) showed that most changes in dark-adapted quantum yield of *S. pistillata* occurred after 10 min. We tested the change in quantum yield following different periods of dark adaptation (10–20 min) prior to the start of the experiment and found that a 15-min period of dark adaptation was suitable for subsequent measurements of dark-adapted quantum yield. To account for a decrease in chlorophyll fluorescence of some fragments due to the loss of pigmentation over the course of 10 days, all measurements were carried out using high instrument settings for both measuring light intensity and electronic signal gain. Due to localized differences in photoacclimation and small-scale variability of the zooxanthellae within the host tissue (Levy et al. 2004), changes in minimal ( $F_o$ ) and maximal fluorescence ( $F_m$ ) yields relevant to photoinhibitory processes can be only interpreted meaningfully if repeated measurements are carried out at exactly the same location. This, however, can lead to localized bleaching (personal observation). To avoid excessive exposure to high light and associated development of photolesions, all measurements were conducted in 2-day intervals. The fibre optic probe of the PAM was clipped to the upper region of the fragment (1 cm below the tip of the branch) using a darkening adapter and modified dark leaf clip (Diving-PAM accessories) while the position of the clip on the coral was marked by three bearing points on the perspex blocks and side of the tanks.

The effect of feeding treatment on the activity of PSII and the NPQ kinetics of *S. pistillata* was explored in two different ways. Firstly, the changes in maximum potential quantum yield [ $F_v/F_m = (F_m - F_o)/F_m$ ], a proxy for the photosynthetic efficiency of PSII, over time were recorded consistently between 1000 and 1100 hours at an average temperature of  $31.2 \pm 0.05^\circ\text{C}$  ( $n = 120$ ; mean  $\pm$  SE) and average light intensity of  $345 \pm 0.05 \mu\text{mol m}^{-2} \text{ s}^{-1}$  ( $n = 120$ ; mean  $\pm$  SE), just before the onset of photoinhibition (established in prior measurements). Each fragment was pulsed with a weak ( $<1 \mu\text{mol m}^{-2} \text{ s}^{-1}$ ) red light to obtain  $F_o$ , followed by a 1-s pulse of saturating actinic light ( $>5,000 \mu\text{mol m}^{-2} \text{ s}^{-1}$ ) to determine  $F_m$ .  $F_v/F_m$  was calculated in the conventional manner as  $F_m - F_o/F_m = F_v/F_m$  (Schreiber 2004).

Secondly, nocturnal recovery of the photosynthetic apparatus from daily exposure to elevated temperatures of 2–3°C above temperatures in situ was determined before dawn (0500–0600 hours) following a 10-h recovery period under dim light ( $\sim 5 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) and in situ temperature (29°C).  $F_o$ ,  $F_m$  and  $F_v/F_m$  were assessed as above. Followed by a 40-s period of darkness, each fragment was exposed to saturating light for 4 min ( $1,000 \mu\text{mol m}^{-2} \text{s}^{-1}$  PAR), after which the fluorescence had reached steady state. The minimum and maximum fluorescence ( $F'$  and  $F_m'$  of light-adapted samples) were recorded and the effective quantum yield determined as the ratio  $\Delta F/F_m'$  where  $\Delta F = F_m' - F'$ . The relative electron transport rates (rETR) of PS II were then calculated as  $\text{rETR} = \Delta F/F_m' \times \text{PAR} \times 0.5$  (Hoegh-Guldberg and Jones 1999) and NPQ as  $\text{NPQ} = F_m - F_m'/F_m'$  (Schreiber 2004).

### Biomass analysis

Coral tissue was stripped from the skeletons under dim light using an airbrush gun and a phosphate buffered saline solution (Sigma Aldrich). The slurry ( $\sim 100$  ml) was homogenized in a hand potter and subsamples of 20 ml frozen for chlorophyll *a* and  $c_2$  analyses. A 10-ml aliquot of the homogenate was preserved in 4% formalin and used to determine zooxanthellae densities (eight replicate counts) using a Neubauer haemocytometer. The mitotic indices were determined from the number of cells appearing as doublets in two samples of 1,000 cells (Jones and Yellowlees 1997). Chlorophyll *a* and  $c_2$  were extracted as described by Gardella and Edmunds (1999) and concentrations calculated according to the equation of Jeffrey and Humphrey (1975). Zooxanthellae densities were normalized to surface area ( $\text{cells cm}^{-2}$ ) and chlorophyll concentrations expressed as micrograms ( $10^6 \text{ cells}^{-1}$ ). The surface area of the coral skeletons were determined photometrically using the dye-dipping method of Hoegh-Guldberg (1988).

### Data analyses

All data were checked for homogeneity of variances using Cochran's *C*-test and, if necessary,  $\ln(x)$  transformed. The variances of maximum temperatures and light intensities among tanks were analysed by a repeated measurements two-way ANOVA (Sokal and Rohlf 1995) using WinGMAV (EICC, University of Sydney, Australia). The data variances of both temperature and light were heterogeneous and failed to stabilize. However, since the data set was large ( $n = 120$ ) and the analysis revealed no significant differences in daily maximum temperature and light intensity between individual tanks, the risk of committing a type I error was eliminated

(Underwood 1997), and the analysis thus regarded as reliable. The effect of feeding versus starvation on changes in the fluorescence responses of zooxanthellae in *S. pistillata* and biomass characteristics after 10 days were analysed using a general linear model with a three-factorial nested design and post-hoc Student Newman Keuls testing using Statistica.

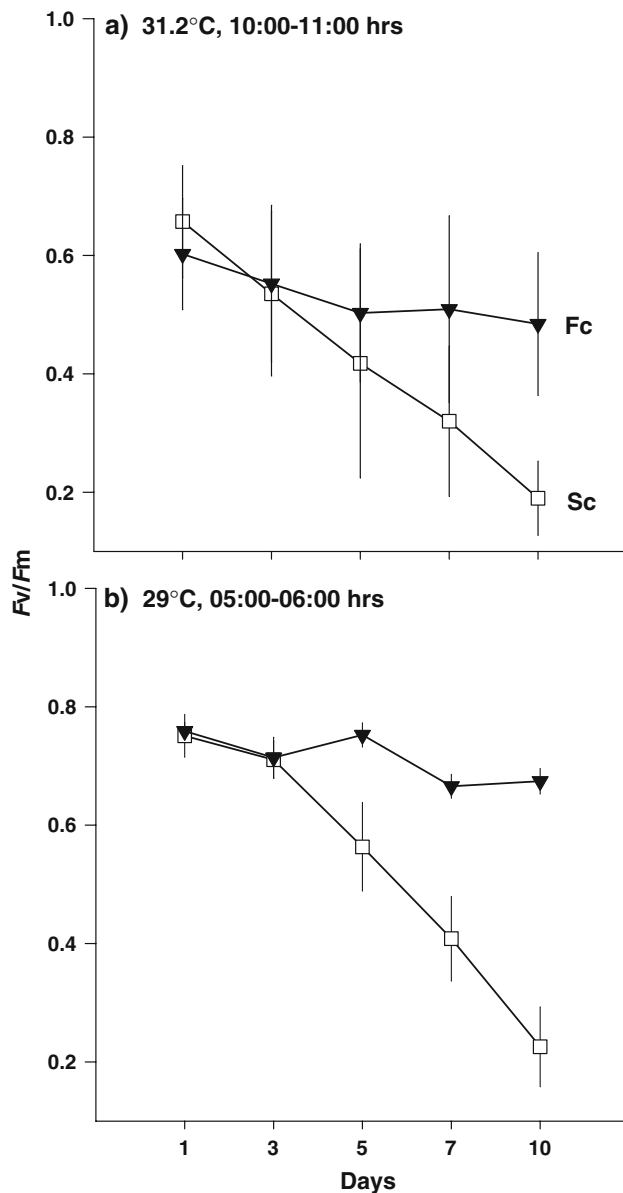
### Results

As would be expected, daily maximum temperatures ( $F_{2,108} = 26.28$ ,  $P = 0.001$ ) and light intensities ( $F_{2,108} = 39.44$ ,  $P = 0.001$ ) of experimental tanks varied significantly among trials. However there was no significant variation in temperature ( $F_{3,108} = 0.39$ ,  $P = 0.75$ ) or light ( $F_{3,108} = 2.01$ ,  $P = 0.11$ ) regime among tanks within each trial.

### Effect of feeding on zooxanthellae chlorophyll fluorescence

There were no significant differences in the effect of feeding treatment between trials and colonies and no significant interaction was detected between trial and treatment (Figs. 1, 2). After 10 days,  $F_v/F_m$  values measured between 1000 and 1100 hours were significantly lower for starved than for fed corals (Fig. 1a). Starvation resulted in a marked decrease in  $F_v/F_m$ , with values dropping from  $\sim 0.6$  at day 1 to  $\sim 0.2$  at day 10, while  $F_v/F_m$  for fed corals did not decrease below  $\sim 0.5$ . Pre-dawn values of  $F_v/F_m$  were similar for starved and fed corals during the first 3 days of the experiment, indicating high rates of nocturnal recovery of the photosynthetic apparatus in all corals. Thereafter,  $F_v/F_m$  values of starved corals declined monotonically, resulting in a  $\sim 70\%$  decrease relative to  $F_v/F_m$  of fed corals, which remained relatively constant for 10 days (Fig. 1b). At day 10  $F_v/F_m$  was significantly lower for starved than for fed corals (Fig. 1b).

Values measured for  $\Delta F/F_m'$  before dawn decreased markedly over time regardless of treatment (Fig. 2a). However, while  $\Delta F/F_m'$  of starved corals declined from  $\sim 0.35$  to almost 0 over the course of 10 days,  $\Delta F/F_m'$  for fed corals declined by only 50%, resulting in a significant difference between fed and starved corals at day 10 (Fig. 2a). Similarly, rETRs of both fed and starved corals declined by an order of magnitude over time (Fig. 2b). At the end of each trial rETRs of starved corals, however, were significantly lower than rETRs of fed corals (Fig. 2b). Feeding significantly increased the coral's ability to engage in NPQ (Fig. 2c). Fed corals sustained high NPQ whereas starved corals displayed a progressive inability to engage in NPQ, culminating in a  $\sim 80\%$  decline in NPQ by day 10 relative to fed corals (Fig. 2c).



**Fig. 1** Maximum potential quantum yield ( $F_v/F_m$ ) of zooxanthellae in dark-adapted *Stylophora pistillata* at **a**  $31.2 \pm 0.05^\circ\text{C}$  between 1000 and 1100 hours, and **b** before dawn (0500–0600 hours) at  $\sim 29^\circ\text{C}$  after daily exposure to elevated temperature for 10 days in three repeat trials. Corals were exposed to either feeding (Fc) or starvation (Sc). Shown are means ( $n = 6$ ) and 95% confidence limits. Effects of feeding treatment on  $F_v/F_m$  after 10 days were significant for **a** 1000–1100 hours ( $F_{1,3} = 461.08$ ,  $P = 0.002$ ) and **b** 0500–0600 hours ( $F_{1,3} = 117.26$ ,  $P = 0.008$ ). There was no effect of colony and trial (1000–1100 hours,  $F_{3,3} = 2.84$ ,  $P = 0.20$ ; 0500–0600 hours,  $F_{3,3} = 0.77$ ,  $P = 0.58$ ) and no significant trial by treatment interactions (1000–1100 hours,  $F_{2,3} = 0.38$ ,  $P = 0.70$ ; 0500–0600 hours,  $F_{2,3} = 1.12$ ,  $P = 0.43$ )

#### Effect of feeding on zooxanthellae densities, mitotic indices and chlorophyll concentrations

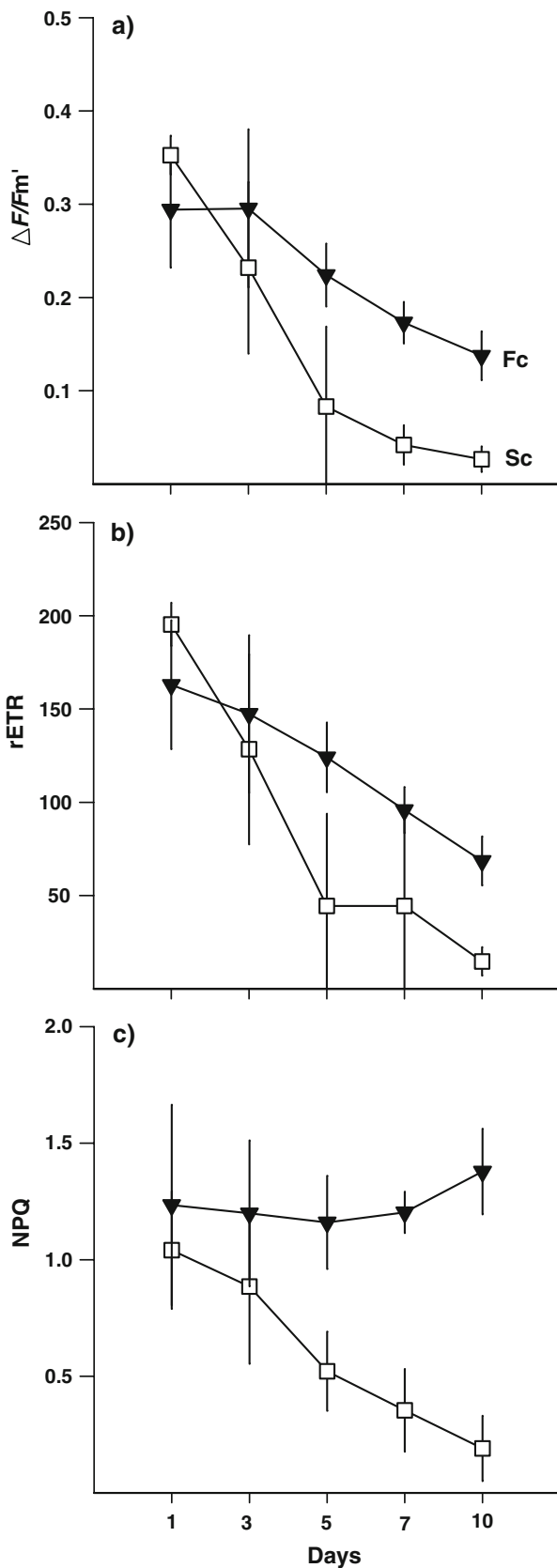
For zooxanthellae densities and mitotic indices there were no significant effects of feeding treatment between trials or

colony, nor was there an interaction between trial and treatment (Fig. 3). Zooxanthellae densities were significantly lower in both fed and starved corals than in reference corals. Although the zooxanthellae densities in fed corals were about 40% higher than zooxanthellae densities in starved corals, this difference was not statistically significant (Fig. 3). By contrast, the mitotic indices of zooxanthellae in fed corals were significantly higher than in both starved and reference corals, but there were no significant differences between starved and reference corals (Fig. 4). Significant trial by treatment interactions were detected for both chlorophyll  $a$  and  $c_2$ , which makes it difficult to evaluate an effect of feeding treatment. Post hoc analyses still distinguished the following differences for chlorophyll  $a$ —trial 1 and 2, reference = feeding = starvation; trial 3, reference = feeding < starvation and for chlorophyll  $c_2$ —trial 1, reference > feeding = starvation; trial 2, starvation > reference = feeding; trial 3, reference = feeding = starvation.

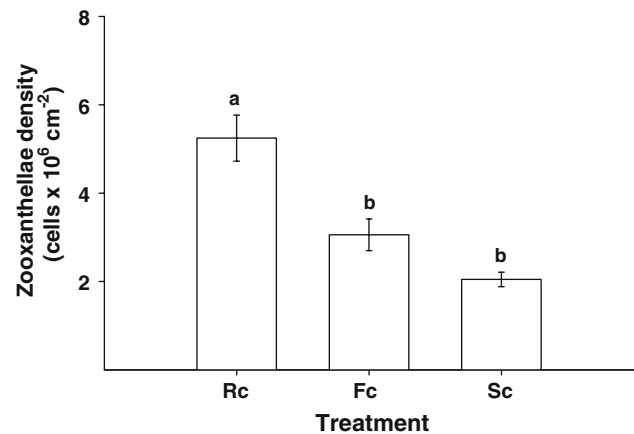
#### Discussion

This is the first experimental study to examine the thermal tolerance of corals in response to exogenous food using chlorophyll fluorescence as a proxy for the photosynthetic activity of PSII of zooxanthellae in *S. pistillata*. Overall our results show a strong correlation between the absences of external food, a decrease in the thermal tolerance of PSII functioning and NPQ processes. As predicted, zooxanthellae in fed corals maintained high photosynthetic efficiency of PSII while starved corals displayed strong signs of chronic photoinhibition. This was reflected in the significant decline in  $F_v/F_m$  values between 1000 and 1100 hours and the concomitant decrease in pre-dawn values for  $F_v/F_m$ ,  $\Delta F/F_m'$ , rETR. This pattern is consistent with previous conclusions (Jones et al. 1998; Jones and Hoegh-Guldberg 2001; Franklin et al. 2004), that photoprotective downregulation of photosynthesis culminates in chronic photoinhibition if nocturnal recovery is incomplete. Although zooxanthellae in fed corals also exhibited signs of loss in overall photosynthetic activity, the high levels of NPQ over time indicate that fed corals sustained the ability to dissipate excess excitation energy as heat, which may have prevented the photoinactivation and photodamage to PSII (Warner 1996, 1999) by reducing the generation of reactive oxygen species (Demmig-Adams and Adams 2002).

Zooxanthellae are believed to utilize and recycle ammonium produced by host catabolism as a nitrogen source (e.g. Falkowski et al. 1993). However, this internal recycling of ammonium cannot proceed indefinitely without “new” input from the outside, and the relative importance of heterotrophy as a means of nitrogen acquisition for cnidarian

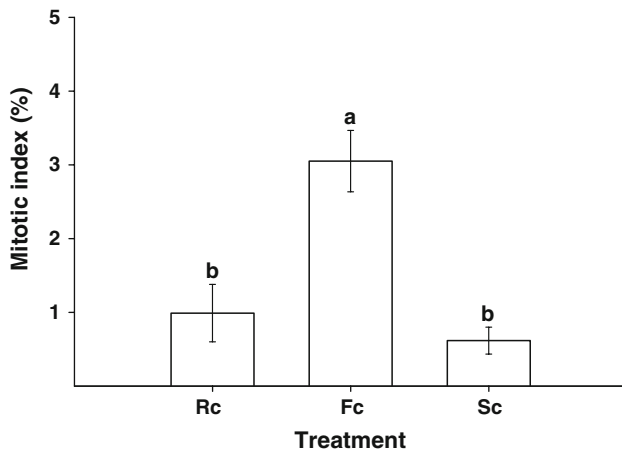


**Fig. 2** *Stylophora pistillata*. Changes in **a** effective quantum yield ( $\Delta F/F_m'$ ), **b** relative electron transport rate (rETR), and **c** non-photochemical quenching (NPQ) of corals before dawn (0500–0600 hours) following 4 min exposure to  $>1,000 \mu\text{mol m}^{-2} \text{s}^{-1}$  over 10 days in three repeat trials. Corals were subjected to either Fc or Sc. Shown are means ( $n = 6$ ) and 95% confidence limits. Effects of feeding treatment after 10 days were significant for **a**  $\Delta F/F_m'$  ( $F_{1,3} = 1,588.77$ ,  $P = 0.000$ ), **b** rETR ( $F_{1,3} = 1,274.60$ ,  $P = 0.000$ ), and **c** NPQ ( $F_{1,3} = 20.68$ ,  $P = 0.04$ ). There was no effect of colony and trial ( $\Delta F/F_m'$ ,  $F_{3,3} = 0.374$ ,  $P = 0.77$ ; rETR,  $F_{3,3} = 0.377$ ,  $P = 0.77$ ; NPQ,  $F_{3,3} = 0.376$ ,  $P = 0.77$ ) and no significant trial by treatment interactions ( $\Delta F/F_m'$ ,  $F_{2,3} = 0.014$ ,  $P = 0.98$ ; rETR,  $F_{2,3} = 0.015$ ,  $P = 0.98$ ; NPQ,  $F_{2,3} = 2.85$ ,  $P = 0.20$ ). For other abbreviations, see Fig. 1



**Fig. 3** Zooxanthellae densities (cells  $\times 10^6 \text{ cm}^{-2}$ ) of *S. pistillata* in reference corals (Rc) and corals after 10 days of exposure to elevated temperature and subjected to either Fc or Sc in three repeat trials (mean  $\pm$  SE,  $n = 6$ ). Effects of feeding treatment were significant ( $F_{2,6} = 78.31$ ,  $P = 0.000$ ). There was no effect of colony and trial ( $F_{3,6} = 2.24$ ,  $P = 0.18$ ) and no significant trial by treatment interactions ( $F_{4,6} = 0.24$ ,  $P = 0.90$ ). Bars that share the same letters are not significantly different [Student Newman Keuls (SNK),  $P < 0.05$ ]. For other abbreviations, see Fig. 1

zooxanthellae has been well documented (McAuley and Cook 1994; Fitt and Cook 2001; Piniak et al. 2003). Host starvation is thought to reduce ammonium assimilation in the zooxanthellae as well as photosynthetic rates, and has been shown to increase cell degradation and reduce cell division and numbers (Fitt and Cook 2001; Titlyanov et al. 2000, 2001). Corals typically maintain high levels of lipids derived from excess photosynthetically fixed carbon and receive substantial amounts of essential fatty acids (Harland et al. 1993), amino acids (Wang and Douglas 1999) and mycosporine-like amino acids (Shick et al. 2005) from their zooxanthellae. In addition, the coral host is believed to obtain essential nutrients for tissue synthesis from heterotrophic sources (Dubinsky and Jokiel 1994; Mueller-Parker et al. 1994; Anthony et al. 2002). Under bleaching conditions where the photosynthetic efficiency of



**Fig. 4** Mitotic indices (%) of zooxanthellae of *S. pistillata* in Rc and corals after 10 days of exposure to elevated temperature and subjected to either Fc or Sc in three repeat trials (mean  $\pm$  SE,  $n = 6$ ). Effects of feeding treatment were significant ( $F_{2,6} = 13.90$ ,  $P = 0.01$ ). There was no effect of colony and trial ( $F_{3,6} = 0.74$ ,  $P = 0.56$ ) and no significant trial by treatment interactions ( $F_{4,6} = 1.25$ ,  $P = 0.38$ ). Bars that share the same letters are not significantly different (SNK,  $P < 0.05$ ). For other abbreviations, see Figs. 1 and 3

zooxanthellae is greatly reduced (Jones et al. 2000; Warner et al. 2002), trophic interactions of the symbiotic association may be therefore uncoupled, resulting in changes in the biochemical composition (Szmant-Froelich 1981; McAuley and Cook 1994) and energy balance of the coral colony (Clayton and Lasker 1984). Thus, in the absence of external food, the temperature tolerance of corals may be lowered as additional stressors such as high temperature are likely to further constrain physiological processes due to increased metabolic costs (Calow 1989; Koehn and Bayne 1989). Unfortunately our results provide no indication of the underlying biochemical mechanisms of how additional resources could have modulated the thermal resistance of the photosynthetic apparatus. We can only speculate that zooplankton may provide a direct source of nitrogen to the zooxanthellae, facilitating enhanced rates of protein repair and re-synthesis of the PSII D1 protein (e.g. Ohad et al. 1984; Warner et al. 1999; Takahashi et al. 2004, but see Smith et al. 2005 for review) or reduce photophysiological damage of the zooxanthellae indirectly by enhancing the capacity of either symbiotic partners to synthesize antioxidant compounds or heat-shock proteins or both (e.g. Downs et al. 2002; Yakovleva et al. 2004).

Contrary to our prediction that fed corals would be less susceptible to bleaching than starved corals, there was no clear correlation between feeding regime, zooxanthellae densities and chlorophyll concentrations. High rates of cell division as were observed for fed corals can be characteristic for the recovery of *in hospite* zooxanthellae populations due to increased space and nutrient availability (Suharsono

and Brown 1992; Jones and Yellowlees 1997). Considering that zooxanthellae numbers in fed corals had decreased by 50% compared to reference corals but displayed rates of cell division that were 3 times as high as in reference corals, the high mitotic indices in fed corals appear odd. The most parsimonious explanation for this observation would be that zooxanthellae of fed corals were dividing at a faster rate after they had been released into the coelenteron compared to those remaining in the tissue (Suharsono and Brown 1992). This suggests that zooxanthellae were released from their hosts at similar rates regardless of feeding treatment, but that zooxanthellae in fed corals were healthier than those expelled from starved corals. Thus zooxanthellae in starved corals may have suffered cellular degradation as has been reported for zooxanthellae in corals during thermal stress in previous studies by Dunn et al. (2002) and Franklin et al. (2004). The low mitotic indices in reference corals, by contrast, are consistent with values reported for healthy *S. pistillata* (Muscatine et al. 1984).

An experimental period of 10 days may have been insufficient to determine clear treatment-dependent changes in zooxanthellae densities and chlorophyll content. Also the water temperatures of the experimental protocol in this study did not entirely match increases of seawater temperatures during natural bleaching events, which commonly feature less diel fluctuations exceeding periods of 10 days (e.g. Gleeson and Strong 1995). The combined results of this study, however, provide an indication that exogenous food can play an important role in reducing the photophysiological damage of zooxanthellae that typically leads to bleaching. This could present an interesting consideration as regards coral communities in turbid, near-shore waters which often provide a potentially rich heterotrophic environment and may therefore benefit some coral species under bleaching conditions (Anthony 2006; Anthony et al. 2007).

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