Coral Reefs (2012) 31:951–960
DOI 10.1007/s00338-012-0925-9

REPORT

Food availability promotes rapid recovery from thermal stress in a scleractinian coral

S. R. Connolly · M. A. Lopez-Yglesias · K. R. N. Anthony

Received: 19 February 2012 / Accepted: 5 June 2012 / Published online: 20 June 2012

Abstract Bleaching in corals due to environmental stress represents a loss of energy intake often leading to an increase in mortality risk. Successful coral recovery from severe bleaching events may depend on the rate of replenishment of algal symbiont populations following the period of thermal stress, the supply of an alternative food source, or both. Here, we explore the role of food availability in promoting the survival and recovery of a common coral (*Acropora intermedia*) following acute experimentally induced thermal stress. Fed corals were provided with live rotifers daily, to maintain densities of zooplankton in tanks that are typical of coral reefs. After a 6-week acclimation phase, heated corals were subjected to a +4 °C thermal anomaly for a 7-day period (bleaching phase) then temperatures were returned to normal for a further 2 weeks (recovery phase). Results demonstrated that heated corals had higher survival when they were provided with heterotrophic food. Fed corals experienced reduced loss of chlorophyll *a*, relative to unfed corals. During the recovery phase, both fed and unfed corals recovered within a few days; however, fed corals recovered to pre-bleaching phase levels of chlorophyll *a*, whereas unfed corals stabilized approximately one-third below this level. Protein levels of fed corals declined markedly during the bleaching phase, but recovered all of their losses by the end of the recovery phase. In contrast, unfed corals had low protein levels that were maintained throughout the experiment. To the extent that these results are representative of corals' responses to thermal anomalies in nature, the findings imply that availability of particulate food matter has the potential to increase corals' capacity to survive thermally induced bleaching and to ameliorate its sub-lethal effects. They also support the hypothesis that different rates of heterotrophy are an important determinant of variation in resilience to thermal stress among reef environments.

Keywords Coral bleaching · Recovery · Heterotrophy · Phototrophy · Nutrients

Communicated by Biology Editor Dr. Anastazia Banaszak

S. R. Connolly (✉) · M. A. Lopez-Yglesias · K. R. N. Anthony
School of Marine and Tropical Biology, James Cook University, Townsville, QLD 4811, Australia
e-mail: sean.connolly@jcu.edu.au

S. R. Connolly
ARC Centre of Excellence for Coral Reef Studies, James Cook University, Townsville, QLD 4811, Australia

Present Address:
K. R. N. Anthony
Australian Institute of Marine Science, Townsville, QLD 4810, Australia

Introduction

Coral bleaching is the loss of symbiotic dinoflagellates from the coral tissue and/or the loss of photosynthetic pigments from the zooxanthellae (Jones 1997). An important consequence of bleaching is reduced photosynthetic capacity (Warner et al. 1996; Jones et al. 2000), potentially leading to a negative energy balance (Nordemar et al. 2003; Anthony et al. 2009). Indeed, levels of remaining energy during and following a bleaching episode are important determinants of mortality risk (Anthony et al. 2007). Moreover, the depletion of energy levels during bleaching events can be long-lasting (e.g., up to 8 months in *Porites compressa*: Rodrigues and Grottoli 2007). This suggests that bleaching may influence subsequent growth and reproductive output, as well as eroding the capacity to survive subsequent bleaching events

(Ward et al. 2000; Baird and Marshall 2002). Thus, the factors that determine the extent of depletion of energy reserves during bleaching events, and the rate of recovery, afterwards, are likely to be critical determinants of the longer-term demographic consequences of changes in the frequency or intensity of bleaching events (Grottoli et al. 2006; Anthony et al. 2009).

Under normal (non-bleaching) conditions, coral symbionts can contribute up to 90 % to the energy balance of the symbiosis (Muscatine 1990). Consequently, the extent of loss and rate of recovery of the symbiont population have important implications for the depletion and replenishment of energy stores of the holobiont. If the extent of the reduction in the symbiont population is large, or if the symbiont population recovers slowly in the aftermath of bleaching, then the restoration of positive energy balance will be impaired. During bleaching, the decline in the photosynthetic capacity of fed corals is often smaller than in unfed corals (Borell and Bischof 2008; Ferrier-Pagès et al. 2010), due at least partly to translocation of heterotrophically acquired carbon to symbionts (Hughes et al. 2010; Tolosa et al. 2011) and greater transfer of limiting nutrients between coral host and symbionts (Anthony et al. 2007). The potential for heterotrophy to promote symbiont recovery rates is less well understood. However, because coral symbioses are generally nutrient-limited (Dubinsky and Jokiel 1994; Muller-Parker et al. 1994; Hoegh-Guldberg 1994), symbiont recovery rate is likely to be a function of the availability of nutrients, and heterotrophy provides one avenue for nutrient uptake (Houlbrèque and Ferrier-Pagès 2009).

Here, we investigated the hypothesis that food availability promotes survival and recovery of thermally bleached corals. In particular, we asked whether corals that had access to a heterotrophic source of nutrition exhibited higher survival during an acute thermal stress event, compared to corals that did not have access to heterotrophic food. We also assessed how food regime influenced the dynamics of symbiont chlorophyll and holobiont protein levels. Specifically, we tested whether, for fed corals, chlorophyll and protein levels (1) had higher baseline levels prior to the onset of thermal stress, (2) declined less during the period of thermal stress, and (3) recovered to higher levels after cessation of thermal stress, compared to unfed corals.

Materials and methods

Study species

We used the branching coral *Acropora intermedia* (Brook, 1891) as our study species. The genus *Acropora* is a ubiquitous constituent of shallow-water reef communities in the Indo-Pacific (Wallace 1999) and is well represented across the continental shelf in the Great Barrier Reef (Veron 2000; DeVantier et al. 2006). Also, most species of *Acropora* are susceptible to bleaching (Marshall and Baird 2000). For example, during the 1998-bleaching event on the Great Barrier Reef, 32–38 % of *Acropora* corals were severely bleached and mortality reached up to 88 % (Baird and Marshall 2002). Despite the large role of *Acropora* species in mass bleaching events throughout much of the Indo-Pacific, they have received relatively little attention in studies of the energetics of bleaching (e.g., Borell and Bischof 2008; Hughes et al. 2010; Tolosa et al. 2011). *A. intermedia* in particular is common in shallow fore-reefs and lagoons in the Indo-Pacific (Veron 2000). It is often a dominant in the community, particularly in lagoonal habitats, where it can provide much of the structural complexity. Thus, its response to thermal bleaching has important implications for ecosystem functioning in these habitats.

Coral collecting and experimental set-up

In November 2004, approximately 500 experimental coral branches (6–8 cm long) were collected from 5 to 8 m depth on the reef slope at the south-eastern end of Pelorus Island, located in the central Great Barrier Reef region (18°33′S, 146°28′E). Because *A. intermedia* is often subjected to fragmentation by physical disturbances, we collected branches over a relatively large area (approximately 200 m × 400 m). Coral branches were then allocated haphazardly to experimental treatments, to minimize any potential artefacts due to genotype-specific responses. The branches were transported while submerged in seawater to aquarium facilities on the nearby Orpheus Island Research Station (OIRS). The corals were distributed among eight experimental tanks (1 m diameter, 30 cm deep) supplied with filtered (<5 μm) running seawater pumped directly from the reef. To minimize the risk of bacterial infections through contact with sediment or tank surfaces, each coral branch was suspended by a thin monofilament nylon line attached to a grid above the tanks. The experiment was divided into three phases: (1) acclimation (6 weeks), (2) bleaching (7 days), and (3) recovery (16 days). At the outset of the acclimation phase, the experimental population was divided into two feeding groups of four tanks each: fed and unfed. The purpose of this was to establish coral groups with two contrasting nutritional states prior to the onset of the experimental bleaching event. The fed group was provided cultures of live rotifers of the genus *Brachionus sp.* enriched with DHA Protein Selco (INVE Aquaculture, Belgium). Corals were fed daily throughout all phases of the experiment. Amounts provided on each

day varied somewhat, due to variation in population growth rates within rotifer cultures and the need to maintain the cultures for the duration of the experiment. Consequently, rotifer concentrations in the tanks varied between 350 and 2,260 l^{-1} (1,316 ± 586 l^{-1} [mean ± SD]), which encompasses the range of zooplankters observed on coral reefs (Roman et al. 1990; Sebens et al. 1998).

During the acclimation phase, the water temperature in all tanks was kept at 27.0 °C, controlled using chiller/heater units (Carrier Systems, Australia), with an accuracy of ~ 0.1 °C. This baseline temperature corresponded to the values recorded at the field site at the time of collecting. The experimental light regime was provided by a metal halide lamp (400 watts, EYE, Japan), suspended 50 cm above each tank and set to a 12:12-h light:dark photoperiod. Throughout the study, irradiance values were kept at approximately 350 µmol photons m^{-2} s^{-1}, which was representative of average daytime field values. At the end of the acclimation phase, two tanks from each feeding treatment were assigned to a high-temperature treatment (31.0 °C), yielding an orthogonal design of two feeding treatments and two temperature groups, each with two tanks. At the end of the acclimation phase, two coral branches were sampled from each tank and frozen immediately on dry ice and kept in darkness at −40 °C for later analysis of chlorophyll *a* and protein. The purpose of these samples was to provide a baseline tissue composition at the onset of the bleaching phase. During the 7-day bleaching phase, two coral branches were extracted from each tank (i.e., 4 corals per treatment) at the beginning of this phase of the experiment, and every 2 days thereafter, frozen immediately on dry ice and kept in darkness at −40 °C for later analysis of tissue components. On the first day of the recovery phase, the high-temperature tanks were reset to 27.0 °C. To estimate rates of recovery of symbiont populations as well as the nutritional status of coral tissues, two branches per tank were sampled every 4 days, frozen and stored for later tissue analyses. After the first 2 weeks of the acclimation phase, coral branches were censused daily for the remainder of the experiment for survival analysis.

Chlorophyll analyses

We used the content of chlorophyll *a* per unit surface area of coral tissues as a measure of bleaching status (Brown 1997; Anthony et al. 2007). The density of chlorophyll *a* provides a useful proxy for photosynthetic capacity because maximum rate of photosynthesis, and thereby colony energetics, scales more closely with chlorophyll *a* than with cell density (Anthony et al. 2009). As a further check on the use of chlorophyll *a* as a measure of photosynthetic capacity, we measured Fv/Fm with a MINI PAM (Walz, Germany). Trends in Fv/Fm were qualitatively very similar to those of chlorophyll *a*, so we focus on the latter here. For chlorophyll *a* measurements, the tip of each coral branch sampled was discarded since tissue components are not evenly distributed along the branch and are usually lower at the tip (Gladfelter et al. 1989). The remainder of each branch was then divided into two central segments, one of which was used for analyses of chlorophyll *a* concentrations of coral tissue, and one for protein analysis. Branch segments used for chlorophyll *a* analyses were ground to a sandy paste using a mortar and a pestle and transferred to cold vials wrapped and capped with aluminium foil, containing approximately 20 ml of cold 100 % acetone. After 12 h at 4 °C, chlorophyll *a* was extracted twice from each vial in darkness and centrifuged at 2,000 rpm for 2 min. Duplicate absorbance readings were measured with a spectrophotometer (Heλios, Thermo Electron Corp.) at 630 and 663 nm, after which the total concentration of chlorophyll *a* was computed according to the formula of Jeffrey and Humphrey (1975).

Protein analyses

Protein content was used as an indicator of nutritional condition (Edmunds et al. 2003; Houlbrèque et al. 2003, 2004). Analyses were performed using standard procedures (e.g. see Leuzinger et al. 2003). Briefly, coral tissues were solubilized in 1 M NaOH at 90 °C for 60 min and then neutralized with hydrochloric acid. Protein standards were established using five volumes (i.e. 0, 1, 2, 3, and 4 ml) of bovine gamma globulin. A protein assay kit (type I) from Bio-Rad (Australia) was used as reagents. Duplicate absorbance readings were performed at 595 nm with a spectrophotometer (Heλios, Thermo Electron Corp.). The tissue surface area of each sample was measured using aluminium foil wrapping (Marsh 1970).

Statistical analysis

We adopted a model selection approach in our statistical analysis, the goal of which is to find the level of model complexity that optimizes the trade-off between precision (which tends to decrease as model complexity increases) and accuracy (which tends to increase with model complexity). Specifically, we conducted model selection using likelihood ratio tests to identify the best-fitting models for each of our analyses, in a backward selection procedure, beginning with the model including a random effect of tank, and including all main and interaction terms for fixed effects that were appropriate for that particular analysis (as specified below). Note that for models without the random effect, model selection by likelihood ratio tests was equivalent to dropping non-significant terms from the final model. All statistical analyses were conducted in R 2.13.1.

For coral survivorship, we used Cox proportional hazards (CPH) models to test for effects of food regime (FOOD: fed vs. unfed), temperature (TEMP: heated vs unheated), and an interaction (FOOD × TEMP), using models with and without a random effect of tank. CPH models impose no assumptions about how mortality rate varies over time. They instead characterize treatment effects as fixed proportional effects on mortality rate; that is, mortality rate in each treatment is always a fixed proportion of the mortality rate in a designated control treatment. Thus, a "significant" treatment effect means that the constant of proportionality for mortality rate in that treatment differs from unity with >95 % confidence. Models without the random effect were fitted using the package "survival" (Therneau and Lumley 2011), and those with the random effect were fitted using the package "coxme" (Therneau 2011), which supplements package "survival" by allowing random effects to be included in CPH models. We tested for violation of the proportional hazards assumption using analysis of scaled Schoenfeld residuals, as implemented in the function cox.zph in package "survival" (Therneau and Lumley 2011). Because corals were allocated to FOOD treatments from the beginning of the acclimation phase, but were not allocated to TEMP treatments until the end of the acclimation phase, we conducted two analyses: one analysis of the acclimation phase, where FOOD was the only fixed effect, and a second analysis of the bleaching and recovery phases, where both FOOD and TEMP were incorporated.

To analyse the decline and recovery of chlorophyll *a* and protein, we used linear mixed-effects models, analysing the bleaching and recovery phases separately. This separation of phases for analysis was necessary because of the strong directional changes—and thus nonlinearity—in the dynamics of chlorophyll *a* and protein over the course of the experiment. Our fixed effects were TIME (treated as a continuous variable), with FOOD and TEMP as categorical treatment variables. Tank was treated as a random effect. However, for the bleaching phase, it is important to note that initial levels of protein and chlorophyll *a* could be due to FOOD, but not TEMP, because corals were allocated to fed and unfed treatments from the beginning of the acclimation phase, but they were allocated to heated and unheated treatments only at the end of the acclimation phase. In order to capture this fact of experimental design in our fitted models, we scaled time to be zero at the end of the acclimation phase, and we fixed the TEMP and FOOD × TEMP effects to be zero in these analyses, but we allowed the interactions TEMP × TIME and FOOD × TEMP × TIME. Thus, initial protein and chlorophyll *a* levels could vary only according to FOOD, or random tank effects, but TEMP could affect the rate of change of chlorophyll *a* or protein over time. For the recovery phase, however, we allowed all main effects and interactions, because we expected the dynamics of our response variables to vary depending upon whether coral branches had been allocated to heated or unheated treatments. We also re-scaled time in the recovery phase analysis to be zero at the conclusion of the experiment, so that the terms omitting a TIME effect could be used to assess differences in the levels of chlorophyll *a* or protein at the end of the recovery phase. Models with random effects were fitted using the function "lme" in package nlme (Pinheiro et al. 2011), and those omitting random effects were fitted using function "lm" in the base R package. For the "lme" fits, we specified "method = ML", to ensure validity of likelihood ratio comparisons for fits using "lm" and "lme" (Pinheiro et al. 2011). We assessed overall goodness-of-fit of the best-fitting model using standard techniques (e.g., qq-plots, Shapiro tests for normality, etc.). Because time was modelled as a continuous, fixed effect, we also plotted autocorrelation functions for residuals, to assess whether there was any evidence of non-independence in residuals over time.

Results

For all survival analyses, there was no support for inclusion of a random tank effect ($\chi^2 \sim 0$; $P > 0.99$ in all cases), so we report results below for corals pooled across tanks within treatments. For the acclimation phase, there was no significant support for an effect of FOOD ($\chi^2 = 0.04$; $P = 0.85$), consistent with the lack of apparent differences in survival among the FOOD treatments (< 0 days in Fig. 1). For the combined analysis of the bleaching and recovery phases, the best-fitting model included effects of FOOD and TEMP, but no interaction term (Table 1). However, while the proportional hazards assumption was met for FOOD in this analysis ($\chi^2 = 0.175$; $P < 0.068$), it was strongly violated for temperature ($\chi^2 = 12.98$; $P < 0.001$). Inspection of scaled Schoenfeld residuals indicated that this was because treatment effects were initially small, and only became apparent by the end of the first week (roughly at the end of the bleaching phase). This can also be deduced from the survivorship curves themselves: they do not begin to separate until late in the bleaching phase (Fig. 1). Therefore, we re-analysed the data using only observations from the recovery phase (time > 7 days in Fig. 1). This approach yielded the same best-fitting model as the original analysis: significant effects of FOOD and TEMP, but no interaction (Table 1). In this case, the proportional hazards assumption was satisfied for both factors (FOOD: $\chi^2 = 1.23$; $P = 0.27$; TEMP: $\chi^2 \approx 0$, $P = 0.99$). Parameter estimates indicated that both effects were large in magnitude: mortality of

unfed corals was nearly 70 % higher than that of fed corals, while heating increased mortality rates more than fourfold (Table 1; Fig. 1).

When models were fitted using untransformed chlorophyll *a* (chl*a*), as the response (dependent) variable, we obtained residual variances that increased strongly with fitted values. However, residuals were well-behaved when chl*a* was log-transformed, so we conducted all chl*a* analyses using ln(chl*a*) as our response variable (Fig. 2a). All treatment groups commenced the bleaching phase with similar levels of chl*a*. Subsequently, the unheated treatments exhibited little systematic change in chl*a* over the course of the experiment, regardless of feeding level. Of the heated treatments, chl*a* of the fed corals declined during the bleaching phase, but recovered to pre-bleaching levels by the end of the recovery phase. In contrast, chl*a* of heated and unfed corals declined further during the bleaching phase and recovered quickly, but stabilized at a lower level, compared to their fed counterparts. These qualitative patterns were reflected in the linear mixed-effects model analysis (Table 2). For the bleaching phase, our best-fitting model for chl*a* included a random effect due to tank, a highly significant TIME effect, significant interactions for both TIME × FOOD and TIME × TEMP, and a significant three-way interaction involving all three fixed effects (Table 2). However, there was no evidence for a significant main effect of FOOD. The absence of a main effect of FOOD indicated that all treatment combinations had a common intercept (i.e., there were no differences between fed and unfed corals in initial levels of chl*a*). The interactions involving TIME were significant because the rate at which chl*a* declined during the bleaching phase varied depending on the particular combination of FOOD and TEMP treatments to which corals were allocated: unfed, heated corals declined the most; fed, heated corals declined by a significantly lesser amount; and unheated corals did not decline, regardless of whether they were fed or unfed.

For the recovery phase, the best-fitting model included main and interactive effects of FOOD and TEMP, but not

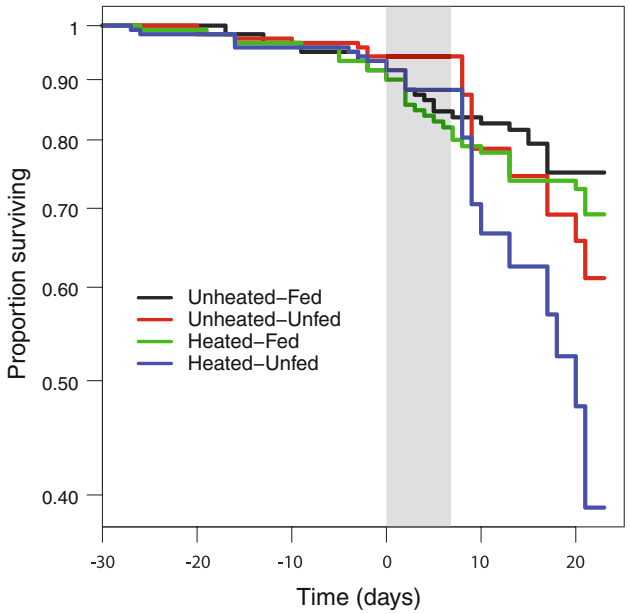

Fig. 1 Coral survivorship. The *lines* show nonparametric (Kaplan–Meier) survival estimates for each treatment combination. The *grey* region spans the bleaching phase (day 0–6) which falls between the 6-week acclimation phase and the recovery phase (day 7–22). Time has been set to zero at the commencement of the bleaching phase. Note that survival analysis was conducted only for the bleaching and recovery phases (day >0), because the temperature treatment was not imposed prior to this time. Also note that survival is plotted on a logarithmic scale

Table 1 Results of Cox proportional hazards (CPH) analysis of coral survival

Fixed effect	Hazard rate	Hazard ratio	*z*	*P*	Likelihood ratio test		
					χ^2	DF	*P*
(a) Bleaching + recovery phase							
FED (unfed)	0.557	1.745	3.028	0.002			
TEMP (heated)	0.833	2.299	4.321	<0.001			
					28.67	2	<0.001
(b) Recovery phase only							
FED (unfed)	0.519	1.681	2.541	0.011			
TEMP (heated)	1.437	4.206	5.829	<0.001			
					47.56	2	<0.001

"Hazard ratio" is the exponential of the hazard rate parameter and expresses the mortality rate of the treatment group as a proportion of the controls (e.g., a value of 1.7 means that mortality rate is 70 % higher than the control; a ratio of 0.8 means that mortality rate is 20 % less than the control). Results of the combined bleaching + recovery analysis should be treated with caution, because the proportional hazards assumption was significantly violated for temperature (see "Results"). Likelihood Ratio tests compare the fit of the CPH model against the null model for which all groups have the same survival. Terms in parenthesis indicate which is considered the "treatment" effect. For instance, TEMP (Heated) indicates that the effect size reported corresponds to hazard for the heated temperature treatment, relative to the unheated one

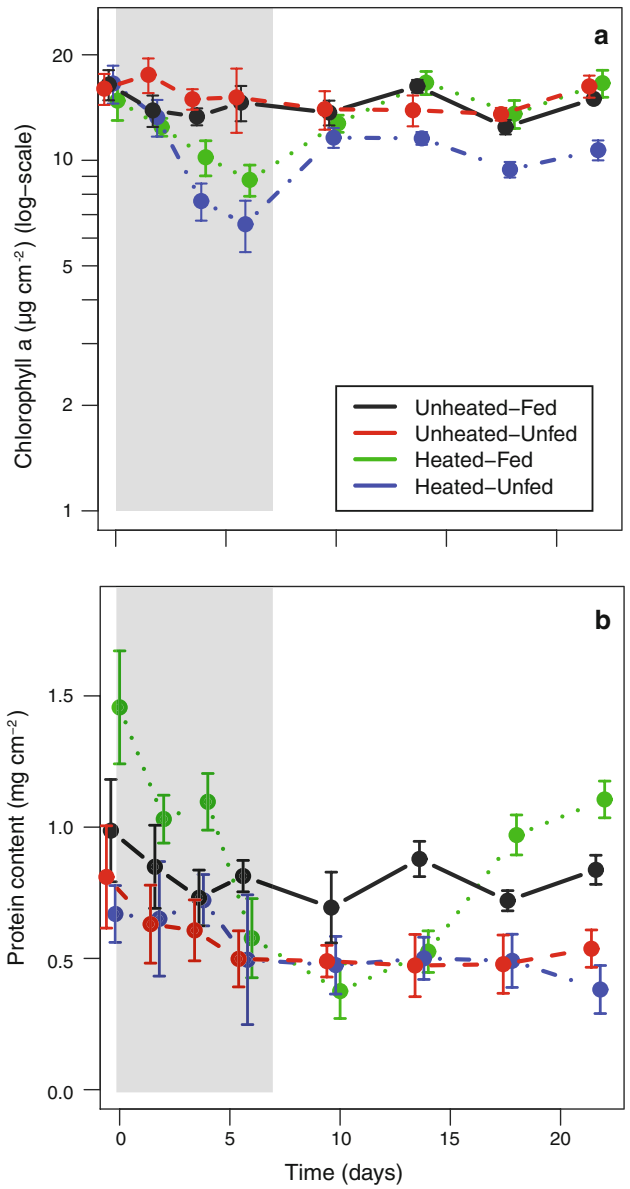

Fig. 2 **a** Chlorophyll *a* concentration (expressed on a logarithmic scale) and **b** protein content over time for the four treatment combinations. *Points* indicate days on which measurements were made. *Error bars* are SE, pooled across tanks for each treatment combination. The *grey* region indicates the bleaching phase (day 0–6), and the *white* region the recovery phase (day 7–22)

TIME, indicating that chl*a* had largely completed any recovery between the end of the bleaching phase and the third day of the recovery phase, when the first recovery phase measurements were made. Interestingly, this level was similar to pre-bleaching chl*a* in the heated and fed corals, but not the heated and unfed corals. Model terms clearly show the differences in final chl*a* apparent in Fig. 2a. Specifically, the "intercept" parameter in Table 2 corresponds to the heated, fed treatment at the conclusion of the experiment. The TEMP effect is not statistically significant (and nearly zero), indicating that the two fed treatments had recovered to virtually identical levels. The fact that the FOOD and FOOD × TEMP terms were of opposite sign and virtually of identical magnitude indicates that the unfed, unheated corals also had very similar levels of chlorophyll *a* to the two fed treatments. In contrast, corals in the heated, unfed treatment had significantly and substantially lower chlorophyll *a* levels than those in the other treatments. Back-transforming the fitted linear model terms from the natural log scale indicates that chl*a* was nearly 50 % higher for the fed, heated corals compared to the unfed, heated corals ($e^{2.68} = 15.6$ µg ml^{-1} vs. $e^{2.77-0.31} = 10.7$ µg ml^{-1}: see INTERCEPT and FOOD terms in Table 2), similar to the observed difference between the raw final measurements of chl*a* (Fig. 2a).

For protein, the behaviour of model residuals was better when the response variable was un-transformed. Corals in fed, heated treatments began with high protein levels, which dropped around 70 % during the bleaching phase of the experiment (Fig. 2b). However, they recovered strongly (from ~ 0.5 to 1 mg cm^{-2}) through the recovery phase. Both unfed treatments had lower protein levels at the beginning of the bleaching phase, then appeared to decline somewhat over the course of the bleaching phase, and ultimately stabilized at these lower levels. Corals in the fed, unheated treatment began the bleaching phase with a higher level of protein than the corals in the unfed treatments (~ 1 mg cm^{-2}), and appeared to remain near this value throughout the bleaching and recovery phases. The linear model analysis suggested that only some of these visually apparent trends in the protein data had strong statistical support. For the bleaching phase, linear mixed-effects model analysis identified a significant main effect of FOOD (~ 30 % lower protein levels at the commencement of the bleaching phase) and a significant negative effect of TIME, consistent with the decreasing trend apparent in most of the treatments during the bleaching phase of the experiment (Table 3; Fig. 2b). However, the apparent differences in rates of decline between treatments were not supported by the linear model analysis: there were no interactions in the best-fitting model (i.e., changes in protein content during the bleaching phase did not occur at significantly different rates between the treatments).

During the recovery phase, the best-fitting model for protein included TIME, FOOD, and TEMP (including all interaction terms), reflecting the fact that fed, heated corals increased substantially in protein content during the recovery phase, but other treatment combinations did not (Fig. 2b; Table 3). This can be seen in the model fit by inspection of the main and interactive effects involving TIME: the main effect (corresponding to the time effect on heated, fed corals) was significantly positive. However,

Table 2 Analysis of chlorophyll *a* for the (a) bleaching and (b) recovery phases

Fixed effect	Value	SE	*t*	*P*
(a)				
INTERCEPT	2.753	0.055	49.59	<0.001
TIME	−0.098	0.019	−5.09	<0.001
TIME:TEMP (unheated)	0.074	0.025	2.92	0.005
TIME:FOOD (unfed)	−0.065	0.025	−2.55	0.014
TIME:TEMP (unheated):FOOD (unfed)	0.073	0.036	2.03	0.047
TANK	0.097			
RESIDUAL	0.195			
(b)				
INTERCEPT	2.684	0.040	66.48	<0.001
FOOD (unfed)	−0.312	0.057	−5.46	<0.001
TEMP (unheated)	−0.030	0.057	−0.53	0.599
TEMP (unheated):FOOD (unfed)	0.311	0.081	3.85	<0.001
RESIDUAL	0.162			

For the bleaching phase, the value for "*TANK*" is the estimated standard deviation of the tank effect, while the "*RESIDUAL*" value is the standard deviation of the residual (within-tank) variation. For the recovery phase, the best-fit model omits a random effect of tank, so the residual term represents the overall standard deviation of residuals. Terms in parenthesis indicate which is considered the "treatment" effect. For instance, TEMP (unheated) indicates that the effect size reported corresponds to the magnitude of the unheated temperature treatment, relative to the heated one

Table 3 Analysis of protein for the (a) bleaching and (b) recovery phases

Fixed effect	Value	SE	*t*	*P*
(a)				
INTERCEPT	1.116	0.075	14.81	<0.001
TIME	−0.058	0.017	−3.34	0.001
FOOD (Unfed)	−0.307	0.077	−3.97	<0.001
RESIDUAL	0.309			
(b)				
INTERCEPT	1.140	0.073	15.60	<0.001
TIME	0.066	0.010	6.75	<0.001
TEMP (unheated)	−0.316	0.103	−3.06	0.003
FOOD (unfed)	−0.721	0.103	−6.98	<0.001
TIME:TEMP (unheated)	−0.059	0.014	−4.28	<0.001
TIME:FOOD (unfed)	−0.073	0.014	−5.30	<0.001
TEMP (unheated):FOOD (unfed)	0.414	0.146	2.84	0.006
TEMP (unheated):FOOD (unfed)	0.070	0.020	3.59	<0.001
RESIDUAL	0.175			

For these analyses, the best-fit models did not include a random effect of tank, so the residual term represents the overall standard deviation of residuals. Terms in parenthesis indicate which is considered the "treatment" effect. For instance, TEMP (unheated) indicates that the effect size reported corresponds to the magnitude of the unheated temperature treatment, relative to the heated one

summing the INTERCEPT term with the various main and interactive effects of FOOD and TEMP yields values close to zero for the other three treatment combinations (e.g., fed and unheated corals: TIME plus TIME × TEMP interaction ≈ 0; unfed, unheated corals: sum of all effects including TIME ≈ 0). In addition, protein levels differed substantially between treatments at the conclusion of the experiment. Among heated corals, for instance, fed corals had nearly three times the protein levels of their unfed counterparts, evidenced by the large negative effect of feeding in the analysis, and consistent with the differences in observed means at the end of the recovery phase (Fig. 2b; Table 3). Somewhat unexpectedly, however, fed corals subjected to heating ended the experiment with slightly higher protein levels than fed corals that were not heated (apparent as a significant TEMP effect).

Discussion

Our results highlight the potential nature and importance of food availability in the recovery of corals following acute thermal stress. For *A. intermedia*, food availability increased the survival of corals both during and in the aftermath of a thermal anomaly. The higher survival of corals in the heterotrophic environment corresponded with patterns of both chlorophyll *a* and protein levels. In the absence of feeding, both of these indicators of physiological coral energetics exhibited substantially less recovery in the 2 weeks following bleaching (Fig. 2). If the responses exhibited by *A. intermedia* in our experiment are indicative of physiological responses of corals to thermal bleaching in nature, then the availability of heterotrophic sources of food is likely to play a key role not only in providing an alternative source of energy during bleaching events, but also in facilitating the recovery of a coral's photosynthetic capacity in its aftermath.

This study is the first, to our knowledge, to experimentally document an effect of food availability on coral survival during and immediately after a period of acute thermal stress. The magnitude of this effect is surprisingly large: approximately 70 % higher mortality rates in unfed corals. This finding supports the hypothesis that temporal and spatial patterns in bleaching (e.g., Berkelmans et al. 2004) and bleaching-induced mortality (Anthony et al. 2009) are partially related to the availability of heterotrophic energy and nutrient sources. For example, inshore (coastal) versus offshore habitats differ strongly in their water quality and, consequently, heterotrophic richness of the water column (Fabricius 2005). Previous work has suggested that taxonomic variation in susceptibility to and recovery from bleaching may be related to taxonomic differences in heterotrophic capacity (e.g., Grottoli et al. 2006). Our findings support the reasoning underpinning this hypothesis and indicate that the importance of heterotrophy as a mediator of the effects of bleaching may extend to within-species variation as well.

There is a growing recognition that heterotrophy is an important determinant of the physiological performance of scleractinian corals (Houlbrèque and Ferrier-Pagès 2009). In particular, our finding that food availability leads to smaller declines in chlorophyll *a* (a proxy for photosynthetic capacity and a measure of coral bleaching state) is consistent with several recent studies on the effects of feeding on physiological performance during bleaching events. For instance, the tissue concentration of chlorophyll *a*, the density of the symbiont population, and rates of photosynthesis in fed corals subjected to thermal stress declined less than they declined in unfed corals, for species from three coral genera (*Stylophora*, *Turbinaria*, and *Galaxea*: Ferrier-Pagès et al. 2010). Similarly, for unfed *Turbinaria reniformis*, Tolosa et al. (2011) reported approximately 50 % lower chlorophyll *a* in corals subjected to thermal stress, but for fed corals, chlorophyll *a* was only 20–25 % lower after thermal stress. Our results also show that, for *A. intermedia*, similar effects of food availability extend to the post-bleaching recovery of photosynthetic capacity. Field studies have suggested that coral species with greater capacity to increase heterotrophic energy and nutrient acquisition in response to bleaching appear to recover better from thermal stress than those with more limited capacity for feeding (Rodrigues et al. 2008) and that translocation of heterotrophically acquired carbon and nutrients to symbionts (zooxanthellae) may facilitate the recovery of photosynthetic capacity (Hughes et al. 2010). A key implication of our study is that differences in the availability of heterotrophic energy and nutrient sources among reef areas can contribute to variation in corals' capacity for rapid recovery following thermal bleaching.

In addition to reducing the extent of decline, the trajectories of chlorophyll *a* recovery in the 2 weeks following the experiment's bleaching phase suggest that impairment of photosynthetic capacity may persist for some time. For instance, for fed corals subjected to thermal stress, chlorophyll *a* concentration recovered to these baseline levels within a few days, a recovery rate that is at the rapid end of the range observed in field studies of other species (weeks to months: Szmant and Gassman 1990; Jones 1997; Hueerkamp et al. 2001; Rodrigues and Grottoli 2007). However, unfed corals subjected to thermal stress recovered only partially. Moreover, chlorophyll *a* appeared to stabilize at these lower levels (Fig. 2), rather than simply recovering more slowly to pre-bleaching levels. Previous work indicates that a one-third decrease in chlorophyll *a* from pre-bleaching levels implies a substantial loss of photosynthetic capacity (e.g., $\sim$ 20–30 % for *Acropora formosa*, another staghorn *Acropora* species: Anthony et al. 2009). This suggests that, in the aftermath of bleaching, corals lacking access to food may suffer a relatively long-lasting loss of photosynthetic capacity, relative to fed corals, with potential implications for colony energetics and demographic performance.

Although feeding influenced the bleaching and recovery dynamics of both protein and chlorophyll *a*, there were some more subtle differences between the dynamics of these two response variables in our experimental *A. intermedia* population. In contrast to chlorophyll *a*, protein levels were lower at the end of the acclimation phase in unfed corals compared to fed corals. Moreover, protein levels of unfed corals remained low throughout the recovery phase, regardless of temperature treatment. The large difference in protein levels of fed and unfed corals at the end of the acclimation phase is consistent with the hypothesis that carbon fixed under high-nutrient conditions is disproportionately directed to protein

synthesis (Taguchi and Kinzie 2001). The lack of a significant effect of temperature on rates of decline in protein levels during the bleaching phase was unexpected, but the dynamics of protein during the recovery phase were more consistent with our expectations. Fed corals that had been subjected to heating increased protein levels rapidly (due, we suspect, to enhanced metabolism fuelling protein synthesis), to near their pre-heating levels, whereas the protein levels of unfed and heated corals remained low throughout the recovery phase.

There is a growing recognition that differences in the rate and extent of recovery from bleaching events will be at least as important as differences in bleaching susceptibility in determining how coral reef assemblages are likely to respond to higher temperatures and more severe thermal anomalies (Pandolfi et al. 2011). In particular, the rates of recovery of physiological state, such as protein and lipid levels, and physiological function, such as photosynthetic capacity, are likely to have demographic consequences by influencing capacity for growth and reproduction (Anthony et al. 2009), as well as vulnerability to other potential sources of mortality, such as subsequent bleaching events (Middlebrook et al. 2010) or pathogens (Bruno et al. 2007). This study shows that, even for a species like *A. intermedia* that is believed to rely heavily on photosynthetically produced carbon for its energy, the availability of heterotrophic food can substantially increase mortality risk, the acute sub-lethal effects of bleaching during acute thermal stress, and the capacity for rapid recovery of physiological function. To the extent that the responses of *A. intermedia* in our experiments are representative of how corals respond to bleaching events in nature, the functional importance of heterotrophy identified here may help to identify those reef environments most likely to be threatened by increases in the frequency and intensity of bleaching, and those for which food availability may confer greater resistance and resilience.

Acknowledgments We thank E. Graham and M. Hisano for assistance with statistical analysis and manuscript formatting, O. Hoegh-Guldberg for kindly permitting use of a MINI PAM, and G. Russ, A. Baird, J. Collins, M. Hoogenboom, and two anonymous reviewers for helpful comments on earlier drafts of this manuscript. This research was supported by the Australian Research Council and James Cook University.

References

Anthony KRN, Connolly SR, Hoegh-Guldberg O (2007) Bleaching, energetics, and coral mortality risk: effects of temperature, light, and sediment regime. Limnol Oceanogr 52:716–726

Anthony KRN, Hoogenboom MO, Maynard JA, Grottoli AG, Middlebrook R (2009) Energetics approach to predicting mortality risk from environmental stress: a case study of coral bleaching. Funct Ecol 23:539–550

Baird AH, Marshall PA (2002) Mortality, growth and reproduction in scleractinian corals following bleaching on the Great Barrier Reef. Mar Ecol Prog Ser 237:133–141

Berkelmans R, De'Ath G, Kininmonth S, Skirving WJ (2004) A comparison of the 1998 and 2002 coral bleaching events on the Great Barrier Reef: spatial correlation, patterns, and predictions. Coral Reefs 23:74–83

Borell EM, Bischof K (2008) Feeding sustains photosynthetic quantum yield of a scleractinian coral during thermal stress. Oecologia 157:593–601

Brown BE (1997) Coral bleaching: causes and consequences. Coral Reefs 16:S129–S138

Bruno JF, Selig ER, Casey KS, Page CA, Willis BL, Harvell CD, Sweatman H, Melendy AM (2007) Thermal stress and coral cover as drivers of coral disease outbreaks. PLoS Biol 5(6):e124. doi:10.1371/journal.pbio.0050124

DeVantier LM, De'ath G, Turak E, Done TJ, Fabricius KE (2006) Species richness and community structure of reef-building corals on the nearshore Great Barrier Reef. Coral Reefs 25:329–340

Dubinsky Z, Jokiel PL (1994) Ratio of energy and nutrient fluxes regulates symbiosis between zooxanthellae and corals. Pac Sci 48:313–324

Edmunds PJ, Gates RD, Gleason DF (2003) The tissue composition of *Montastraea franksi* during a natural bleaching event in the Florida Keys. Coral Reefs 22:54–62

Fabricius KE (2005) Effects of terrestrial runoff on the ecology of corals and coral reefs: review and synthesis. Mar Pollut Bull 50:125–146

Ferrier-Pagès C, Rottier C, Beraud E, Levy O (2010) Experimental assessment of the feeding effort of three scleractinian coral species during a thermal stress: effect on the rates of photosynthesis. J Exp Mar Biol Ecol 390:118–124

Gladfelter EH, Michel G, Sanfelici A (1989) Metabolic gradients along a branch of the reef coral *Acropora palmata*. Bull Mar Sci 44:1166–1173

Grottoli AG, Rodrigues LJ, Palardy JE (2006) Heterotrophic plasticity and resilience in bleached corals. Nature 440:1186–1189

Hoegh-Guldberg O (1994) Population dynamics of symbiotic zooxanthellae in the coral *Pocillopora damicornis* exposed to elevated ammonium [(NH4)2 SO4] concentrations. Pac Sci 48:263–272

Houlbrèque F, Ferrier-Pagès C (2009) Heterotrophy in tropical scleractinian corals. Biol Rev Camb Philos Soc 84:1–17

Houlbrèque F, Tambutté E, Ferrier-Pagès C (2003) Effect of zooplankton availability on the rates of photosynthesis, and tissue and skeletal growth in the scleractinian coral *Stylophora pistillata*. J Exp Mar Biol Ecol 296:145–166

Houlbrèque F, Tambutté E, Allemand D, Ferrier-Pagès C (2004) Interactions between zooplankton feeding, photosynthesis and skeletal growth in the scleractinian coral *Stylophora pistillata*. J Exp Biol 207:1461–1469

Hueerkamp C, Glynn PW, D'Croz L, Mate JL, Colley SB (2001) Bleaching and recovery of five eastern Pacific corals in an El Nino-related temperature experiment. Bull Mar Sci 69:215–236

Hughes AD, Grottoli AG, Pease TK, Matsui Y (2010) Acquisition and assimilation of carbon in non-bleached and bleached corals. Mar Ecol Prog Ser 420:91–101

Jeffrey SW, Humphrey GF (1975) New spectrophotometric equations for determining chlorophylls a, b, c1 and c2 in higher plants, algae and natural phytoplankton. Biochem Physiol Pflanz 167:191–194

Jones RJ (1997) Changes in zooxanthellar densities and chlorophyll concentrations in corals during and after a bleaching event. Mar Ecol Prog Ser 158:51–59

Jones RJ, Ward S, Amri AY, Hoegh-Guldberg O (2000) Changes in quantum efficiency of Photosystem II of symbiotic dinoflagellates

of corals after heat stress, and of bleached corals sampled after the 1998 Great Barrier Reef mass bleaching event. Mar Freshw Res 51:63–71

Leuzinger S, Anthony KRN, Willis BL (2003) Reproductive energy investment in corals: scaling with module size. Oecologia 136:524–531

Marsh JA (1970) Primary productivity of reef-building calcareous red algae. Ecology 51:255–263

Marshall PA, Baird AH (2000) Bleaching of corals on the Great Barrier Reef: differential susceptibilities among taxa. Coral Reefs 19:155–163

Middlebrook R, Anthony KRN, Hoegh-Guldberg O, Dove S (2010) Heating rate and symbiont productivity are key factors determining thermal stress in the reef-building coral *Acropora formosa*. J Exp Biol 213:1026–1034

Muller-Parker G, McCloskey L, Hoegh-Guldberg O, McAuley P (1994) Effect of ammonium enrichment on animal and algal biomass of the coral *Pocillopora damicornis*. Pac Sci 48:273–283

Muscatine L (1990) The role of symbiotic algae in carbon and energy flux in reef corals. In: Dubinsky Z (ed) Coral reefs ecosystems of the world. Elsevier, Amsterdam, pp 75–87

Nordemar I, Nystrom M, Dizon R (2003) Effects of elevated seawater temperature and nitrate enrichment on the branching coral *Porites cylindrica* in the absence of particulate food. Mar Biol 142:669–677

Pandolfi JM, Connolly SR, Marshall DJ, Cohen AL (2011) Projecting coral reef futures under global warming and ocean acidification. Science 333:418–422

Pinheiro JC, Bates MD, DebRoy S, Sarkar D, R Development Core Team (2011) nlme: linear and nonlinear mixed effects models. R package version 3:1–102

Rodrigues LJ, Grottoli AG (2007) Energy reserves and metabolism as indicators of coral recovery from bleaching. Limnol Oceanogr 52:1874–1882

Rodrigues LJ, Grottoli AG, Pease TK (2008) Lipid class composition of bleached and recovering *Porites compressa* Dana, 1846 and *Montipora capitata* Dana, 1846 corals from Hawaii. J Exp Mar Biol Ecol 358:136–143

Roman MR, Furnas MJ, Mullin MM (1990) Zooplankton abundance and grazing at Davies Reef, Great Barrier Reef, Australia. Mar Biol 105:73–82

Sebens KP, Grace SP, Helmuth B, Maney EJ Jr, Miles JS (1998) Water flow and prey capture by three scleractinian corals, *Madracis mirabilis*, *Montastrea cavernosa* and *Porites porites*, in a field enclosure. Mar Biol 131:347–360

Szmant AM, Gassman NJ (1990) The effects of prolonged "bleaching" on the tissue biomass and reproduction of the reef coral *Montastrea annularis*. Coral Reefs 8:217–224

Taguchi S, Kinzie RA III (2001) Growth of zooxanthellae in culture with two nitrogen sources. Mar Biol 138:149–155

Therneau T (2011) coxme: mixed effects cox models. R package version 2.1-3

Therneau T, Lumley T (2011) survival: survival analysis, including penalised likelihood. R package version 2.36-5

Tolosa I, Treignier C, Grover R, Ferrier-Pages C (2011) Impact of feeding and short-term temperature stress on the content and isotopic signature of fatty acids, sterols, and alcohols in the scleractinian coral *Turbinaria reniformis*. Coral Reefs 30: 763–774

Veron JEN (2000) Corals of the world, vol 1–3. Australian Institute of Marine Science, Townsville, Australia

Wallace CC (1999) Staghorn corals of the world: a revision of the genus *Acropora*. CSIRO Publishing, Collingwood

Ward S, Harrison PJ, Hoegh-Guldberg O (2000) Coral bleaching reduces reproduction of scleractinian corals and increases susceptibility to future stress. Proc 9th Int Coral Reef Symp 2:1123–1128

Warner ME, Fitt WK, Schmidt GW (1996) The effects of elevated temperature on the photosynthetic efficiency of zooxanthellae in hospite from four different species of reef coral: a novel approach. Plant Cell Environ 19:291–299