Author for correspondence:
Verena Schoepf
e-mail: schoepf.4@osu.edu

THE ROYAL SOCIETY
PUBLISHING

Annual coral bleaching and the long-term recovery capacity of coral

Verena Schoepf[1,2], Andréa G. Grottoli[1], Stephen J. Levas[1,3], Matthew D. Aschaffenburg[4], Justin H. Baumann[1,5], Yohei Matsui[1] and Mark E. Warner[4]

[1]School of Earth Sciences, The Ohio State University, Columbus, OH, USA
[2]Australian Research Council Centre of Excellence for Coral Reef Studies, School of Earth and Environment, and UWA Oceans Institute, The University of Western Australia, Crawley, Western Australia, Australia
[3]Department of Geography and the Environment, Villanova University, Villanova, PA, USA
[4]School of Marine Science and Policy, University of Delaware, Lewes, DE, USA
[5]Department of Marine Sciences, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

Mass bleaching events are predicted to occur annually later this century. Nevertheless, it remains unknown whether corals will be able to recover between annual bleaching events. Using a combined tank and field experiment, we simulated annual bleaching by exposing three Caribbean coral species (*Porites divaricata*, *Porites astreoides* and *Orbicella faveolata*) to elevated temperatures for 2.5 weeks in 2 consecutive years. The impact of annual bleaching stress on chlorophyll *a*, energy reserves, calcification, and tissue C and N isotopes was assessed immediately after the second bleaching and after both short- and long-term recovery on the reef (1.5 and 11 months, respectively). While *P. divaricata* and *O. faveolata* were able to recover from repeat bleaching within 1 year, *P. astreoides* experienced cumulative damage that prevented full recovery within this time frame, suggesting that repeat bleaching had diminished its recovery capacity. Specifically, *P. astreoides* was not able to recover protein and carbohydrate concentrations. As energy reserves promote bleaching resistance, failure to recover from annual bleaching within 1 year will likely result in the future demise of heat-sensitive coral species.

1. Introduction

Climate change is one of the main threats to coral reefs today [1], and mass bleaching events due to periods of elevated ocean surface temperatures have increased in frequency over the past decades [2,3]. Furthermore, annual severe bleaching is expected to occur worldwide later this century, putting more than 90% of reefs at risk of long-term degradation [4]. In the Caribbean, this is projected to occur as early as 2040 [5]. Yet, the effects of annually recurring bleaching on coral physiology, recovery capacity and resilience remain largely unstudied.

There is mounting evidence that thermal history has strong impacts on coral bleaching susceptibility [6–8]. However, the first study to experimentally address annual coral bleaching was Grottoli *et al.* [9], which showed that bleaching stress occurring in 2 consecutive years can dramatically alter the thermal tolerance of coral, and that while some species were able to acclimate rapidly to repeat bleaching events, others became more susceptible. Although this study was a major step forward, it did not address the impacts of annual bleaching on long-term recovery (i.e. more than 1.5 months). It is currently unknown if corals can recover between annual bleaching events, or if their recovery capacity is overwhelmed by such frequent bleaching stress.

During bleaching, corals lose a significant portion of their algal endosymbionts (*Symbiodinium* spp.) and/or photosynthetic pigments, giving the colony a pale (hence bleached) appearance [10,11]. As healthy corals typically meet most of their daily energy requirements via photosynthesis by their endosymbiotic algae (e.g. [12]), bleaching results in severe reductions in algal photosynthesis

and translocation of photosynthate to the coral host accompanied by dramatic changes in physiology. Thus, bleached corals typically decrease calcification (e.g. [13,14]) and catabolize energy reserves (e.g. [15–17]), and some species may increase heterotrophy [18]. Generally, the size of energy reserves (i.e. lipids, protein, carbohydrates), endosymbiont type, as well as heterotrophic capacities are critical in promoting bleaching resilience and recovery [9,18–21].

In addition to physiological measurements, coral tissue isotopes can be useful indicators of bleaching recovery. Specifically, the carbon isotopic composition of the animal host ($\delta^{13}C_h$) and endosymbiont ($\delta^{13}C_e$) track changes in the allocation of auto- and heterotrophically derived C to the tissues, whereas their nitrogen isotopic composition ($\delta^{15}N_h$ and $\delta^{15}N_e$) records sources and rate of nitrogen incorporation into coral tissues during bleaching and recovery [14,22–24]. Detailed physiological measurements coupled with isotopic analyses are therefore powerful tools to study the short- and long-term recovery from annual coral bleaching.

This study is the first to assess short- and long-term recovery from annual coral bleaching in three Caribbean corals using both physiological and biogeochemical methods. We conducted a 2-year-long study to determine (i) if the coral holobiont can recover from annually recurring bleaching within 1 year, and (ii) which traits are associated with short- and long-term recovery.

2. Material and methods

(a) Repeat bleaching experiment

A detailed description of this experiment can be found in Schoepf *et al.* [25], and a flow diagram of the experimental design is shown in the electronic supplementary material, figure S1. Briefly, coral fragments of *Porites divaricata*, *Porites astreoides* and *Orbicella faveolata* were collected in July 2009 near Puerto Morelos, Mexico (20°50′ N, 86°52′ W region; see [9] for details). Peak sea surface temperatures (SST) at this location typically occur in August/September with the maximum monthly mean (MMM) SST being 29.0°C; the bleaching threshold SST is therefore 30.0°C (MMM + 1°C, NOAA Coral Reef Watch Virtual Station Puerto Morelos, Mexico). Corals were then buoyantly weighed, and allowed to recover in shaded outdoor flow-through seawater tanks for 5 days. In mid-July, temperature in half of the tanks was gradually raised to 31.5 ± 0.20°C (single bleaching treatment) over 7 days and then maintained at the elevated temperature, while the remaining tanks received ambient reef water (controls; 30.6 ± 0.24°C). After 15 days, all corals were placed on the reef in a random arrangement for 1 full year at ambient reef temperatures (electronic supplementary material, figures S1 and S2). During the time on the reef, corals were considered naturally fed but were not artificially fed during the tank portion of the study.

In late July/early August 2010, the experiment was repeated (repeat bleaching treatment) and the treatment corals from the previous year were exposed to elevated temperatures again (31.6 ± 0.24°C) while the control fragments from 2009 were maintained at ambient temperature (30.4 ± 0.23°C) (electronic supplementary material, figures S1 and S2). After 17 days, coral fragments were visually assessed for their health status [17] (see definition of each health status category in the electronic supplementary material, figure S3), buoyantly weighed and then one third of all fragments were frozen for physiological and isotopic analyses (i.e. 0 months on the reef). The remaining fragments were placed back on the reef in a random arrangement

at ambient reef temperatures (electronic supplementary material, figures S1 and S2). To assess short- and long-term recovery from repeat bleaching, half of the remaining fragments were collected after 1.5 and 11 months on the reef, respectively. Fragment health status was visually assessed, they were buoyantly weighed and then frozen for physiological and isotopic analyses (see below).

Seawater temperatures during the tank and field portion of the study are shown in the electronic supplementary material, figure S2. On average, treatment corals were exposed to estimated heat stress levels of 3–4 degree heating weeks (DHW), which is similar to those recorded in the Caribbean in 1998 but much lower than during the severe regional bleaching event in 2005, where up to 16 DHW were recorded in some locations [26].

(b) Physiological and isotopic analyses

Chlorophyll *a* was determined according to Jeffrey & Humphrey [27] and standardized to surface area [28]. Chlorophyll *a* values at 0 and 1.5 months on the reef following repeat bleaching are from Schoepf *et al.* [29]. Total soluble lipids, animal soluble protein and animal soluble carbohydrate were determined on ground, frozen coral fragments using established methods [17,30] and then converted to Joules per gram ash-free dry weight [31] (see the electronic supplementary material for more information). Net calcification was determined using the buoyant weight technique [32] and standardized to surface area. Calcification rates at 0 and 1.5 months on the reef following repeat bleaching are from Grottoli *et al.* [9].

Tissue C and N isotopic analyses were performed on separated animal host and endosymbiont fractions using established methods [14,33]. The difference between $\delta^{13}C_h$ and $\delta^{13}C_e$ (i.e. $\delta^{13}C_{h-e}$) was calculated to determine the relative contribution of photoautotrophic versus heterotrophic carbon to the coral [14,23]. Repeated measurements of commercial standards (USGS-24, IAEA-N2) had a standard deviation of ± 0.04‰ for $\delta^{13}C$ ($n = 55$) and ± 0.11‰ for $\delta^{15}N$ ($n = 51$).

(c) Statistical analyses

Multivariate one-way analysis of similarity (ANOSIM) was used to test whether the overall bleaching and recovery response differed significantly between the three species. As there was a significant species effect (see Results), two-way ANOSIMs were conducted individually for each species to test for treatment (control, treatment) and time (0, 1.5 and 11 months on the reef) effects. Furthermore, SIMPER analyses were conducted to determine which variables contributed most to the observed physiological changes due to treatment and time effects. Non-metric multidimensional scaling (NMDS) was used to graphically represent relationships between response variables, species and treatments in multidimensional space. In order to prevent bias from including all five isotopic variables, only $\delta^{13}C_{h-e}$ and $\delta^{15}N_e$ were included in the multivariate analyses along with chlorophyll *a*, lipid, protein, carbohydrate and calcification.

Univariate three-way analysis of variance (ANOVA) was then used to test the effects of temperature, time and genotype for each species individually for all physiological and isotopic variables. *Post hoc* slice tests (e.g. tests of simple effects [34]) determined if the control and treatment averages differed significantly within each time interval and species. If a variable was significantly different between treatment and control averages at 0 and/or 1.5 months of recovery but no longer differed at 1.5 and/or 11 months on the reef, it was deemed to be fully recovered from repeat bleaching. Conversely, if one or more variables still had significantly lower treatment averages than the control after 11 months on the reef, this was deemed indicative of impaired long-term recovery capacity. More detailed information is provided in the electronic supplementary material.

Table 1. Results from two-way ANOSIMs testing the effects of temperature and time on the reef on the overall bleaching and recovery response of each of the three study species following repeat bleaching. Significant p-values ($p \leq 0.05$) are highlighted in bold.

	global R-statistic	p-value	possible no. permutations	actual no. permutations	no. permuted statistics $\geq$ global R
Porites divaricata					
temperature	0.173	**0.020**	20 490 624	999	19
time	0.504	**0.001**	large number	999	0
Porites astreoides					
temperature	0.567	**0.001**	large number	999	0
time	0.387	**0.001**	large number	999	0
Orbicella faveolata					
temperature	0.108	**0.041**	large number	999	40
time	0.395	**0.001**	large number	999	0

3. Results

(a) Multivariate analyses

Over the entire 11 months following repeat bleaching, all three species significantly differed from each other (one-way ANOSIM $R = 0.39$, $p = 0.001$). *Porites divaricata* was most distinct from the other two species (*P. d.* versus *O. f.*: $R = 0.70$; *P. d.* versus *P. a.*: $R = 0.39$; *P. a.* versus *O. f.*: $R = 0.19$; all $p < 0.05$), consistent with the NMDS results (electronic supplementary material, figure S3 and table S2).

For each species, significant treatment and time effects were also observed (table 1). Only *P. astreoides* showed clear separation of the treatment and control groups ($R = 0.567$; table 1; electronic supplementary material, figure S3), which was largely driven by lower chlorophyll a concentrations, higher $\delta^{15}N_e$ and lower calcification rates in the treatment corals (electronic supplementary material, table S1 and figure S3). In contrast, *P. divaricata* showed the greatest change in responses over time ($R = 0.504$; table 1), largely due to differences in chlorophyll a concentration (0 versus 2 months), $\delta^{15}N_e$ and carbohydrate concentration (0 versus 11 months), as well as lipid concentration (2 versus 11 months and 2 versus 11 months) (electronic supplementary material, table S1). In *O. faveolata*, significant overlap among treatment and time groups was observed ($R < 0.5$; table 1; electronic supplementary material, figure S3); thus the response of each variable is best assessed individually for each time point (see below).

(b) Physiology following repeat bleaching

(i) Porites divaricata

Chlorophyll a concentrations of treatment corals were 42% lower immediately after repeat bleaching, but had fully recovered after 1.5 months on the reef (figure 1*a*; electronic supplementary material, table S3) [29]. Lipid concentrations of treatment corals did not differ from controls immediately after repeat bleaching, but were 30% lower at 1.5 months on the reef and had fully recovered after 11 months (figure 1*b*; electronic supplementary material, table S3). Protein and carbohydrate concentrations were the same for both treatment and controls in the year following repeat bleaching, though carbohydrate concentrations increased steadily (figure 1*c,d*; electronic supplementary material, table S3).
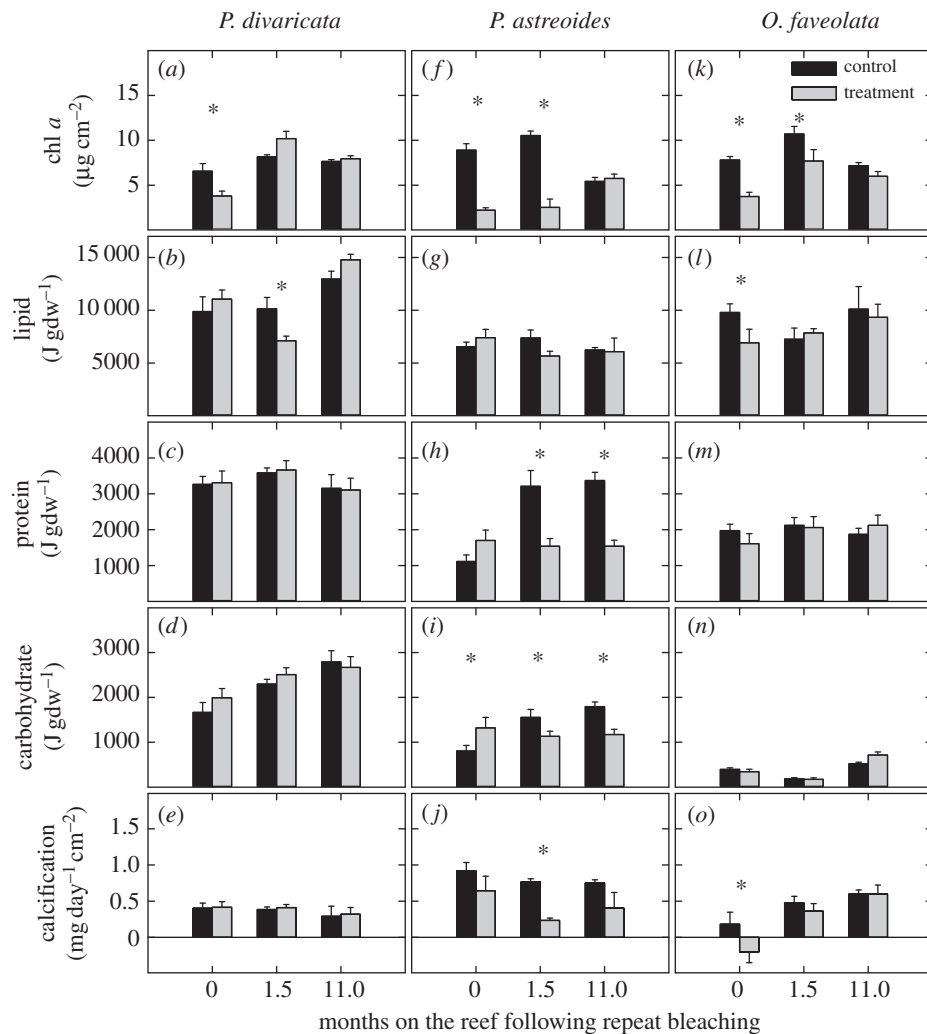
Calcification rates were the same for both treatment and control corals throughout the 11 months following repeat bleaching (figure 1*e*; electronic supplementary material, table S3) [9].

(ii) Porites astreoides

Chlorophyll a concentrations of treatment corals were 75% and 76% lower than in controls after both 0 and 1.5 months on the reef, respectively [29], but were fully recovered after 11 months (figure 1*f*; electronic supplementary material, table S3). Lipid concentrations were the same for both treatment and control corals throughout the 11 months following repeat bleaching (figure 1*g*; electronic supplementary material, table S3). In contrast, protein concentrations of treatment corals were 52% and 54% lower after 1.5 and 11 months on the reef, respectively (figure 1*h*). Carbohydrate concentrations of treatment corals were initially 64% higher than in controls, but then were 27% and 35% lower than in controls after 1.5 and 11 months on the reef, respectively (figure 1*i*; electronic supplementary material, table S3). Thus, neither protein nor carbohydrate concentrations were fully recovered by the end of the study. Calcification rates of treatment corals were the same as in controls immediately following bleaching, but were 69% lower after 1.5 months on the reef (figure 1*j*; electronic supplementary material, table S3) [9]. Although calcification rates were still 46% lower in the treatment corals after 11 months on the reef, this was not statistically significant due to high variability between fragments ($p < 0.43$) (figure 1*j*; electronic supplementary material, table S3).

(iii) Orbicella faveolata

Chlorophyll a concentrations were 52% and 28% lower in treatment corals compared with controls after 0 and 1.5 months on the reef, respectively [29], but had fully recovered after 11 months (figure 1*k*; electronic supplementary material, table S3). Chlorophyll a also showed seasonal variability with higher concentrations after 1.5 months than at the other two sampling points. Lipid concentrations were 29% lower in treatment than control corals immediately after repeat bleaching, but were fully recovered after 1.5 months on the reef (figure 1*l*; electronic supplementary material, table S3). Protein and carbohydrate concentrations did not differ

Figure 1. Average chlorophyll a (chl a), lipid, protein, carbohydrate and calcification rates of ($a-e$) *Porites divaricata*, ($f-j$) *Porites astreoides* and ($k-o$) *Orbicella faveolata* after 0, 1.5 and 11 months on the reef following repeat bleaching. Averages are shown ± 1 s.e. Asterisks indicate significant differences between treatment and control corals within a time point and species. Sample size per average ranges from 5–9. Statistical results shown in the electronic supplementary material, table S2. Chl a and calcification values at both 0 and 1.5 months on the reef are reproduced from Schoepf *et al.* [29] and Grottoli *et al.* [9], respectively.

between treatment and control corals at any point following repeat bleaching (figure 1m,n; electronic supplementary material, table S3), though carbohydrate concentrations varied over time. In contrast, calcification rates of treatment corals were significantly compromised (-212%) and net skeletal dissolution was observed immediately after repeat bleaching (figure 1o; electronic supplementary material, table S3) [9]. However, calcification rates had fully recovered 1.5 months later [9] and remained no different from the controls after 11 months (figure 1o).

Results from the visual assessment of health status at each time point are given in the electronic supplementary material, Additional results and figure S4. Lipid, protein and carbohydrate values in gram per gram ash-free dry weight are shown for each species in the electronic supplementary material, figure S5 to facilitate comparison with other studies.

(c) Tissue isotopes following repeat bleaching

Results for tissue C and N isotopes for all three species (figure 2; electronic supplementary material, table S4) are detailed in the electronic supplementary material, Additional results.

4. Discussion

(a) Evidence for impaired long-term recovery capacity after repeat bleaching in *Porites astreoides*

Mounding *P. astreoides* has recently increased in abundance throughout the Caribbean, possibly in part due to its history of high thermal tolerance [35] and the ability to survive disturbance better than other species [36]. This is consistent with previous published findings that *P. astreoides* was only modestly impacted by single bleaching stress and had fully recovered from single bleaching within 1.5 months [9]. In stark contrast, *P. astreoides* was much more severely affected by repeat bleaching [9] (table 2) and did not fully recover all variables within 11 months (figures 1h–j, 2h; electronic supplementary material, figure S4b). This is further corroborated by the multivariate analyses showing that the overall physiology of treatment *P. astreoides* strongly differed from the controls for the 11 months following repeat bleaching (table 1; electronic supplementary material, figure S3). As annual bleaching significantly increased the bleaching susceptibility of this species [9], it is therefore not surprising that the resulting cumulative damage overwhelmed its capacity to recover within 1 year.
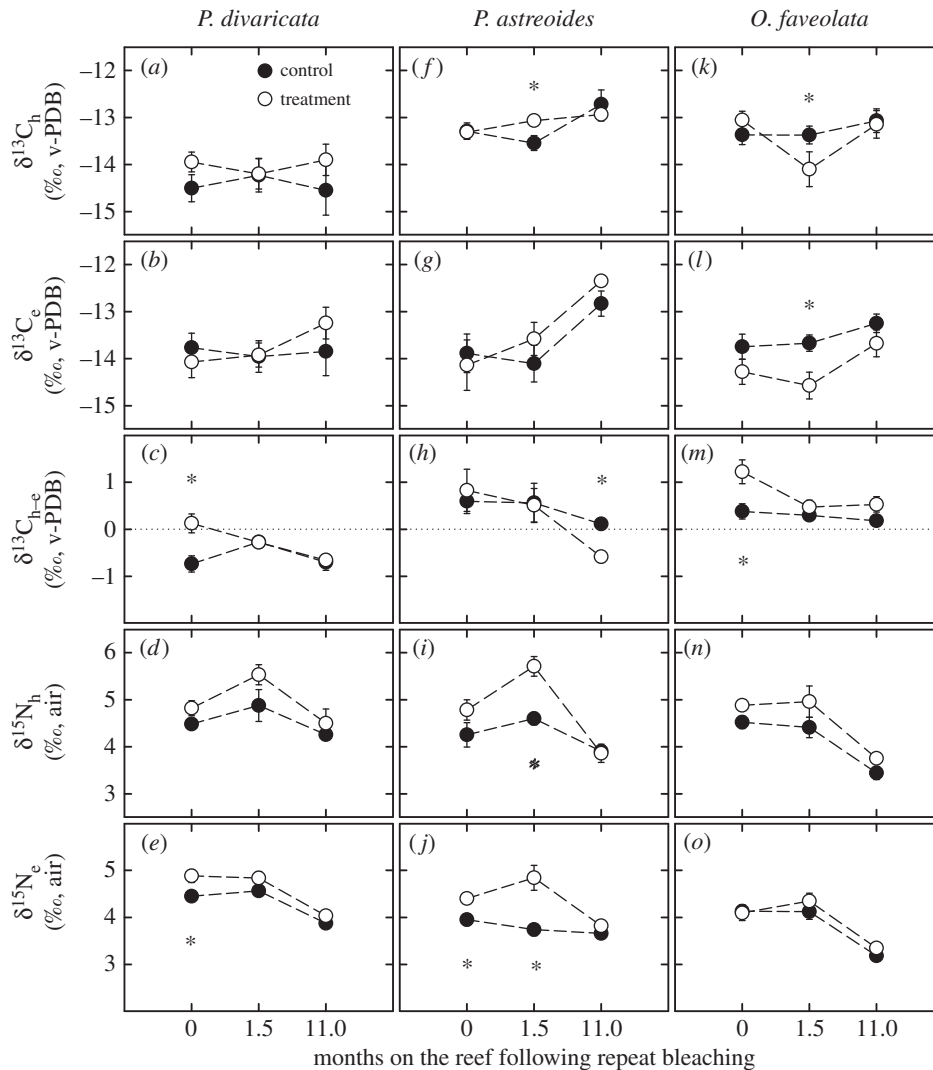
Figure 2. Average stable carbon isotopes of the animal host ($\delta^{13}C_h$) and endosymbiont ($\delta^{13}C_e$), $\delta^{13}C_{h-e}$ and the stable nitrogen isotopes of the animal host ($\delta^{15}N_h$) and endosymbiont ($\delta^{15}N_e$) of (a–e) *Porites divaricata*, (f–j) *Porites astreoides* and (k–o) *Orbicella faveolata* after 0, 1.5 and 11 months on the reef following repeat bleaching. For $\delta^{13}C_{h-e}$, heterotrophic C is preferentially incorporated into tissues when the difference is less than 0, whereas photosynthetic C is preferentially incorporated when the difference is greater than or equal to 0. Averages are shown ± 1 s.e. Asterisks indicate significant differences between treatment and control corals within a time point and species. Sample size per average ranges from 3 to 9. Statistical results shown in the electronic supplementary material, table S3.

Overall, treatment *P. astreoides* was characterized by a combination of lower chlorophyll *a*, protein and carbohydrate concentrations, lower calcification rates and higher $\delta^{15}N_e$ values (electronic supplementary material, table S1 and figure S3). Critically, both protein and carbohydrate concentrations were still 54% and 35% lower than in the controls after 11 months on the reef (figure 1h,i), respectively, and had thus not fully recovered within the study period. In contrast, Grottoli *et al.* [9] showed that *P. astreoides* did not catabolize total energy reserves in response to single bleaching stress. Similarly, calcification rates declined by 69% after repeat bleaching and showed a trend of still being lower (−46%) than the controls after 11 months on the reef (figure 1j). Further, the relative contribution of heterotrophic versus photoautotrophic C ($\delta^{13}C_{h-e}$) indicated that treatment *P. astreoides* was incorporating a disproportionate amount of heterotrophic C into its tissues even after 11 months on the reef (figure 2h), suggesting that they needed additional energy to promote the ongoing recovery process. This is consistent with findings from single bleaching studies that showed that heterotrophic C can play a vital role in the long-term recovery of tissue and energy reserves from bleaching [39,40].

Grottoli *et al.* [9] suggested that the increased bleaching susceptibility of *P. astreoides* was due to a combination of a low flexibility to associate with different genetic types of *Symbiodinium* and low overall total energy reserve concentrations compared with the other species studied. Here, we show that the low overall energy reserves were due to lower concentrations of lipid reserves compared with the other two species (figure 1b,g,l) and also the failure to recover protein and carbohydrates during the 11 months of recovery on the reef. As lipids have the highest energetic value when compared with protein and carbohydrates [31], their low concentrations in *P. astreoides* have a disproportionate effect on total energy reserves. We hypothesize that the low baseline levels of lipids in this species force it to catabolize protein and carbohydrates when bleached. The low lipid levels could be related to the reproductive cycle of *P. astreoides*, which is a brooder with a long reproductive season lasting from January through September [41]. Consequently, lipid levels are expected to be lowest towards the end of this period, which coincides with peak SST at our study site. Such unfortunate timing likely influences resistance to heat stress [19] and may contribute to the increased bleaching

Table 2. Months after single and repeat bleaching when response variables no longer differed significantly between treatment and control corals of *Porites divaricata* (*P. d.*), *Porites astreoides* (*P. a.*) and *Orbicella faveolata* (*O. f.*). The capacity to recover from annual bleaching was defined as impaired if one or more variables were not fully recovered within 11 months after repeat bleaching. The symbol '−' denotes no statistically significant difference between treatment and control corals at any time and '>11' indicates that bleached corals had not recovered by 11 months.

	single bleaching			repeat bleaching		
variable	P. d.	P. a.	O. f.	P. d.	P. a.	O. f.
symbiont density (cells cm^{-2})[a]	1.5	1.5	1.5	—	11	1.5
chlorophyll a (μg cm^{-2})[b]				1.5	11	11
calcification (mg day^{-1} cm^{-2})[c]	1.5	1.5	11	—	11[f]	1.5
total energy reserves (J gdw^{-1})[d]	11	—	—	—	>11	1.5
$\delta^{13}C_{h-e}$ (‰)[e]				1.5	>11	1.5
$\delta^{15}N_e$ (‰)[e]				1.5	11	—
capacity to recover from annual bleaching				not impaired	impaired	not impaired

[a]0 and 1.5 months data from [9], 11 months data from [37].
[b]0 and 1.5 months data from [29] (figure 1a,f,k).
[c]0 and 1.5 months data from [9]; 11 months data single bleaching from [38] (figure 1e,j,o).
[d]0 and 1.5 months data from [9], 11 months data single bleaching from [38] (figure 1).
[e]Figure 2.
[f]Treatment corals not statistically significant from controls but still 46% lower after 11 months on reef (figure 1j).

susceptibility and impaired recovery capacity of this species in response to annual bleaching.

These findings highlight that following repeat bleaching, recovery of the animal can take much longer than the recovery of the endosymbiont. Both chlorophyll a concentrations and endosymbiont densities of treatment *P. astreoides* were fully recovered 11 months after repeat bleaching (this study; [37]), which is also reflected in the N isotopic data (figure 2d,e). $\delta^{15}N_h$ and $\delta^{15}N_e$ typically increase in bleached and/or recovering corals as the remaining endosymbionts are released from nitrogen limitation and take up more dissolved inorganic nitrogen to promote growth and chlorophyll a recovery [15], thus leading to less discrimination against ^{15}N during uptake [14]. This trend was generally observed in treatment corals of *P. astreoides* as well as the other two coral species (figure 2; electronic supplementary material, figure S3 and table S1), although it was not always statistically significant, and is thus consistent with other studies showing $\delta^{15}N$ enrichment in singly bleached corals [14,24,42].

Overall, this suggests that even when translocation of photosynthetic carbon to the animal has been restored, recovery of energy reserves and possibly also calcification rates often requires additional time and energy or even depends on additional factors. This is consistent with other, single bleaching studies from the Caribbean and the Pacific [14,15,17,43]. Therefore, in a future with annual bleaching stress, failure to fully recover energy reserves and calcification within a year could negatively affect coral reproduction (e.g. [44]), compromise resistance to further stress events (e.g. [19]) and diminish a coral's capacity to compete for space.

(b) Evidence for sustained long-term recovery capacity after repeat bleaching in *Porites divaricata* and *Orbicella faveolata*

In contrast to *P. astreoides*, branching *P. divaricata* and mounding *O. faveolata* were both able to fully recover from repeat bleaching stress within 11 months (figures 1 and 2), consistent with their response to single bleaching [9] (table 2). This is corroborated by the multivariate analyses showing that repeat bleaching affected the overall physiology of these two species much less than that of *P. astreoides* (table 1; electronic supplementary material, figure S3). *Porites divaricata* was particularly distinguished due to its high resistance to repeat bleaching and consequent rapid recovery (electronic supplementary material, figure S3). Nevertheless, the underlying mechanism of their recoveries was quite different and highlights the species-specific recovery from annual bleaching stress (electronic supplementary material, figure S3).

P. divaricata was barely affected by repeat bleaching, only showing declines in chlorophyll a immediately after repeat bleaching and declines in lipid at 1.5 months on the reef (figure 1a,b). Further, both chlorophyll a and lipid were fully recovered after 1.5 and 11 months on the reef, respectively (figure 1a,b). A substantial shift in *Symbiodinium* dominance from C47 to A4 coupled with high total energy reserve concentrations appeared to be the underlying mechanism for this rapid acclimation and recovery [9]. Interestingly, all three energy reserve pools were higher in this species compared with *P. astreoides* or *O. faveolata* (figure 1b–d; electronic supplementary material, figure S3), possibly because peak reproductive output in this brooding species occurs in spring [45], thus leaving time to rebuild lipid reserves before water temperatures reach their maximum. Overall, it is likely that the remarkable acclimation and recovery capacity of this species will make *P. divaricata* significantly more competitive than many other species in a future of annual bleaching events.

Orbicella faveolata also showed a remarkable capacity to recover from repeat bleaching, especially since Grottoli *et al.* [9] showed that this species was much more severely affected by repeat bleaching compared with single bleaching stress. Even so, *O. faveolata* recovered most variables within the first 1.5 months and the remainder after 11 months on the

reef (figures 1k–o and 2k–o). For example, calcification stopped completely immediately after repeat bleaching resulting in net dissolution of skeletal material but had fully recovered only 1.5 months later (figure 1o). Similarly, lipids declined by 29% immediately after repeat bleaching, but were fully recovered 1.5 months later (figure 1l). High lipid levels at the time of the repeat bleaching (late July/early August) may have contributed to the remarkable recovery capacity and were probably linked to the reproductive cycle of this species, which typically spawns in August/September [41] and thus when water temperatures reach their maximum.

Only chlorophyll a levels took up to 11 months on the reef to fully recover (figure 1k). This was also evident in the $\delta^{13}C_e$ values, which were systematically lower than in the controls for large parts of the study (figure 2l; electronic supplementary material, table S4). Decreases in $\delta^{13}C_e$ are typically indicative of a reduction in the photosynthetic rate and/or the incorporation of photosynthetically derived C into the endosymbiont cells [14,23]. Although all measured variables of treatment O. faveolata were fully recovered by the end of the study, these systematically lower values could indicate that their endosymbionts (incl. a higher prevalence of Symbiodinium trenchii [46]) had associated physiological trade-offs compared with the original A and B types [9,47,48]. Thus, although O. faveolata overall appears to be able to recover from repeat bleaching within 1 year, additional studies are needed to fully test this over several years of annual bleaching.

(c) Importance of energy reserves and heterotrophy for long-term recovery from annual bleaching

Resilience to and recovery from coral bleaching may be promoted by heterotrophic plasticity, shifts in endosymbiont type and the size of energy reserves [9,18–21]. This study confirms that high levels of energy reserves play an important role in long-term recovery from annual bleaching stress as the species with the highest energy reserves, P. divaricata, was only minimally affected by repeat bleaching and was able to recover rapidly (table 2). However, it also highlights that location-specific, seasonal dynamics of different reproductive strategies and cycles, lipid levels and SST interact and significantly influence how species recover from bleaching. Additionally, the specific energy reserve pool catabolized during recovery from repeat bleaching appears to be highly species-specific with P. astreoides exclusively catabolizing protein and carbohydrates (figure 1g–i) and P. divaricata and O. faveolata catabolizing only lipid (figure 1b,l). This demonstrates that coral lipids are not necessarily the most important source of energy reserves as is often assumed, which has important implications for coral energy budgets.

In contrast to the size of energy reserves, heterotrophic plasticity did not play an important role in promoting short-term recovery from annual bleaching in these corals. None of the three species increased zooplankton feeding rates immediately after repeat bleaching, and zooplankton feeding of treatment corals contributed less than 10% to daily animal respiration [9]. Although corals may have accessed other sources of heterotrophic carbon such as bacteria and dissolved and particulate organic matter (e.g.

[24,49,50]), this was probably not a significant contribution to the fixed carbon pool given the positive $\delta^{13}C_{h-e}$ values for all treatment corals at 0 months on the reef (figure 2c,h,m) indicating that autotrophic C was preferentially incorporated into tissues.

However, heterotrophy appears to play an important role in the long-term recovery from annual bleaching as observed in $\delta^{13}C_{h-e}$ of P. astreoides 11 months after repeat bleaching (figure 2h). Increased heterotrophy for up to 11 months post-bleaching has also been observed in singly bleached corals [39,40], and it has been debated whether this is a sign of prolonged stress and impaired recovery or indicates acclimation and increased resistance to future bleaching [39]. The current study is the first to show that this mechanism likely indicates prolonged stress, at least in repeat bleached P. astreoides given its incomplete recovery 11 months after repeat bleaching.

(d) Implications for the future of coral reefs

Overall, this study highlights that annually recurring bleaching events will disproportionately affect the long-term recovery of different coral species (table 2), which will likely change future coral community composition, diversity and reef functioning. It is encouraging that two of the species studied here were able to fully recover within a year, and acclimation combined with the capacity to recover between annual bleaching events will likely result in significantly fewer severe bleaching events and coral mortality by 2100 (e.g. [51]). However, it needs to be cautioned that the temperature treatments in this study simulated short bleaching events relative to naturally occurring events and that one of the species studied here nevertheless experienced cumulative damage and impaired long-term recovery capacity. The resilience of corals to future annual bleaching could also depend on potential interactive effects of heat and pCO_2 stress although recent evidence suggests that ocean acidification does not affect bleaching susceptibility [52]. Importantly, models should integrate a range of trajectories to account for species-specific responses to annual bleaching (e.g. unaltered versus impaired recovery capacity) to provide more realistic predictions of future coral mortality, reef degradation and occurrence of mass bleaching events.

References

1. Hoegh-Guldberg O et al. 2007 Coral reefs under rapid climate change and ocean acidification. *Science* **318**, 1737–1742. (doi:10.1126/science.1152509)

2. Hoegh-Guldberg O. 1999 Climate change, coral bleaching and the future of the world's coral reefs. *Mar. Freshwater Res.* **50**, 839–866. (doi:10.1071/MF99078)

3. Eakin CM, Lough JM, Heron SF. 2009 Climate variability and change: monitoring data and evidence for increased coral bleaching stress. In *Coral bleaching: patterns, processes, causes and consequences* (eds MJH van Oppen, JM Lough), pp. 41–67. Berlin, Germany: Springer.

4. Frieler K, Meinshausen M, Golly A, Mengel M, Lebek K, Donner SD, Hoegh-Guldberg O. 2013 Limiting global warming to 2°C is unlikely to save most coral reefs. *Nat. Clim. Change* **3**, 165–170. (doi:10.1038/nclimate1674)

5. van Hooidonk R, Maynard JA, Liu Y, Lee S-K. 2015 Downscaled projections of Caribbean coral bleaching that can inform conservation planning. *Glob. Change Biol.* **21**, 3389–3401. (doi:10.1111/gcb.12901)

6. Maynard JA, Anthony KRN, Marshall PA, Masiri I. 2008 Major bleaching events can lead to increased thermal tolerance in corals. *Mar. Biol.* **155**, 173–182. (doi:10.1007/s00227-008-1015-y)

7. Guest JR, Baird AH, Maynard JF, Muttaqin E, Edwards AJ, Campbell SJ, Yewdall K, Affendi YA, Chou LM. 2012 Contrasting patterns of coral bleaching susceptibility in 2010 suggest an adaptive response to thermal stress. *PLoS ONE* **7**, e33353. (doi:10.1371/journal.pone.0033353)

8. Middlebrook R, Hoegh-Guldberg O, Leggat W. 2008 The effect of thermal history on the susceptibility of reef-building corals to thermal stress. *J. Exp. Biol.* **211**, 1050–1056. (doi:10.1242/jeb.013284)

9. Grottoli AG, Warner M, Levas SJ, Aschaffenburg M, Schoepf V, McGinley M, Baumann J, Matsui Y. 2014 The cumulative impact of annual coral bleaching can turn some coral species winners into losers. *Glob. Change Biol.* **20**, 3823–3833. (doi:10.1111/gcb.12658)

10. Hoegh-Guldberg O, Smith GJ. 1989 The effect of sudden changes in temperature, light, and salinity on the population density and export of zooxanthellae from the reef coral *Stylophora pistillata* Esper and *Seriatopora hystrix* Dana. *J. Exp. Mar. Biol. Ecol.* **129**, 279–303. (doi:10.1016/0022-0981(89)90109-3)

11. Jokiel PL, Coles SL. 1990 Response of Hawaiian and other Indo-Pacific reef corals to elevated temperature. *Coral Reefs* **8**, 155–162. (doi:10.1007/BF00265006)

12. Muscatine L, McCloskey LR, Marian RE. 1981 Estimating the daily contribution of carbon from zooxanthellae to coral animal respiration. *Limnol. Oceanogr.* **26**, 601–611. (doi:10.4319/lo.1981.26.4.0601)

13. Leder JJ, Szmant AM, Swart P. 1991 The effect of prolonged 'bleaching' on skeletal banding and stable isotopic composition in *Montastraea annularis*. *Coral Reefs* **10**, 19–27. (doi:10.1007/BF00301902)

14. Rodrigues LJ, Grottoli AG. 2006 Calcification rate and the stable carbon, oxygen, and nitrogen isotopes in the skeleton, host tissue, and zooxanthellae of bleached and recovering Hawaiian corals. *Geochim. Cosmochim. Acta* **70**, 2781–2789. (doi:10.1016/j.gca.2006.02.014)

15. Fitt WK, Spero HJ, Halas J, White MW, Porter JW. 1993 Recovery of the coral *Montastraea annularis* in the Florida Keys after the 1987 Caribbean 'bleaching event'. *Coral Reefs* **12**, 57–64. (doi:10.1007/BF00302102)

16. Porter JW, Fitt WK, Spero HJ, Rogers AD, White MW. 1989 Bleaching in reef corals: physiological and stable isotopic responses. *Proc. Natl Acad. Sci. USA* **86**, 9342–9346. (doi:10.1073/pnas.86.23.9342)

17. Rodrigues LJ, Grottoli AG. 2007 Energy reserves and metabolism as indicators of coral recovery from bleaching. *Limnol. Oceanogr.* **52**, 1874–1882. (doi:10.4319/lo.2007.52.5.1874)

18. Grottoli AG, Rodrigues LJ, Palardy JE. 2006 Heterotrophic plasticity and resilience in bleached corals. *Nature* **440**, 1186–1189. (doi:10.1038/nature04565)

19. Anthony KRN, Hoogenboom MO, Maynard JF, 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. (doi:10.1111/j.1365-2435.2008.01531.x)

20. 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. (doi:10.1016/j.jembe.2010.05.007)

21. Connolly SR, Lopez-Yglesias MA, Anthony KRN. 2012 Food availability promotes rapid recovery from thermal stress in a scleractinian coral. *Coral Reefs* **31**, 951–960. (doi:10.1007/s00338-012-0925-9)

22. Reynaud S, Ferrier-Pages C, Sambrotto R, Juillet-Leclerc A, Jaubert J, Gattuso JP. 2002 Effect of feeding on the carbon and oxygen isotopic composition in the tissues and skeleton of the zooxanthellate coral *Stylophora pistillata*. *Mar. Ecol. Prog. Ser.* **238**, 81–89. (doi:10.3354/meps238081)

23. Muscatine L, Porter JW, Kaplan IR. 1989 Resource partitioning by reef corals as determined from stable isotope composition. I. δ^{13}C of zooxanthellae and animal tissue vs. depth. *Mar. Biol.* **100**, 185–193. (doi:10.1007/BF00391957)

24. Levas SJ, Grottoli AG, Hughes AD, Osburn CL, Matsui Y. 2013 Physiological and biogeochemical traits of bleaching and recovery in the mounding species of coral *Porites lobata*: implications for resilience in mounding corals. *PLoS ONE* **8**, e63267. (doi:10.1371/journal.pone.0063267)

25. Schoepf V, Levas SJ, Rodrigues LJ, McBride MO, Aschaffenburg M, Matsui Y, Warner M, Hughes AD, Grottoli AG. 2014 Kinetic and metabolic isotope effects in coral skeletal carbon isotopes: a re-evaluation using experimental coral bleaching as a case study. *Geochim. Cosmochim. Acta* **146**, 164–178. (doi:10.1016/j.gca.2014.09.033)

26. Eakin CM et al. 2010 Caribbean corals in crisis: record thermal stress, bleaching, and mortality in 2005. *PLoS One* **5**, e13969. (doi:10.1371/journal.pone.0013969)

27. 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. Pflanzen* **167**, 191–194.

28. Marsh JA. 1970 Primary productivity of reef-building calcareous red algae. *Ecology* **51**, 255–263. (doi:10.2307/1933661)

29. Schoepf V, McCulloch MT, Warner ME, Levas SJ, Matsui Y, Aschaffenburg M, Grottoli AG. 2014 Short-term coral bleaching is not recorded by skeletal boron isotopes. *PLoS One* **9**, e112011. (doi:10.1371/journal.pone.0112011)

30. Schoepf V et al. 2013 Coral energy reserves and calcification in a high-CO$_2$ world at two temperatures. *PLoS ONE* **8**, e75049. (doi:75010.71371/journal.pone.0075049)

31. Gnaiger E, Bitterlich G. 1984 Proximate biochemical composition and caloric content calculated from elemental CHN analysis: a stoichiometric concept. *Oecologia* **62**, 289–298. (doi:10.1007/BF00384259)

32. Jokiel PL, Maragos JE, Franziket L. 1978 Coral growth: buoyant weight technique. In *Coral reefs: research methods* (eds DR Stoddart, RE Johannes), pp. 529–541. Paris, France: UNESCO.

33. 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. (doi:10.3354/meps08866)

34. Winer BJ. 1971 *Statistical principles in experimental design*. New York, NY: McGraw-Hill.

35. Warner ME, LaJeunesse TC, Robison JD, Thur RM. 2006 The ecological distribution and comparative photobiology of symbiotic dinoflagellates from reef corals in Belize: potential implications for coral bleaching. *Limnol. Oceanogr.* **51**, 1887–1897. (doi:10.4319/lo.2006.51.4.1887)

36. Green DH, Edmunds PJ, Carpenter RC. 2008 Increasing relative abundance of *Porites astreoides* on Caribbean reefs mediated by an overall decline in coral cover. *Mar. Ecol. Prog. Ser.* **359**, 1–10. (doi:10.3354/meps07454)

37. Aschaffenburg M. 2012 The physiological response of *Symbiodinium* spp. to thermal and light stress: a comparison of different phylotypes and implications for coral reef bleaching. PhD thesis, University of Delaware.

38. Levas SJ. 2012 Biogeochemistry and physiology of bleached and recovering Hawaiian and Caribbean

9

rspb.royalsocietypublishing.org   *Proc. R. Soc. B* **282**: 20151887

corals. PhD thesis. The Ohio State University, Columbus, OH, USA.

39. Hughes AD, Grottoli AG. 2013 Heterotrophic compensation: a possible mechanism for resilience of coral reefs to global warming or a sign of prolonged stress? *PLoS ONE* **8**, e81172. (doi:10.1371/journal.pone.0081172)

40. Baumann J, Hughes AD, Matsui Y, Grottoli AG. 2014 Photoautotrophic and heterotrophic carbon in bleached and non-bleached coral lipid acquisition and storage. *J. Exp. Mar. Biol. Ecol.* **461**, 469–478. (doi:10.1016/j.jembe.2014.09.017)

41. Szmant AM. 1986 Reproductive ecology of Caribbean reef corals. *Coral Reefs* **5**, 43–54. (doi:10.1007/BF00302170)

42. Bessell-Browne P, Stat M, Thomson D, Clode PL. 2014 *Coscinaraea marshae* corals that have survived prolonged bleaching exhibit signs of increased heterotrophic feeding. *Coral Reefs* **33**, 795–804. (doi:10.1007/s00338-014-1156-z)

43. Fitt WK, McFarland FK, Warner ME, Chilcoat GC. 2000 Seasonal patterns of tissue biomass and densities of symbiotic dinoflagellates in reef corals and relation to coral bleaching. *Limnol. Oceanogr.* **45**, 677–685. (doi:10.4319/lo.2000.45.3.0677)

44. Ward S, Harrison PJ, Hoegh-Guldberg O. 2000 Coral bleaching reduces reproduction of scleractinian corals and increases susceptibility to future stress. *In Proc. 9th Int. Coral Reef Symp., Bali, Indonesia, 23–27 October 2000*, pp. 1123–1128.

45. McDermond J. 2014 Reproduction and population of *Porites divaricata* at Rodriguez Key: the Florida Keys, USA. Master thesis, Nova Southeastern University.

46. LaJeunesse TC, Wham DC, Pettay DT, Parkinson JE, Keshavmurthy S, Chen CA. 2014 Ecologically differentiated stress-tolerant endosymbionts in the dinoflagellate genus *Symbiodinium* (Dinophyceae) clade D are different species. *Phycologia* **53**, 305–319. (doi:10.2216/13-186.1)

47. Pettay DT, Wham DC, Smith RT, Iglesias-Prieto R, LaJeunesse TC. 2015 Microbial invasion of the Caribbean by an Indo-Pacific coral zooxanthella. *Proc. Natl Acad. Sci. USA* **112**, 7513–7518. (doi:10.1073/pnas.1502283112)

48. Jones A, Berkelmans R. 2010 Potential costs of acclimatization to a warmer climate: growth of a reef coral with heat tolerant versus sensitive symbiont types. *PLoS ONE* **5**, e10437. (doi:10.1371/journal.pone.0010437)

49. Tremblay P, Naumann MS, Sikorski S, Grover R, Ferrier-Pagès C. 2012 Experimental assessment of organic carbon fluxes in the scleractinian coral *Stylophora pistillata* during a thermal and photo stress event. *Mar. Ecol. Prog. Ser.* **453**, 63–77. (doi:10.3354/meps09640)

50. Anthony KRN. 2000 Enhanced particle-feeding capacity of corals on turbid reefs (Great Barrier Reef, Australia). *Coral Reefs* **19**, 59–67. (doi:10.1007/s003380050227)

51. Logan CA, Dunne JP, Eakin CM, Donner SD. 2014 Incorporating adaptive responses into future projections of coral bleaching. *Glob. Change Biol.* **20**, 125–139. (doi:10.1111/gcb.12390)

52. Noonan SHC, Fabricius KE. 2015 Ocean acidification affects productivity but not the severity of thermal bleaching in some tropical corals. *ICES J. Mar. Sci.* (doi:10.1093/icesjms/fsv127)