Bleaching, energetics, and coral mortality risk: Effects of temperature, light, and sediment regime

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Abstract

The most severe outcome of coral bleaching is colony mortality. However, the risk of mortality is one of the least understood consequences for reef corals under climate-change scenarios. Specifically, links among combinations of temperature anomalies, varying solar irradiance, reduced water quality, and mortality risks are unclear. Here, we analyze the effects of high temperature, irradiance, and sediment loading on coral survivorship in a controlled tank experiment using *Acropora intermedia* from the inner Great Barrier Reef lagoon. Survival analyses based on the fate of 1600 subcolonies revealed that temperature and sediment exerted strong effects on coral mortality risk. As expected, high temperature increased mortality risk at all light and sediment levels. However, high sediment reduced mortality under high temperature and/or high light, potentially by alleviating light pressure and by providing an alternative food source for bleached corals. A survivorship model using coral energy status (lipid stores) as a predictor variable provided an excellent fit to the data, suggesting that much of the variation in survivorship among treatments and over time can be explained by colony energetics. Our study provides a new framework for predicting coral mortality risk under complex bleaching scenarios in which multiple environmental variables are involved.

The environmental causes and ecological consequences of coral bleaching (the loss of pigment associated with dinoflagellate symbionts) are major current issues in coral reef ecology and reef management worldwide (e.g., Hoegh-Guldberg 2004). It has been well established that large sea-temperature anomalies are the key causes of coral mass bleaching worldwide (for reviews, *see* Brown 1997*a,b*; Hoegh-Guldberg 1999, 2004). Also, recent studies have demonstrated that high levels of solar irradiance may exacerbate bleaching during periods of thermal stress (e.g., Glynn 1996; Hoegh-Guldberg 1999; Mumby et al. 2001) and may cause bleaching at temperatures that would otherwise not elicit bleaching (Brown et al. 1994; Dunne and Brown 2001). However, while relationships among temperature, light, and bleaching risk are increasingly well-calibrated, our knowledge of mortality risk incurred under

bleaching events of differing severity is based almost exclusively on surveys of coral cover conducted following bleaching events (but *see* Edmunds 2005 for an exception). Also, the extent to which varying water quality interacts with light and temperature to affect the outcome of bleaching events is unknown, but it has major significance for the fate of coastal reefs under climate change. To understand the effects of increasing temperatures on the structure and dynamics of scleractinian coral populations, particularly along environmental gradients, it is important to understand how other key environmental stressors such as irradiance and water quality (e.g., turbidity and sedimentation) interact with temperature to influence bleaching severity and the widespread colony mortality that can result.

Photosynthesis by symbiotic algae is the principal source of energy for reef-building corals (e.g., Muscatine 1990; Anthony and Fabricius 2000). The partial or complete loss of symbiont populations (e.g. Jones 1997; Grottoli et al. 2004) as well as the reduced photosynthetic efficiency of remaining symbionts (Iglesias-Prieto et al. 1992; Lesser 1996; Jones et al. 2000; Warner et al. 2002) consequently impair the photosynthetic capacity of the coral colony, and thus may greatly impact on a coral colony's energy balance and tissue biomass (Fitt et al. 2000). In the present study, we examine the hypothesis that a key mechanism for mortality risk associated with bleaching is the shift in colony energy balance from positive to negative, i.e., into a state of starvation induced by bleaching (e.g., Grottoli et al. 2006). In general, reductions in an animal's energetic status (as apparent, for instance, from lowered lipid content: e.g., Anthony and Fabricius 2000; Wilmer et al. 2000) may affect a range of life functions, including growth

Table 1. Summary of environmental conditions for experimental populations of the coral *Acropora intermedia* before and during the bleaching experiment. ''Field'' refers to conditions in situ during the month prior to collection, ''acclimation'' refers to the 4-week period in the experimental setup under baseline conditions (see Fig. 1), and ''low'' and ''high'' refer to the eight combinations of high and low temperature, light, and concentrations of suspended sediment (turbidity) during the 6-week experimental phase. Each treatment level used duplicate tanks, and each tank held between 90 and 105 coral branches.

Treatments	Field	Acclimation	Low (control)	High
Temperature (°C)	28.7±0.3	27.8±0.5	27.7±0.5	30.4±0.8
Light (μmol m^{-2} s^{-1})	179.8±81.3	138.0±26.7	90.8±24.1	372.1±93.4
Sediment (mg L^{-1})	3.8±2.4	0.3±0.4	0.2±0.4	10.2±3.7

and reproduction (Maltby 1999; Kooijman 2000; Nisbet et al. 2000), and they may increase its susceptibility to physiological dysfunction, leading to increased mortality (Finstad et al. 2004). Because coral energy balance is a function of phototrophy (which is influenced by bleaching status and light regime: e.g., Jones et al. 1998) and heterotrophy and energy loss (which are functions of temperature, light, and particle regime: e.g., Anthony and Connolly 2004; Grottoli et al. 2006), the effect of environmental variables on mortality risk is likely to be mediated by their combined impact on physiological energetics. If mortality risk can be characterized explicitly as a function of environmental and physiological variables, our ability to predict the consequences of bleaching for coral populations will be substantially enhanced. In the present study, we analyze experimentally the effects of temperature, light, sediment regime, and their interactions on mortality risk of a common species of *Acropora* under eight environmental scenarios in a large controlled tank experiment. Also, to explicitly examine the mechanistic basis of coral mortality risk, we examine the extent to which environmental effects on survivorship can be explained through the functional relationship between physiological status (i.e., bleaching and energy status) and mortality risk.

Methods

Experimental design and setup—We used *Acropora intermedia*, one of the most abundant and widespread species in the Indo-Pacific (Wallace 1999), as our study species. The propensity of *A. intermedia* to propagate by fragmentation makes this species especially amenable to experimental manipulation: colony fragments collected in the field readily reattach to their stands in experimental tanks. Moreover, the genus *Acropora* represents more than one-third of the coral species in the Indo-Pacific (Veron 2000), and it is among the first to bleach during thermal stress events (Marshall and Baird 2000). By choosing this common and species-rich genus, the results of this study have relevance for bleaching and mortality patterns for a large proportion of Indo-Pacific reef assemblages.

Four weeks prior to the experiment, 1600 branches ($\sim$8-cm long) of *A. intermedia* were collected at 8–12-m depth at the SE corner of Pelorus Island, located $\sim$15 km off the coast of north Queensland, Australia. The corals were collected in January 2003 so that the experimental period would coincide with the season during which bleaching

events are most likely to occur in situ (*see* Berkelmans and Oliver 1999). Within one hour of collection, the corals were transported (shaded and submerged in seawater) to Orpheus Island Research Station. Coral branches were attached to polypropylene pins (4-mm thick, 60-mm long) using cable ties and then placed in racks on the bottom of the holding tanks. The branches were then distributed haphazardly and evenly among 16 large flow-through tanks (200 cm long by 100 cm wide by 30 cm deep). The experimental setup was covered by neutral-density screens to mimic downwelling irradiances at the depth and site of collection (daily means of $\sim$200 μmol m^{-2} s^{-1} and noon maxima of $\sim$400 μmol m^{-2} s^{-1}). These values corresponded to the irradiance regime recorded continuously near the coral colonies in situ during the month prior to collection (*see* Anthony et al. 2004). During the subsequent acclimation phase (12 January to 28 February), the water temperature in the tanks was kept at 28 $\pm$ 0.5°C using a central temperature control system (C023, Carrier Systems, NSW, Australia). Each tank was equipped with a large circulation pump (Eheim 1260) that generated natural flow velocities, which approximated 3–5 cm s^{-1} as estimated visually by particle tracking. The water volume of each tank ($\sim$800 liters) was turned over approximately 10 times daily. During the acclimation period, mortality was less than 0.1%, and all corals overgrew their polypropylene pins.

In the treatment phase (28 February to 2 April 2003), eight different combinations of temperatures, light regimes, and sediment concentrations were established using two replicate tanks for each treatment level. The baseline (control) temperature was set to 28 $\pm$ 0.5°C, which is well below the documented threshold for local populations of genus *Acropora* (Berkelmans 2002). The high temperature was set at 31.0 $\pm$ 0.5°C (Table 1), which was expected to cause bleaching within days (Berkelmans 2002). During the treatment phase, the low and high experimental light regimes (daily averages) were kept at 100–130 and 400–500 μmol m^{-2} s^{-1}, respectively (Fig. 1). Both values are well within the range of irradiances to which *A. intermedia* was exposed in situ (depths of $\sim$10 and 4 m, respectively). Temperature and downwelling irradiance were monitored continuously in all tanks throughout the acclimation period and the treatment phase using automated data loggers (Dataflow Systems, Cooroy, Australia).

Fine suspended sediment was delivered to eight of the tanks using a technique described previously (Anthony
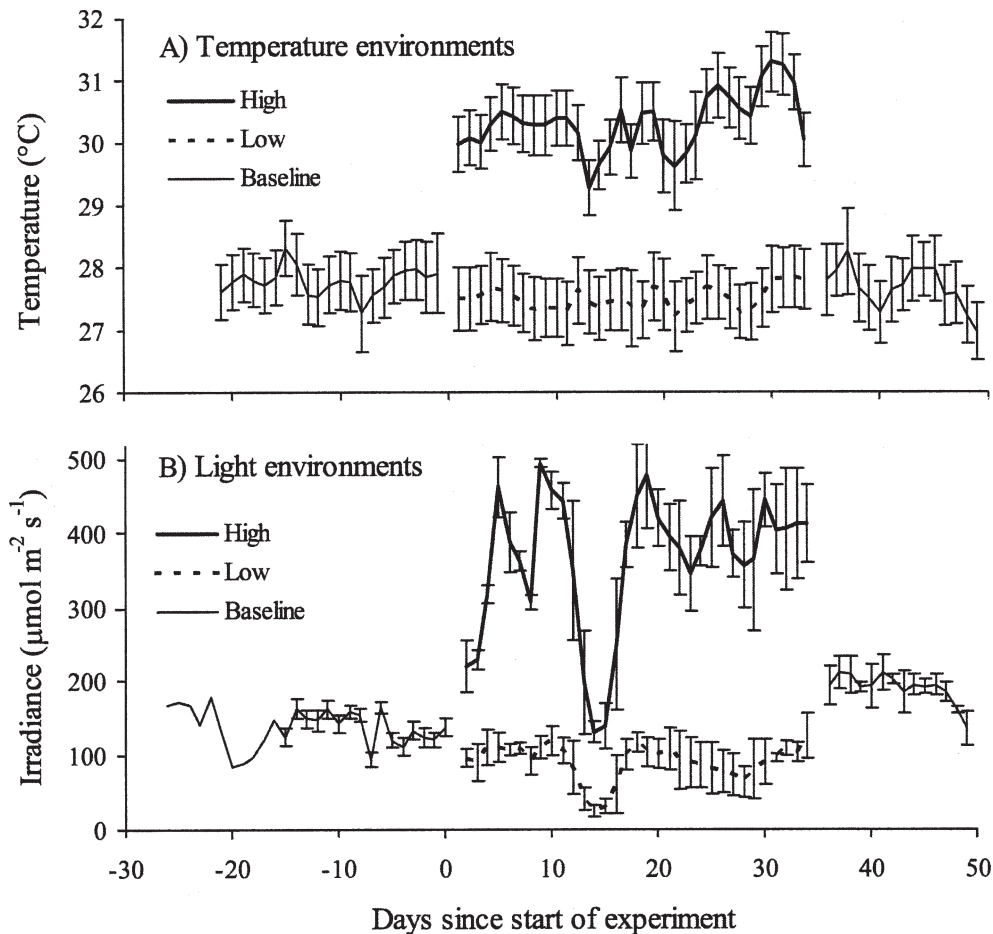
Fig. 1. (A) Temperature and (B) irradiance regimes before, during, and after the tank experiment. Irradiance values are reported as daily means of values measured at 30-min intervals between 08:00 h and 18:00 h. Error bars for temperature regimes are standard deviations of hourly readings in each tank and among tanks, and error bars for irradiances are standard deviations of daily means among tanks.

1999). The rate of sediment delivery was in this study set to ~10 mg L^{-1} (Table 1). This value mimicked conditions at some of the most turbid reef zones in the inner Great Barrier Reef lagoon (Larcombe et al. 1995; Anthony et al. 2004) and was 5- to 10-fold higher than average turbidity levels recorded at the field site. Background concentrations in the remaining eight tanks (sediment controls) were approximately 0.2 mg L^{-1}. Sediment concentrations were monitored daily using a water-quality analyzer (Model 611, Yeo-Kal Electronics, Australia) calibrated against known standards of the experimental sediment in suspension.

Survivorship—A census of the experimental coral population in each tank was undertaken every three days during the first two weeks of the experiment and every day during the last two weeks. A coral branch was scored as dead when tissue was visibly sloughing off from more than 50% of its surface area. Because all coral branches with tissue losses of more than 30–50% lost their remaining tissues within one or two days, this cutoff was adequate to ensure that total colony mortality was imminent. Survivorship of each tank population was expressed as the percentage of live branches relative to the size of the tank population at day 1, adjusted for the weekly reduction in population size due to sampling (see next).

Estimates of bleaching and energy status—To determine temporal changes in bleaching and energy status, tank populations were sampled weekly for concentrations of chlorophyll *a* and total lipids. Five coral branches were sampled randomly from each tank on day 1 (28 February 2003) and subsequently at weekly intervals for five weeks. All samples were snap-frozen in liquid nitrogen and stored in darkness at $-70°C$ until analyzed. For each sampling occasion, chlorophyll was extracted from a subsample (~5 cm^2) of each of the five coral branches. To minimize within-branch variability in photosynthetic pigments (Oliver 1984) and lipids (Stimson 1987), only central branch sections (15–20 cm^2 tissue surface area) were used. Prior to processing, the tissue surface area of each subsample was determined by foil wrapping (Marsh 1970). Subsamples were then ground to a paste in liquid nitrogen under dim light, and extractions were undertaken in darkness using cold (4°C) acetone (100%) in three sets, each of 4–6-h

duration. Absorbances were determined at 630 and 663 nm, and concentrations of chlorophyll *a* were calculated using a standard formula (Jeffrey and Humphrey 1975).

To estimate changes in energy status, the remaining subsample of each branch was assayed for content of total lipids. Previous studies have demonstrated a close link between resource environment, physiological energetics, and lipid reserves in corals (Anthony and Fabricius, 2000; Anthony et al., 2002; Grottoli et al. 2006). Following measurements of surface area (see previous), each subsample was ground to a paste, and the total lipid was extracted using a method modified from a standard procedure (Leuzinger et al. 2003). In summary, the lipid was extracted in three sets of chloroform-methanol (2:1 V/V), filtered through a Whatman GF/C filter, the extract was washed in 0.88% KCl and methanol-H_2O (1:1 V/V), and the chloroform was then evaporated until the weight of the sample remained constant (i.e., consisted of lipid only).

Data analysis—Effects of treatments on coral survivorship were estimated using survival analysis (Muenchow 1986), specifically the Cox's Proportional Hazard (CPH) model (Cox 1972). The CPH model framework builds on the premise that the hazard (i.e., mortality) rate is a log-linear function of the covariates, in this case the environmental or physiological variables. It assumes, further, that the effect of a covariate on survivorship does not vary over time, i.e., the effect of covariates on the hazard rate is proportional to the baseline hazard (Muenchow 1986). In other words, if an experimental treatment confers a 2-fold increase in mortality risk at one time, it will do so at other times also, regardless of whether or how the baseline mortality risk is also varying over time. The survival model was fitted using the function "coxph" in the "survival" library of the software program *R* (R Core Development Team 2005; Therneau and Lumley 2005). This provided estimates of effect sizes and significance levels for each term. Transformed residuals from these fits were used for graphical assessments of violation of model assumptions. Statistical tests for violation of the proportional hazards assumption were also conducted, using the function "cox.zph." Mortality due to sampling for bleaching status (chlorophyll *a*) and energy reserves (lipid contents) was corrected for in the schedule by adjusting the effective population size over time. Due to contamination of the water-supply system to three of the tanks from low-temperature treatments, these tanks were omitted from the survival analyses.

Effects of temperature, light and sediment treatments on chlorophyll and lipid contents were analyzed using a four-factor analysis of variance (ANOVA) with time (sampling occasion) as repeated measure and tanks (means of five coral samples) as replicates. Although this approach reduces the number of degrees of freedom, it eliminates any concerns of temporal pseudo-replication within tanks. Due to the low number of replicate tanks ($n = 2$ per treatment), all tanks were used in this analysis.

Two different sets of survival analyses were conducted. To directly assess the effects of environmental variables on

Table 2. Results of survivorship analysis (Cox's Proportional Hazard model) for *Acropora intermedia* using temperature, light, and sediment regime as time-dependent covariates. β is the covariate (regression) coefficient indicating the effect of the treatment on the log-hazard, and thus $\exp(\beta)$ is the proportional change in hazard associated with the treatment relative to the control (low temperature, low sediment, low light). *LRT* indicates the likelihood ratio statistic of the model against the null hypothesis of no covariate effect (higher values indicate greater support for the model), and SE is standard error.

| Covariates | Survival analysis | | | |
	β	$\exp(\beta)$	SE	p
Temperature (T)	2.75	15.66	0.46	<**0.001**
Light (L)	−1.74	0.18	1.1	0.110
Sediment (S)	−1.82	0.16	1.1	0.098
T × L	1.62	5.07	1.11	0.140
T × S	1.03	2.79	1.11	0.350
L × S	3.34	28.35	1.52	**0.028**
T × L × S	−3.86	0.02	1.54	**0.012**

LRT=339, df=7, p<0.001, n=1,170.

mortality risk, survival was modelled as a function of temperature, light, and sediment using the treatment levels as categorical variables, as in conventional ANOVA. Then, to determine the extent to which physiological condition could explain the variation in mortality risk among treatments and over time, we conducted survival analyses in which chlorophyll *a* and lipid concentrations, rather than experimental treatments, were used as covariates in the CPH model. Before fitting the model, chlorophyll and lipid values were standardized by centering: subtracting the mean covariate value from each measured value and dividing the result by the standard deviation of the measured values. Each covariate thus had a mean value of zero and a standard deviation of one. Because a census of the populations was conducted daily for survivorship, whereas sampling for chlorophyll and lipids was conducted weekly, chlorophyll and lipid values used in the survival analysis were interpolated between days of sampling.

Results

Survivorship analysis: Temperature, light, and sediment analysis—The results of fitting the CPH model to the full data set are presented in Table 2. This analysis suggests a complex, three-way interaction among temperature, light, and sediment. However, goodness of fit testing suggests that these results should be interpreted with caution. First, onset of high mortalities in most of the high-temperature and high-light treatments occurred during the latter half of the experiment (days 20–30; Fig. 2), most likely because of a gradual buildup of stress effects following implementation of treatments. In other words, the treatment effects were larger in the latter part of the experiment than in the initial phase. This delay in mortality responses led to strong violation of assumptions of the CPH model ($\chi^2 = 60$, $p < 0.0001$), which meant that the effect on mortality risk of being in a particular treatment changed over the course of the experiment. Secondly, there
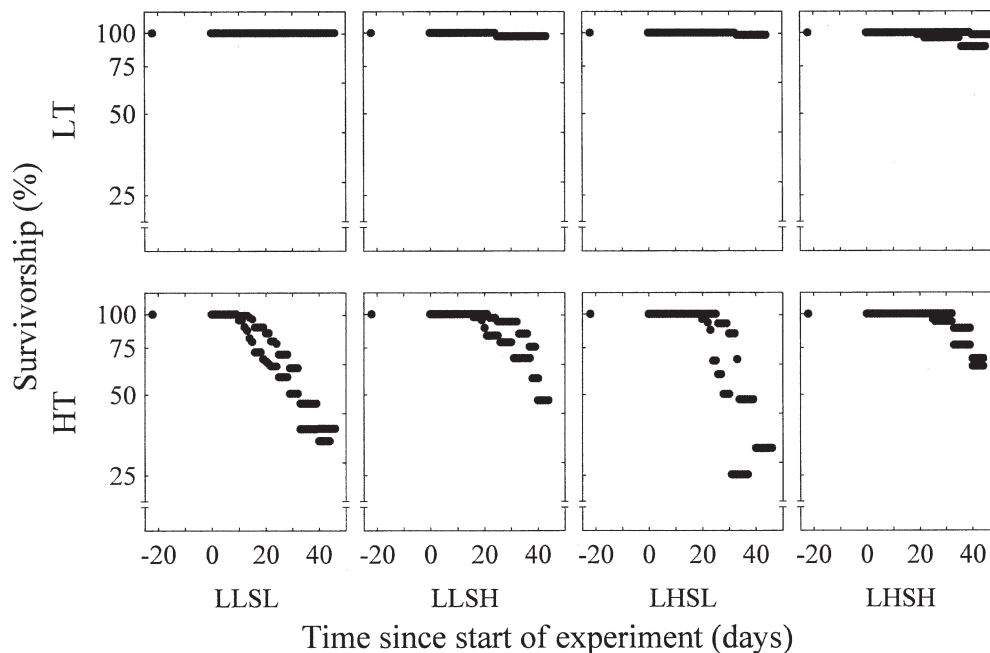
Fig. 2. Survivorship curves for *Acropora intermedia* during the 6-week experiment. The treatment phase was conducted from 28 February (day 1) to 2 April (day 33) 2003. Duplicate tanks are shown for each combination of temperature, light, and sediment regime (duplicate tanks were pooled in the analyses). Each tank held 90–105 coral branches, from which five branches were sampled weekly. Survivorship curves were adjusted for changes in population sizes due to sampling. See Table 1 and Fig. 1 for details of temperature, light, and turbidity treatments and Tables 2, 3, and 5 for results of survival analyses. Key to symbols: HT, high temperature; LT, low (control) temperature; LL, low light; HL, high light; LS, low (sediment) turbidity; HS, high sediment regime.

was minimal mortality in all low-temperature treatments; for instance, less than 2% of the coral branches died in the low-temperature, high-light, low-sediment treatment. As a result, these events had a strong effect on the model parameter estimates, as was evidenced by plots of transformed score residuals (Klein and Moeschberger 2003; Therneau and Lumley 2005).

To assess the robustness of the results of the full analysis, we reanalyzed the data in two ways. First, we tested the robustness of the strong main effect of temperature (apparent in Table 2, and also from visual inspection of the survivorship curves in Fig. 2) by stratifying on light and sediment (Klein and Moeschberger 2003). This is somewhat analogous to an ANOVA in which one tests for a temperature effect by treating each combination of light and sediment as a blocking factor. This analysis not only supported the importance of temperature, but the magnitude of the estimated temperature effect ($\beta = 2.7 \pm 0.3, p < 0.001$) was comparable to that estimated in the full model (indicating that mortality risk was approximately 15 times higher in the high-temperature versus low-temperature treatments: $e^{2.7} = 14.9$). Moreover, the proportional hazards assumption was satisfied ($\chi^2 = 0.395, p = 0.53$), and plots of transformed score residuals indicated that no individual observations had particularly strong effects on the estimated temperature effect (the most influential observation changed the temperature effect by <2.5% of

its estimated value, compared with ~100% in the original analysis).

Second, to test for effects of light and sediment, we omitted the low-temperature treatments from our analysis (this eliminated the potential for very rare mortality events in the low-temperature treatments to bias the results), and tested only for effects of light and sediment and their interaction. This analysis identified a significant main effect of sediment, but no significant interaction or main effect of light (Table 3A). However, as in the original analysis, the proportional hazards assumption was violated ($\chi^2 = 57, p < 0.0001$), suggesting that the effects of light and temperature varied over time. Therefore, we fit this model a second time using only the latter half of the experiment (days 21–42). This analysis also found a significant effect of sediment, but it identified a significant light-sediment interaction as well (Table 3B). Again, the proportional hazards assumption was violated, though much less severely ($\chi^2 = 11.8, p < 0.01$). Because model assumptions were not satisfied for any of these analyses (full model, light-sediment for the full time series, light-sediment for the second half of the experiment), we calculated the estimated proportional hazards from each of the models to determine which results, if any, might be robust (Fig. 3). This analysis suggested that high sediment reduces mortality risk at high temperature and that this beneficial effect of sediment is greatest in high-light treatments. Inspection of the observed

Table 3. Summary results of survival analyses for *Acropora intermedia* using light and sediment regimes as covariates in the high-temperature tanks only, for (A) the full time series, and (B) the second half of the time series only (days 21+; see text for justification). β and $\exp(\beta)$ are the covariate coefficient and proportional change in hazard, respectively, associated with the treatment relative to the baseline hazard, which, here, is the hazard under high temperature, low sediment and low light. *LRT* indicates the likelihood ratio statistic of the model against the null hypothesis of no covariate effect (higher values indicate greater support for the model), and SE is standard error.

	Covariates	β	$\exp(\beta)$	SE	p
A: Days 1–45	Light (L)	−0.12	0.89	0.15	0.420
	Sediment (S)	−0.79	0.46	0.17	**0.000**
	L × S	−0.52	0.59	0.26	**0.040**
LRT=79.9, df=3, *p*<0.001, *n*=720.					
B: Days 21–45	Light (L)	0.37	1.45	0.18	**0.033**
	Sediment (S)	−0.45	0.64	0.20	**0.021**
	L × S	−0.90	0.41	0.28	**0.001**
LRT=59.2, df=3, *p*<0.001, *n*=553.					

survivorship curves (Fig. 2) can help us to understand the assumption violations in this case. Survival in the high-sediment treatments is higher than in the corresponding low-sediment treatments for all high-temperature tanks (bottom row of Fig. 2). However, the light effects are complicated by the fact that mortality begins sooner under low light and low sediment (leftmost panel in bottom row of Fig. 2) but is more severe in the corresponding high-light treatment toward the end of the experiment (third panel in bottom row of Fig. 2). This makes the estimated light effect (and thus any associated interactions) highly sensitive to the time window that is analyzed (e.g., compare Fig. 3, panels B and C). For instance, for the full time series, high light decreases hazard under both low- and high-sediment conditions (Fig. 3B, compare bars 1 and 3, and 2 and 4). However, for the latter half of the experiment, high light increases hazard under low-sediment conditions (Fig. 3C, compare bars 1 and 3), but it decreases hazard under high-sediment conditions (Fig. 3C, compare bars 2 and 4).

Survivorship analysis: Responses of chlorophyll and lipids—The pattern of chlorophyll *a* concentrations over time reflected the survivorship curves for the high-temperature and high-light groups, with significant effects of temperature and temperature-light interactions (Fig. 4; Table 4). In the high-temperature treatments, chlorophyll *a* concentrations declined monotonically, and approximately linearly, over time from the start of the treatment phase (day 1). However, coral populations in all eight high-temperature treatment tanks produced similar responses, regardless of light and sediment regime: a steady decrease in chlorophyll *a* toward 1–3 μg cm^{-2}. Results of the ANOVA indicated that sediment treatments had no effect on chlorophyll levels (Table 4) and did not interact significantly with temperature or light. During the acclimation phase (days −22 to 1), chlorophyll *a* concentrations remained constant across treatment tanks, indicating that bleaching status was unaffected by sampling, handling, or by the tank environment.
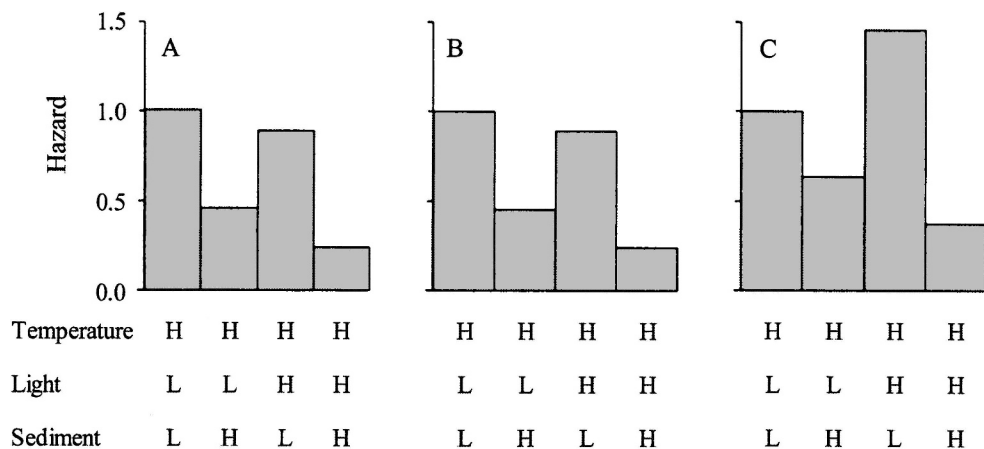

Fig. 3. Proportional hazards of *Acropora intermedia* for all combinations of high (H) and low (L) light and sediment regimes, as estimated by CPH models. Proportional hazards are scaled so that the low-light, low-sediment treatment has a proportional hazard of 1.0. (A) Proportional hazards from the full three-factor model (temperature, light, and sediment). (B) Proportional hazards from the light × sediment model, using only high-temperature treatments. (C) Proportional hazards from the same model as in (B), but using only the latter half of the experiment (days 21–42).
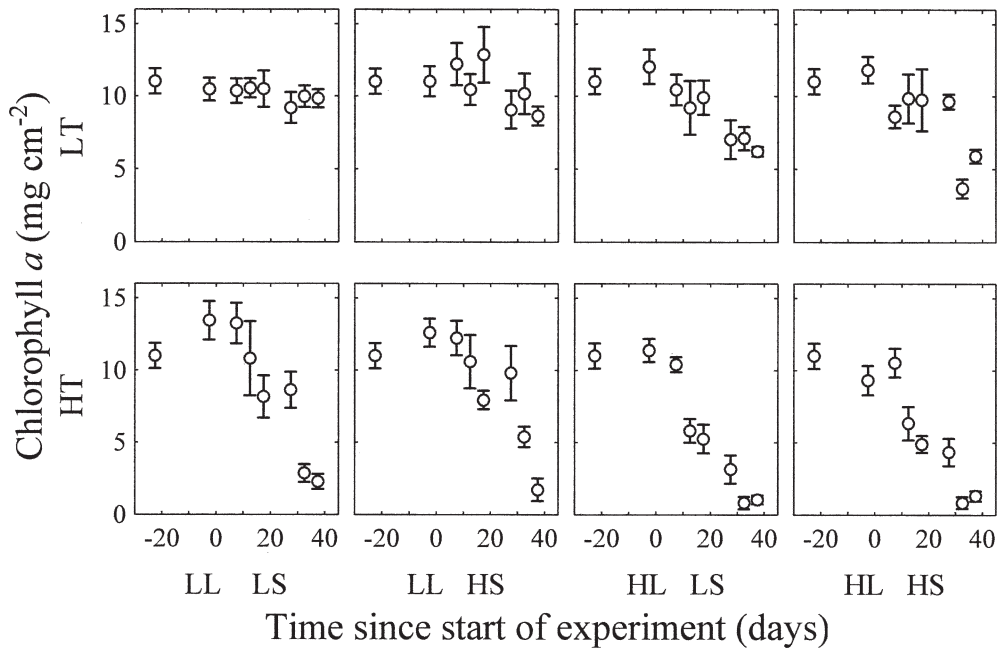
Fig. 4. Time-course of chlorophyll *a* concentrations of *Acropora intermedia* during the experiment. Error bars are standard errors of 8–12 samples. See Table 4 for results of analyses. See Fig. 2 for key to symbols.

Lipid concentrations followed a pattern largely similar to that of the chlorophyll *a*, except for a significant decline during the acclimation phase (Fig. 5) and the lack of a significant temperature-light interaction over time (Table 4). Also, the response of lipids to the experimental treatments was generally weaker than that of chlorophyll *a*. First, lipid levels dropped 30–50% during the three-week acclimation phase, probably due to energy costs associated with tissue repair following collection. Light levels during the acclimation phase were 30% lower than at the field site (Table 1), and water supply was low in particulate food, both of which potentially led to reduced lipid reserves. Second, whereas chlorophyll *a* concentrations in corals in the high-temperature treatments declined 80–90% during the course of the experiment, lipid levels fell only 60–70% during the experiment. This was reflected in a weaker time effect for lipids relative to chlorophyll (Table 4). Last, contrary to the pattern for chlorophyll *a*, lipid concentra-

tions in the low-temperature–high-light–high-sediment treatment increased nearly 50% during the experiment (to a level similar to that at the time of collection), indicating that the combination of high light and high sediment load was nutritionally favorable under nonbleaching temperatures.

Time-dependent covariates—Survival analyses of tank populations of *Acropora intermedia* indicated that bleaching (i.e., decreased chlorophyll *a* concentration) and energy status (lipid concentrations) were both strong predictors of survivorship. Because chlorophyll *a* and lipid were significantly correlated (Pearson $r = 0.526$; $p < 0.001$) across time and among tanks, we modelled their effects separately. Our analysis indicated a negative effect of chlorophyll *a* on hazard rate (Table 5). However, the proportional hazard assumption was strongly violated ($\chi^2 = 253$, $p < 0.001$); this result was caused by the tendency for the chlorophyll

Table 4. Summary results of repeated ANOVA measures for chlorophyll *a* and lipid contents in *Acropora intermedia* in response to the 42-d experiment of high and low temperature, light, and sediment treatments. The mean of five corals from each of two tanks sampled weekly was used as repeated measure (factor time, $n=7$), thereby using tanks as replicates.

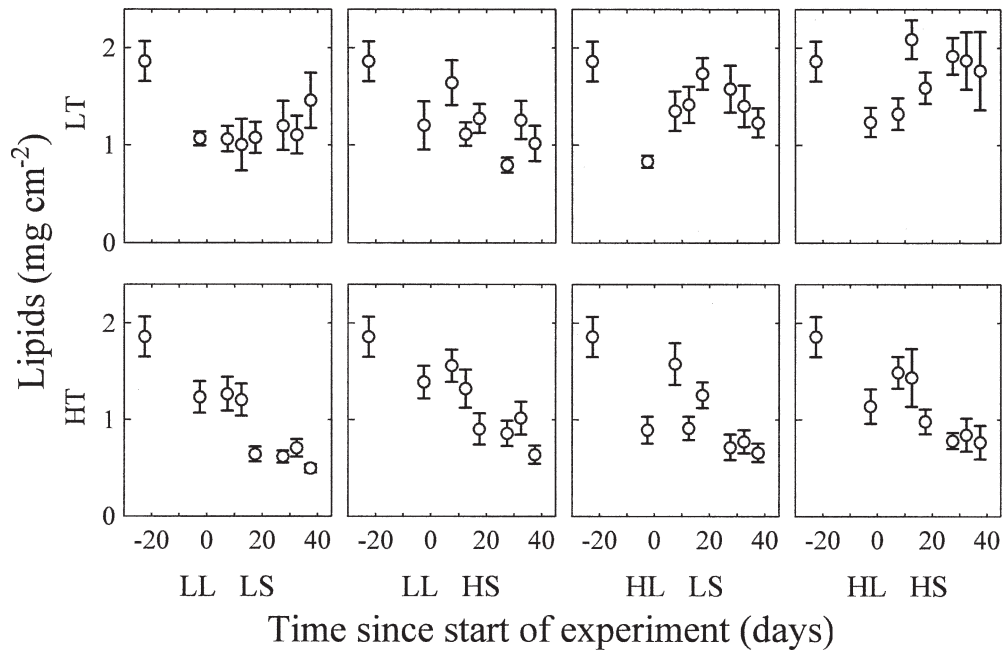| Source of variation	df	Chlorophyll *a*		Lipid	
		F	p	F	p
Time	6	29.06	**<0.001**	3.48	**0.007**
Temperature (T) × time	6	8.22	**<0.001**	6.01	**<0.001**
Light (L) × time	6	2.02	0.084	2.06	0.079
Sediment (S) × time	6	0.67	0.678	0.90	0.502
T × L × time	6	2.34	**0.048**	1.09	0.381
T × S × time	6	0.64	0.697	0.42	0.860
L × S × time	6	0.97	0.457	0.62	0.712
T × L × S × time	6	0.85	0.536	0.16	0.987

Fig. 5. Time-course of the concentrations of total lipid in *Acropora intermedia*. Error bars are standard errors of 8–12 samples (duplicate tanks pooled). See Fig. 2 for key to symbols and Table 4 for results of analyses.

effect to become increasingly negative over time. For instance, the chlorophyll effect increased nearly 4-fold in magnitude when only the second half of the experiment (days 21+) was analyzed (compare β estimates for the two analyses in Table 5). This can also be seen in a Schoenfeld residuals plot (Fig. 6A), which shows a clear trend of the chlorophyll effect becoming increasingly negative over time.

Lipids provided a much better time-integrated predictor of mortality risk than chlorophyll *a* (Table 5; compare likelihood ratio statistics for the two models). In particular, there was a strong negative effect of lipid levels on mortality (Table 5). Although the proportional hazards assumption was also violated ($\chi^2 = 9$, $p < 0.001$), inspection of Schoenfeld residuals (Fig. 6B) indicates that the increase in the lipid effect over time was much smaller

in magnitude than for chlorophyll *a*. This plot also suggests that violation of the proportional hazards assumption was confined to the initial stages of the experiment, when the lipid effect was less negative during the first two weeks (suggested by the positive residuals early in the experiment, as shown in Fig. 6B). Indeed, when only the latter half of the experiment is analyzed (days 21+), the violation of the proportional hazards assumption disappears ($\chi^2 = 1.08$, $p = 0.3$), and the magnitude of the estimated lipid effect remains comparable to that estimated from the full time series (Table 5).

Discussion

This study provides the first formal experimental analysis of coral mortality risk (hazard) associated with

Table 5. Results of survival analyses (Cox's Proportional Hazard model) using chlorophyll *a* and total lipid concentrations in coral tissues as time-dependent covariates. Similar to Table 3, analyses were run (A) with and (B) without the first half of the time series due to delayed onset of mortalities. β is here the regression coefficient indicating the effect of a unit change in the covariate on the log-hazard, i.e., $\exp(\beta)$ is the proportional change in hazard associated with a change of one standard deviation in the covariate. *LRT* indicates the likelihood ratio statistic of the model against the null hypothesis of no covariate effect (higher values indicate greater support for the model), and SE is standard error.

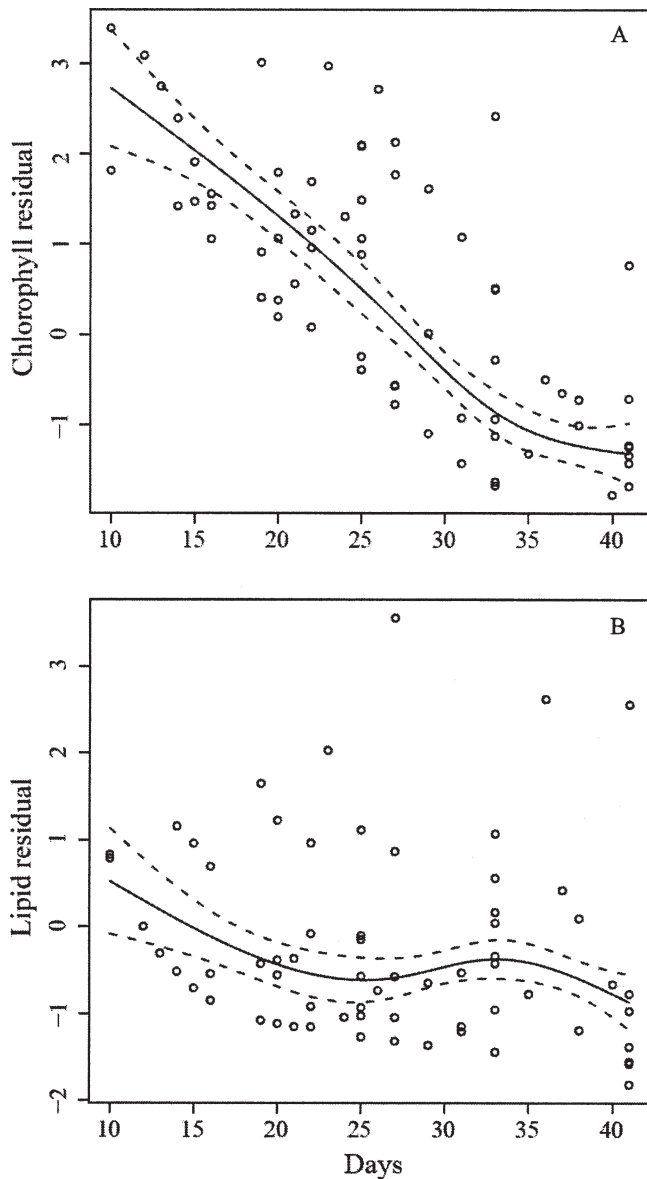|	Covariates	β	$\exp(\beta)$	SE	p
A: Days 1–42	Chlorophyll *a*	−0.18	0.83	0.07	**0.009**
	LRT=6.9, df=1, *p*=0.009, *n*=1,170				
	Total lipid	−0.48	0.62	0.07	**<0.001**
	LRT=59.5, df=1, *p*=<0.001, *n*=927				
B: Days 21–42	Chlorophyll *a*	−0.60	0.55	0.09	**<0.001**
	LRT=53.9, df=1, *p*=<0.001, *n*=1,170				
	Total lipid	−0.56	0.57	0.07	**<0.001**
	LRT=66.7, df=1, *p*=<0.001, *n*=927				

Fig. 6. Plots of scaled Schoenfeld residuals (points) for the Cox Proportional Hazards model, with fitted smoothing spline (solid line), and 95% confidence limits (dashed lines). The model used (A) chlorophyll *a* and (B) lipids as time-dependent covariates (see Table 5 for results of analyses). Positive residuals indicate that the effect of chlorophyll (or lipid) at that time is greater (generally less negative) than average, while negative residuals indicate that the chlorophyll (or lipid) effect is more negative than average.

bleaching events and its relationship to changes in the physiology of corals. Our results, based on a large experimental design involving ~1600 coral branches in a controlled tank environment, demonstrated two important points. First, coral mortality risk during bleaching events is a function of multiple environmental factors, such as temperature, sediment, and possibly light intensity, all of which affected coral survival. Second, mortality risk is closely coupled to the key physiological response variables: bleaching status (chlorophyll concentrations) and, in particular, energy status (lipid stores). These results underscore the need for a multivariate approach to predicting the coral mortality risks associated with bleaching events, and they show how population-level responses are in part dictated by physiological processes. To date, predictive bleaching risk models have tended to be based on temperature anomalies only and have focused only on bleaching severity as the key response variable (Hoegh-Guldberg 1999, 2004; Berkelmans 2002; Strong et al. 2004). The relationships among environmental stress, bleaching, and coral mortality risk are critical to projecting how increasing environmental challenges from global climate change will affect coral reefs.

Surprisingly, high sediment loads had a strong positive effect on coral survivorship under high-temperature as well as high-light conditions, and less so under high temperature alone. These results have strong implications for the consequences of bleaching events on coastal, high-turbidity reefs because they indicate that turbidity may alleviate bleaching stress induced by high temperature and light conditions. Although the high-light treatment was less than 400 μmol photons m^{-2} d^{-1} (daily average), it was a significant increase from field conditions (Table 1). These findings may be related to two key processes: (1) nutrition enhancement under high turbidity, and/or (2) the secondary effects of turbidity on the underwater light regime. First, feeding on particles at high concentrations may provide corals with nutrients and consequently facilitate tissue growth and increase lipid levels (Anthony and Fabricius 2000; Anthony et al. 2002), thereby reducing the risk of mortality due to starvation (see also Grottoli et al. 2006). In particular, the significant increase in lipid content of corals in the low-temperature, high-light, high-sediment treatment suggests that high-sediment treatments reduce nutrient limitation of tissue growth. Second, high turbidity acts as a light filter (e.g., Mobley 1994) and can reduce damaging levels of ultraviolet (UV) radiation even in shallow water (Bracchini et al. 2004). Interestingly, the evidence for effect of light on survival responses was minimal, with light effects only apparent in the high-temperature, high-sediment treatment combinations. One possible explanation for this is that the high-light treatment was not sufficiently stressful to exacerbate bleaching. However, the interactions among temperature, light, and time in the bleaching response (i.e., the analysis of chlorophyll concentrations) are in accordance with the photoinhibition model of the mechanisms of bleaching (Jones et al. 1998), and they suggest that high light levels did exacerbate thermal bleaching in our experiment (*see also* Brown et al. 2002).

Survival analysis based on the failure-time principle provides a framework for formally examining the relationships among environmental variables, organism properties, and survivorship or mortality risk (e.g., Muenchow 1986; Dungan et al. 2003; Wahlqvist et al. 2005). This study is the first to use this framework to analyze the linkage among environmental factors, in particular bleaching scenarios, physiological responses, and mortality risk in corals. Our results support the hypothesis that coral survivorship is affected by bleaching via its influence on energy status.

Specifically, reduced photosynthetic capacity, via bleaching, leads to reduced energy reserves or tissue biomass (*see also* Fitt et al. 2000; Grottoli et al. 2004), which consequently impacts on energy reserves for maintenance and growth. Corals contain large lipid stores under normal (nonbleaching) conditions (Stimson 1987; Spencer-Davies 1991). However, our results indicate that 30–50% depletion of those reserves may occur during a bleaching event of moderate duration (less than six weeks). During the course of a bleaching event, energy reserves may thus fall to the point at which resources for maintenance and growth are compromised, leading to increased risk of mortality.

The fact that the effect of lipid levels on mortality risk was much more consistent over time than effects of chlorophyll supports our hypothesis that lipid stores themselves serve as a time-integrated indicator of mortality risk. In this study, a lipid level of 0.5–1.0 mg cm^{-2} (around day 20 for high-temperature groups) appeared to be the threshold triggering high mortalities. The large magnitude of the lipid effect (~50% increase in hazard with a drop in lipid levels of one standard deviation below the mean level) observed in this study was somewhat surprising given that lipid levels were highly variable in our study (Fig. 5).

To evaluate conservatively whether observed lipid losses in bleached coral can be explained by changes in energy balance, we estimated the lipid equivalents of a 40-d period of respiration and excretion (i.e., assuming a negligible rate of photosynthesis). Using data on sediment-enhanced respiration and excretion for *Acropora valida* (Anthony and Connolly 2004) and recent data on temperature-enhanced respiration in *Acropora aspera* (unpubl. data), maximum daily rates of respiration would approximate 20 μg O$_2$ cm^{-2} d^{-1}. Assuming that 1 mg of lipid corresponds to the respiration and excretion of 2.2 mg of carbon (based on a specific enthalpy of -39.5 kJ g^{-1} and a respiratory quotient of 0.72; Gnaiger and Bitterlich 1984), maximum rates of lipid loss during the 40-day period would be ~0.5 mg of lipid cm^{-2}. This estimate corresponds to the lower end of the observed lipid losses in the high-temperature treatments of this study (0.5–1.0 mg cm^{-2}). Given that this estimate is highly conservative (by assuming zero photosynthesis), it suggests that more lipids are being lost due to bleaching than can be accounted for by oxygen fluxes alone. The difference may be made up of enhanced lipid excretion associated with the expulsion of symbiont cells and loss of host cells from the endodermic layer.

Overall, our results suggest that coral mortality risk from multivariate bleaching scenarios is, in part, a deterministic function of the physiological responses to the environment, specifically energy status. These findings contribute to an improved mechanistic understanding of coral mortality risks in multivariate bleaching scenarios and a greater ability to forecast such risks.

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