Spatial variation in the biochemical and isotopic composition of corals during bleaching and recovery

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Abstract

Ocean warming and the increased prevalence of coral bleaching events threaten coral reefs. However, the biology of corals during and following bleaching events under field conditions is poorly understood. We examined bleaching and postbleaching recovery in *Montipora capitata* and *Porites compressa* corals that either bleached or did not bleach during a 2014 bleaching event at three reef locations in Kāne'ohe Bay, O'ahu, Hawai'i. We measured changes in chlorophylls, tissue biomass, and nutritional plasticity using stable isotopes (δ^{13}C, δ^{15}N). Coral traits showed significant variation among periods, sites, bleaching conditions, and their interactions. Bleached colonies of both species had lower chlorophyll and total biomass, and while *M. capitata* chlorophyll and biomass recovered 3 months later, *P. compressa* chlorophyll recovery was location dependent and total biomass of previously bleached colonies remained low. Biomass energy reserves were not affected by bleaching, instead *M. capitata* proteins and *P. compressa* biomass energy and lipids declined over time and *P. compressa* lipids were site specific during bleaching recovery. Stable isotope analyses did not indicate increased heterotrophic nutrition in bleached colonies of either species, during or after thermal stress. Instead, mass balance calculations revealed that variations in δ^{13}C values reflect biomass compositional change (i.e., protein : lipid : carbohydrate ratios). Observed δ^{15}N values reflected spatiotemporal variability in nitrogen sources in both species and bleaching effects on symbiont nitrogen demand in *P. compressa*. These results highlight the dynamic responses of corals to natural bleaching and recovery and identify the need to consider the influence of biomass composition in the interpretation of isotopic values in corals.

Scleractinian corals in association with dinoflagellate endosymbiont algae (family : Symbiodiniaceae, formerly *Symbiodinium* spp.; LaJeunesse et al. 2018) are important primary producers on coral reefs, which through biogenic processes create the complex calcium carbonate framework of the reef milieu. The coral–algae symbiosis can be disturbed under environmental stress, leading to the reduction of symbiotic algae in coral tissue (i.e., coral bleaching; Weis 2008). Depending on the severity or duration of stress, bleaching causes coral mortality, although some corals survive and recover their symbionts postbleaching (Fitt et al. 1993; Cunning et al. 2016). The strength and frequency of bleaching events have increased over the last three decades from a

combination of progressive seawater warming (Heron et al. 2016) and climatic events (i.e., El Niño Southern Oscillation [ENSO]; Hughes et al. 2017). It is therefore critical to advance an understanding of the environmental conditions and biological mechanisms that underpin the physiological resilience of corals to thermal stress.

The resistance and recovery of corals from bleaching stress is influenced by associations with thermally tolerant symbionts (Sampayo et al. 2008), tissue biomass abundance (Thornhill et al. 2011) and energetic quality (i.e., lipid content), and the capacity to maintain positive energy budgets through nutritional plasticity (Anthony et al. 2009). Coral nutrition is largely supported by fixed carbon derived from endosymbiont algae; however, particle feeding (Mills et al. 2004), plankton capture (Sebens et al. 1998), and the uptake of dissolved compounds from seawater and sediments (Mills and Sebens 2004; Grover et al. 2006; collectively, "heterotrophy") can account for < 15–50% of energy demands (Porter 1976; Houlbrèque and Ferrier-Pagès 2009) and > 100% of respiratory carbon demand in bleached corals (Grottoli et al. 2006; Palardy et al. 2008; Levas et al. 2016). Facultative shifts from autotrophic to heterotrophic nutrition are often linked to reduced

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Additional Supporting Information may be found in the online version of this article.

symbiont photosynthesis in response to periodic light attenuation (i.e., turbidity) and/or environmental stress (Houlbrèque and Ferrier-Pagès 2009). As such, nutritional plasticity is an important acclimatization mechanism shaping the physiological niche of corals (Anthony and Fabricius 2000) and supporting the resilience of reef-building corals to changing environments and resource availability (Grottoli et al. 2006; Ferrier-Pagès et al. 2010; Connolly et al. 2012; Hughes and Grottoli 2013).

Heterotrophic nutrition is a fundamental process in the metabolism and growth of corals (Palardy et al. 2008; Houlbrèque and Ferrier-Pagès 2009; Hughes and Grottoli 2013). In some corals, thermal stress and bleaching results in an increased feeding on zooplankton (Grottoli et al. 2006; Ferrier-Pagès et al. 2010; Hughes and Grottoli 2013; Levas et al. 2013) and suspended particles (Anthony and Fabricius 2000) and stimulates coral uptake of diazotroph-derived nitrogen (Bednarz et al. 2017) and dissolved organic carbon (Levas et al. 2016). Periods of stress or resource limitation, however, do not facilitate shifts toward heterotrophic nutrition in all corals (Anthony and Fabricius 2000; Schoepf et al. 2015); instead, energetic demands are met by the catabolism of energy-rich biomass (i.e., proteins, lipids, and carbohydrates; Fitt et al. 1993; Grottoli et al. 2006; Schoepf et al. 2015). Considering the limited size of biomass reserves, corals capable of increasing the acquisition of heterotrophic energy may experience a fitness advantage during times of stress and symbiosis disruption, as well as increased rates of physiological recovery (Rodrigues and Grottoli 2007; Connolly et al. 2012; Grottoli et al. 2014).

Elevated temperature effects on corals are also mediated by co-occurring environmental factors, including ultraviolet (Shick et al. 1996) and photosynthetically active radiation (PAR; Coles and Jokiel 1977), the concentration (Vega Thurber et al. 2014) and stoichiometry of dissolved nutrients (e.g., nitrogen and phosphorous; Wiedenmann et al. 2012), and water motion (Nakamura and van Woesik 2001). For instance, elevated light levels and chronic nutrient loading can exacerbate thermal stress (Coles and Jokiel 1977; Vega Thurber et al. 2014), whereas high water motion and seawater turbidity can reduce bleaching severity and mortality (Nakamura and van Woesik 2001; Anthony et al. 2007). In addition, enhanced nutrition from heterotrophic feeding preceding and following thermal stress can replenish lipid biomass (Baumann et al. 2014), reduce bleaching severity and coral mortality (Anthony et al. 2009; Ferrier-Pagès et al. 2010), and promote postbleaching recovery of the host and symbiont (Marubini and Davies 1996; Connolly et al. 2012). Spatiotemporal variation in abiotic conditions that affect coral performance and resource availability/demand, therefore, can influence coral holobiont response trajectories and outcomes to physiological stress (Hoogenboom et al. 2011; Connolly et al. 2012; Scheufen et al. 2017). Considering reef corals may experience bleaching effects > 12 months following initial thermal stress and well beyond the return of normal tissue pigmentation (Fitt et al. 1993; Baumann et al. 2014; Grottoli et al. 2014; Levitan et al. 2014; Schoepf et al. 2015), it is important to consider the environmental effects and physiological mechanism(s) that facilitate or hinder postbleaching recovery.

The occurrence of large-scale coral bleaching episodes has been historically rare in the Main Hawaiian Islands, being limited to 1996 (Jokiel and Brown 2004). However, coastal seawater in Hawai'i is warming ($0.02°C$ yr^{-1}, annual mean 1956–2014; Bahr et al. 2015) and the frequency and severity of global bleaching events is increasing (Hughes et al. 2017). From September to October 2014, the Hawaiian Archipelago experienced a protracted period of elevated sea surface warming. Degree heating weeks (DHW) for the Main Hawaiian Islands began to accumulate on 15 September, peaking at 7 DHW on 20 October and declining below < 7 DHW after 08 December (NOAA Coral Reef Watch 2018). Water temperatures (29–30.5°C; Bahr et al. 2015) exceeded O'ahu mean summertime maximum temperatures (ca. 28°C; Jokiel and Brown 2004) and resulted in a rare coral bleaching event spanning the archipelago (Bahr et al. 2017; Couch et al. 2017) with extensive bleaching in Kāne'ohe Bay, O'ahu (62–100% of coral cover across reef habitats; Bahr et al. 2015). This event provided a rare opportunity to track the biology of bleaching resistant and susceptible corals during and after thermal stress under natural field conditions, with the potential to monitor the mechanisms of bleaching recovery among reef habitats.

In this study, the physiology underpinning two different phenotypes of bleaching response (bleached vs. nonbleached) was examined for two dominant Kāne'ohe Bay coral species (*Montipora capitata* and *Porites compressa*; Fig. 1). *M. capitata* and *P. compressa* can differ in the physiological responses to experimental bleaching and recovery, with *M. capitata* increasing heterotrophic feeding and *P. compressa* catabolizing tissue reserves (Grottoli et al. 2006; Rodrigues and Grottoli 2007). Coral fragments were collected from bleached and nonbleached individuals of each species during peak bleaching and 3 months following thermal stress (Supporting Information Fig. S1a) from three patch reefs within an environmental gradient of decreasing oceanic influence (Lowe et al. 2009) and terrigenous nutrient perturbations (Smith et al. 1981), which allowed an examination of the spatial variance and environmental influence (temperature, light, sedimentation, and dissolved nutrients) on corals after thermal stress. We tested (1) whether photopigments, coral biomass (total biomass, protein, lipid, carbohydrate concentrations, and energy content), and contributions of heterotrophic nutrition ($\delta^{13}C$ and $\delta^{15}N$ values) differed among time periods, reef sites, or bleaching conditions and (2) whether environmental conditions influenced bleaching severity and mechanisms of physiological recovery.

Materials and methods

Site description

Naturally bleached and nonbleached corals were identified from three patch reefs (Fig. 1a): one in northern (Reef 44: 21°28′36.4″N, 157°50′01.0″W), central (Reef 25: 21°27′40.3″N, 157°49′20.1″W), and southern (Hawai'i Institute of Marine Biology [HIMB]: 21°26′06.0″N, 157°47′27.9″W) Kāne'ohe Bay, O'ahu, Hawai'i (*see* Cunning et al. [2016] for more detail). Reef sites were identified for their location within the longitudinal axis of
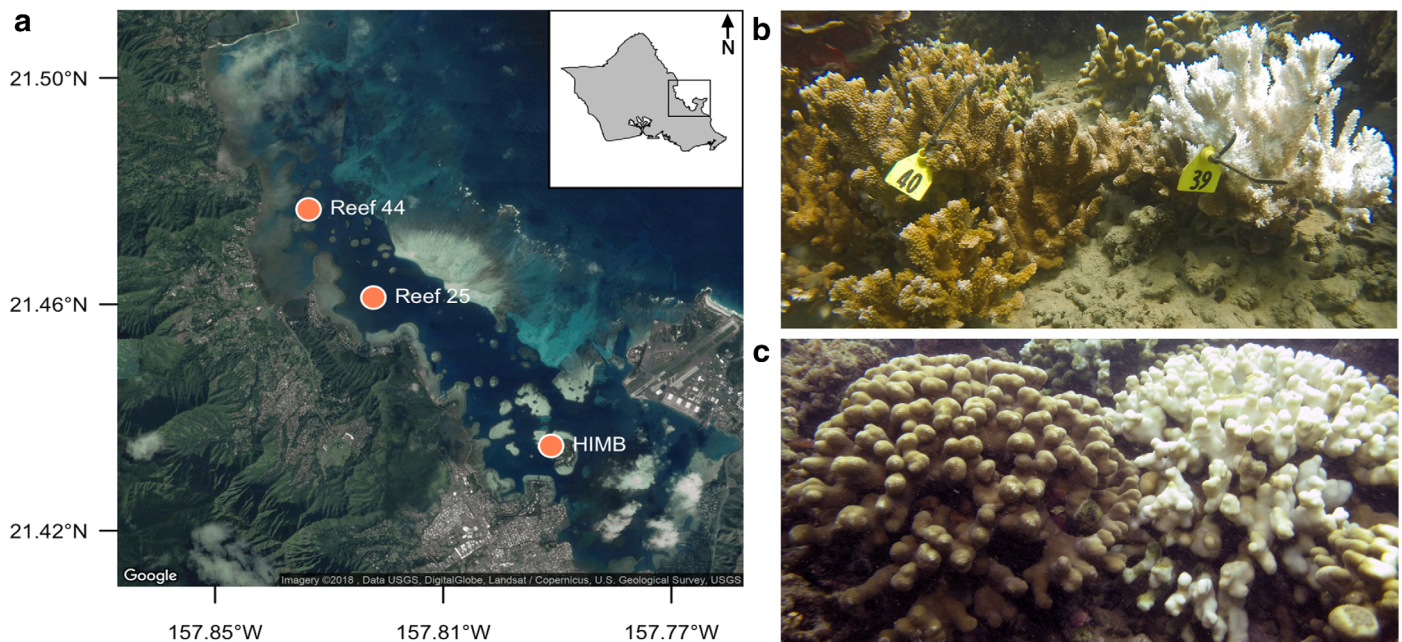
Fig. 1. (**a**) Map of Kāneʻohe Bay on the windward side of Oʻahu, Hawaiʻi, U.S.A., showing study sites Reef 44, Reef 25, and HIMB. Bleached and nonbleached (**b**) *M. capitata* and (**c**) *P. compressa* during a regional thermal stress event in October 2014. Photo credit (**b**, **c**): CB Wall.

Kāneʻohe Bay, which spans a north–south hydrodynamic gradient of seawater residence times (north: < 2 d; south: 30–60 d) and oceanic influence (high in north and low in south; Lowe et al. 2009).

Environmental data

Dissolved inorganic nutrients (DINs) in seawater were measured on samples collected (ca. 100 mL) from surface waters (< 1 m) at each reef site once every 2 weeks from 04 November 2014 to 04 February 2015. In total, 10 seawater samples were analyzed for each reef site over the study period. Additional samples were also collected to determine the $\delta^{15}N$ value of seawater nitrate using the bacterial denitrifier method (*see* Supporting Information). Seawater was filtered (0.7 μm) and stored in 0.1 mol L^{-1} HCl-washed bottles and frozen at $-20°C$ until analyzed. DINs (ammonium $[NH_4^+]$, nitrate + nitrite $[NO_3^- + NO_2^-$; i.e., N + N], phosphate $[PO_4^{3-}]$, and silicate $[Si\{OH\}_4]$) in seawater were measured by the University of Hawaiʻi at Mānoa School of Ocean and Earth Science and Technology (SOEST) Laboratory for Analytical Biogeochemistry using a Seal Analytical AA3 HR nutrient autoanalyzer and expressed as μmol L^{-1}. PAR and temperature data were continuously recorded at 15 min intervals at 2 m depth at each reef site using cross-calibrated Odyssey PAR loggers (Dataflow Systems) and Hobo Pendant UA-002-08 loggers ($\pm$ 0.53°C accuracy, Onset Computer; *see* Supporting Information). PAR and temperature loggers at Reef 25 experienced mechanical errors; therefore, only data from Reef 44 and HIMB are presented. Instantaneous PAR values were used to calculate the daily light integral (DLI) for each site (mol photons m^{-2} d^{-1}). Rates of sedimentation at the three sites were measured using sediment traps collected each month and expressed as g sediment d^{-1} (*see* Supporting Information).

Coral collection and tissue analysis

During peak bleaching in October 2014, colonies of *M. capitata* (Dana, 1846) and *P. compressa* (Dana, 1846) exhibiting different bleaching conditions—tissue paling (bleached) and fully pigmented (nonbleached; Fig. 1b,c)—were identified and tagged (depth: < 1–3 m) with cattle tags and zip ties. In each species, neighboring colonies of each condition (bleached and nonbleached) were selected and are referred to as conspecific colony pairs (Fig. 1b,c). Fragments (4 cm in length) from each conspecific colony pair (five pairs per species) were collected from the three reefs sites (detailed above) during bleaching (24 October 2014) and ca. 3 month following peak seawater temperatures during postbleaching recovery (14 January 2015; Supporting Information Fig. S1). Fragments were immediately frozen in liquid nitrogen and stored at $-80°C$ until processing.

All biomass assays were performed on holobiont tissues (host + symbionts), following established procedures (Wall et al. 2017). Additional methodology information can be found in the Supporting Information. Coral tissues were removed from skeletons using an airbrush filled with filtered seawater (0.2 μm). The tissue slurry was briefly homogenized and stored on ice. Total chlorophyll ($a + c_2$) was used as a metric of bleaching (Grottoli et al. 2006), and symbiont densities (symbiont : host cell ratio) were measured in a parallel study (Cunning et al. 2016). Chlorophyll in algal symbionts was extracted in 100% acetone and measured by spectrophotometry (Jeffrey and Humphrey 1975). Pigment concentrations were normalized to skeletal surface area (cm^2) determined by the wax-dipping technique (Stimson and Kinzie 1991).

Total tissue biomass was determined from the difference of dry (60°C) and combusted (4 h, 450°C) masses of an aliquot of tissue extract and expressed as the ash-free dry weight (AFDW) of

biomass per centimeter square. Total protein (soluble + insoluble) was measured spectrophotometrically following the Pierce BCA Protein Assay Kit (Pierce Biotechnology) using a bovine serum albumin standard curve (Smith et al. 1985). Tissue lipids were quantified on lyophilized tissue slurry in a 2 : 1 chloroform : methanol solution followed by 0.88% KCl and 100% chloroform washes. The lipid extract was evaporated in precombusted (450°C, 4 h) aluminum pans and measured to nearest 0.0001 g (Wall et al. 2017). Carbohydrates were measured by the phenol–sulfuric acid method using glucose as a standard (DuBois et al. 1956). Finally, changes in tissue biomass reserves were assessed energetically (Lesser 2013) using compound-specific enthalpies of combustion (Gnaiger and Bitterlich 1984). Proteins, lipids, carbohydrates, and biomass kilojoules (i.e., energy content) were normalized to g AFDW of the tissue slurry (*see* Supporting Information).

Stable isotope analysis

Skeletal carbonates were filtered from the tissue slurry (Maier et al. 2010) and host and symbiont tissues were separated by centrifugation ($2000 \times g \times 3$ min) with filtered seawater (0.2 μm) rinses (Muscatine et al. 1989). Tissues were filtered onto precombusted 25 mm GF/F filters (450°C, 4 h), dried overnight (60°C), and packed in tin capsules. Carbon (δ^{13}C) and nitrogen (δ^{15}N) isotopic values and molar ratios of carbon : nitrogen (C : N) for coral host ($\delta^{13}C_H$, $\delta^{15}N_H$, and C : N_H) and algal symbiont ($\delta^{13}C_S$, $\delta^{15}N_S$, and C : N_S) tissues were determined using a Costech elemental combustion system coupled to a Thermo-Finnigan Delta Plus XP Isotope Ratio Mass-Spectrometer. Analytical precision of δ^{13}C and δ^{15}N values of samples was < 0.2‰ determined by analysis of laboratory reference material run before and after every 10 samples. Isotopic data are reported in delta values (δ) using the conventional permil (‰) notation and expressed relative to Vienna–Pee Dee Belemnite (V–PDB) and atmospheric N_2 standards (air) for carbon and nitrogen. The relative differences in isotopic values in the host and symbiont for carbon ($\delta^{13}C_{H\text{-}S}$ = $\delta^{13}C_H - \delta^{13}C_S$) and nitrogen ($\delta^{15}N_{H\text{-}S}$) were calculated to evaluate changes in the proportion of heterotrophic carbon to coral host nutrition (i.e., $\delta^{13}C_{H\text{-}S}$) and changes in trophic enrichment among host and symbiont (i.e., $\delta^{15}N_{H\text{-}S}$; Rodrigues and Grottoli 2006; Reynaud et al. 2009; *see* Supporting Information).

An isotope mass balance was used to model the effect of changes in tissue biomass composition on holobiont (defined here as the combined host + symbiont tissues) δ^{13}C values during bleaching recovery, following Hayes (2001). First, the isotopic composition of the holobiont ($\delta^{13}C_{Holobiont}$) was modeled for each time period:

$$\delta^{13}C_{Holobiont} = \left(m_H \times \delta^{13}C_H\right) + \left(m_S \times \delta^{13}C_S\right)$$

where *m* is the estimated proportion of host (m_H) and symbiont (m_S) tissues in holobiont biomass (g AFDW), and δ^{13}C (defined above) are measured isotopic values of tissues. Second, the δ^{13}C value of biomass reflects the distribution of ^{13}C among the major classes of compounds, therefore biomass composition (i.e., % of proteins, lipids, and carbohydrates) and $\delta^{13}C_{Holobiont}$ values were used to estimate compound class-specific isotopic values ($\delta^{13}C_{Compound}$) for each compound class in corals during the bleaching period of October 2014, using Eq. 5 in Hayes (2001) (*see* Supporting Information). We assume that the δ^{13}C value of proteins is 1‰ higher that the δ^{13}C value of carbohydrates and lipids are depleted in ^{13}C by 6‰ relative to carbohydrates (*see* Hayes [2001] and references therein). $\delta^{13}C_{Compound}$ values for each colony were then applied to the same colonies in January 2015 using measurements of tissue composition and $\delta^{13}C_{Holobiont}$ values (i.e., observed-$\delta^{13}C_{Holobiont}$) to calculate expected-$\delta^{13}C_{Holobiont}$ values—representing the predicted value of the holobiont as a product of a fixed, colony-specific $\delta^{13}C_{Compound}$ value applied to a new biomass composition. The relationship between observed and expected $\delta^{13}C_{Holobiont}$ was evaluated using a linear regression (*see* Supporting Information).

Statistical analysis

A matrix of all biological response variables for *M. capitata* and *P. compressa* was first analyzed using a permutational multivariate analysis of variance (PERMANOVA) with periods (October 2014 and January 2015), sites (Reef 44, Reef 25, and HIMB), and colony-level physiological condition observed in October 2014 (i.e., bleached or nonbleached) as main effects. δ^{13}C values were incorporated into the data matrix by transforming to absolute values (i.e., $|\delta^{13}C|$). Sum of squares were partitioned according to Bray–Curtis dissimilarity matrix, and sequential tests were applied on 1000 model permutations using *adonis2* in R package *vegan* (Oksanen et al. 2017; R Development Core Team 2018), with pairwise comparisons over an additional 1000 permutations in *RVAideMemoire*. Results of PERMANOVA were applied to distinguish the hierarchy of main effects between coral species and to holistically evaluate postbleaching recovery. Multivariate relationships among periods, sites, and bleaching conditions were visualized for each species separately using nonmetric multidimensional scaling (NMDS) plots with ellipses representing standard errors (SEs) of point means. NMDS plots were used to visualize differences among reefs and bleaching conditions (i.e., site × condition) and among bleached and nonbleached corals across all sites with vectors representing significant biological responses ($p \leq 0.05$).

Environmental data (temperature, light, dissolved nutrients, and sedimentation) from each reef were analyzed to test for site-specific conditions, influencing bleaching and recovery responses. Environmental data were analyzed using a linear mixed effect model using *lmer* in package *lme4* (Bates et al. 2015) with reef site as a fixed effect and date of sample collection as a random effect. Biological response variables for individual species were used to test for differences among time periods, reef locations, and bleaching conditions. Physiology and isotopic data were analyzed using three-way linear mixed effect models in *lme4* with period, site, and condition as fixed effects and coral colony and colony pairs as random effects. Model selection was performed on candidate models using a combination of Akaike information criterion (AIC) and likelihood ratio tests (Akaike 1978). Where significant interactions

were observed, pairwise post hoc slice tests of main effects by least-square means were performed in package *lsmeans* (Lenth 2016). ANOVA tables for all environmental and biological metrics were generated using Type II sum of squares with Satterthwaite approximation of degrees freedom using *lmerTest* (Kuznetsova et al. 2017). Environmental data from these reefs are publically available (Ritson-Williams and Gates 2016*a,b,c*; Ritson-Williams et al. 2019). All analyses were performed in R version 3.4.3 (R Development Core Team 2018); materials (data, R code) to reproduce tables, figures, and analyses are archived at Zenodo (Wall 2019).

Results

Environmental data

Kāneʻohe Bay reef flats sustained a maximum seawater temperatures of ca. 31°C (Bahr et al. 2015). Peak seawater warming at HIMB spanned 15–24 September 2014 with temperatures ranging from 29.8°C to 30.2°C ($\pm$ 0.2°C accuracy, $\pm$ 0.1°C resolution; NOAA 2017; Supporting Information Fig. S1a). Seawater temperatures at Reef 44 and HIMB declined from peaks in mid-October ($\leq$ 29.2°C $\pm$ 0.5°C), declining thereafter, and seawater temperatures from October 2014 to January 2015 (mean, maximum, and minimum) were comparable, with among sites differences (ca. 0.01°C) below logger resolution ($\pm$ 0.14°C) and accuracy ($\pm$ 0.53°C; Supporting Information Fig. S1; Supporting Information Table S1). Light values integrated over 24 h (i.e., DLI mol photons m^{-2} d^{-1}) were 4.5 mol photons m^{-2} d^{-1} greater at HIMB compared to Reef 44 (p < 0.001; Supporting Information Fig. S1; Supporting Information Table S1).

The concentrations of dissolved inorganic nutrients were low during most of the study, but differences among the three reefs were detected (Fig. 2a–d; Supporting Information Table S1). Phosphate was lowest at Reef 25 (p = 0.019), although this effect was small (difference < 0.02 μmol L^{-1}). Ammonium concentrations were equivalent among reefs (p = 0.161; ca. 0.5 μmol L^{-1}) but most variable at Reef 44 (transient increases of up to 2.0 μmol L^{-1}), and nitrate + nitrite concentrations at Reef 44 were two-fold higher than other sites (p = 0.002; 0.35–0.42 μmol L^{-1}). Silicate (p = 0.724) and short-term sedimentation rates (p = 0.161; Fig. 2e) did not differ among sites; however, silicate tended to be higher at Reef 44, and an extended monitoring of sedimentation rates (December 2014–January 2016) shows that annual sedimentation rates at Reef 44 and HIMB were greater and more variable than rates at Reef 25 (p = 0.041; Fig. 2f). δ^{15}N values for nitrate ranged from 3.8‰ to 4.9‰ (Supporting Information Table S2), however, low [N + N] reduced sample sizes for δ^{15}N-nitrate analysis (n = 1–2 samples per site).

Coral physiology

Multivariate analysis of 16 response variables in *M. capitata* and *P. compressa* revealed significant changes in corals among time periods (p < 0.001), between bleached and nonbleached corals (p $\leq$ 0.004) and in response to the period × condition interaction (p $\leq$ 0.029; Supporting Information Table S3). Reef sites

significantly influenced *M. capitata* condition (p = 0.006), especially during October 2014 (Fig. 3a), whereas *P. compressa* colonies were less influenced by site (p = 0.099) and instead predominantly affected by bleaching condition (Fig. 4a). NMDS plots showed differences in bleached and nonbleached colonies of both species during October 2014 (post hoc: p $\leq$ 0.008), where bleaching correlated with reductions in chlorophyll concentration (Chl) and biomass (Figs. 3b, 4b) and lower host and symbiont C : N in *P. compressa* (Fig. 4b). By January 2015, the physiological condition of previously bleached *M. capitata* (post hoc: p = 0.337) and *P. compressa* colonies (post hoc: p = 0.125) was indistinguishable from nonbleached conspecifics, indicating a convergence of physiological properties in corals across bleaching histories and a rapid physiological recovery from bleaching (Figs. 3c,d, 4c,d). A summary of significant effects for all response variables can be found in Table 1.

M. capitata total chlorophyll (p = 0.041) and tissue biomass (p = 0.011) were affected by the interaction of period × condition (Supporting Information Table S4), and these responses did not vary among sites (p $\geq$ 0.222). In October 2014, bleached *M. capitata* had 63% less chlorophyll and 30% less tissue biomass than nonbleached phenotypes (Fig. 5a,b). By January 2015, however, *M. capitata* chlorophyll and tissue biomass were equivalent among bleached and nonbleached corals, having increased 255% and 95% in bleached phenotypes and 54% and 37% in nonbleached colonies, respectively, from October 2014 levels (Fig. 5a,b). Over the recovery period, *M. capitata* protein biomass (g gdw^{-1}) declined by 20% (p = 0.010) but did not differ among sites (p = 0.461) or between bleached and nonbleached colonies (p = 0.267; Fig. 6a; Supporting Information Table S4). *M. capitata* tissue lipids, carbohydrates, and energy content did not differ among periods (p $\geq$ 0.073), sites (p $\geq$ 0.065), or between bleached and nonbleached colonies (p $\geq$ 0.291; Fig. 6b–d).

P. compressa chlorophyll content differed according to period × condition (p < 0.001) and site × condition (p = 0.011) interactions (Fig. 5c; Supporting Information Fig. S5). In October 2014, chlorophyll in bleached *P. compressa* was 84% (Reef 44), 78% (Reef 25), and 92% (HIMB) lower than nonbleached colonies. By January 2015, chlorophyll was equivalent between all *P. compressa* at Reef 25 and Reef 44, but chlorophyll recovery was suppressed in colonies at HIMB, with 25% less chlorophyll in previously bleached colonies. *P. compressa* total biomass was on average 19% higher in nonbleached relative to bleached colonies (p = 0.025) but did not differ among periods or sites (p $\geq$ 0.173; Fig. 5d).

P. compressa protein biomass (g gdw^{-1}) was affected by period × condition (p = 0.011; Fig. 6e; Supporting Information Table S5), but in post hoc tests, protein was not different among bleached and nonbleached colonies during October 2014 or January 2015. Tissue lipids and energy content were affected by the period × site interaction (p $\leq$ 0.008), but not bleaching conditions (p $\geq$ 0.179). At the time of bleaching in October 2014, *P. compressa* lipids and biomass energy content was equivalent among sites (Fig. 6f,h), but by January 2015, tissue lipids and energy content declined by ca. 27% and 18%, respectively, from
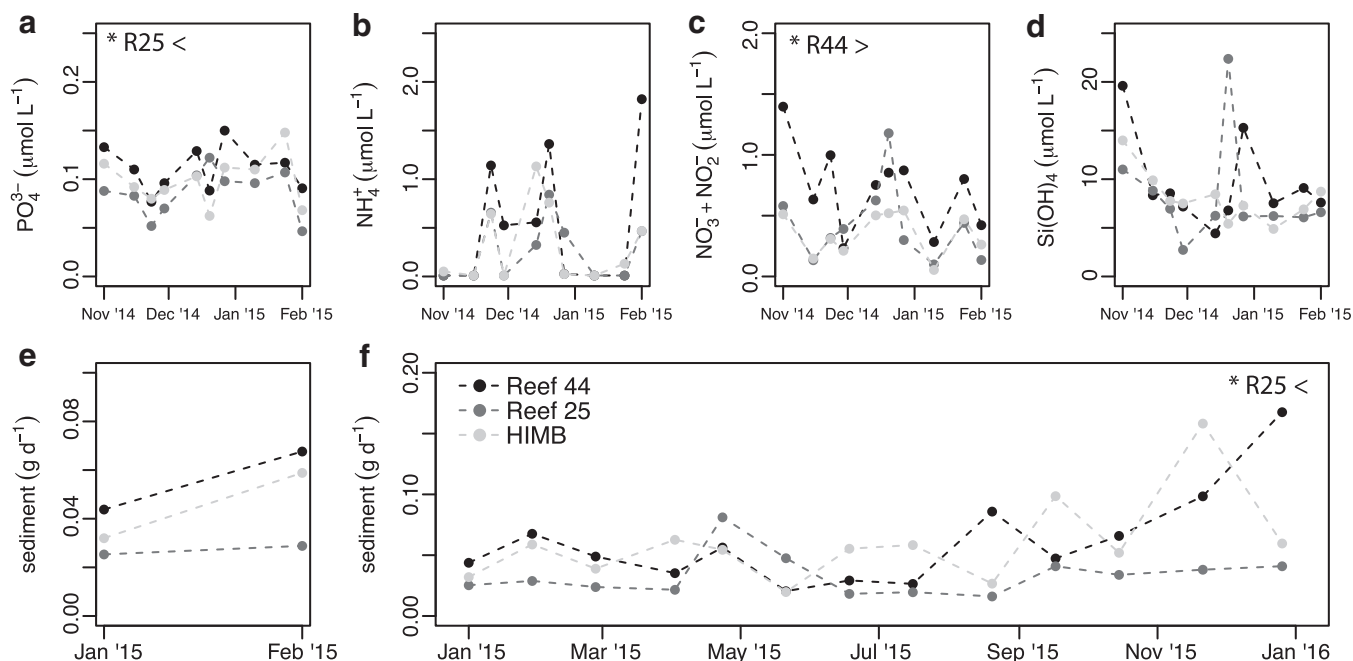
Fig. 2. DIN concentrations (November 2014–February 2015) and sedimentation rates (January 2015–January 2016) at Reef 44, Reef 25, and HIMB in Kāneʻohe Bay. (**a**) Phosphate (PO_4^{3-}), (**b**) ammonium (NH_4^+), (**c**) nitrate + nitrite ($NO_3^- + NO_2^-$), and (**d**) silicate ($Si[OH]_4$) concentrations in seawater and the (**e**) short-term and (**f**) annual sedimentation rates at the three reef sites. Symbols (*) indicate significant site effects ($p < 0.05$).

October 2014 levels. In particular, declining lipid biomass in recovering *P. compressa* was limited to Reef 44 and Reef 25 colonies, whereas lipids in HIMB corals remained high. Carbohydrate biomass showed no significant differences ($p \geq 0.114$; Fig. 6g).

Tissue isotopic compositions

Differences in the carbon isotopic composition of *M. capitata* host ($\delta^{13}C_H$) tissues varied according to bleaching condition ($p = 0.022$), with higher values in bleached colonies, although these differences were small (0.7‰; Fig. 7a). Symbiont $\delta^{13}C$ values varied over time, being lower (0.7‰) during bleaching in October 2014 compared to January 2015 ($p = 0.001$; Fig. 7b). The relative difference in *M. capitata* host and symbiont $\delta^{13}C$ values ($\delta^{13}C_{H-S}$)—a metric for greater proportion of autotrophic- (positive values) and heterotrophic- (negative values)-derived carbon—changed over time, with higher $\delta^{13}C_{H-S}$ values in October 2014 and a decline in $\delta^{13}C_{H-S}$ values in January 2015 ($p = 0.001$; Fig. 7c); $\delta^{13}C_{H-S}$ were slightly higher in bleached colonies (0.3‰; $p = 0.050$). Nitrogen isotopic composition of *M. capitata* host ($\delta^{15}N_H$) tissues differed among reef sites ($p = 0.043$), being ^{15}N-enriched (1‰) at HIMB (5.4‰ ± 0.1‰, mean ± SE) relative to other sites (Fig. 7d). Symbiont $\delta^{15}N$ and $\delta^{15}N_{H-S}$ values showed no statistically significant effects ($p \geq 0.066$; Fig. 7e,f). *M. capitata* C : N_H increased over time ($p < 0.001$) and was higher in bleached relative to nonbleached colonies in January 2015 ($p = 0.046$), but differences across time and conditions were small (< 8% change; Supporting Information Fig. S2a). C : N_S ($p \geq 0.060$) was unaffected across the study (Supporting Information Fig. S2b; Supporting Information Table S6).

P. compressa host $\delta^{13}C$ values were comparable among all colonies in October 2014. In January 2015, effects on $\delta^{13}C_H$ values were limited to HIMB alone, where previously bleached colonies were ^{13}C enriched (2‰) relative to nonbleached colonies ($p = 0.032$; Fig. 7g; Table 1; Supporting Information Table S7). Similarly, symbiont $\delta^{13}C$ values in January 2015 were higher (1‰) in previously bleached colonies, driven largely by higher $\delta^{13}C$ values in colonies at HIMB ($p = 0.048$; Fig. 7h). *P. compressa* $\delta^{13}C_{H-S}$ values did not differ over the study ($p \geq 0.136$; Fig. 7i). *P. compressa* $\delta^{15}N_H$ values were slightly lower (0.4‰) in October 2014 ($p = 0.014$) but were largely spatially influenced ($p = 0.002$), being ^{15}N enriched (1‰) in colonies from HIMB compared to other sites (Fig. 7j). Interactive effects of period × condition on $\delta^{15}N_H$ ($p = 0.033$) were not significant in a priori post hoc contrasts ($p \geq 0.078$). Similarly, *P. compressa* symbiont $\delta^{15}N$ became progressively ^{15}N enriched (ca. 1.2‰) from northern Reef 44 to southern HIMB ($p = 0.024$; Fig. 7k). Additionally, $\delta^{15}N_S$ was higher (1.1‰) in bleached relative to nonbleached *P. compressa* in October 2014, but not January 2015 ($p = 0.009$), corresponding to lower $\delta^{15}N_{H-S}$ values ($p = 0.001$) for bleached relative to nonbleached *P. compressa* ($p = 0.001$) in October 2014 during thermal stress (Fig. 7l). *P. compressa* C : N_H increased over time ($p < 0.001$) and was lower (October 2014) and higher (January 2015) in bleached relative to nonbleached colonies ($p < 0.001$; Supporting Information Table S7), although these effects were small (< 10% change); C : N_H site × condition effects ($p = 0.004$) were not significant in post hoc contrasts. C : N_S showed no significant effects ($p \geq 0.085$; Supporting Information Fig. S2d).
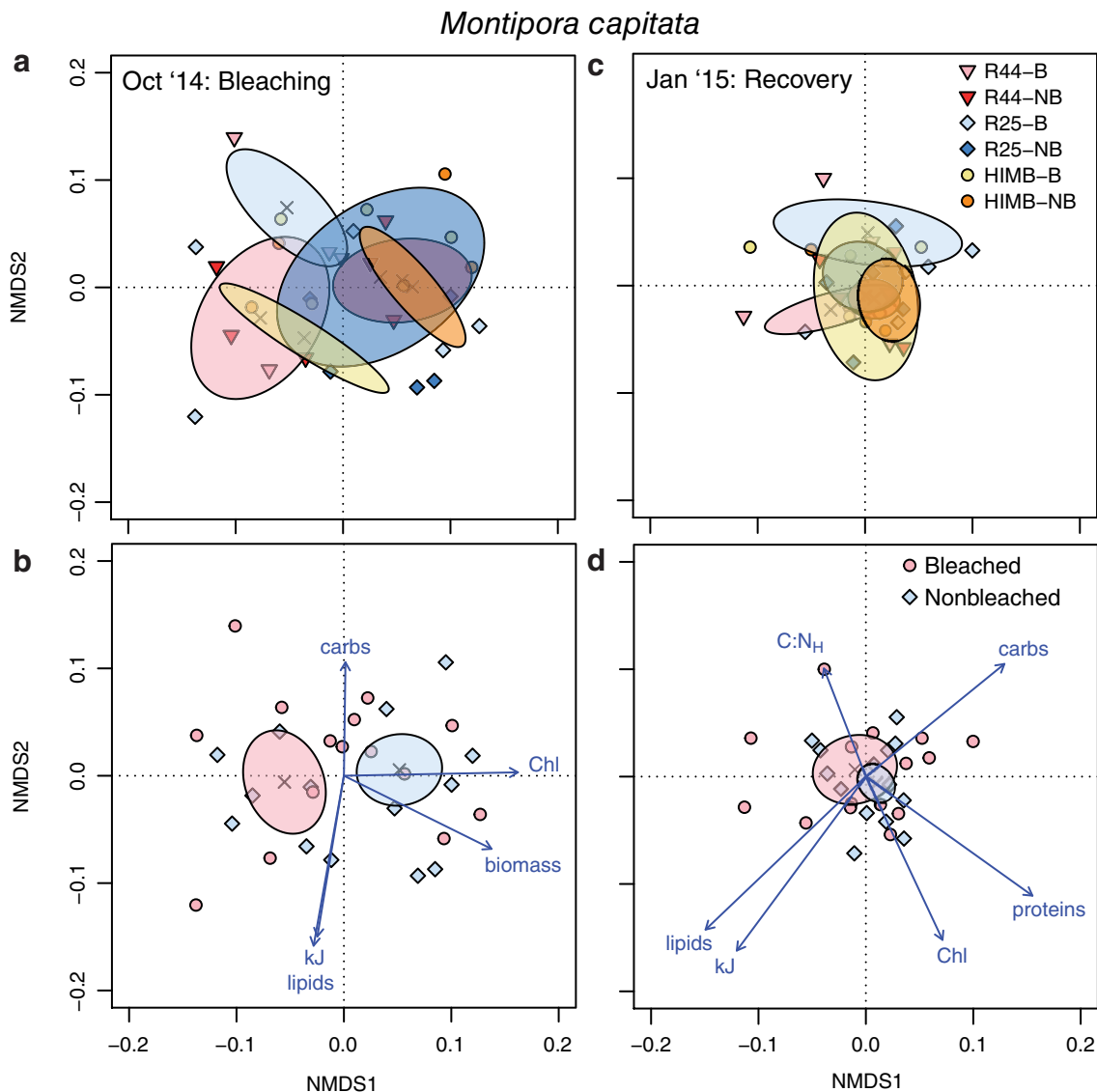
Fig. 3. Multivariate NMDS plots for bleached (B) and nonbleached (NB) *M. capitata* at three reefs (Reef 44 [R44], Reef 25 [R25], and HIMB) during bleaching (left panel) and recovery (right panel) from a regional bleaching event. Polygons are SE of point means (x symbols). (**a**, **c**) NMDS with site × condition effect. (**b**, **d**) NMDS with condition effect alone, with vectors showing significant responses ($p < 0.05$) among bleached and nonbleached corals.

To reconcile small changes in tissue $\delta^{13}C$ values in host and symbiont fractions across the three scales tested here (i.e., period, site, and condition), an isotope mass balance was used. Measurements of total biomass and compound class concentrations (i.e., proteins, lipids, and carbohydrates; Hayes 2001) were used to estimate compound class-specific $\delta^{13}C$ values (i.e., $\delta^{13}C_{Compound}$) for all coral holobionts (i.e., $\delta^{13}C_{Holobiont}$) at the time of thermal stress in October 2014 (Supporting Information Fig. S3). Using colony-specific $\delta^{13}C_{Compound}$ estimates for corals in October 2014 and applying these estimates to the measured proportion of tissue compounds produces an expected $\delta^{13}C_{Holobiont}$, which should explain observed $\delta^{13}C_{Holobiont}$ if $\delta^{13}C_{Compound}$ values have not been substantially altered by the incorporation of different carbon

sources or changes in residual $\delta^{13}C_{Compound}$ from metabolic effects. Expected $\delta^{13}C_{Holobiont}$ values provided a good estimate of observed $\delta^{13}C_{Holobiont}$, which ranged from $\delta^{13}C$ of −19‰ to −13‰ (Fig. 8). The range in $\delta^{13}C$ values is important, as it shows a considerable range in holobiont $\delta^{13}C$ from biological and environmental effects on corals and Symbiodiniaceae. The relationship between the expected $\delta^{13}C_{Holobiont}$ and the observed $\delta^{13}C_{Holobiont}$ values in all corals (i.e., those recovered from bleaching and non-bleached) was significant for both *M. capitata* ($R^2 = 0.88$, $p < 0.001$) and *P. compressa* ($R^2 = 0.56$, $p < 0.001$; Fig. 8), indicating a significant influence of protein : lipid : carbohydrate ratios in explaining variance in $\delta^{13}C$ values in both species during bleaching recovery.
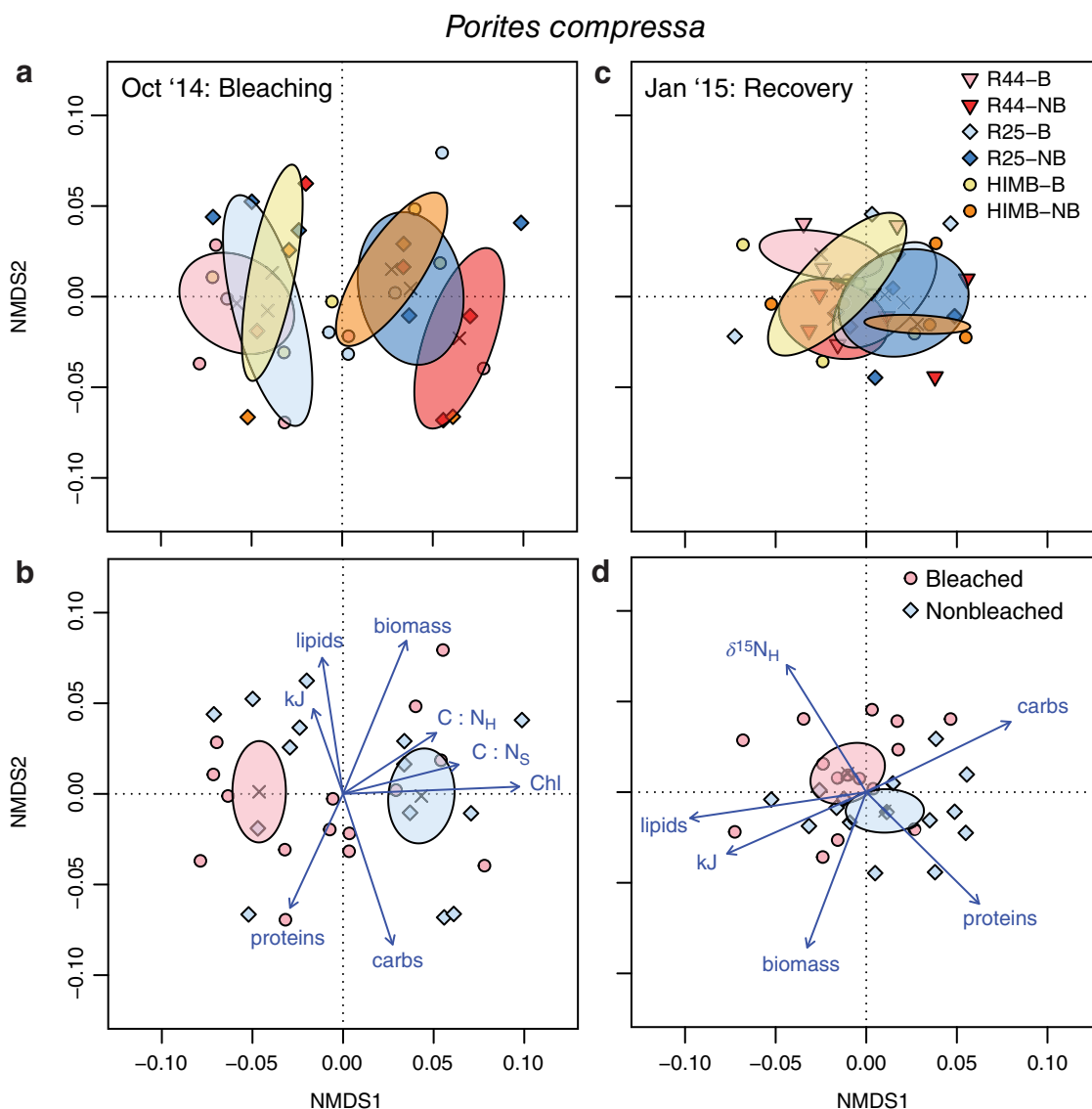
Fig. 4. Multivariate NMDS plots for bleached (B) and nonbleached (NB) *P. compressa* at three reefs (Reef 44 [R44], Reef 25 [R25], and HIMB) during bleaching (left panel) and recovery (right panel) from a regional bleaching event. Polygons are SE of point means (x symbols). (**a, c**) NMDS with site × condition effect. (**b, d**) NMDS with condition effect alone, with vectors showing significant responses ($p < 0.05$) among bleached and nonbleached corals.

Discussion

Few studies have monitored changes in coral physiology and nutritional plasticity during and after large-scale natural bleaching events (Fitt et al. 1993; Edmunds et al. 2003; Rodrigues et al. 2008; Grottoli and Rodrigues 2011) or evaluated local environmental effects on physiological conditions that shape bleaching recovery (Cunning et al. 2016). Using *M. capitata* and *P. compressa* colonies from three reefs spanning 6.3 km along Kāneʻohe Bay, we observed variable tissue biomass and chlorophylls among bleaching conditions and through time, but energy reserves were unaffected by bleaching stress. Furthermore, evidence suggests that relatively small changes in coral tissues composition across space and time and not changes in heterotrophic nutrition explain patterns in $\delta^{13}C$ values of both coral species during

bleaching recovery. Taken together, these results shed light on coral physiology during and after thermal stress and identify the need to quantify tissue composition effects on isotopic values in corals, as this may provide insight into the performance of corals across a continuum of physiological conditions and ecological scales.

Environmental context, bleaching, and recovery

Seawater temperatures during and after bleaching in October 2014 were comparable among the three reefs, but light availability was lower and dissolved nutrients and sedimentation tended to be higher at Reef 44 in northern Kāneʻohe Bay (Fig. 1a). These observations correspond with a combination of greater discharge of subterranean groundwater, watershed/stream inputs, and the unique

Table 1. Statistical analysis of bleached and nonbleached *M. capitata* and *P. compressa* at three Kāne'ohe Bay patch reefs during bleaching and recovery.

Response variable	M. capitata Oct 2014: Bleaching	M. capitata Jan 2015: Recovery	P. compressa Oct 2014: Bleaching	P. compressa Jan 2015: Recovery
Chlorophylls	B < NB	—	B < NB	HIMB: B < NB
Biomass	B < NB	—	B < NB	
Proteins	2014 > 2015		—	—
Lipids	—		—	HIMB > R44 = R25
Carbohydrates	—		—	
Energy content	—		2014 > 2015	
$\delta^{13}C_H$	B > NB		—	HIMB: B > NB
$\delta^{13}C_S$	2014 < 2015		—	B > NB
$\delta^{13}C_{H-S}$	2014 > 2015		—	
$\delta^{15}N_H$	HIMB > R25		2014 < 2015 / HIMB > R44 = R25	
$\delta^{15}N_S$	—		HIMB > R44	
			B > NB	—
$\delta^{15}N_{H-S}$	—		B < NB	—
C : N$_H$	2014 < 2015		2014 < 2015	
	—	B > NB	B < NB	B > NB
C : N$_S$	—		—	

Table information shows significant model effects and post hoc comparisons ($p < 0.05$); dashed lines indicate no significant effects ($p > 0.05$). Periods are October 2014 bleaching and January 2015 recovery. Sites (north to south) are Reef 44 (R44), Reef 25 (R25), and the HIMB. Corals are described according to their physiological condition in October 2014, being bleached (B) or nonbleached (NB); condition designators from October (i.e., B/NB) were retained in January after corals regained pigmentation. Subscripts indicate either host (H) or symbiont (S) tissues, or their relative difference (H − S).

hydrology (short seawater residence) at this location (Drupp et al. 2011; Dulai et al. 2016). While physiological stress from high light (Anthony et al. 2007) and nutrient enrichment (Wiedenmann et al. 2012) can exacerbate thermal stress, bleaching severity (assessed from chlorophyll density) was similar among the three reef sites, and N : P ratios (range: 0.6–10.5) were below those reported in cases where nutrients negatively affected corals (i.e., bleaching and tissue loss; N : P of 255 : 1 [Rosset et al. 2017], 22 : 1 and 43 : 1 [Wiedenmann et al. 2012]). Excess nutrient enrichment is detrimental to coral reefs (Vega Thurber et al. 2014; Silbiger et al. 2018), yet moderate nutrient enrichment and stochastic nutrient perturbations can benefit corals by stimulating symbiont growth (Sawall et al. 2014) and increasing concentrations of dissolved organic carbon (Levas et al. 2016), suspended particles, and prey (Mills et al. 2004; Mills and Sebens 2004; Selph et al. 2018) to the benefit of coral energy acquisition (Fox et al. 2018). Therefore, site-specific patterns in light and nutrient concentrations in the present study did not appear to affect bleaching responses but may have influenced postbleaching trajectories of physiological recovery and symbiont repopulation (see also Cunning et al. 2016).

Three months after a regional bleaching event (i.e., January 2015), bleached colonies had regained photopigmentation and were indistinguishable from nonbleached conspecifics, with the exception of moderately lower chlorophyll in bleached *P. compressa* at

HIMB. Recovery from the 2014 bleaching event may have been hastened by seawater cooling initiated by the passage of Hurricane Ana by the Hawaiian Islands (ca. 17–23 October 2014; NOAA 2018) days before our sampling (24 October 2014), serving to mitigate further physiological thermal stress in October 2014 (Supporting Information Fig. S1a; Manzello et al. 2007). Rapid recovery rates observed here over short periods, however, do not negate possible long-term effects of bleaching. For instance, in many coral species, bleaching effects can reduce long-term reproductive capacity (Levitan et al. 2014), alter tissue biochemistry (Rodrigues and Grottoli 2007; Baumann et al. 2014; Schoepf et al. 2015), and alter gene expression for several months (Pinzón et al. 2015) to a year after the onset of thermal stress (Thomas and Palumbi 2017). Moreover, effects of repeat bleaching events can be complex and multiplicative, reducing the physiological resilience of corals in the long term (Grottoli et al. 2014). Therefore, it is important to recognize short-term recovery of pigmentation and biomass (Fig. 5) as one part of the bleaching condition, while acknowledging the uncertainty in long-term effects of bleaching on coral biology after symbiont repopulation.

Physiological impacts of bleaching and recovery

Bleaching sensitivity is affected by the capacity for cellular and genetic properties of Symbiodiniaceae and host genotypes to mitigate cellular damage (Weis 2008; Kenkel et al. 2013). *P. compressa* is
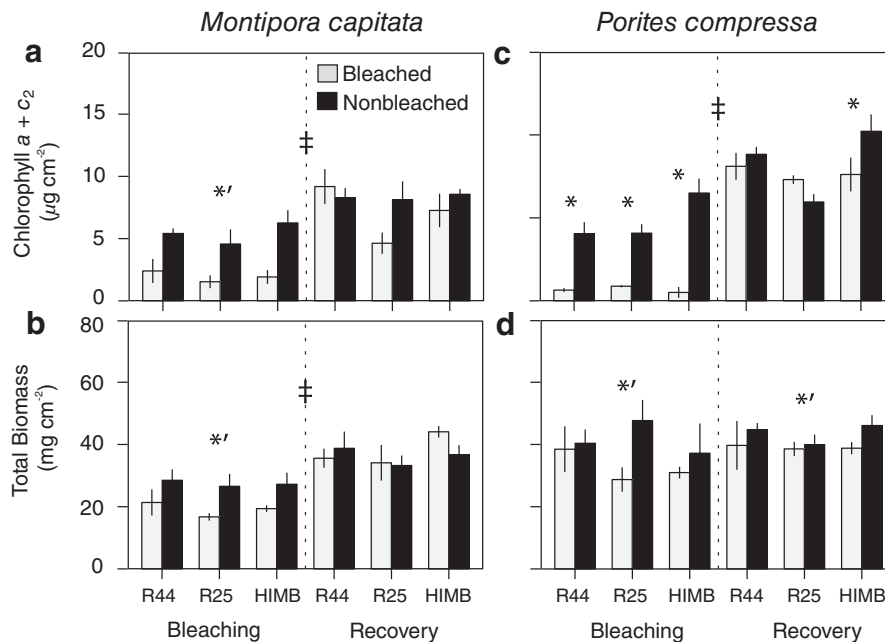
Fig. 5. Total chlorophyll and total biomass in bleached (*gray*) and nonbleached (*black*) *M. capitata* (left panel) and *P. compressa* (right panel) at three reefs (Reef 44 [R44], Reef 25 [R25], and HIMB) during bleaching and recovery. Area-normalized (**a**, **c**) chlorophyll ($a + c_2$) and (**b**, **d**) AFDW of tissue biomass. Values are mean $\pm$ SE ($n = 5$). Symbols indicate significant differences ($p < 0.05$) between periods (‡) and bleached and nonbleached corals within a period (*′) and within a site (*).

a symbiont specifist, hosting only one species of *Cladocopium* sp. (formerly, clade C) symbionts (ITS2 Type C15; LaJeunesse et al. 2004). *M. capitata*, however, exhibits flexible symbiont partnerships that partition across habitats (Innis et al. 2018) and influence bleaching responses (Cunning et al. 2016). In a parallel study of *M. capitata* in Kāneʻohe Bay following the 2014 bleaching event, bleached colonies were always dominated by *Cladocopium* sp. symbionts (ITS2 Type C31), whereas nonbleached colonies could be dominated by *Cladocopium* sp. or *Durusdinium glynnii* (formerly, *Symbiodinium glynii* [ITS2 Type D1-4-6]; Cunning et al. 2016). Thus, symbiont communities alone cannot explain the distinct bleaching phenotypes observed in either *M. capitata* or *P. compressa* during the 2014 bleaching event but instead point to physiological acclimatization (Kenkel and Matz 2016) or genetic mechanism(s) (Palumbi et al. 2014) on behalf of host and symbiont genotypes or their combination as supporting holobiont thermal tolerance (Sampayo et al. 2008).

Coral host biomass quantity (i.e., total biomass), quality (i.e., % lipids and energy content), and thickness are important determinants for stress resilience and postbleaching survival (Loya et al. 2001; Anthony et al. 2009; Thornhill et al. 2011). In the present study, bleached colonies of both species had between 25% and 30% less biomass than nonbleached corals, and during postbleaching recovery, changes in tissue biomass were species specific and dependent on bleaching history. In previous studies, tissue biomass (i.e., mg AFDW cm^{-2}) has been shown to decline 34–50% during and after thermal stress (Porter et al. 1989) as a result of tissue catabolism (Fitt et al. 1993; Grottoli et al. 2006; Rodrigues and Grottoli 2007) and/or cellular detachment during bleaching (Gates

et al. 1992). Postbleaching, *M. capitata* recovered biomass quickly (< 3 months; Fig. 5); in contrast, biomass in previously bleached *P. compressa* colonies remained low (17% less than nonbleached colonies) at both time periods. These results agree with laboratory experiments, where bleaching quickly reduced *M. capitata* and *P. compressa* biomass, but *P. compressa* tissues took much longer to recover (4–6 months postbleaching) compared to *M. capitata* (1.5 months; Grottoli et al. 2006; Rodrigues and Grottoli 2007). The cause for different biomass recovery rates is uncertain, but can indicate the extent of physiological stress, energetic demands, and differences in rates of tissue growth and metabolism between the two species (Coles and Jokiel 1977).

During the natural bleaching event and subsequent recovery, changes in the biomass composition were independent of bleaching history, and instead varied according to periods in both *M. capitata* (proteins) and *P. compressa* (energy content) and among sites during recovery for *P. compressa* (lipids; Fig. 6). Bleaching-independent changes in biomass composition and energy observed here (Fig. 6; Supporting Information Fig. S2) can also relate to shared physiological challenges confronting both bleaching susceptible and resistant corals (i.e., gene regulation and stress protein synthesis; Kenkel et al. 2013) and complex seasonal (Fitt et al. 2000) and site-specific environmental contexts (i.e., light availability; Patton et al. 1977; Anthony 2006) juxtaposed atop bleaching stress. Indeed, while tissue composition (i.e., % proteins, lipids, and carbohydrates) did not differ among bleached and nonbleached colonies at either time point, total biomass (mg cm^{-2}) was lower in all colonies in October 2014 regardless of bleaching condition (Fig. 5). Therefore, thermal stress may reduce the total biomass production in both bleaching
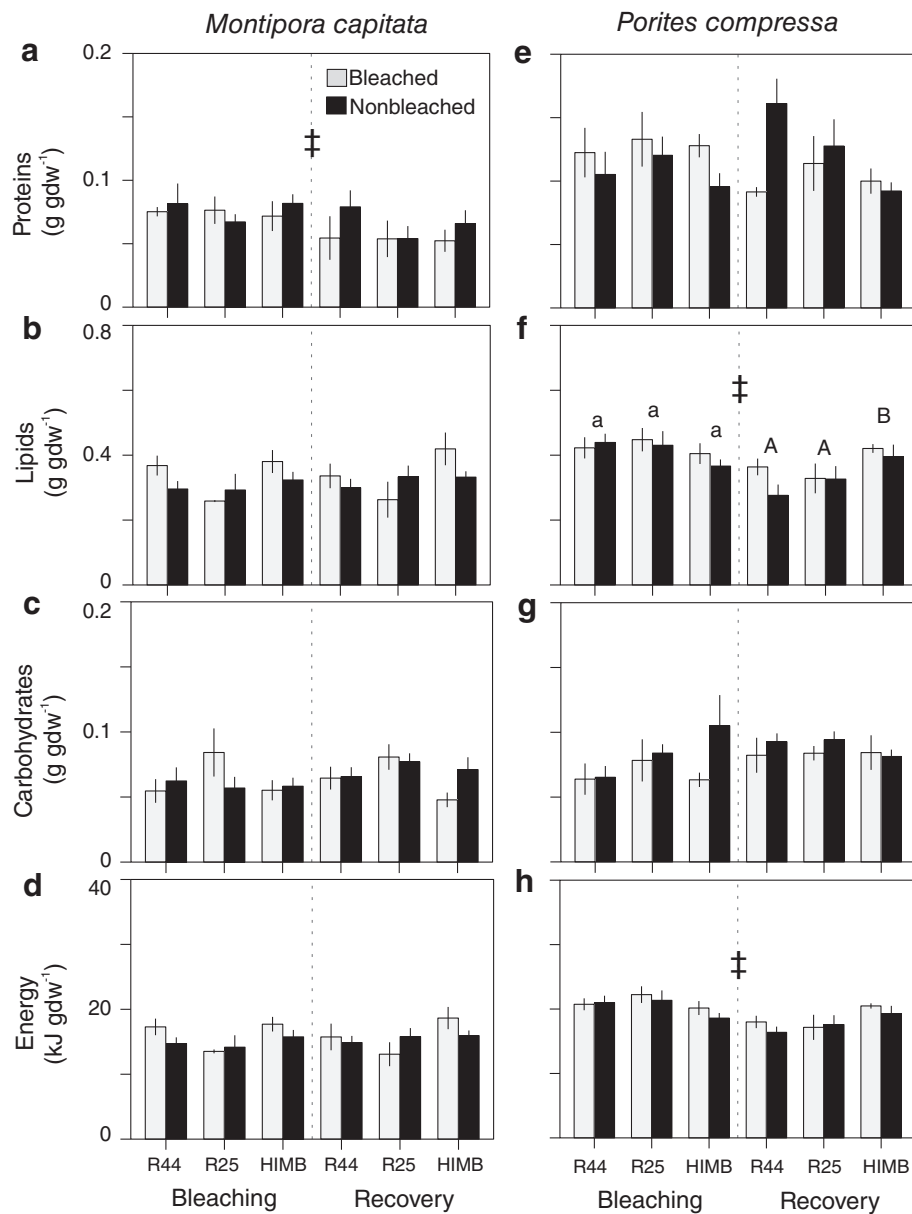
Fig. 6. Biomass composition and energy content in bleached (gray) and nonbleached (*black*) *M. capitata* (left panel) and *P. compressa* (right panel) at three reefs (Reef 44 [R44], Reef 25 [R25], and HIMB) during bleaching and recovery. (**a, e**) proteins, (**b, f**) lipids, (**c, g**) carbohydrates, and (**d, h**) energy content (kJ) normalized to grams of AFDW (gdw^{-1}). Values are mean ± SE (n = 4–5). Symbols indicate significant (p < 0.05) period effects (‡); letters indicate differences between sites within periods of bleaching (lowercase) or recovery (uppercase).

susceptible and resistant corals, and tissue biomass in bleached corals may remain low for several months postbleaching.

Nutritional plasticity and tissue isotopic composition

The isotopic values of an organism are linked to the constitutive biochemical composition of the tissues and substrates acquired through its diet and broken down in metabolism (Minagawa and Wada 1984; Hayes 2001). Isotopic inferences on nutritional plasticity in corals are also complicated by the translocation/recycling of metabolites between symbiotic partners (Reynaud et al. 2002; Einbinder et al. 2009), kinetic isotope fractionation in biological reactions (i.e., metabolic isotope effects; Land et al. 1975), and the isotopic composition of internal and external nutrient pools (Swart et al. 2005*b*) which are influenced by rates of production and growth, among other processes. For instance, in Symbiodiniaceae and other microalgae, elevated rates of photosynthesis and growth produce carbon limitations (Laws et al. 1995; Swart et al. 2005*a*) that reduce isotopic discrimination and increase δ^{13}C values. Conversely, light attenuation and low rates of photosynthesis (Muscatine et al. 1989; Laws et al. 1995; Swart et al. 2005*b*; Maier et al. 2010) can decrease both δ^{13}C and δ^{15}N values in corals (but see also Rost et al. 2002). Lower
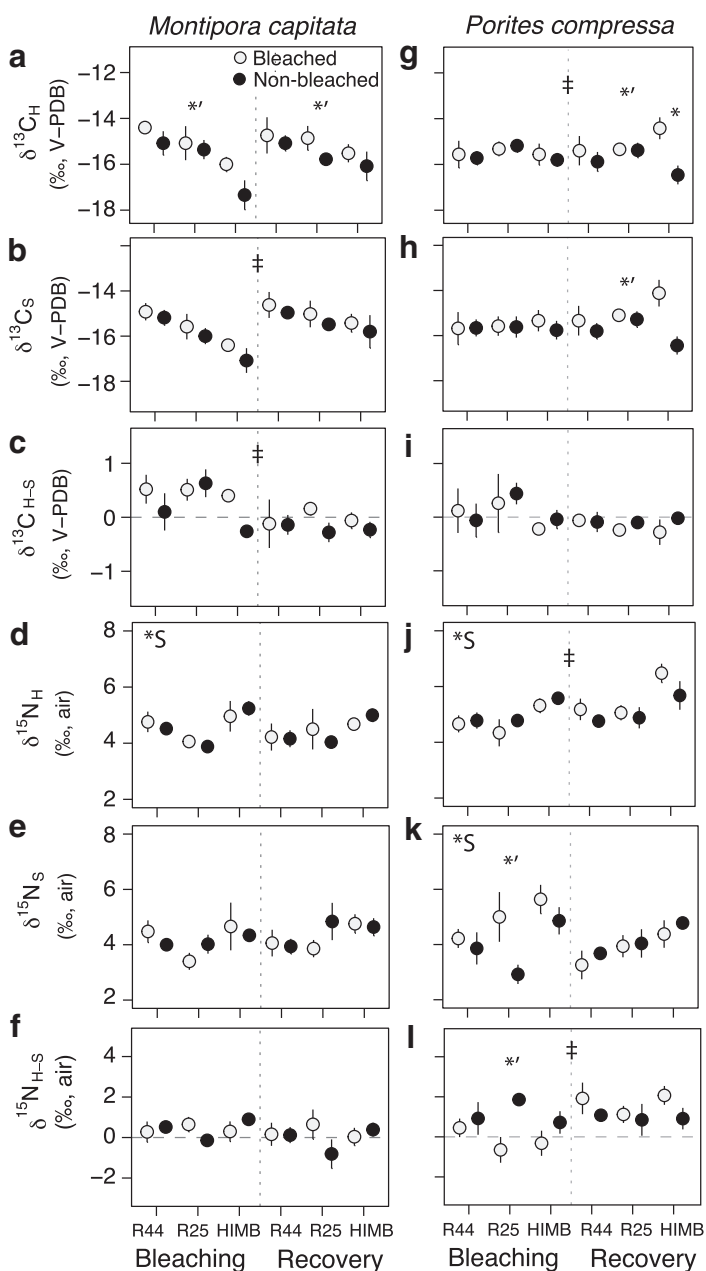
Fig. 7. Isotopic analysis of bleached (gray) and nonbleached (black) *M. capitata* (left) and *P. compressa* (right) host and symbiont tissues at three reefs (Reef 44 [R44], Reef 25 [R25], and HIMB) during bleaching and recovery. Carbon (δ^{13}C) and nitrogen (δ^{15}N) isotopic values for (**a, g, d, j**) coral host ($\delta^{13}C_H$, $\delta^{15}N_H$), (**b, h, e, k**) symbiont algae ($\delta^{13}C_S$, $\delta^{15}N_S$), and (**c, i, f, l**) their relative difference ($\delta^{13}C_{H-S}$, $\delta^{15}N_{H-S}$). Values are permil (‰) relative to standards for carbon (V–PDB) and nitrogen (air). Values are mean ± SE (*n* = 5); small SE may be masked by points. Symbols indicate significant (*p* < 0.05) period (‡) and site effects (*S) and differences among bleached and nonbleached corals within a period (*′) or a site (*).

δ^{13}C values can also result from greater feeding on particles (i.e., plankton and organic particles; Levas et al. 2013; Grottoli et al. 2017) and the preferential utilization of heterotrophic nutrition in lipid biosynthesis (Alamaru et al. 2009; Baumann et al. 2014).
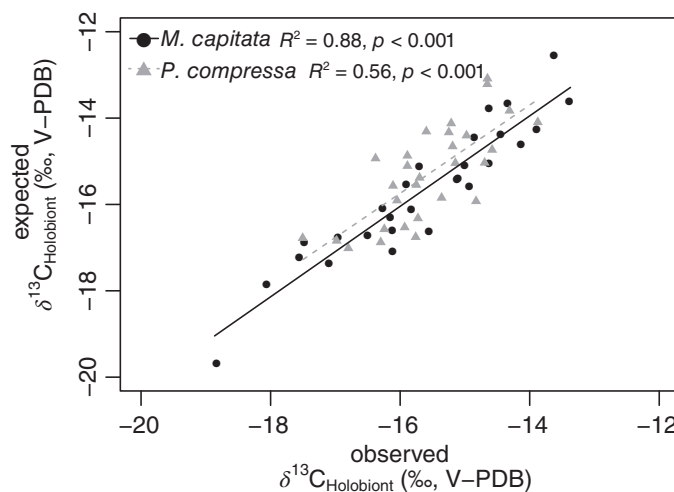
Fig. 8. Relationship between observed- and expected-$\delta^{13}C_{Holobiont}$ for *M. capitata* (black circles) and *P. compressa* (gray triangles) during post-bleaching recovery. Lines represent linear regression for *M. capitata* (solid line) and *P. compressa* (dotted line).

Short-term increases in heterotrophic nutrition can be difficult to verify, however, due to uncertainty in rates of tissue turnover and changes in tissue composition, especially following physiological stress (Rodrigues and Grottoli 2006; Logan et al. 2008). For instance, the recovery of tissue biomass reserves in bleached corals is compound specific (Rodrigues and Grottoli 2007; Schoepf et al. 2015) and the nutritional inputs (i.e., autotrophy vs. heterotrophy) responsible for biomass growth can differ among species and according to time postbleaching (Baumann et al. 2014).

Throughout the study, *M. capitata* $\delta^{13}C_H$ values were higher in bleached corals, whereas symbiont δ^{13}C values were lower in October 2014 during bleaching relative to January 2015 during recovery (Fig. 7a,b). *M. capitata* $\delta^{13}C_{H-S}$ values were also consistently higher in October 2014 relative to January 2015 and slightly more positive in bleached corals. Effects on *P. compressa* δ^{13}C values were limited to postbleaching recovery in January 2015, where previously bleached colonies had higher $\delta^{13}C_S$ values at all sites and higher $\delta^{13}C_H$ values at HIMB alone, although the differences were very small (< 1‰). In all these cases, host and symbiont δ^{13}C and $\delta^{13}C_{H-S}$ values do not support a greater reliance on heterotrophy in bleached corals. Lower $\delta^{13}C_H$ values in nonbleached colonies (*M. capitata* overall and *P. compressa* at HIMB in January 2015) instead can be explained by changes in host biomass properties (i.e., protein : lipid : carbohydrate ratios) and not greater feeding on ^{13}C-depleted prey. In contrast, *M. capitata* $\delta^{13}C_S$ values varied across time independent of bleaching history, perhaps as a result of temperature (or seasonal) effects on symbiont production, growth, and nitrogen demand. Similarly, thermal effects, seasonality, and/or symbiont repopulation may explain higher $\delta^{13}C_S$ values in previously bleached *P. compressa* in January 2015. In total, δ^{13}C values provided poor support for nutritional plasticity in both species in this study, whereas changes in

biomass properties may offer a unifying hypothesis to explain variance in δ^{13}C values at the multiple scales within this study (period, site, and condition).

Organism bulk δ^{13}C values are affected by their biochemical compositions (Logan et al. 2008; Alamaru et al. 2009). Isotope mass balance calculations show that the majority of variance in *M. capitata* and *P. compressa* δ^{13}C$_{Holobiont}$ values (88% and 55%, respectively; Fig. 8) can be explained by changes in the relative proportions of compounds (i.e., proteins, lipids, and carbohydrates), despite individual compounds not differing among bleaching and nonbleached colonies of either species. However, it should be acknowledged that δ^{13}C values of compounds—particularly, lipids—in corals may change in response to physiological stress (Grottoli and Rodrigues 2011) and are shaped by biosynthesis sources and rates of tissue growth/metabolism (Alamaru et al. 2009; Baumann et al. 2014). Reef corals are considered lipid rich (ca. 30% of biomass; Patton et al. 1977), and lipids are depleted in ^{13}C relative to bulk tissues (Hayes 2001; Supporting Information Fig. S3). The breakdown of lipids, therefore, is expected to lead to small increases in δ^{13}C values of remaining lipid fraction and organism δ^{13}C values (DeNiro and Epstein 1977). However, corals can catabolize isotopically light lipids during bleaching, resulting in residual lipid ^{13}C enrichment (Grottoli and Rodrigues 2011). Should tissue lipids in bleached colonies depart from predicted isotopic relationships (Hayes 2001)—being either 3‰ lower or higher than lipids in nonbleached colonies—the predictive power of our modeled relationship in observed vs. expected δ^{13}C values for corals during the recovery period in January 2015 is lessened (48% and 67% [*M. capitata*] and 27% and 36% [*P. compressa*] variance explained, respectively). Therefore, using a constant relationship of compound class-specific δ^{13}C values relative to whole tissue δ^{13}C values, we infer changes in the relative proportions of proteins, lipids, and carbohydrates and not their isotopic composition best explain patterns in the bulk δ^{13}C values of corals in this study. Although few examples of compound-class or compound-specific isotope values for coral tissues exist (lipids [Alamaru et al. 2009; Grottoli and Rodrigues 2011] and coral skeletal organic matrix [Muscatine et al. 2005]), changes in biomass composition can effectively explain the patterns in δ^{13}C values of both species used in this study, albeit an understanding of baseline isotopic values for coral tissue compounds is needed to better discern effects of habitat, environment, and nutrition in reef corals.

Unlike most predator–prey relationships (Minagawa and Wada 1984), greater heterotrophic nutrition in corals does not lead to appreciable higher δ^{15}N values in coral tissue relative to its symbiont algae (Reynaud et al. 2009); instead, coral δ^{15}N values often relate to sources at the base of the food web (Heikoop et al. 2000; Dailer et al. 2010). *M. capitata* and *P. compressa* δ^{15}N values were within the range of δ^{15}N-nitrate values in Kāne'ohe Bay (4–5‰; Supporting Information Table S2) and higher at HIMB relative to other sites. Similar patterns of higher δ^{15}N values in southern Kāne'ohe Bay were also seen in juvenile brown stingray (*Dasyatis lata*) known to have a fairly constant diet (Dale et al. 2011), indicating spatial variability in the sources and isotopic values of DIN

δ^{15}N values that permeate the food web of Kāne'ohe Bay (Heikoop et al. 2000; Nahon et al. 2013). These spatial effects are expected to result from a combination of greater subterranean groundwater discharge in northern Kāne'ohe Bay (Dulai et al. 2016), high stream input (30% of bay total), and legacy effects of sewage dumping (1951–1978) in southern Kāne'ohe Bay (Smith et al. 1981). Higher δ^{15}N$_H$ values in all *P. compressa* in January—driven largely by corals at HIMB—may also be influenced by nitrogen acquisition deficits, as well as changes in amino-acid synthesis/deamination and nitrogen concentration of heterotrophic (Haubert et al. 2005) and autotrophic resources (Tanaka et al. 2006).

P. compressa δ^{15}N$_S$ values differed from the host, being higher in October 2014 relative to January 2015, and in particular, 2‰ higher in nonbleached Reef 25 *P. compressa* relative to bleached colonies in October. At the same time, the predicted +1.5‰ enrichment (i.e., δ^{15}N$_{H-S}$) for consumers relative to their food source reversed and was negative for bleached *P. compressa* at Reef 25 and HIMB colonies (October 2014), suggesting disruption of nitrogen recycling (Wang and Douglas 1998) in bleached colonies and/or contributions of nitrogen not originating from animal metabolism. These low δ^{15}N$_S$ values may indicate a greater utilization of a ^{15}N-depleted DIN source, possibly from N_2 fixation by coral-associated diazotrophs (Bednarz et al. 2017) or decreased rates of growth and nitrogen demand in nonbleached coral symbionts (Heikoop et al. 1998; Baker et al. 2013). δ^{15}N values of Symbiodiniaceae are predicted to increase when growth rates are elevated and nitrogen availability is limited (Rodrigues and Grottoli 2006), although this depends on whether rates of photosynthesis and growth are balanced (Granger et al. 2004). Increased δ^{15}N$_S$ values in bleached *P. compressa* agree with other studies (Rodrigues and Grottoli 2006; Bessell-Browne et al. 2014; Schoepf et al. 2015) suggesting elevated rates of mitotic cell division, and photopigment synthesis postbleaching increases symbiont nitrogen demand, thereby reduced nitrogen isotope fractionation (Heikoop et al. 1998). An increase in δ^{15}N$_S$ values at the time of bleaching is intriguing, as this suggests that symbiont repopulation proceeds rapidly following peak thermal stress. The capacity for rapid nitrogen assimilation in symbionts postbleaching may be an important factor in physiological resilience of corals and may be shaped by the functional diversity of Symbiodiniaceae (Baker et al. 2013), properties of the coral host (Loya et al. 2001), and the extent of physiological stress.

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Conflict of Interest

None declared