

Research Article

Tradeoffs to Thermal Acclimation: Energetics and Reproduction of a Reef Coral with Heat Tolerant *Symbiodinium* Type-D

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The photo-physiological characteristics of thermo-tolerant *Symbiodinium* types have been postulated to have negative effects on the energetics of the reef corals by reducing fitness. To investigate this, two key and inextricably coupled indicators of fitness, lipids and reproduction, were monitored in colonies of the broadcast-spawning coral *Acropora millepora* over a two-year period that included a natural bleaching event. In the absence of bleaching ITS1-type clade D predominant colonies had 26% lower stored lipids compared to C2 colonies. At spawning time, this correlated with 28% smaller eggs in type-D colonies. This energetic disparity is expected to have reduced larval duration and settlement-competency periods in type-D compared to type-C2 colonies. More importantly, irrespective of the effect of genotype, the fitness of all corals was adversely affected by the stress of the bleaching event which reduced prespawning lipids by 60% and halved the number of eggs compared to the previous year. Our results extend work that has shown that direct temperature stress and symbiont change are likely to work in concert on corals by demonstrating that the lipids and reproduction of the reef building corals on tropical reefs are likely to be impaired by these processes as our climate warms.

1. Introduction

Reef-building corals are vulnerable to climate change as they live in a narrow thermal range and are extremely sensitive to variations. An increase of only 1–2°C above the coral's normal summer maximum, in combination with light, can cause expulsion of the endosymbiotic algae (zooxanthellae) that live within their tissues, resulting in coral bleaching [1]. Such extremes are expected to occur regularly on reefs within the next century meaning that corals must adapt or acclimatize at a rate of 0.2°C every decade if they are to keep pace with even the lowest predicted temperature rise of 2°C by 2100 [2]. One way in which corals can increase their heat tolerance is by changing their endo-symbiont community to more thermally tolerant types like *Symbiodinium* type D [3]. Hosting a viable population of heat tolerant symbionts may give corals a better chance of surviving repeated bleaching events [4]. A recent study utilizing novel molecular methods has revealed previously undetectable levels (<5–10%) of

thermally tolerant symbionts types in four species of reef corals [5], raising the possibility that many species may be able to acclimatize to climate change in this way [6, 7]. So far, symbiont changes have been transitory [8, 9], and it remains to be seen whether they will become permanent as sea temperatures increase and the return time between anomalously warm summers decreases. The phenomenon of symbiont change raises the question: what effects will symbiont change have on holobiont (coral + endosymbionts) energetics and what will this mean to the capacity for coral reefs to recover following disturbance?

The overall energy budget of reef-building corals is influenced by the genotype of their algal endosymbionts. Shallow water reef corals rely in large part on energy derived from symbiont photosynthesis [10]. The symbionts use photochemical energy to fix carbon to produce low molecular weight carbon compounds such as glycerol and triglycerides, a large proportion of which are then translocated to their host as lipids [11]. The lipids are used as an energy store

for processes such as tissue growth [12], skeletal growth [13], and reproduction [14]. Genotypic differences have been found in the translocation rates of photosynthetically fixed ^{14}C to the tissues of juvenile *A. millepora* [15]. In laboratory studies of isolated coral zooxanthellae, the genetic identity of the symbionts influenced the amounts of ^{14}C fixed and released by the host [16]. These and other studies suggest that symbiont genotype may affect host growth rates in juvenile [17, 18] and adult corals [19]. Since skeletal growth is affected by symbiont type it follows that host energetics and fecundity may also vary with symbiont type. However, until now, this link and the magnitude of any such dependence have not been demonstrated.

There is strong evidence from higher plants that there is a link between energetics and thermal tolerance, a feature which is also likely to exist in marine autotrophs like *Symbiodinium* because of shared photosystem mechanisms. For example, analysis of chloroplast thylakoid membranes revealed that the critical threshold temperature separating thermally tolerant from sensitive species of *Symbiodinium* is determined by the saturation of the lipids [20]. Just as in plants, increased saturation state of the thylakoid membranes influences the temperature at which the photosystems become unstable [21]. The most significant consequence of increased polysaturation of the thylakoid membranes for marine autotrophs is that in plants this trait has been shown to reduce the amount of energy available for growth [21]. If *Symbiodinium* utilise similar mechanisms for heat tolerance then it follows that there may be downstream effects on coral energetics. A productivity cost as a result of thermal tolerance may in fact have more than one source: first, reduced photosynthetic activity due to increased polyunsaturation of the thylakoid membranes and second, the now limited energetic resources are diverted to cope with stress [22]. This suggests that although increased thermal tolerance represents a survival benefit, it might not represent an energetic benefit because it may reduce the physical fitness of the organism, or in the case of corals, the holobiont.

Reef-building, or scleractinian, corals form the physical structure of tropical reefs, providing habitat and food for a variety of other marine organisms. Following disturbance, reef regeneration is provisional upon the recruitment and regrowth of individuals of the key structural species [23]. Processes that are energy costly such as thermal stress [24], low salinity [25], and spawning [26] can increase the risk of disease and mortality and impair energetic processes following environmental disturbance [9, 27–29]. In particular, in corals, key regeneration processes like reproduction [29] and larval dispersal and settlement [30], are dependent on the availability of stored lipids. If lipid stores are depleted by metabolic processes involved in recovery from stress then less lipids are available for egg production [31]. The situation is potentially compounded if the coral recovers from a disturbance with a more thermally tolerant, but less autotrophically efficient symbiont type. In this case there could be a compounding effect of symbiont identity and the direct effects of the stress on host energetics and reproduction, potentially affecting the capacity of a reef to regenerate by recruitment.

This study quantifies stored lipids and reproduction; two factors which are critical for coral community regeneration following mass bleaching. *A. millepora* was chosen for the study because it is a common reef builder on leeward shores of the islands in the Keppel region of the southern Great Barrier Reef [32]. Colonies on these reefs naturally host mixed or uniform communities of thermally sensitive type C2 and thermally tolerant type D *Symbiodinium* [3] making this an ideal location to study the downstream effect of symbiont genotype on lipid stores and reproductive output during normal conditions and stress such as bleaching. A bleaching event during the study provided an opportunity to investigate the lipids and reproduction of colonies during their recovery.

2. Materials and Methods

Storage lipids, egg size, and egg number from tagged, adult *A. millepora* colonies with known predominant symbiont genotype were measured seasonally over 24 months to investigate the links between predominant symbiont community identity, bleaching resistance, and colony fitness. The study was conducted before, during, and after a major bleaching event in February 2006 which caused significant mortality of colonies on reef flats and slopes in the Keppel region [33]. Before the bleaching event, colonies of *A. millepora* at this site naturally hosted *Symbiodinium* type C2 with the occasional occurrence of type D, or mixtures of both C2 and D. Following the bleaching event, 71% of surviving colonies exhibited a shift in symbiont community from C2 to D and another potentially thermally tolerant type, C1 [8].

2.1. Sampling Design. The study took place at Miall Island (23°09'S 150°54'E) in the Keppel Island group in the southern inshore Great Barrier Reef. In March 2005, 24 colonies of the Indo-Pacific stony coral *A. millepora* (Ehrenberg, 1834) of roughly the same size and at the same depth were chosen based on their predominant symbiont genotype. The *Symbiodinium* type in the colonies was verified just before the start of the experiment and at each sampling occasion in March and July 2005 and January, May, and August 2006 using Single Stranded Conformational Polymorphism (SSCP) analysis of the ITS1 region of algal nuclear ribosomal DNA. Samples from colonies with intense single SSCP bands were considered to host monomorphic (predominant) symbioses although the presence of other genotypes below 5–10% relative abundance could not be ruled out [34]. Samples with multiple bands matching C2, D, and C1 were considered mixed symbioses. SSCP profiles were assigned to symbiont type by cloning representative samples per SSCP profile, followed by DNA sequencing as described in [19]. Twenty-four colonies with single, intense type C2 or type D SSCP bands were chosen for the study. Colonies were initially divided into two treatment groups: 12 colonies with predominantly type C2 symbionts and 12 with predominantly type D symbionts. Colonies were not regentyped in November 2005, February 2006, or November 2006.

The symbiont community was relatively stable before the bleaching event except for two colonies which changed

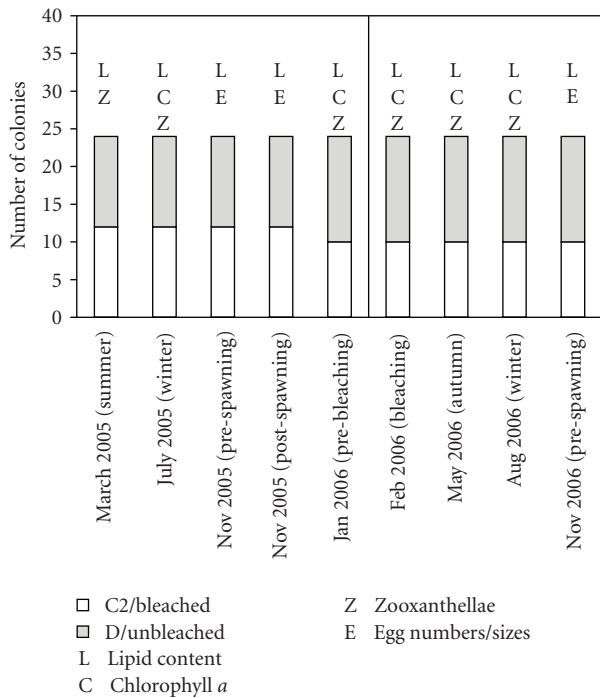


FIGURE 1: Sampling design for the study of storage lipids (L), algal chlorophyll *a* content (C), symbiont densities (Z), and egg sizes and numbers (E) in colonies of *Acropora millepora* sampled before (grey bars = D, white bars = C2) and after (grey bars = unbleached, white bars = bleached) a bleaching event in February 2006 (shown as a thick line between January and February 2006).

from predominantly C2 symbionts in March and July 2005 to host predominantly type D symbionts in February 2006. The colonies were sampled again three months later in July 2005, then before and after spawning in November 2005, in January 2006 (at the onset of bleaching), and in February 2006 at the peak of the bleaching (Figure 1). The bleaching state of each colony was recorded throughout February 2006 by visually estimating the loss of colony colouration compared to the prebleaching state (white = bleached, normal colouration = unbleached). At this time, all 10 type C2 colonies had lost their normal colouration and all 14 type D colonies appeared normally coloured. After the peak of the bleaching, the same colonies were re-sampled in May 2006, August 2006, and before the annual spawning event in November 2006. The symbiont community changed dramatically following the bleaching (see Section 4.1).

At each of the three monthly sampling occasions, two branches were removed from the centre of each colony. A small piece of one branch was placed in 100% ethanol for symbiont identification and the remaining samples were snap-frozen in liquid nitrogen and subsequently stored at -80°C until processed. One branch was used for the analysis of lipids and the second branch for the determination of chlorophyll *a* content, and symbiont densities. Algal chlorophyll *a* content is a proxy for bleaching severity and recovery rates in corals [35].

In addition to the three monthly sampling occasions, two additional branches were removed from each tagged colony before and after spawning in November 2005 and before spawning in November 2006. A maximum diameter was recorded for each colony to determine whether colony size had any influence on reproductive output. One branch was snap-frozen in liquid nitrogen and stored at -80°C for the analysis of lipids, and the second branch was stored in 10% formalin in seawater and used for the determination of reproductive output (egg number, and egg size).

2.2. Lipids. Lipid analysis was performed on dried, ground, whole coral tissue samples including zooxanthellae and skeleton. The samples were ground under liquid nitrogen [36] to produce a fine homogenous powder and placed in acetone-washed and preweighed 25 mL sintered glass vials. The vials containing the frozen ground samples were freeze-dried and weighed to four decimal places and the total dry weight of the sample (DWS) recorded. Subsamples of known weight (approx 0.1–0.5 mg) were removed for lipid analysis. Lipid measurements were standardized to coral tissue surface area using the 3D digital image analysis methods described in Jones et al. [37].

The total lipid content of each branch was determined using two consecutive chloroform: methanol extractions following the technique described by Folch et al. [38] and Harland et al. [39]. The organic phase was washed once with 0.88% KCl and three times with methanol: water (1 : 1), evaporated and dried in an oven at 60°C . The lipid content was expressed as the difference between the weight of the pan, and the weight of the pan plus sample, multiplied by the proportion of the total DWS. Total lipids were calculated by multiplying the lipid content per dry weight of the subsample by the total dry weight of the whole branch.

2.3. Symbiont Densities and Chlorophyll *a* Pigments. To determine the influence of symbiont densities and the photosynthetic pigment chlorophyll *a* on coral lipids and reproduction, frozen branches were stripped of tissue using an air gun and the resultant slurry was macerated with a tissue homogenizer for 20 s. The homogenate volume was recorded, and a 9 mL aliquot was drawn off and preserved with 1 mL of formalin (32% w·w⁻¹). Symbiont counts were made on eight independent drops (0.0001 mm³) from each sample using a New Improved Nuebauer haemocytometer under a compound light microscope. Symbiont numbers were standardized to coral tissue surface area using the 3D digital image analysis method described in Jones et al. [37].

A separate 10 ml aliquot was drawn from the remaining tissue homogenate and the algal pellet was separated from the host tissue by centrifugation (3000 g for 5 minutes) at 4°C . Chlorophyll was extracted overnight from the algal pellet using 100% methanol at 4°C . The first 10 samples were extracted three times to determine the extraction efficiency. Absorbance at 668 nm and 635 nm was measured with a spectrophotometer (Hitachi U-3200). Total branch chlorophyll *a* was calculated from the equation of Jeffrey and Haxo [40] after adjustment for extraction efficiency and standardized to algal cells.

2.4. Reproduction. Branches that had been stored in 10% formaldehyde and seawater were decalcified in 15 mL Falcon tubes using 6% hydrochloric acid and 2% formaldehyde in reverse osmosis (RO) purified water. The decalcifying solution was changed twice over three days until no skeletal material remained. Samples were then washed in RO water and stored in 15 mL Falcon tubes in 5% formaldehyde and RO water.

Six polyps were haphazardly selected from each decalcified coral branch and removed from the branch under a stereomicroscope [41]. Individual polyps were then dissected under a light microscope with a calibrated eyepiece micrometer (0.25 mm, [42, 43]) to expose the mesenteries. Maximum “length” and “width” measurements were taken for each egg [41, 44]. The size was calculated as the width multiplied by the length. The total number and the size of each egg and sperm bundle (hereafter referred to as eggs) were recorded for each of the six polyps.

3. Statistical Analysis

3.1. Lipids, Symbiont Densities and Chlorophyll *a* Content. To investigate the effects of symbiont type and sampling occasion on lipid stores and symbiont densities before the bleaching, data were analysed with a multivariate repeated measure ANOVA using symbiont type (fixed, two levels) as the predictor variable and sampling occasion (random, two levels) as the repeated measure in the analysis.

To investigate the effects of symbiont type on the chlorophyll *a* content in *A. millepora* colonies before the bleaching, data were analysed with univariate ANOVA using symbiont type (fixed, two levels) as the predictor variable in the analysis. Postbleaching samples were not included in these analyses because the adoption of multiple types and the dynamic nature of the symbiont community prevented a robust statistical analysis given the small sample sizes involved.

The symbiont changes following the bleaching prevented a direct comparison of lipids, symbiont densities and chlorophyll *a* content of colonies with respect to symbiont type. To investigate the effects of the bleaching on lipid stores, chlorophyll *a* content and symbiont densities were analysed with a repeated measure ANOVA using colony bleaching condition (fixed, two levels) as the predictor variable and sampling occasion (random, four levels) as the repeated measure in the analysis.

3.2. Reproduction. To investigate the effects of symbiont genotype on the reproductive output of *A. millepora* colonies before the bleaching event, data for prespawning lipids, egg number, and egg size were analysed with a single factor multivariate ANOVA using symbiont type (two levels) as the fixed predictor variable in the model. Model II regression analysis was used to investigate significant relationships between prespawning lipids, lipid allocation (prespawning lipids minus postspawning lipids), colony size, and egg number and egg size.

The symbiont changes following the bleaching prevented a direct comparison of colony reproductive output with

respect to symbiont type. To investigate the effects of the bleaching event on the reproductive output of *A. millepora* colonies, data for prespawning lipids, egg number and egg size were analysed with a multivariate repeated measures ANOVA using bleaching condition (two levels) as the fixed predictor variable and year (random, two levels, before and after the bleaching event) as the repeated measure in the model.

In all analyses described above, scatterplots of the unstandardized residuals and standardized predicted values were used to verify the normality of the data and Levene's tests were used to verify the homogeneity of variances. Wherever significant interactions between predictor variables were found, simple, pairwise comparisons were performed using Sidak's adjustment for multiple comparisons [45]. In all repeated measures analyses if sphericity of the data could not be assumed, significance values were adjusted using the Greenhouse-Geisser epsilon. All statistical analyses in the study were performed using SPSS version 17.0.

4. Results

4.1. Symbiont Identification. The onset of the bleaching occurred just prior to January 2006 when the *A. millepora* colonies in the study had begun to appear pale or “fluorescent”. The bleaching peaked in February 2006 when water temperatures remained at $>29^{\circ}\text{C}$ for over two weeks [11-year mean summer temperature (December–February) = $27.0 \pm 0.5^{\circ}\text{C}$, mean \pm S.D; R. Berkelmans, unpublished data]. As a result of heat stress, 10 of the original 12 colonies with C2 symbionts bleached white due to loss of symbionts. The remaining two original C2 colonies were unbleached because they had changed to host predominantly type D symbionts between November 2005 and January 2006. All 12 of the original type D colonies and these two colonies retained their normal prebleaching colouration. All 24 colonies survived the bleaching and had returned to their normal prebleaching colouration by May 2006. Of the 10 prebleaching (January 2006) type C2 colonies, two changed to type D upon recovery, one colony recovered with a mix of C2 and D, one colony with a mix of D and C1, three with a mix of C2 and C1, and three colonies changed to a monomorphic C1 symbiont community. Ten of the original 12 D colonies retained their D symbionts, one colony changed to C1 and one colony gained C1 as “background” symbionts (Figure 2). The two colonies that changed from C2 to D before the bleaching retained their new D symbiont types throughout and after the bleaching event. These symbiont changes provided an opportunity to investigate the lipids and reproduction of specific colonies during their recovery but prevented a direct comparison of the differential effects of bleaching on the lipids and reproduction with respect to symbiont type (D versus C2 colonies).

4.2. Prebleaching

4.2.1. Lipids. Before the bleaching event, lipid content in *A. millepora* colonies varied significantly with the predominant symbiont genotype ($P < .05$). The mean annual lipid content

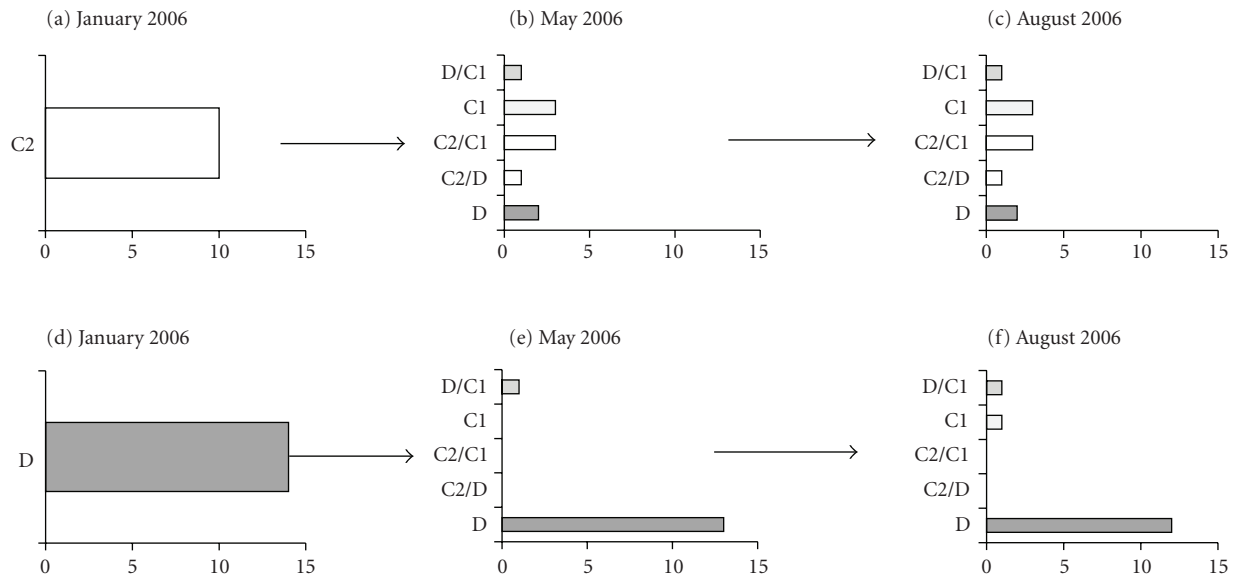


FIGURE 2: Symbiont community changes in colonies of *Acropora millepora* following a bleaching event in February 2006. (a)–(c). Two of the original 12 C2 colonies changed to type D symbionts, one colony recovered with C2 and D, one colony changed to D and C1, three colonies changed to C1, and three colonies recovered with both C2 and C1 symbionts. (d)–(f). Ten of the original D colonies retained their D symbionts, one colony changed to C1, and one colony gained C1 symbionts.

of type D colonies was $2.5 \pm 0.3 \text{ mg cm}^{-2}$ (mean \pm S.E.), 26% lower than that of type C2 colonies which was $3.4 \pm 0.3 \text{ mg cm}^{-2}$ (mean \pm S.E., Figure 3(a), Table 1, $P < .05$).

4.2.2. Symbiont Densities and Chlorophyll *a* Pigments. Chlorophyll *a* content in *A. millepora* colonies also varied significantly with symbiont genotype before the bleaching event (based on data from July 2005, Figure 3(b), Table 2). The mean chlorophyll *a* content of symbionts for type D colonies was $28.5 \pm 1.9 \times 10^{-6} \mu\text{g cell}^{-1}$, 21% lower than for type C2 colonies which had $35.9 \pm 1.9 \times 10^{-6} \mu\text{g cell}^{-1}$ (mean \pm S.E., $P < .05$).

Symbiont densities of *A. millepora* colonies were not significantly different between symbiont genotypes before the bleaching (Figure 3(c)). However, type D colonies had 71% higher densities in the winter following the bleaching ($3.1 \pm 0.3 \times 10^6 \text{ cells cm}^{-2}$, mean \pm S.E.) compared to summer densities ($1.8 \pm 0.2 \times 10^6 \text{ cells cm}^{-2}$, mean \pm S.E., $P < .05$).

4.2.3. Reproduction. Lipids and egg sizes varied significantly with predominant symbiont genotype before the bleaching (Table 3). In the lead up to the annual mass spawning in 2005, lipids reached their highest levels just before spawning in November; an increase of 37% compared to July ($P < .05$), and decreased by 38% following spawning ($P < .05$). Prespawning lipids were positively correlated with egg numbers ($r^2 = 0.01$, $P < .001$) and negatively correlated with egg sizes ($r^2 = 0.05$, $P = .017$, Figure 4). There was no significant correlation between lipid allocation to spawning (pre- minus postspawning lipids) and eggs (number or size).

Type D colonies had $3.1 \pm 0.4 \text{ mg cm}^{-2}$ (mean \pm S.E.) stored lipids before the annual spawning, 34% less than

C2 colonies which had $4.6 \pm 0.8 \text{ mg cm}^{-2}$ (mean \pm S.E., $P < .05$, Figure 5(a)). The eggs in type D colonies measured $4.2 \pm 0.5 \text{ mm}^2$ mean \pm S.E., 28% smaller than the eggs of C2 colonies which measured $5.9 \pm 0.6 \text{ mm}^2$ (mean \pm S.E., $P < .05$, Figure 5(b)). All the polyps dissected from C2 colonies contained egg bundles. Two of the type D colonies contained no eggs in any of the six haphazardly selected polyps. There was no significant effect of symbiont genotype on egg numbers (Figure 5c).

4.3. Postbleaching

4.3.1. Lipids. The bleaching event in early 2006 had a profound effect on lipid levels in *A. millepora* (Figure 3(a), Tables 4 and 5). The storage lipids in the tagged colonies followed a similar pattern to symbiont densities, and chlorophyll *a* content, declining to ~59% of January levels by May 2006, three months after the peak of the bleaching (Figures 3(b)–3(c)). Lipids remained 21% lower than prebleaching levels (based on the mean annual lipid content) in August 2006, six months after the peak of bleaching ($P < .05$).

Although the lipids of all colonies (irrespective of prebleaching symbiont type or conditions) were affected by the temperature stress, colonies that originally had C2 symbionts that bleached white were affected more severely than those with type D symbionts that appeared unbleached ($P < .005$). The 10 bleached C2 colonies lost 67% of their lipids between February and May 2006 ($P < .05$), whereas the 14 unbleached type D colonies lost only 43% of their lipids which for those months was not significantly different to their prebleaching levels. The time-frame for this decline in lipid content is consistent with that described by Anthony et al. [28] for acroporid corals two months after bleaching. Recovering

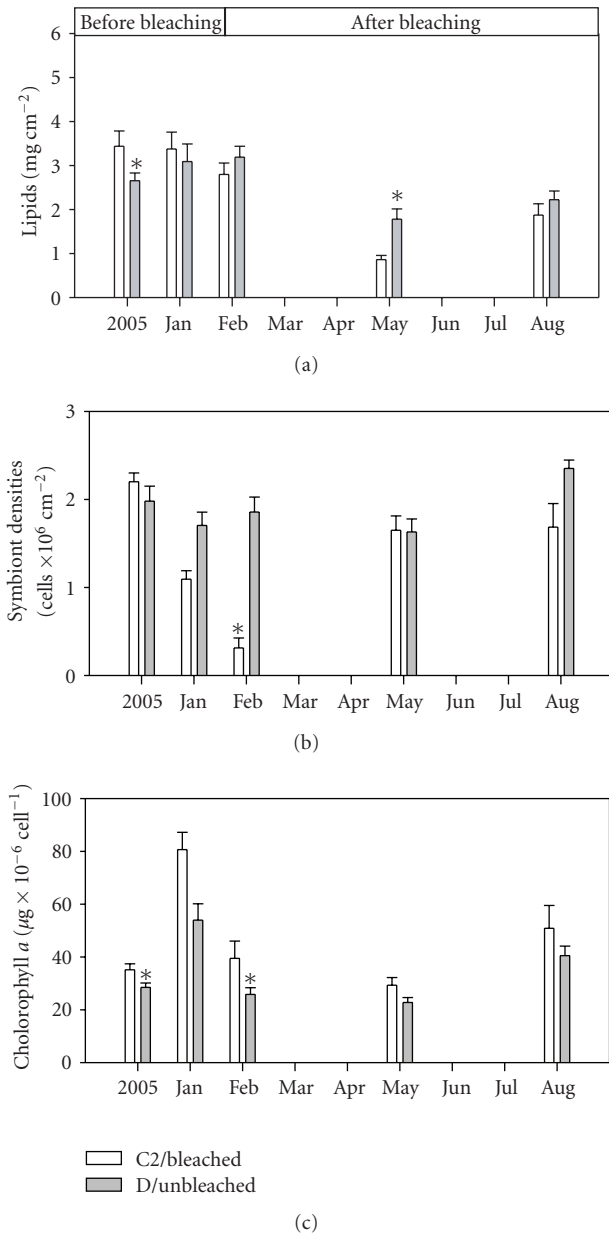


FIGURE 3: (a) Lipids, (b) chlorophyll *a* content, and (c) symbiont densities of *Acropora millepora* in the year before and after a bleaching event that occurred in February 2006. White bars represent colonies that had predominantly C2 symbionts before the bleaching and bleached white and grey bars represent colonies with predominantly D symbionts that did not bleach. Whisker bars above the bars represent the standard error of the mean. * represent significant differences.

bleached colonies (which now had a mixture of C1, D, and C2 symbionts) had regained 82% of their lipids between May and August, six months after the event ($P < .05$).

4.3.2. Symbiont Densities and Chlorophyll *a* Pigments. Colonies that had type C2 symbionts before the bleaching lost 72% of their symbionts between January and February 2006 ($P < .05$, Figure 3(b)). In contrast, symbiont densities

in unbleached type D colonies were not significantly affected. Between February and May 2006, bleached, original type C2 colonies (which now had mostly C1 and D symbionts) regained 82% of their prebleaching symbiont densities ($P < .001$).

Symbiont chlorophyll *a* concentrations in all colonies were 53% lower in February compared to the levels in January but had recovered by May and August 2006 ($P < .05$, Figure 3(c)). There were no significant differences in the symbiont chlorophyll *a* content of colonies with respect to their new symbiont types or their condition during the bleaching event.

4.3.3. Reproduction. Temperature stress affected the pre-spawning lipids and egg size of *A. millepora* colonies irrespective of their condition (bleached or unbleached, $P < .05$, Figures 5(a)–5(c), Table 6). In November 2006, the mean pre-spawning lipids of the colonies in the study was 1.51 ± 0.19 mg cm⁻² (mean \pm S.E.), 62% lower compared to levels for the same period in 2005 ($P < .05$, Figure 5(a)). In spite of the significant difference between the sizes of eggs in C2 and D colonies before the bleaching ($P < .05$, Figure 5(b)) there were no differences with respect to symbiont type (C2 or D) or colony condition in egg sizes after the bleaching. The mean number of eggs in colonies irrespective of their prebleaching symbiont type was 4.1 ± 0.3 eggs polyp⁻¹ (mean \pm S.E.), 41% less than numbers in 2005 ($P < .05$, Figure 5(c)).

5. Discussion

Our results show that climate change may diminish the energy stores and fecundity of reef corals in two separate and independent processes. First, the stored lipids will be diminished and the egg sizes decreased in colonies that host predominantly type D symbionts under normal conditions. Second, the lipids and egg numbers will be depleted by the very stress event that caused symbiont shuffling to more thermally tolerant types in the first place. While the effects may in part be due to changed symbiont types, the decrease in lipids and egg numbers post-bleaching was much greater than could be accounted for by symbiont type based on prebleaching data. Under normal conditions, *A. millepora* that host thermally tolerant type D symbionts had 26% lower lipid stores and 28% smaller eggs compared to C2 colonies. A severe bleaching event in 2006 [46] resulted in a significant *Symbiodinium* community shift from type C2 to types D and C1 symbionts in the *A. millepora* colonies at the study site. The shift may have resulted in at least temporarily greater resistance to heat stress [3], but the tradeoff was reduced energy stores leading to lower gamete numbers for all colonies, irrespective of either symbiont genotype before the bleaching or condition during the bleaching. While bleached colonies fared much worse than unbleached colonies in their post-bleaching capacity to produce and store lipids and ultimately in the numbers of gametes they produced, even unbleached colonies were affected by the heat stress with an estimated overall reduction of 55% in pre-spawning lipids and 29% in egg numbers compared to the previous season.

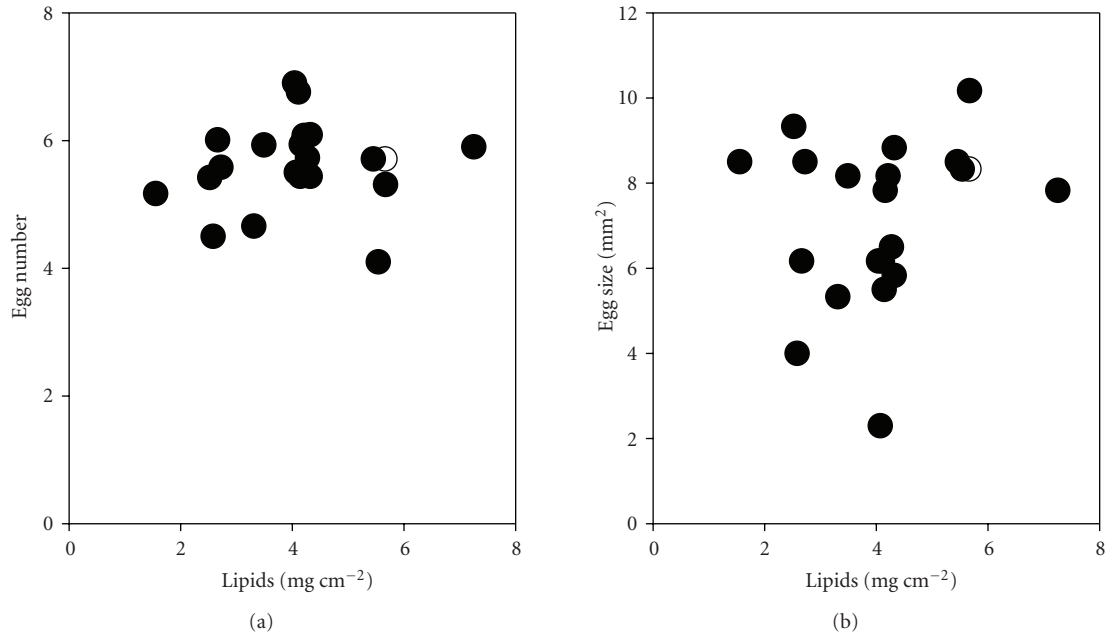


FIGURE 4: Scatterplots showing the significant correlations between prespawning lipids and (a) egg numbers ($r^2 = 0.01$, $P < .001$) and (b) egg sizes ($r^2 = 0.05$, $P = .017$) in *Acropora millepora* colonies in the year before a bleaching event.

TABLE 1: The results of a multivariate repeated measure ANOVA showing the significant between-subject effect of type (C2 or D) on lipid stores of *Acropora millepora* colonies before a bleaching. *indicate significant results.

	Measure	S.S.	df	Mean square	F	Sig.
Intercept	Lipids	428.53	1	428.53	256.6	0.000
	Symbiont densities	275.91	1	275.91	465.3	0.000
Type	Lipids	9.68	1	9.68	5.8	0.025*
	Symbiont densities	0.33	1	0.33	0.6	0.464
Error	Lipids	36.74	22	1.67		
	Symbiont densities	13.05	22	0.59		

Coral bleaching results in the loss of a readily available source of photosynthetically fixed carbon to the coral host. Until it recovers its symbiont community, the coral has to survive temporarily on its stored energy reserves. By far the most important source of this energy is lipids which can account for 10–40% of dry tissue weight [47–49] and can provide ~78–90% metabolic energy requirements of the coral [14, 50, 51]. In zooxanthellate corals without heterotrophic-autotrophic plasticity stored lipids are depleted following bleaching, without being replenished as they are gradually used for cellular repair and metabolism [52]. The loss of autotrophy as a direct result of symbiont expulsion clearly contributed to the depletion of lipid stores in bleached type C2 colonies. Two phenomena are remarkable here. The first is that stored lipids of bleached C2 colonies rapidly recovered to levels comparable with those of their unbleached counterparts in spite of recovering with the less photosynthetically efficient type D and C1 symbionts [15, 17–19]. The second is that unbleached type

D colonies, which did not lose their symbionts, still had drastically reduced lipids compared to prebleaching levels for up to 9 months following the bleaching. This is supported by Grottoli et al. [53] who found that even unbleached *Porites compressa* and *Montipora verrucosa* colonies had ~16% and 24% less stored lipids, respectively, following a bleaching event in Hawaii in 1996, compared to the same season in previous nonbleaching years. Clearly there are factors at play other than the loss of symbionts. However, even more importantly, the temporary loss or interruption of their lipid production and storage capacity can place corals at risk of mortality after a thermal stress event regardless of their symbiont community or bleaching status [27, 28].

This study is one of several to demonstrate that factors other than the temporary loss of the symbionts are key determinants of the way that reef corals respond to temperature stress. Abrego et al. [54] demonstrated that light utilization efficiency, photosynthetic reaction centre integrity, and light prehistory may all play a part in the physiological response of

TABLE 2: The results of a univariate ANOVA showing the significant effect of predominant symbiont type (C2 or D) on the chlorophyll *a* content of *Acropora millepora* colonies in winter (July 2005) before a bleaching. The corrected model shows the variation in the dependent variable by other effects (other than the intercept) after correction for the mean. *indicate significant results.

	S.S.	df	Mean Square	F	Sig.
Corrected Model	331.00 ^a	1	331.00	7.4	0.013
Intercept	24837.84	1	24837.84	551.9	0.000
Type	331.00	1	331.00	7.4	0.013*
Error	990.02	22	45.00		
Total	26158.86	24			
Corrected Total	1321.02	23			

(^a) R Squared = 0.251 (Adjusted R Squared = 0.217).

TABLE 3: ANOVA table showing the significant effect of symbiont Type (C2, D) on pre- and postspawning lipids and egg size (mm²) in *Acropora millepora* colonies before the bleaching. The corrected model shows the variation in the dependent variable by other effects (other than the intercept) after correction for the mean. *indicate significant results.

	Measure	S.S.	df	Mean square	F	Sig.
Corrected model	Lipids	14.25 ^(a)	1	14.25	8.4	0.009
	Egg size	16.18 ^(b)	1	16.18	6.8	0.017
	Egg number	21.08 ^(c)	1	21.08	3.1	0.095
Intercept	Lipids	334.63	1	334.63	197.1	0.000
	Egg size	594.46	1	594.46	249.2	0.000
	Egg number	969.94	1	969.94	140.3	0.000
Type	Lipids	14.25	1	14.25	8.4	0.009*
	Egg size	16.18	1	16.18	6.8	0.017*
	Egg number	21.08	1	21.08	3.1	0.095
Error	Lipids	35.65	21	1.70		
	Egg size	50.09	21	2.39		
	Egg number	145.13	21	6.91		
Total	Lipids	391.20	23			
	Egg size	670.43	23			
	Egg number	1150.48	23			
Corrected total	Lipids	49.90	22			
	Egg size	66.27	22			
	Egg number	166.21	22			

(^a) R Squared = 0.286 (Adjusted R Squared = 0.252).

(^b) R Squared = 0.244 (Adjusted R Squared = 0.208).

(^c) R Squared = 0.127 (Adjusted R Squared = 0.085).

TABLE 4: Results of repeated measures multivariate ANOVA showing the significant effect of sampling occasion (Jan, Feb, May, and Aug) on symbiont chlorophyll *a* content and lipids and the significant interaction between sampling occasion and bleaching condition on the symbiont densities of *Acropora millepora* colonies following a bleaching event in February 2006. *indicate significant results.

	Measure	S.S	df	Mean square	F	Sig.
Sampling occasion	Symbiont densities	6.62	3	2.21	5.2	0.004*
	Chlorophyll <i>a</i>	14876.64	3	4958.88	11.5	0.000*
	Lipids	46.74	2	22.94	14.3	0.000*
Sampling occasion* bleaching condition	Symbiont densities	7.85	3	2.62	6.2	0.001*
	Chlorophyll <i>a</i>	269.09	3	89.70	0.2	0.891
	Lipids	3.50	2	1.72	1.1	0.356
Error (sampling occasion)	Symbiont densities	19.01	45	0.42		
	Chlorophyll <i>a</i>	19477.60	45	432.84		
	Lipids	49.02	31	1.60		

TABLE 5: The between-subject effects of repeated-measures multivariate ANOVA showing the significant effect of condition (bleached or unbleached in February 2006) on the symbiont densities and algal chlorophyll *a* content of *Acropora millepora* colonies. *indicate significant results.

	Measure	S.S.	df	Mean square	F	Sig.
Intercept	Symbiont densities	206.51	1	206.51	229.4	0.000
	Chlorophyll <i>a</i>	122068.33	1	122068.33	507.1	0.000
	Lipids	384.88	1	384.88	242.9	0.000
Bleaching condition	Symbiont densities	10.72	1	10.72	11.9	0.004*
	Chlorophyll <i>a</i>	1616.52	1	1616.52	6.7	0.020*
	Lipids	2.82	1	2.82	1.8	0.202
Error	Symbiont densities	13.50	15	0.90		
	Chlorophyll <i>a</i>	3610.80	15	240.72		
	Lipids	23.77	15	1.58		

TABLE 6: The results of a multivariate ANOVA showing the significant within-subject effect of year on the lipids and egg numbers in bleached C2 and unbleached D colonies of *Acropora millepora* before and after a bleaching event in February 2006. There was no significant effect of year on egg size and no significant interactions between bleaching condition and year. *indicate significant results.

	Measure	S.S.	df	Mean Square	F	Sig.
Year	Lipids	50.92	1	50.92	51.6	0.000*
	Egg size	0.40	1	0.40	0.3	0.569
	Egg number	58.73	1	58.73	14.8	0.001*
Year* bleaching condition	Lipids	3.02	1	3.02	3.1	0.098
	Egg size	3.07	1	3.07	2.6	0.126
	Egg number	8.06	1	8.06	2.0	0.172
Error (year)	Lipids	16.78	17	0.99		
	Egg size	20.14	17	1.18		
	Egg number	67.39	17	3.96		

different coral host-symbiont combinations. Warmer conditions during bleaching could increase host cell metabolism, placing a heavier metabolic demand on the stored energy reserves [55, 56]. If the host's respiratory demands remained high throughout the bleaching (and possibly longer to help cope with the stress), stored lipids are used faster than they are synthesized by the recovering symbiont population. This could occur even in unbleached colonies [57, 58]. An alternative explanation is that in the colonies that retained their symbionts, the photosystems were still affected by the heat stress [59]. Reversible uncoupling of normal photosynthetic function, or photoinhibition, can potentially reduce carbon fixation under these conditions [60, 61]. Diverting energy to heat protects the photosystems from excess excitation pressure. Downregulation of photosynthesis can also be achieved in corals by reducing the pigment content of algal cells which was observed in unbleached *A. millepora* colonies as reduced algal chlorophyll *a* content during and immediately following the bleaching. A fourth explanation for the decrease in lipids in unbleached corals is that host factors play a part in lipid depletion through increased mucous production in response to stress [62]. It remains unclear exactly which of these factors or combination of factors contributed to the lower lipid stores of type D colonies in the year following the bleaching. What is clear

is that in spite of increasing bleaching resistance, the thermal tolerance of the symbionts does not protect the coral from the detrimental but sublethal effects of the heat stress itself, especially in terms of post-bleaching lipid stores and gamete production.

Symbiont identity affects reproductive output of the holobiont in a different way to that of direct thermal stress. Under normal conditions, in the lead-up to the annual spawning event, the colonies with type D symbionts had less stored lipids available to contribute to egg production, leading to smaller eggs than type C2 colonies. For most reef-building corals the energy required for the development of planulae larvae is mainly derived from stored lipids: primarily triacylglycerides and esters [48, 63]. *A. millepora* produce azooxanthellate larvae which acquire symbionts from the surrounding environment after settlement. Once acquired, the symbionts can provide 13–27% of the energetic requirements of the larvae via photosynthesis [64]. Until the symbiosis is established the larvae are dependent on the stored energy provided by the parent. These energy stores control two key factors of reproductive success, larval duration and settlement-competency periods [64]. Under nonstressful conditions *A. millepora* colonies with type D symbionts may be diverting smaller quantities of storage lipids to egg production than type C2 colonies because

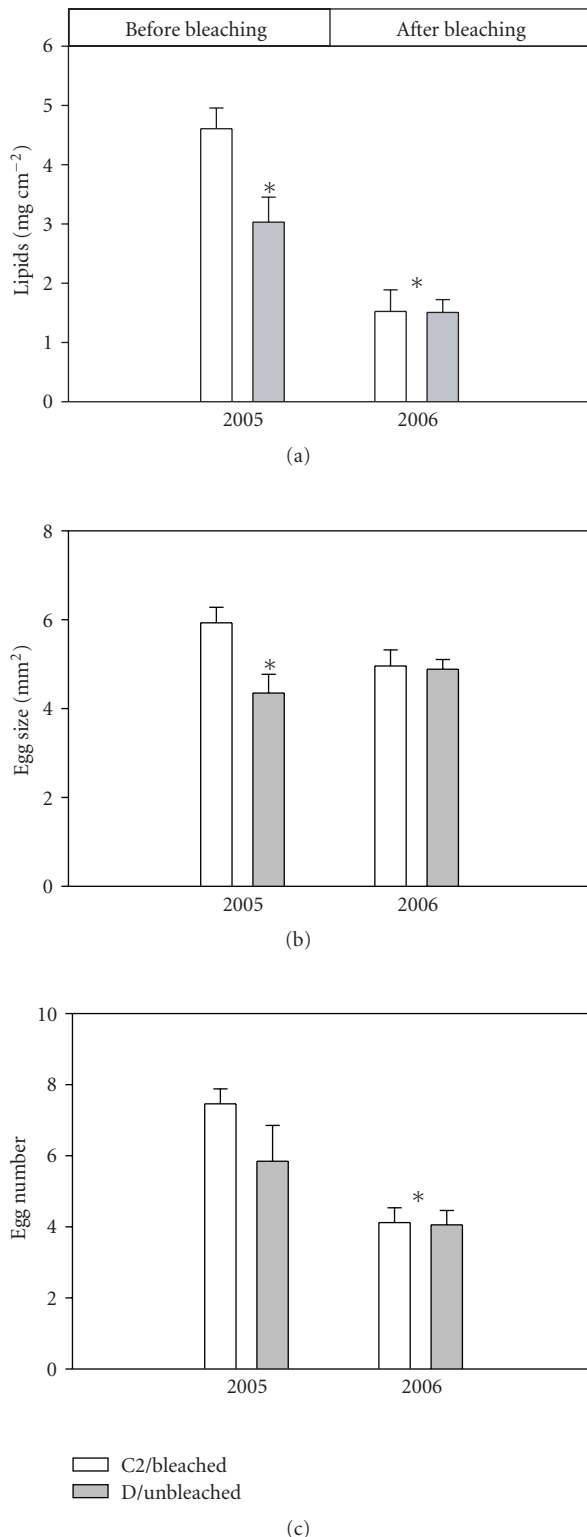


FIGURE 5: (a) Lipids, (b) egg size, and (c) egg numbers in colonies of *Acropora millepora* before and after a bleaching event that occurred in February 2006. White bars represent colonies with predominantly C2 symbionts before the bleaching that bleached white and grey bars represent colonies with predominantly D symbionts before the bleaching that were unbleached during the event. Whisker bars above the bars represent the standard error of the mean. *represent significant differences.

smaller quantities are available to begin with. To accommodate for the reduced lipids available for egg generation, type D colonies appear to maintain egg numbers at a cost to egg size, maintaining the numerical odds of successful self-proliferation but at a cost to larval competency and subsequently, reducing the potential extent of geographic distribution [65]. During reproduction following a bleaching however, the opposite occurs. *A. millepora* colonies that have undergone temperature stress, regardless of their bleaching condition, produce fewer eggs but maintain egg sizes. Perhaps this ensures that adequate energy is available for the now limited number of larvae to survive longer and improves settlement-competency periods, reducing the numerical odds of self-proliferation but maintaining the range of geographic distribution.

This study has provided some insights into the synergistic effects and magnitude of symbiont genotype and thermal stress on the lipid stores and reproduction of *Acropora* corals. These two influences are likely to have significant implications for the bleaching resistance, mortality risk, and reproduction of corals that are capable of shuffling their symbionts as ocean temperatures warm with climate change. Not all corals can change their symbionts, and it has been argued that those that can, such as the *Acropora*, may be the exception rather than the rule [66, 67]. However, it must be acknowledged that to date our understanding of symbiont shuffling is poor, especially in relation to which species (and populations within species) and the circumstances in which this process occurs. Novel molecular methods are revealing previously hidden symbiont diversity in many reef corals suggesting that under favorable conditions, the potential exists for different types to multiply and outcompete other types [68]. For corals that can shuffle, the results of this study suggest that the thermal tolerance of the predominant symbiont genotype can be detrimental to energetics and reproduction, and that this will be compounded by the long-term effects of severe heat stress even if they do survive. For those corals, acclimation by symbiont shuffling in response to warmer and more stressful conditions therefore may not represent as much of an ecological benefit as has previously been suggested [3]. However, (and more importantly) if sustained warmer conditions cause wholesale symbiont community change to more thermally tolerant types, then there will be compounding effects of symbiont identity and direct thermal stress on coral energy stores and reproduction.

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