

Long-term growth rates and effects of bleaching in *Acropora hyacinthus*

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Abstract Understanding the response of coral growth to natural variation in the environment, as well as to acute temperature stress under current and future climate change conditions, is critical to predicting the future health of coral reef ecosystems. As such, ecological surveys are beginning to focus on corals that live in high thermal stress environments to understand how future coral populations may adapt to climate change. We investigated the relationship between coral growth, thermal microhabitat, symbionts type, and thermal acclimatization of four species of the *Acropora hyacinthus* complex in back-reef lagoons in American Samoa. Coral growth was measured from August 2010 to April 2016 using horizontal planar area of coral colonies derived from photographs and in situ maximum width measurements. Despite marked intraspecific variation, we found that planar colony growth rates were significantly different among cryptic species. The highly heat tolerant *A. hyacinthus* variant “HE” increased in area an average of 2.9% month⁻¹ (0.03 cm average mean radial extension month⁻¹). By contrast, the three less tolerant species averaged 6.1% (0.07 cm average mean radial extension month⁻¹). Planar growth rates were 40% higher on average

in corals harboring Clade C versus Clade D symbiont types, although marked inter-colony variation in growth rendered this difference nonsignificant. Planar growth rates for all four species dropped to near zero following a 2015 bleaching event, independent of the visually estimated percent area of bleaching. Within 1 yr, growth rates recovered to previous levels, confirming previous studies that found sublethal effects of thermal stress on coral growth. Long-term studies of individual coral colonies provide an important tool to measure impacts of environmental change and allow integration of coral physiology, genetics, symbionts, and microclimate on reef growth patterns.

Keywords Coral growth · Coral bleaching · *Acropora hyacinthus* · Symbiont · Thermal stress · American Samoa

Introduction

Coral growth is markedly affected by both natural and anthropogenic environmental stressors (Edmunds et al. 2004; Pratchett et al. 2015). Sedimentation, nutrient loading, algal overgrowth, coral symbiont type, ocean acidification, and high temperature have all been implicated in reducing coral growth rates (Pratchett et al. 2015). The effects of high temperature on corals are particularly concerning as extended periods of elevated sea temperatures often cause acute thermal stress and bleaching in many coral species, and such events are increasing both in severity and duration (Baker et al. 2008; Doney et al. 2012).

However, not all corals are equally susceptible to thermal stress. Some back-reef species appear to thrive under conditions of low water flow, shallow depths, and high temperatures (Oliver and Palumbi 2009). In particular, Acroporid corals in distinct isolated back-reef pools in Ofu,

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American Samoa, often experience noon-time temperatures above 34 °C, over 1 °C above the bleaching threshold for many reef building corals (Barshis et al. 2013). Common garden reciprocal transplant experiments demonstrate that populations of *Acropora hyacinthus* have the ability to acclimate and adapt to these challenging thermal environments. Specifically, genetic analysis of coral transplants shows that *A. hyacinthus* corals living in these warm back-reef lagoons can both acclimatize through changes in phenotypic expression and adapt through changes in genotypic profiles to thermal stress, resulting in a decrease in bleaching frequency despite exposure to periods of significant thermal stress (Palumbi et al. 2014).

In addition to changes in the coral animal, thermal resilience is also linked to *Symbiodinium* clades harbored within the coral (Berkelmans and van Oppen 2006; Oliver and Palumbi 2011a). Specifically, corals that harbor *Symbiodinium* Clade D often have higher thermal resilience and bleaching resistance than those with Clade C (Rowan 2004), leading to the hypothesis that bleaching may be an adaptive response to thermal stress to facilitate symbiont switching (Baker et al. 2004). Although switching to Clade D symbionts can result in increased thermal tolerance (Berkelmans and van Oppen 2006), symbiont switching may also come at an energetic cost as Clade D symbionts have a daily net carbon gain 6–10% lower than Clade C symbionts (Rowan 2004). As such, there is a physiological trade-off where higher thermal tolerance results in slower coral growth, a prediction borne out by lab studies but seldom in the field (Little et al. 2004; Jones and Berkelmans 2011; Cunning et al. 2015).

Branching corals of the genus *Acropora* are among the fastest growing taxa on most coral reefs; they also have highly variable symbionts. As such, *Acropora* is highly susceptible to thermal stress and bleaching (Linares et al. 2011), with concomitant impacts on growth. For example, for the table top coral *A. hyacinthus*, average growth rates range from < 3 to 10 cm diameter increase per year, with much of this variation thought to be a response to temperature, in addition to competition and other abiotic and biotic factors (Tomascik et al. 1996; Wakeford et al. 2008; Linares et al. 2011).

On Ofu, American Samoa, *A. hyacinthus* is a species complex comprised of several distinct cryptic species denoted as HA, HC, HD, and HE, based on genotyping (Ladner and Palumbi 2012; Sheets and Palumbi 2018) (Fig. 1). These species fall into distinct gene pools while living in sympatric populations, such as Ofu, but also have been found across the Pacific. Species HA, HC, and HD are the most widespread. Species HE is most common in the southwestern Pacific in the Samoan archipelago and Cook Islands (Ladner and Palumbi 2012). The HE cryptic species dominates back-reef pools that experience the most

extreme temperature variation (highly variable pool) (Oliver and Palumbi 2011a) and demonstrates higher thermal tolerance than the other three *A. hyacinthus* lineages (Barshis et al. 2013; Rose et al. 2017). Recently, species HE and HC have been further compared genetically at Ofu: they show widespread differentiation at polymorphic SNPs, showing an F_{ST} across the genome of 0.18. They also show distinct habitat preferences, different patterns of gene expression in stress-related gene networks, different associations with symbiont types, and different responses to natural bleaching events (Rose et al. 2017). This set of divergent traits bolsters the conclusion of cryptic species status suggested by previous genotyping and has been suggested as a possible example of ecological differentiation among distinct genetic lineages (Rose et al. 2017).

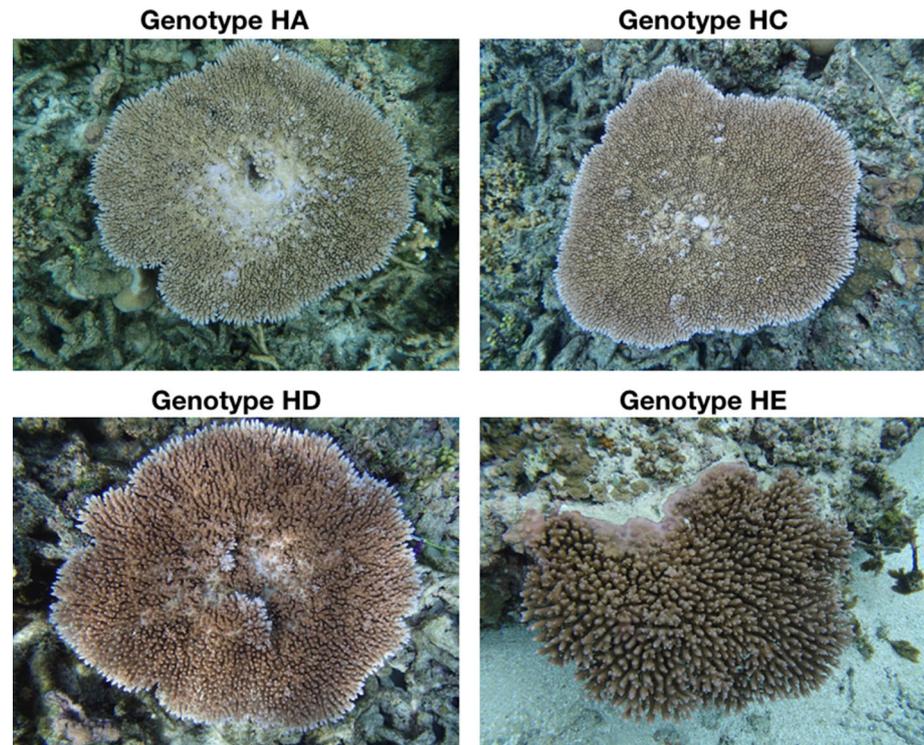
Here we examine the growth rates of cryptic *A. hyacinthus* species on Ofu, American Samoa. Specifically, we follow the growth rate of individual *A. hyacinthus* colonies over a period of 6 yrs in back-reef environments with distinct thermal variability profiles. We then compared growth rates across colonies based on coral species and *Symbiodinium* composition to determine whether increased thermal tolerance resulted in reduced growth. In addition, we estimate the impact of bleaching temperatures on subsequent growth following a significant local bleaching event.

Methods

Cryptic species

To examine how cryptic species, *Symbiodinium* community composition, and environmental variation impact the growth rate of *A. hyacinthus*, we measured the growth rate of 92 coral colonies on Ofu, American Samoa, from February 2010 to April 2016. All of the coral colonies included in this study have been intensely studied (Barshis et al. 2013; Palumbi et al. 2014; Bay and Palumbi 2015; Seneca and Palumbi 2015), and the majority have been genomically characterized by measuring SNPs across the transcriptome. For all colonies, we further characterized cryptic species status by genotyping colonies at 195 SNPs used by Ladner and Palumbi (2012) to detect discrete gene pools in sympatric populations. To identify the cryptic species of each *A. hyacinthus* coral colony, we sequenced eight exons and the mtDNA control region following the methods in Ladner and Palumbi (2012), although in the present study multiple SNPs within amplified haplotypes were not phased (Sheets and Palumbi 2018). We then ran genetic assignment tests using GenAIEx v6.5 using genotype likelihoods based on population allele frequencies (Paetkau et al. 1995, 2004; Peakall and Smouse 2012). We subsequently assigned coral colonies to one of the four

Fig. 1 *Acropora hyacinthus* cryptic species. Photographs of the four cryptic species of *A. hyacinthus*



cryptic gene pools based on the likelihood of an individual colony's genotype belonging to a reference population (Paetkau et al. 2004). Finally, we verified the accuracy of cryptic species assignment by comparing the original assignments in Ladner and Palumbi (2012) to the cryptic species assignments of these individuals using the SNP assignment method (Sheets and Palumbi 2018).

Coral growth

We followed coral colonies living in two distinct back-reef lagoons with different thermal variability as defined by Oliver and Palumbi (2011b). We measured 65 colonies from a moderately variable pool (14.178935S, – 169.653959W) characterized by maximum summer time temperatures of 33 °C. We also measured 27 colonies in a highly variable pool (14.180483S, – 169.656351W) characterized by maximum summer time temperatures of 35 °C (Oliver and Palumbi 2011b). We tagged each coral colony with an identification number and recorded its GPS coordinates. Colonies were sampled every 4–6 months starting in February 2010. 58 corals, 44 in the moderately variable pool and 14 in the highly variable pool, had sufficient growth, genetic, and symbiont data to be evaluated by December 2014.

Sample size of the highly variable pool was lower due to the smaller size of this back-reef habitat. Since colonies were selected before cryptic species or symbiont types were accurately identified, all species and symbiont types were

not equally represented. Given the discovery of *A. hyacinthus* cryptic species, a concerted effort was made to diversify the species mapped and followed in August 2012. For example, there was an addition of nine colonies of species HD from the highly variable pool to the study in this field season. Furthermore, as coral colonies were measured as part of several different studies, the time period that coral growth was measured varied from 2 to 6 yrs.

To calculate coral growth rate, we measured planar change in the living tissue area of each colony following Neal et al. (2015). Although in situ growth of branching corals is often measured using individual branch linear extension or staining of coral colonies (Bak et al. 2009; Anderson et al. 2015; Pratchett et al. 2015), these methods often inadvertently cause damage or mortality to studied corals (Pratchett et al. 2015) making them unsuitable for this study. In contrast, planar area techniques are noninvasive and previous research has demonstrated that measurements of planar colony growth are comparable to more invasive measurements, allowing for long-term observations (Neal et al. 2015; Pratchett et al. 2015). However, a significant caveat of the planar area measurement approach is the inability to capture three-dimensional growth, making the method ill-suited for complex coral morphologies (Madin et al. 2012). Although *A. hyacinthus* colonies grow vertically through the production of multiple overlapping plates, growth is in the horizontal axis. Moreover, we observed limited vertical growth in the colonies studied as most colonies sampled were young (< 100 cm maximum

diameter) with few or no overlapping plates. As such, two-dimensional planar photographs provide a useful estimation of total colony growth (Neal et al. 2015; Pratchett et al. 2015).

We photographed individual coral colonies using a hand held Olympus TG-5 underwater camera (Model Number V104190RU000), ensuring that the camera was parallel to the colony plate surface to prevent parallax error. We visually inspected all photographs and applied stringent quality controls to remove all images that failed to capture the full extent of the colony and had qualitatively inferred high parallax error. To further validate photographic measurements, we measured maximum colony diameter to the nearest half centimeter using a transect tape, unless otherwise noted. In these cases, we measured the maximum diameter of a single plate for a few colonies in the field.

To calculate the planar area of each coral colony, we set the maximum diameter of each coral colony within the ImageJ software (Schneider et al. 2012) and then carefully outlined the colony accounting for all branches and gaps between branches and plates. We then calculated the total area (cm^2) and perimeter (cm) of corals using a simple linear transformation of the number of pixels to the preset length (Schneider et al. 2012). We corrected for camera barrel eye distortion using a linear transformation based on underwater photographs taken of standards with known areas (Pratchett et al. 2015) (Supplementary Figures 1 and 2).

We calculated average planar growth rates ($\text{cm}^2 \text{ month}^{-1}$) by subtracting initial coral size from the subsequent coral size and then dividing by the number of months in between measurements. Although few coral growth studies report percent growth rates, relative growth rates are commonly used in plant and community ecology literature as a standard size metric because it accounts for the effects of individual size on growth (Grime and Hunt 1975). As such, we calculated average percent growth rates ($\% \text{ month}^{-1}$) by dividing the average planar growth rate by the initial coral size. We then used the *nlme* library (Pinheiro et al. 2009) in R (R Core Team 2014) to fit linear regressions of log transformed planar colony size across time to obtain an independent exponential growth metric for each colony for linear model analysis (Supplemental Table 1). In addition, we calculated the arithmetic mean radius (cm) by dividing the planar area by the perimeter of the colony and estimated the change in radial extension (cm month^{-1}) by subtracting initial arithmetic mean radius from the subsequent observed arithmetic mean radius (Pratchett et al. 2015).

Due to the nature of combining multiple data sets across multiple independent studies, not all corals were sampled in every field season. Therefore, percent growth and radial extension rates were calculated over specific time periods, with each time period ending on the same sampling date. For

example, the growth rates for the time period ending in August 2013 all shared the same final August 2013 measurement but have initial size measurements from different starting dates (e.g., from December 2012 or August 2012). Although reporting growth rates in this manner masks intra-annual variability, it provides a standardized time metric for comparing average growth rates across colonies and factors while including all available growth data collected for each colony. However, given the biases associated with this method, this type of time period averaged data was not used for statistical analyses to compare coral between cryptic species, symbiont, and pool factors or over time.

To specifically test for patterns of intra-annual variability in coral growth patterns we fit a linear mixed effects model on time standardized percent growth data with matching starting and ending time points. The library *lme4* in R was used to fit linear mixed effects models using percent growth as the fixed effect (Bates et al. 2014). For a random effect, we used time periods as a categorical variable, nested within colony.

We then conducted parallel statistical analysis on both arithmetic mean radius estimates and exponential growth coefficients. To test for the combined effects of repeated measures, pool, symbiont type, and species on coral growth, we fit linear regression models on exponential growth coefficients and then fit linear mixed effects models on arithmetic mean radius estimates. The library *nlme* in R was used to fit linear regression models using exponential growth coefficients as the dependent variable (Pinheiro et al. 2009; R Core Team 2014). As independent variables, we used ordinal time, cryptic species, symbiont type, and pool. In addition, we included the interaction of symbiont–pool and species–symbiont to specifically identify additional drivers of coral growth. In contrast, the library *lme4* in R was used to fit linear mixed effects models using arithmetic mean radius as the fixed effect (Bates et al. 2014). As random effects, we used ordinal time, cryptic species, symbiont type, and pool, nested within colony. In addition, we included the interaction of time and the three other factors in these analyses to control for repeated measures, and specifically identify drivers of coral growth rate differences over time.

The intercept of the linear mixed model represents the differences in initial arithmetic mean radius within factors, while the interaction between time and the three factors (species, symbiont, pool) indicates differences in the growth rates between different factor levels (i.e., species HE vs. HA). Thus, we are specifically interested in the interaction terms between time and the three other factors to compare growth rates between cryptic species, symbiont type, and pool. We conducted exponential growth and arithmetic mean radius model analyses on both the entire data set, as well as on the two distinct back-reef pools and

cryptic species separately, to determine which factors, if any, are most important in predicting coral growth trends. To test for the specific differences within factors and factor combinations, we conducted a post hoc analysis using restricted maximum likelihood (REML) *t* test comparisons in R (Bates et al. 2014; R Core Team 2014). Despite imbalance in the study design, linear mixed model methods are not strongly affected by differences in treatment sample size, and thus allow for their use in these statistical analyses (Nelder and Baker 2004; Bates et al. 2014).

Symbiont type

Symbiont type was recorded by amplifying a region of the cp23 gene and viewing the symbiont type specific product size on an electrophoretic gel as in Oliver and Palumbi (2011a).

Impacts of bleaching on growth rate

A severe El Niño bleaching event occurred in January 2015. To quantify the impact of this bleaching event on subsequent coral growth, we measured coral growth four times after the bleaching event (August 2015, December 2015, February 2016 and April 2016). Coral growth was measured for 17 of the surviving colonies including one colony which was not used for pre-bleaching growth analysis due to inconclusive symbiont typing. In addition, the percent area of bleaching was visually estimated for each colony immediately after the bleaching event in May 2015. Unpigmented tissue surface area for each colony was visually estimated into 10% bins. Quantitative symbiont cell counts were conducted on a subset of the bleached colonies up to a year after the bleaching event (Thomas and Palumbi 2017). These results demonstrated strong concordance between qualitative visual estimates of bleaching and low symbiont cells counts (Thomas and Palumbi 2017). To assess how this bleaching event affected subsequent coral growth rate, we calculated average percent growth before the bleaching event (time point ending in December 2014) and at four times after (August 2015, December 2015, February 2016, April 2016). In addition, we calculated the arithmetic mean radius for each colony before and at four time points after the bleaching event. Time intervals were standardized with the same ending time period. We then compared growth rates between all four time intervals: pre-bleaching, post-bleaching 1, post-bleaching 2, post-bleaching 3, and post-bleaching 4, and statistical analysis was performed using Wilcoxon signed rank tests between time points. Mortality rates before and after the bleaching event were compared using a proportions test.

Results

Coral growth

In total, 58 corals had sufficient growth points to analyze the effects of species, symbiont, and pool on growth rate (Supplemental Table 2). Each of these 58 colonies was measured an average of eight times (Max = 14, Min = 3) over an average of 40.9 months (Max = 74, Min = 12). Error estimation of repeated ImageJ measurements were low, allowing for calculation of subsequent growth metrics (Supplementary Table 3). Prior to the bleaching event, the average absolute horizontal planar growth rate was $35 \text{ cm}^2 \text{ month}^{-1}$ from February 2010 to December 2014, corresponding to an average linear growth rate of $0.73 \text{ cm month}^{-1}$ (8.7 cm yr^{-1}). The highest absolute growth rate was $458 \text{ cm}^2 \text{ month}^{-1}$ for 3 months, and highest linear growth rate was $4.3 \text{ cm month}^{-1}$ over 4 months.

The fastest percent growth rate among all colonies before the 2015 bleaching event was observed between November 2011 and March 2012 ($10.9\% \text{ month}^{-1}$), while the slowest percent growth rate was observed between August 2014 and December 2014 ($2.9\% \text{ month}^{-1}$, linear mixed effects model, $P < 0.0001$) (Supplemental Table 4). There was no clear seasonal trend in observed growth rates. The three highest growth periods included one that ended in summer, one in winter, and one in fall. The three lowest growth periods included two that ended in summer and one that ended in fall (Supplemental Figure 3). Radial extension rates were also lowest between August 2014 and December 2014 ($-0.04 \text{ cm month}^{-1}$), while the fastest observed linear growth rate occurred ending in February 2010 and August 2010 ($0.15 \text{ cm month}^{-1}$).

Results from the exponential growth model applied to 62 colonies showed generally high correlations between size and time (Supplemental Table 1). As such, we extracted the model exponent as an overall measure of horizontal planar growth. This exponent had a high correlation to the average percent growth rate of each colony across all time intervals ($r^2 = 0.891$) (Supplemental Table 1).

The mean percent growth rate for all individual colonies across the pre-bleaching study period (Supplementary Table 5) was a $5.2\% \text{ month}^{-1}$ increase in size (range across time intervals: 0.01–16.7%; range of the average growth across colonies: 1–12%). The mean radial extension for individual colonies was $0.071 \text{ cm month}^{-1}$ (range across time intervals: -0.793 to $0.755 \text{ cm month}^{-1}$; range of average growth across colonies -0.058 to $0.255 \text{ cm month}^{-1}$).

Colonies genotyped to be members of cryptic species HA, HC, and HD showed mean average percent growth rates month^{-1} of 6.8%, 5.3%, $6.5\% \text{ month}^{-1}$, respectively, and were significantly higher than those for HE, $2.9\% \text{ month}^{-1}$

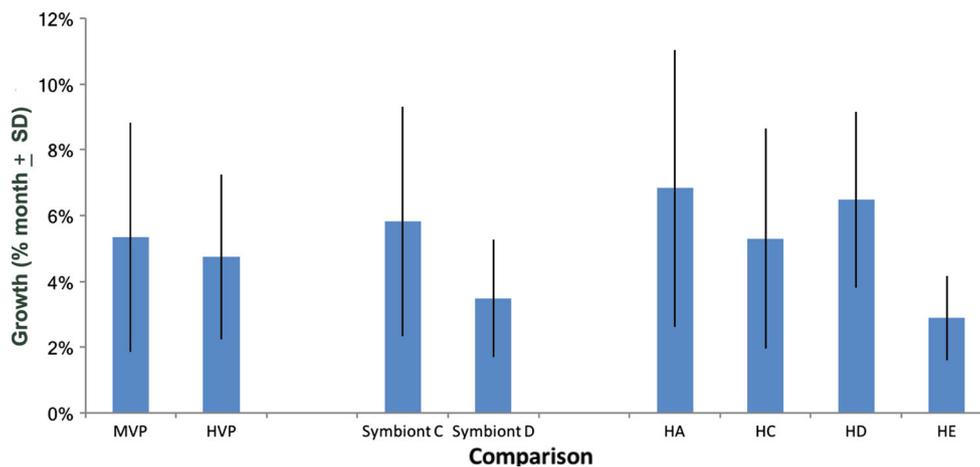


Fig. 2 Mean average growth rates across pools, symbiont type, and cryptic species. Colonies genotyped to be members of cryptic species HA, HC, and HD had significantly higher growth rates than those for HE (linear mixed effects model, $P < 0.05$). Growth rates were 40%

higher on average in corals harboring Clade C versus Clade D symbiont types, although marked inter-colony variation in growth rendered this difference nonsignificant. Vertical bars represent standard deviation (SD)

(linear mixed effects model, $P < 0.05$, Fig. 2, Supplemental Table 6). We found a similar trend based on exponential growth curves with exponents of 0.11, 0.08, 0.12, and 0.04 for colonies of cryptic species HA, HC, HD, and HE, respectively. HA, HC, and HD colonies also displayed mean average radial extension rates month⁻¹ of 0.081, 0.075, and 0.062 cm month⁻¹, respectively, that were significantly higher than those for HE, 0.030 cm month⁻¹ (linear mixed effects model, $P < 0.05$, Supplementary Table 7).

Between pools, growth rates tended to be very similar. HE colony growth rates were similar between the moderately variable and highly variable pool, 2.9% versus 2.8% month⁻¹ and 0.026 versus 0.035 cm month⁻¹ for percent growth and average mean radial extension, respectively (% growth linear mixed effects model, $P = 0.59$, radial extension linear mixed effects model $P = 0.13$, Supplemental Tables 8 and 9). Other than HE colonies, only HD colonies occurred in sufficient numbers in the highly variable pool for meaningful analysis. With our percent growth analysis, HD colonies in the highly variable pool grew as fast as HD colonies in the moderately variable pool 6.5% versus 6.5% month⁻¹, respectively (linear mixed effects model, $P = 0.59$, Supplemental Table 8). However, the arithmetic mean radius extension analysis found that HD colonies grew faster in the moderately variable pool, 0.087 cm month⁻¹, than colonies in the highly variable pool, 0.044 cm month⁻¹ (linear mixed effects model, $P = 0.05$, Supplemental Table 9).

Effect of symbionts

Across all coral colonies, corals with symbiont type D had lower average percent area growth rates, 3.5% month⁻¹, than did colonies with type C symbionts, 5.8% month⁻¹. A

similar pattern was observed for the exponential growth rate, 0.060 in type D versus 0.095 for type C, and for the mean radial extension rate, 0.041 month⁻¹ in type D versus 0.070 cm month⁻¹ in type C.

However, these above values are averaged over all cryptic species and locations. In general, symbiont type D-containing colonies are more common in the highly variable pool where species HE dominates (Supplemental Table 5). Thus, the slower growth rate among symbiont types was due to the high proportion of D-containing colonies that were the slow growing species HE. When we excluded HE colonies and colonies from the highly variable pool, we find that non-HE colonies with Clade C symbionts grew about 40% faster than those with Clade D (6.2 vs. 4.4% and 0.086 vs. 0.052 cm month⁻¹, respectively). However, high variance in colony growth and low sample size of colonies with type D symbionts in the moderately variable pool ($N = 6$) resulted in no significant difference for these values (linear regression and linear mixed effects models, $P > 0.2$ for both growth metrics, Supplemental Tables 10 and 11). Within species HE, colonies with symbiont type D had the same exponential growth in comparison to colonies with type C symbionts, 2.92% and 2.89% month⁻¹, respectively (linear mixed effects model, $P > 0.5$) (Supplemental Table 12). These HE type D colonies had a significantly higher arithmetic mean radius extension, 0.033 cm month⁻¹, than did HE colonies with type C symbionts, 0.025 cm month⁻¹ (linear mixed effects model, $P = 0.005$) (Supplemental Table 13).

Impacts of bleaching on growth rate

In May 2015, 19 of 21 surviving *A. hyacinthus* colonies in the moderately variable pool showed visible signs of

bleaching. On average, bleaching affected $23.6 \pm 20.2\%$ (mean \pm SD) of the colony surface for these corals (range 0–80%). Visual censuses showed that normal pigmentation had returned to all but one colony by August 2015 (Thomas and Palumbi 2017) (Supplemental Table 14). Colony sizes in August 2015 were on average 17% smaller than in December 2014 due to post-bleaching, partial mortality. Ten of 13 colonies showed a 27% decline in colony area in the time period ending in August 2015. The other three colonies, two HC and one HD cryptic species, all with Type C symbionts, posted 2, 10 and 15% growth in the eight months leading up to the August 2015 census. Across all individuals post-bleaching growth rates from December 2014 to August 2015 averaged $-1.0\% \pm 3.3\%$ month⁻¹ (mean \pm SD) due to partial mortality and were significantly lower than the average of $2.9\% \pm 4.0\%$ month⁻¹ growth rate prior to bleaching (Wilcoxon signed rank test, $P < 0.005$, $n = 16$) (Fig. 3).

In December 2015, five of 13 colonies declined in size, by an average of 37%. However, eight colonies grew during this period, averaging 8.5% growth over 4 months. Averaged across all colonies, significantly lower growth rates persisted from August 2015 to December 2015, $-2.9\% \pm 5.6\%$ month⁻¹ (Wilcoxon signed rank test, $P < 0.001$, $n = 14$). By February 2016 five colonies lost an average of 21% of their areas in the 2 months after December, but five other colonies gained an average of 10%, close to the normal monthly growth rate. Most colonies returned to near normal growth rates after February 2016: all colonies grew substantially in the 2 months from February to April 2016, averaging 11.5% gain in this 2 month period. Monthly growth, $5.7\% \pm 13.3\%$ month⁻¹ was not significantly different than the pre-bleaching average in April 2016 (Wilcoxon signed rank test, $P > 0.2$, $n = 14$) (Fig. 3). We found similar patterns of slow recovery in the February 2016 arithmetic mean radius measurements, 6.85 ± 3.85 cm, (Wilcoxon signed rank test, $P < 0.10$, $n = 13$), followed by

near normal arithmetic mean radius size by April 2016, 7.04 ± 4.89 cm (Wilcoxon signed rank test, $P > 0.2$, $n = 14$).

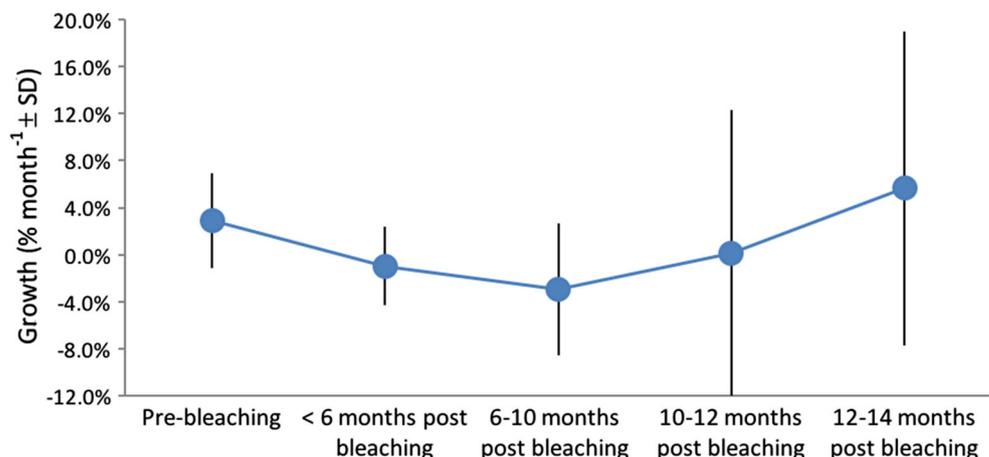
Colony mortality rates were significantly higher after the bleaching event (proportions test, $P < 0.02$). Pre-bleaching event mortality was 0.27 colonies month⁻¹ (cumulative 18 of 92 colonies) over 66 months. By contrast, post-bleaching event mortality was 2.83 colonies month⁻¹ (cumulative 23 of 68 colonies) over 8 months. Pre-bleaching mortality was predominately caused by coral disease including white band syndrome ($n = 7$), storm damage leading to inverted or damaged colonies ($n = 4$), summer heat stress ($n = 3$), and unknown causes leading to overgrowth from algae ($n = 4$). In contrast, post-bleaching mortality was predominantly caused by severe bleaching stress ($n = 15$), with a few corals also succumbing to white band syndrome ($n = 2$), storm damage ($n = 2$), overgrowth ($n = 1$), and unknown causes ($n = 3$).

Discussion

Colonies of *A. hyacinthus* in American Samoa showed strongly divergent growth rates among distinct cryptic species with only moderate effects of colony, pool, or symbiont type. Results showed that the cryptic species HE had the lowest growth rate, about twofold lower than the other three cryptic species (Fig. 2). These species specific differences in growth rate occurred similarly in both the highly variable pool, experiencing more temperature extremes, and the milder moderately variable pool.

Despite clear differences in coral growth rate between HE and the other three cryptic species, we observed marked variation in growth across individuals with HA, HC, and HD genotypes, indicating complex intra-individual and temporal heterogeneity (Supplementary Figure 4). For example, colonies 112 and 117, (species HC with

Fig. 3 Average coral growth rates across the 2015 bleaching episode. Post-bleaching coral growth rates until 12–14 months after the bleaching event was significantly lower than pre-bleaching growth rates (December 2014 to August 2015 and August 2015 to December 2015, Wilcoxon signed rank tests, $P < 0.005$). Vertical bars represent standard deviation (SD)



symbiont clade C) grew by a factor of 4–6 from August 2011 to August 2012. By contrast, colonies 106, 111, 113, and 116 (species HA, HC, and HD with symbiont clade C) did not even double in size in that period despite all six of these colonies being within 20 m of each other in the moderately variable pool. Individual-to-individual variation in growth is associated with marked genetic variation between colonies in these species (Bay and Palumbi 2014). Microhabitat variation, including depth, light, and flow, can affect growth of individual corals (e.g., (Edmunds et al. 2004)). However, emerging data from common garden reciprocal transplant experiments suggests that individual growth differences can be a specific feature of a colony. For example, Morikawa and Palumbi (2018) show that over one-third of the variance in growth among coral clones in a set of common garden nurseries is attributable to coral genotype.

Variation in radial extension growth metrics was higher than percent growth rates. This pattern was due to the wide variation in perimeter shape and growth of coral colonies, which was not as standard as planar area between time points (Supplemental Figure 5). For example, Colony 13 had widely fluctuating perimeter estimates from August 2011 to 2012 (106–171.5 cm) yet only grew 54 cm² over the course of the same year. Thus, calculated radial extension rates maintained much higher variability (– 0.17 to – 0.02 cm) than percent growth rates (0.4–1.6%) during the same time period. However, despite this marked difference in variance between the two growth metrics, we found strong concordance between these two methods. The similarity between findings of percent growth rates and radial extension data sets suggests robust results. Combining redundant growth analyses provides an internal benchmark to determine the sensitivity of conclusions to an employed growth metric.

Strong differences are apparent in growth rate among cryptic species

Colonies assigned to the gene pool of the cryptic species HE had the lowest growth rates of all four cryptic species. This difference persisted whether HE was growing in the highly variable pool or in the moderately variable pool or whether HE was growing with symbiont C or D in the moderately variable pool. Cryptic species HE was most common in the highly variable pool, and previous research has indicated that species HE is more thermally tolerant (Rose et al. 2017).

This result may seem to indicate that slow growth rate is a constraint associated with increased thermal tolerance. However, slower growth of HE corals may also be related to basic morphological differences or ontogenetic stage of coral colonies. HE colonies have stouter, thicker branches, and a

more robust skeleton than most individuals of the other cryptic species in the *A. hyacinthus* complex. As a result, growth of HE colonies may require more calcification.

In addition, the HE colonies we monitored are known to be older (though not always larger) than most of the colonies of the other cryptic species we tracked. In this study, we observed a slight slowdown in growth with increasing colony size. Therefore, we used prior data sets on very small transplanted colonies to estimate the impact of size on growth. Based on transplanted colonies of cryptic species HE, we saw a strong decline in growth with increasing size up, but this trend disappears above the minimum colony size monitored in the current study (Supplementary Figure 6) (Bay and Palumbi 2014). Above this size, rates were indistinguishable from those measured from the generally larger, wild colonies studied here. As a result, there appears to be a marked slowdown in growth with size, but the shift occurs at very small colony sizes rarely observed in this study. Thus, differences in growth rates between species HE are likely due to thermal tolerance trade-offs or morphological differences, not differences in ontogenetic stage between the distinct species observed. Further investigation into the specific thermal tolerance and growth trade-off in this cryptic species is clearly warranted.

The above results show that cryptic species of *A. hyacinthus* exhibit differences in heat tolerance, growth, and dominance in different parts of the Ofu Island back reef, suggestive of ecological differentiation. Similar ecological differentiation is observed in other reef cnidarians. For example, the two genetically distinct lineages of the gorgonian *Eunicea flexuosa* segregate incompletely over a depth gradient in the Caribbean (Prada and Hellberg 2013; Prada et al. 2014). Likewise, Warner et al. (2015) found cryptic species differences among populations of *Seriato-pora hystrix* living in different microclimates on the Great Barrier Reef. In addition, the *Orbicella* species complex (Knowlton and Weil 1992) was originally defined as three separate, ecologically distinct species that were subsequently synonymized (Fukami et al. 2004). Overall, ecological speciation has received little attention in marine taxa, but recent work suggests that it may be a powerful factor in local species divergence (Rocha et al. 2005; Bird et al. 2011).

Cryptic species in *A. hyacinthus* are defined by statistically robust genetic differences of populations sampled in sympatric populations (Ladner and Palumbi 2012). This approach originally defined cryptic species in the *Orbicella* (*Montastrea*) complex (Knowlton and Weil 1992), which has been followed up by discovery of subtle morphological differences and spawning time shifts among these species in some sympatric locations (Fukami et al. 2004). The gene pool of the cryptic species *A. hyacinthus* HE has been

found in Samoa and the Cook Islands (see Bay et al. 2017) and lives on Ofu reefs in broad association with colonies of HC, HD, and HA (Ladner and Palumbi 2012; Rose et al. 2017). Rose et al. (2017) showed that colonies assigned to species HE had higher heat tolerance, a different distribution in back-reef pools, and higher likelihood of harboring symbiont type D than did colonies assigned to other species. Full, low coverage genome sequences of these species reveal strong genetic differentiation ($F_{ST} = 0.18$) between HE and HC: 2334 loci showed F_{ST} between species greater than 0.5. There were also strong gene expression differences between species. However, there were few fully reciprocally monophyletic loci ($n = 16$), suggesting either very recent divergence or a low level of continuing gene flow between species (Rose et al. 2017). No ecological work has yet been done demonstrating the mechanism of reproductive isolation in this species complex. The data presented here add divergent growth rates to the list of differences between colonies assigned to different species and further opens this widespread complex to research on patterns of speciation and ecological specialization in the hyper-diverse Acroporid corals.

Symbiont associations

Colonies of *A. hyacinthus* with symbiont type C had higher percent growth rates than corals with symbiont type D (6.2% vs. 4.4% month⁻¹ among HA, HC, and HD colonies) after standardizing for species and pool. These results are consistent with previous field and laboratory studies (Little et al. 2004; Mieog et al. 2009; Jones and Berkelmans 2010, 2011; Gillette 2012; Cunning et al. 2015). However, in our study, the high variance across non-HE colonies in the moderately variable pool and low sample size of Clade D individuals ($n = 6$) resulted in low confidence in this comparison (linear mixed effects model, $P > 0.2$). This high variance is likely a function of one quarter of the colonies with C clade symbionts in the moderately variable pool having exceptionally high growth rates (8–17% month⁻¹), resulting in intra-clade growth variation exceeding inter-clade variation. These comparisons are further complicated by skewed sample size (Clade C $n = 36$, Clade D $n = 6$).

Other cryptic species associations have a stronger link to symbiont type than we find here. Cryptic species in the gorgonian *E. flexuosa* do not share symbiont clades, for example, though in this case symbionts are passed from mother to offspring. Likewise Pinzon and LaJeunesse (2011) found that allopatric, cryptic species of *Pocillopora* harbored different symbiont strains, and Bongaerts et al. (2010) showed a similar pattern in *S. hystrix*. In our system, virtually all coral colonies in the *A. hyacinthus* complex that we have tested in American Samoa harbor both

Symbiodinium clades C and D (Palumbi et al. 2014), but the proportion of these clades varies across pools (Oliver and Palumbi 2011b). In addition, because HE is more common in the highly variable pool than the other species, and because *Symbiodinium* Clade D is also more common there, there appears to be an association of symbionts and species. This association breaks down when we examine only colonies in the moderately variable pool, where growth rate, microhabitat, and cryptic species are all independent of symbiont clade.

A long timescale for bleaching recovery

Although it is unsurprising to find reduced coral growth during periods of coral bleaching, we found a significant decrease in coral growth rate even 1 yr following the 2015 El Niño bleaching event (Fig. 3). This result shows that intense short-term thermal stress leads to a lasting impact on the growth patterns and health of surviving coral colonies (Anthony et al. 2009; Jones and Berkelmans 2010). Previous experimental and field studies have found prolonged reductions in coral growth rates as a response of bleaching stress, with corals not fully recovering pre-bleaching growth rates up to 18 months after the bleaching event (Baird and Marshall 2002; Rodrigues and Grottoli 2007). Furthermore, previous studies have found prolonged effects of bleaching on the reproductive abilities of corals up to 2 yrs after the onset of coral bleaching (Mendes and Woodley 2002; Charuchinda and Hylleberg 2009).

Mechanistically, long-term effects of bleaching have been linked to a decrease in coral's metabolic energy reserves, including significant loss in both lipid and carbohydrate storage (Rodrigues and Grottoli 2007; Anthony et al. 2009). In some cases, it can take corals up to 8 months to recover pre-bleaching lipid and carbohydrate concentrations (Rodrigues and Grottoli 2007), limiting the available energy for colony growth (Baird and Marshall 2002; Jones and Berkelmans 2011). Parallel work at Ofu also shows that gene transcription profiles changed dramatically during the bleaching event and did not return to normal for 8–12 months (Thomas and Palumbi 2017). Genes involved in carbohydrate metabolism were particularly slow to regularize.

This study highlights the growth of cryptic species of *A. hyacinthus* and the importance of direct in situ measurements of coral colony growth over both intra- and inter-annual timescales. Our findings indicate that corals can maintain growth rates in high thermal stress environments. However, our results also hint at trade-offs to maintain thermal resilience in species HE, which displayed both high heat tolerance and slow average growth. Coral colonies and populations are known to both acclimate and adapt to the different back-reef pools of our study site (Bay and

Palumbi 2014, 2015; Palumbi et al. 2014). However, growth rates are similar across pools. This result is potentially a reflection of the success of acclimation and adaptation to maintain homeostasis and growth potential in the face of environmental variation. Ultimately, the observed decrease in growth rates a year after the El Niño event provides further evidence that thermal stress has significant effects on coral health and that under current and future climate change coral reefs may be severely threatened by intensifying bleaching events.

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