Coral Reefs
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REPORT

Coral host physiology and symbiont dynamics associated with differential recovery from mass bleaching in an extreme, macro-tidal reef environment in northwest Australia

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Abstract As marine heatwaves increasingly threaten coral reefs worldwide, some extreme reef environments naturally expose corals to high-temperature fluctuations and can therefore provide important insights into the mechanisms underlying coral heat tolerance. Coral reefs in the Kimberley region in northwest Australia experience the world's largest tropical tides and are therefore exposed to highly fluctuating temperatures in the intertidal. In contrast, the subtidal remains mostly submerged, resulting in moderate daily temperature fluctuations. A marine heatwave in 2016 triggered wide-spread bleaching in the Kimberley. Intertidal corals bleached less and recovered faster than adjacent subtidal corals; however, the mechanisms underlying this differential bleaching and recovery response remain poorly understood. Here we assessed both host- and symbiont-based indicators of bleaching resilience in the coral *Acropora aspera*. We tagged visibly healthy and bleached colonies from both environments in April 2016 and measured symbiont community composition, cell density, chlorophyll *a*, total biomass and host tissue energy reserves (lipids, protein and carbohydrates) during bleaching in April and in November 2016. Bleaching severity was higher in the subtidal than in intertidal, and while

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Cladocopium dominated all corals, symbiont community compositions differed significantly between environments and between bleached and healthy subtidal corals. Interestingly, bleaching resilience seemed decoupled from energy reserves, even though high levels of energy reserves and/or sufficient consumption during bleaching are widely thought to increase resistance to and recovery from bleaching. Although all bleached/recovered corals showed a general pattern of catabolizing protein reserves, distinct environment-specific trends were observed: subtidal corals that suffered extensive mortality also catabolized energy-poor carbohydrate reserves. In contrast, intertidal corals recovered rapidly after bleaching and maintained energy reserves. Total biomass remained unchanged between bleached and healthy corals in both environments. Overall, the findings of this study demonstrate that the consumption of energy reserves during bleaching is not always a reliable indicator of bleaching resilience.

Keywords Coral bleaching · Recovery capacity · Energy reserves · Symbiont dynamics · Extreme reef environments · Kimberley region

Introduction

Coral reefs provide a variety of ecosystem goods and services (Spalding et al. 2017) and serve as a habitat for more than one-third of all marine species, despite only covering 0.2% of the world´s oceans (Fisher et al. 2015). However, coral reefs are severely threatened by ocean warming and increasingly frequent and severe marine heatwaves (Hughes et al. 2018). This raises the question whether coral reefs will be able to persist into the future, and whether corals can recover from bleaching events fast enough to keep pace with climate change. In particular, we need to better understand which traits increase coral resistance to heat stress events and enable them to recover quickly from bleaching (i.e., the breakdown of the coral-algae symbiosis).

The symbiosis between dinoflagellate algae (Family Symbiodiniaceae; LaJeunesse et al. 2018) and the coral host is crucial for the establishment of coral reef structures because the symbionts provide healthy corals with up to 100% of their daily metabolic energy needs (Muscatine and Cernichiari 1969; Muscatine et al. 1984). Excess photosynthetically fixed carbon is stored as energy reserves in the form of lipids or proteins in the coral host (Muscatine and Cernichiari 1969). During bleaching, the breakdown of the symbiosis significantly limits both the transfer and the quality of essential photosynthetic products due to a loss of endosymbionts and/or photosynthetic pigments (Grottoli et al. 2004; Hillyer et al. 2018). This reduces both metabolic and growth rates, tissue biomass and energy reserves (Fitt et al. 2000; Grottoli et al. 2004; Rodrigues and Grottoli 2007) and ultimately threatens the survival of the coral (Rogers 1979).

Naturally extreme reef environments expose resident coral populations to environmental conditions that often exceed those predicted to occur under future climate change (Camp et al. 2018). They can therefore provide new insights into the mechanisms that may enable coral resistance to various climate change stressors, including high temperatures. A recent review on extreme reef systems highlighted that frequent exposure to environmental extremes can indeed increase the resistance of corals to various abiotic stressors (Camp et al. 2018). For example, high-temperature fluctuations in thermally variable reefs have been shown to promote coral heat resistance (Oliver and Palumbi 2011; Palumbi et al. 2014; Schoepf et al. 2015a, 2020) and reduce the risk of bleaching (Safaie et al. 2018). However, significant knowledge gaps remain, particularly associated with how these extreme environments promote not only bleaching resistance, but enhanced recovery from heat stress events.

Coral reefs along the macro-tidal Kimberley region in NW Australia are exposed to a naturally extreme environment where they experience the world's largest tropical tides (up to 12 m during spring tides) (Rosser and Veron 2011; Dandan et al. 2015; Richards et al. 2015) and strong daily temperature fluctuations of up to 7 °C (Dandan et al. 2015; Schoepf et al. 2015a). Recent studies have shown that corals from a thermally variable intertidal environment were more heat tolerant (Schoepf et al. 2015a, 2020), bleached less (Le Nohaïc et al. 2017; Schoepf et al. 2020) and recovered remarkably better (Schoepf et al. 2020) than corals from the adjacent but less thermally variable subtidal environment during the unprecedented mass bleaching event in 2016.

One important factor associated with coral susceptibility to, and recovery from, bleaching is the presence of or shifts to more heat-tolerant algal symbiont types (Berkelmans and Van Oppen 2006; Stat and Gates 2011; Grottoli et al. 2014). However, little is known about the symbiont communities in these intertidal communities (Thomas et al. 2014). Although corals from both intertidal and subtidal environments host symbionts of the genus *Cladocopium* (Schoepf et al. 2015a, 2020), it remains unclear how the presence of species within this genus, or other genera in low abundance, with inherent physiological differences (Baker 2001) could influence the thermal tolerance of the coral (Howells et al. 2012). This highlights the need for investigating both symbiont dynamics and community composition at greater community resolution.

In addition to symbiont community composition, both biomass quantity (total ash free dry weight (AFDW) cm^{-2})

and quality (% lipid, protein and carbohydrate per g biomass), as well as nutritional flexibility of the coral host, can greatly influence the resistance to and recovery from bleaching (Grottoli et al. 2006). High levels of energy reserves typically promote bleaching resistance and recovery (e.g., Grottoli et al. 2014; Schoepf et al. 2015b; Wall et al. 2019) because they can provide an alternative source of carbon to resource-limited bleached corals. Both energy reserve catabolism during bleaching and their overall concentration can differ strongly between species and have been linked to their differential bleaching resilience (Schoepf et al. 2015b). Total biomass is generally a good indicator of coral health (Fitt et al. 2000). Corals with high biomass levels and high tissue thickness typically have higher survival rates after bleaching (Thornhill et al. 2011). The metabolic demand can also be met via heterotrophic feeding on zooplankton and dissolved and particulate organic particles (DOM and POM, respectively) (Grottoli et al. 2006; Houlbrèque and Ferrier-Pagès 2009; Goldberg 2018). If the transfer of photosynthetic carbon is reduced (e.g., during bleaching), heterotrophic carbon can become a crucial, alternative energy source (Grottoli et al. 2006; Houlbrèque and Ferrier-Pagès 2009).

The aim of this study was to explore potential mechanisms underlying the differential environment-specific trends in bleaching and recovery observed during unprecedented mass bleaching in 2016 (Le Nohaic et al. 2017, Schoepf et al. 2020). We investigated key indicators of bleaching resilience for the dominant coral holobiont at our study site, *Acropora aspera*, by comparing healthy and bleached corals from both subtidal and intertidal environments during peak bleaching and after seven months of recovery. We hypothesized that higher bleaching resistance and rapid recovery, as indicated by greater retention of symbionts and chlorophyll *a* levels (Warner et al. 1996; Schoepf et al. 2015a), is associated with the presence of a unique community of algal symbionts and/or high host energy reserve levels or sufficient energy reserve consumption during bleaching.

Material and Methods

Study site and coral sampling

The study site was located at Shell Island, Cygnet Bay (16°28′46.8″S, 123°2′36.6″E), in the macro-tidal Kimberley region (up to 12 m tidal range) in northwestern Australia. A detailed site description can be found in Schoepf et al. (2015a, 2020). Briefly, the intertidal becomes a shallow tide pool during low tide with a slack water period for up to four hours, leading to high light levels (up to 2400 µmol m^{-2} s^{-1}), daily temperature fluctuations of up to 7 °C (min. 22 °C, max. 38 °C short-term temperatures, Fig. S1) and frequent aerial exposure of the corals (Dandan et al. 2015; Schoepf et al. 2015a). In contrast, corals in the adjacent subtidal experience more moderate light (up to 1800 µmol m^{-2} s^{-1}) and temperature levels, with daily fluctuations of up to 3 °C (min. 23 °C, max. 34 °C short-term temperatures, Fig. S1) and are only rarely exposed to air during the most extreme spring low tides (Dandan et al. 2015; Schoepf et al., 2015a). Heat stress experiments and surveys conducted during the 2016 mass bleaching event in this region showed that intertidal corals have a higher heat tolerance and recovery capacity than subtidal corals (Schoepf et al. 2015a, 2020; Le Nohaïc et al. 2017).

In April 2016, at the peak of a marine heatwave, both healthy and pale/bleached colonies from both environments were randomly tagged using cattle tags epoxied to the coral colony (Z-Spar) (intertidal: 11 healthy and eight bleached/pale colonies; subtidal: 12 healthy and ten bleached/pale colonies). The visual health status of all coral colonies was surveyed in April 2016 and again after 7 months of recovery in November 2016 using the Coral Health Chart (Siebeck et al. 2008). Each coral colony was labeled as either "bleached" (category 1–3.5) or "healthy" (category 3.6–6). Changes in brightness of at least two units symbolize a significant change in symbiont density and chlorophyll *a* content (i.e., the bleaching state of the coral) (Siebeck et al. 2006).

Four branch tips ($\sim$ 3 cm in length) were collected from all tagged colonies in April and November 2016, respectively, for physiological and symbiont sequence analyses (see below). Coral mortality in both environments during the heat stress period led to a reduction of surviving tagged colonies by November 2016 (n = 1–10 per health group and environment, Fig. S2, Table S3). The collected coral fragments were stored at − 80 °C until further processing.

Symbiont typing

DNA from coral branch tips was extracted using a DNeasy Blood and Tissue kit (Qiagen). Symbiodiniaceae internal transcribed spacer 2 (ITS2) marker genes in algal symbionts from a total of 53 coral colonies (April: intertidal: healthy: 8, bleached: 6; subtidal: healthy: 9, bleached: 9; November: intertidal: healthy: 6, bleached: 7; subtidal: healthy: 7, bleached: 1), PCR-negative controls (without DNA) and extraction blanks (without tissue) were then amplified via PCR using oligonucleotides that included the ITSD (5′-GTGAATTGCAGAACTCCGTG-′3) and ITS2-rev2 (5′-CCTCCGCTTACTTATATGCTT-′3) primers (Pochon et al. 2001). PCR annealing temperatures differed for April (60 °C) and November (52 °C) samples as different library building methods were utilized (see Supplementary Material for details). Amplicon libraries were

sequenced at the TrEnD Laboratory at Curtin University, Perth, Australia, and the Australian Genome Research Facility (AGRF). Raw sequence data are available at https://doi.org/10.17605/OSF.IO/ZGUDR. Raw FASTQ files for both time points were analyzed through Symportal (Hume et al. 2019). This pipeline was designed specifically for the analysis of Symbiodiniaceae ITS2 metabarcoding data to overcome the issues associated with interpreting complex communities against a background of the intragenomic variation characteristic of the ITS2 marker. Symportal uses a minimum entropy decomposition (MED)-based approach which groups raw sequence data into distinct sequence nodes (Eren et al. 2015). In contrast to the operational taxonomic unit (OTU) approach, which uses a fixed 97% similarity threshold, these sequence nodes are based on the position of biologically informative sequences (Eren et al. 2015). Symportal analyzes all of the different ITS2 sequences within a sample to define complex ITS2 profiles ('defining intragenomic variants', DIVs) that comprise the most abundant ITS2 sequences based on the presence of multimodal distributions within an ITS2 set of sequences. DIVs are indicative of a genetically differentiated Symbiodiniaceae community and can distinguish between very low abundant ITS2 sequences that would have been below detection limit when using, for example, a 97% (OTU) approach (Hume et al. 2019). We focused on these DIVs for the analysis of our data.

Physiological analyses

For total biomass analysis, coral tissue was separated from one branch tip using an airbrush. The tissue slurry was then homogenized and dried to constant weight at 50 °C for at least 24 h. The dried tissue was then burnt in a muffle furnace at 450 °C for at least four hours. The AFDW of the sample was standardized to surface area. See supplementary material for more information.

To determine chlorophyll *a* concentration, a subsample of the airbrushed tissue slurry was centrifuged (2 × 10 min at 3000 *g*) to separate host and symbiont cells. Chlorophyll *a* was extracted in 100% acetone in the dark at − 20 °C for 24 h, and the concentration determined spectrophotometrically (Jeffrey and Humphrey 1975) and then standardized to both surface area (data reproduced from Schoepf et al. 2020) and symbiont cell density (this study). Symbiont density was calculated using five replicate counts on a Neubauer hemocytometer. The surface area was calculated using the formula ($y = 9.4871 \cdot x^{0.7729}$) derived from a previously determined relation between the skeletal mass (x) and the respective computer tomography-determined surface areas (y) of *A. aspera* skeletons from Shell Island, Cygnet Bay (Schoepf et al. 2020).

Soluble protein and carbohydrates were extracted from the host tissue (one whole ground branch tip; ~ 1 cm), and lipids from both host and symbiont tissue (another whole ground branch tip; ~ 2 cm), and determined after established methods (Dubois et al. 1956; Folch et al. 1957; Smith et al. 1985; Grottoli et al. 2004; Schoepf et al. 2013), converted to kilojoules (kJ) (Gnaiger and Bitterlich 1984) and then standardized to the AFDW of the fragment. In order to determine total energy reserves, the energetic value (kJ) of protein, carbohydrates and lipids was summed up. See supplementary material for more information.

Statistical analyses

Sequencing counts for ITS2 sequences and DIVs per sample were converted to square root-transformed percent abundance data. Differences in symbiont community composition (based on both all detected ITS2 sequences and ITS2 profiles represented as DIVs) between environment (intertidal and subtidal), health (healthy and bleached as determined in April 2016) and time (April and November) were tested using two-way Permutational Multivariate Analysis of Variance (PERMANOVA) and the Bray–Curtis similarity index with 9999 iterations in PAST, version 3.15. Principal component analyses were performed, and changes in diversity and evenness of the symbiont community composition were calculated using Shannon and Simpson diversity indices in PAST, version 3.15. For all physiological parameters, generalized linear mixed effect models were used to test for the above effects using SAS software version 9.4. The distribution of the physiological raw data was visually assessed using the quantile–quantile plot of the residuals. Raw data of both chlorophyll *a* normalized to symbiont cell and symbiont density were square root transformed. Biomass data were log transformed. No transformation was performed on the raw data of protein, carbohydrates and lipids. When main effects were significant, Tukey adjusted p values were used for post hoc tests. For each significant interaction, multiple post hoc pairwise comparisons were made by using Tukey adjusted p values. Differences between healthy and bleached corals in their respective environments at each time point were tested a priori. p values ≤ 0.05 were considered significant. Data for statistical analyses in PAST and SAS are available with code at https://doi.org/10.17605/OSF.IO/ZGUDR.

Results

Sequencing results and clustering

Amplicon sequencing returned 1,389,180 reads, resulting in 25,419 ± 10,042 (mean ± SD) reads per sample (Table S1), from the 53 colonies of intertidal (27 colonies) and subtidal

(26 colonies) *A. aspera*. After running the samples through the Symportal quality control pipeline and MED, we identified 80 unique ITS2 sequences and two DIVs. Five sequences from an extraction blank and two sequences from PCR-negative controls were identified as C3. However, the sequence found in high abundance in all samples was C3 (see below), and the number of reads in the controls was low compared to the number of reads in each coral sample. Therefore, the low level of cross-contamination detected was determined to have not influenced the results.

The two identified DIVs consisted of the six and five, respectively, most abundant ITS2 sequences (Hume et al. 2019): DIV1: C3-C1-1345_C-C1n-1329_C-1346_C; DIV2: C3-C1-C1n-C3v-C1q. *Cladocopium* C3 was the most abundant ITS2 sequence and dominant in all colonies (46% of reads from all samples; Fig. S3, Table S2). The second- and third-most abundant ITS2 sequences (C1 and C1n) accounted for 12% and 7%, respectively, of the total symbiont sequence data (Fig. S3, Table S2). The average Simpson index of 0.753 and an average Shannon index of 2.146 among all samples confirmed that the overall symbiont diversity was similar between colonies (Table S1).

Symbiont community composition

The symbiont community composition based on DIVs was characterized by a significant effect of health and environment (Table 1). DIVs differed significantly between intertidal and subtidal environments in April and between healthy and bleached corals in both environments in April 2016 (Table 1). In the intertidal communities of April, DIV1 was identified in all healthy and in 67% of bleached corals (Fig. 1, Fig. S3). One-third of the bleached intertidal corals were assigned DIV2 (Fig. 1, Fig. S3). In contrast, in the subtidal communities in April, the majority of the bleached corals (78%) and 22% of the healthy corals were assigned DIV2, whereas DIV1 was identified in 78% of healthy corals (Fig. 1, Fig. S3).

The symbiont community composition of all corals based on ITS2 sequences was also characterized by significant effects of health and environment and by an interactive effect of environment and health (Table 1). In contrast to the DIVs composition, ITS2 sequences were significantly different between April and November (Table 1, Fig. S4). However, the difference was driven by rare ITS2 sequences representing $< 1\%$ of the sequence data (Table 1). See supplementary material for more details.

Symbiont physiology

We identified contrasting physiological responses between subtidal and intertidal corals during the bleaching event in 2016. Chlorophyll *a* data normalized to symbiont cell density (chl *a*/cell) showed that both bleaching and recovery of both intertidal and subtidal *A. aspera* were driven by significant interactive effects of environment, health and time (Table S4). In April, bleached subtidal corals lost 56% of their chl *a*/cell compared to the healthy corals, whereas bleached intertidal corals fully retained their chl *a*/cell (Fig. 2a). In November 2016, the one surviving bleached/recovered subtidal coral colony showed a trend of 36% higher chl *a*/cell compared to corals that were visibly healthy during the bleaching event (Fig. 2b). However, this difference was not statistically significant as only one bleached subtidal colony had survived/recovered from bleaching. In the intertidal, chl *a*/cell did not differ between healthy and bleached/recovered corals at the same time point (Fig. 2b).

In April 2016, significantly more symbionts (88%) were lost in bleached vs. healthy corals in the subtidal, leading to significant interactive effects of environment and health (Table S4). Comparatively, bleached intertidal corals only showed a trend of 54% lower symbiont densities (Fig. 2e). By November 2016, symbiont densities had fully recovered in the formerly bleached intertidal corals (Fig. 2f). In contrast, in the subtidal, the one surviving bleached/recovered colony tended to have 36% lower symbiont densities compared to healthy corals in this environment. However, this difference was not statistically significant as only one bleached subtidal colony had survived/recovered from bleaching (Fig. 2f). In addition, symbiont densities were overall 55% lower in April than November 2016 (Fig. 2e, f, Table S4).

Host physiology

Tissue biomass of subtidal corals was 63% lower in April than in November 2016 which likely drove the observed significant interactive effect of time and environment (Fig. 3i, k, Table S4). In contrast, intertidal corals maintained constant levels of tissue biomass between April and November 2016 (Fig. 3i, k). In addition, tissue biomass did not differ between healthy and bleached/recovered corals within their respective environment at both time points (Fig. 3i, k).

Protein concentrations were significantly higher ($+$ 15%) in healthy compared to bleached/recovered corals (Fig. 3a, b, Table S4). In the intertidal, corals maintained similar levels of protein concentrations between April and November 2016. In contrast, protein levels in subtidal corals declined by 26% between April and November (Fig. 3a, b) which drove the observed significant interactive effects of environment and time (Table S4). At both time points, protein concentrations did not differ between healthy and bleached/recovered corals within their

Table 1 Multivariate Permutational Analyses of Variance (PERMANOVA) testing the effect of environment, health and time on Symbiodiniaceae ITS2 types of *Acropora aspera*

Multivariate analysis	Factor	*df*	*F* statistic	*p* value DIV	*F* statistic	*p* value ITS2 sequences
Two-way PERMANOVA	Health	1	14.863	**0.0004**	8.741	**0.0002**
	Environment	1	6.176	**0.0188**	4.852	**0.0047**
	Interaction	1	0.658	0.1013	2.706	**0.0082**
Two-way PERMANOVA	Health	1	12.650	**0.0012**	8.095	**0.0004**
	Time	1	0.223	0.5446	5.971	**0.0012**
	Interaction	1	−1.611	0.1434	−2.591	0.3215
Two-way PERMANOVA	Time	1	0.181	0.5718	5.197	**0.0018**
	Environment	1	4.284	**0.0402**	3.911	**0.0107**
	Interaction	1	−4.441	0.7223	−5.472	0.9503
One-way PERMANOVA	Environment April (IT vs. ST)	1	4.840	**0.0466**	4.129	**0.0221**
One-way PERMANOVA	Health April (IT: NB vs. BL)	1	3.429	**0.0232**	2.268	0.072
One-way PERMANOVA	Health April (ST: NB vs. BL)	1	7.122	**0.0236**	6.347	**0.0057**
One-way PERMANOVA	Environment November (IT vs. ST)	1	0.467	0.6018	1.016	0.3434

Effects with p values ≤ 0.05 are highlighted in bold. Table shows F statistic and p values for both defining intragenomic variants (DIVs) and individual ITS2 sequences. df = numerator degrees of freedom, IT = intertidal, ST = subtidal, NB = healthy, BL = bleached

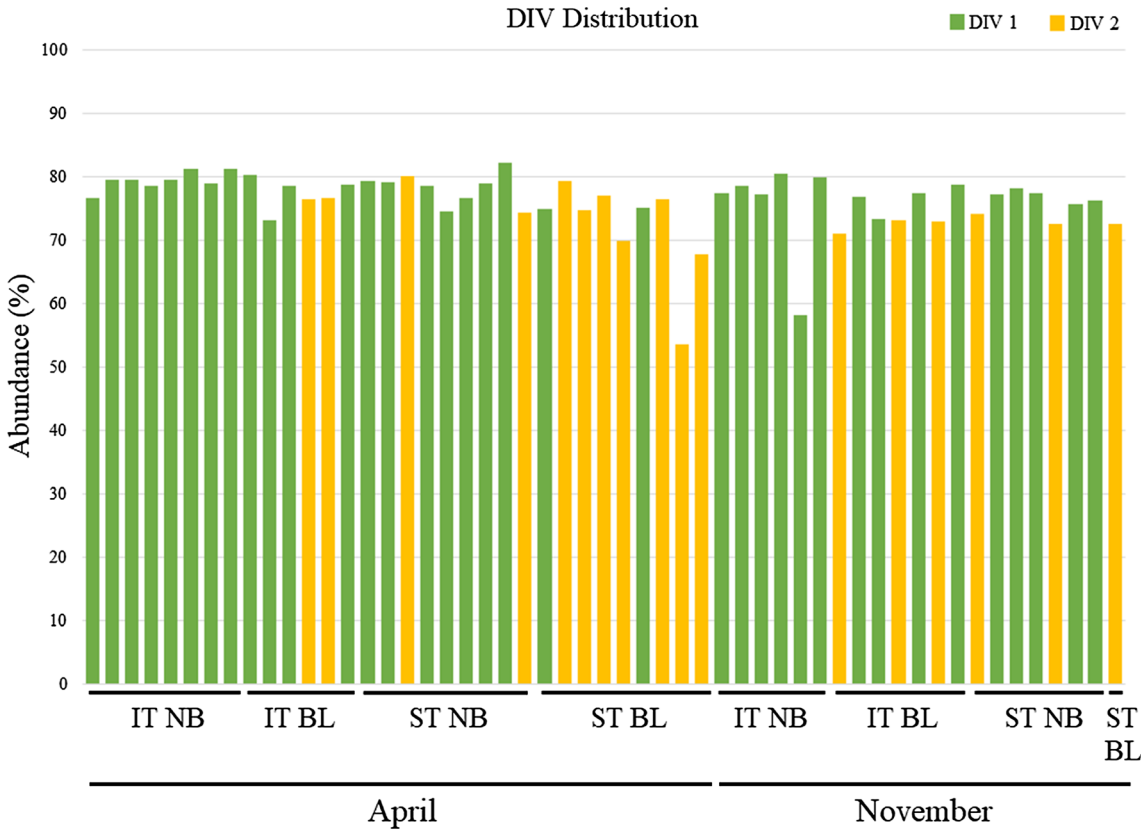

Fig. 1 Relative proportion of the defining intragenomic variants (DIVs) within each sample for healthy and bleached intertidal and healthy and bleached subtidal corals in April and November 2016, respectively. DIV1: C3-C1-1345_C-C1n-1329_C-1346_C; DIV2: C3-C1-C1n-C3v-C1q. IT: intertidal; ST: subtidal; NB: healthy; BL: bleached. Note that in November, BL stands for bleached/recovered. Note: DIVs only comprise the six and five, respectively; most abundant ITS2 types of all ITS2 types present in each sample. Therefore, the abundances do not add up to 100%

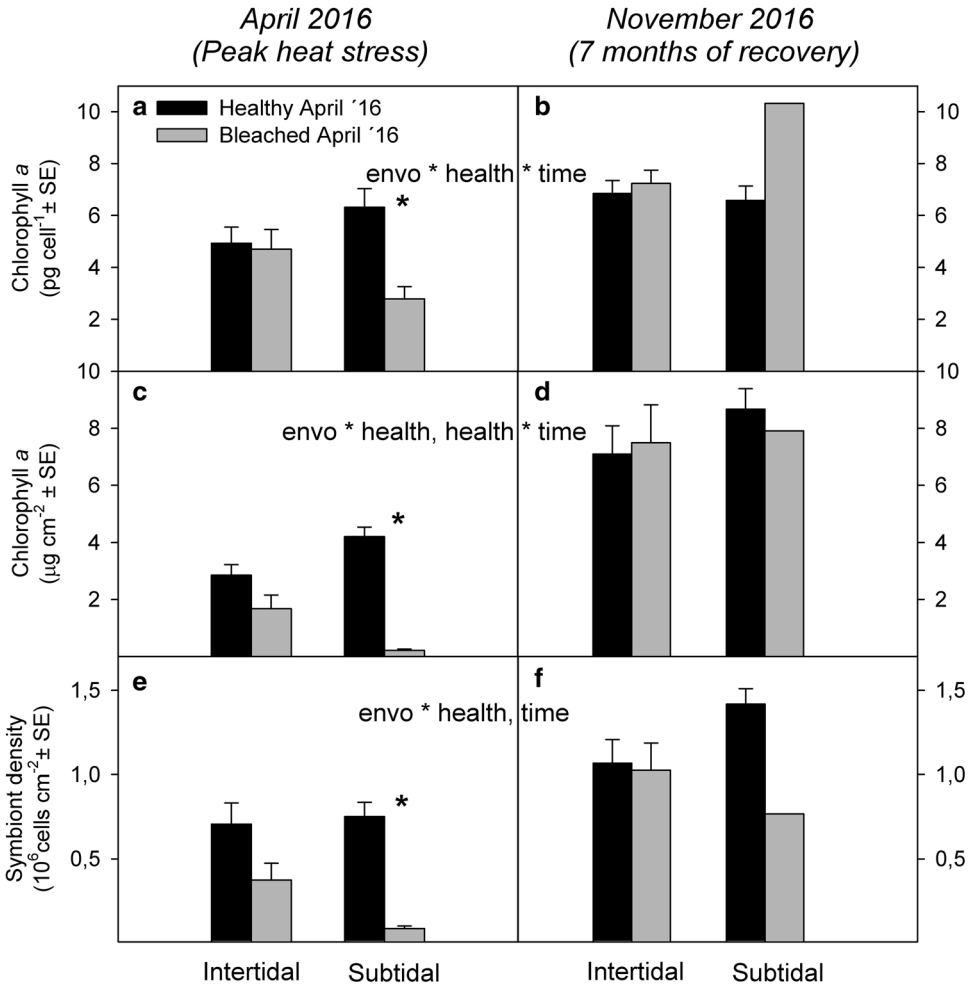

Fig. 2 Symbiont physiology. Cell density-normalized chlorophyll *a* concentrations (**a**, **b**), area-normalized chlorophyll *a* concentrations (**c**, **d**; reproduced from Schoepf et al. 2020) and symbiont density (**e**, **f**) ± standard error (SE) of *Acropora aspera*. SE is missing for bleached/recovered subtidal corals in November 2016 as sample size was one (Table S3). Asterisks indicate significant differences between healthy and bleached/recovered corals within each environment. Significant environment (envo), health and time effects are indicated when present (Table S4). Note that the health status refers to the assessment in April 2016

respective environment (Fig. 3a, b); the 30% decline in protein concentrations in bleached compared to healthy subtidal corals in November was not significant.

A significant interactive effect of environment, health and time was observed for carbohydrate concentrations (Table S4). In the subtidal, bleached/recovered corals showed significantly lower carbohydrate concentrations compared to healthy corals in both April and November 2016 (33% and 46%, respectively) (Fig. 3c, d). In contrast, both healthy and bleached/recovered intertidal corals maintained similar levels of carbohydrates irrespective of time (Fig. 3c, d). Carbohydrate concentrations in bleached intertidal corals were 33% lower in April than in November 2016, whereas no difference was observed in bleached subtidal corals between the two time points (Fig. 3c, d).

Lipid stores significantly differed between time points (Table S4) as they were 41% lower in April than in November 2016, which was primarily because bleached corals had substantially less lipids than healthy corals during peak bleaching (Fig. 3e, f). In April 2016, lipid concentrations in bleached versus healthy corals declined three times more in the intertidal than subtidal (30% vs. 11%, respectively); however, this trend was not significant (Fig. 3e).

Overall, total energy reserves of corals were significantly lower in April than in November 2016 (-20%). More specifically, total energy reserves of bleached corals showed a non-significant decline of 23% compared to healthy corals in the intertidal in April, but were fully recovered by November 2016 (Fig. 3g, h). No difference in total energy reserves was observed between healthy and bleached subtidal corals at either time points (Fig. 3g, h).

Discussion

In 2016, a marine heatwave caused unprecedented bleaching in the extreme macro-tidal Kimberley region in northwestern Australia (Le Nohaïc et al. 2017; Gilmour et al. 2019; Schoepf et al., 2020). Intertidal coral communities at our study site fully recovered, whereas the majority of adjacent subtidal coral communities did not survive this extreme climatic event (Schoepf et al. 2020, this study). Here, we investigated potential drivers of

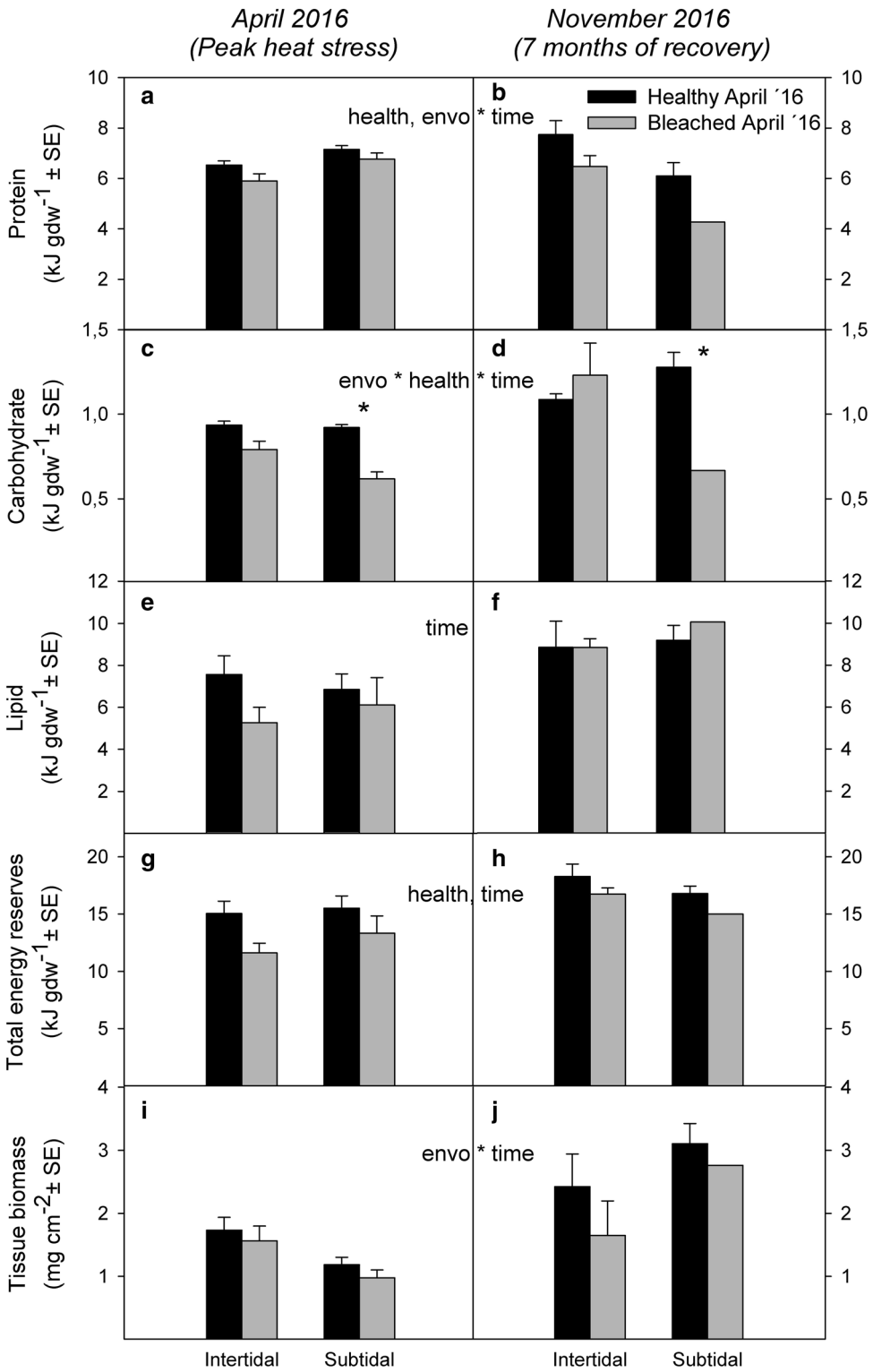

Fig. 3 Energy reserves. Protein (**a**, **b**), carbohydrate (**c**, **d**), lipid (**e**, **f**), total energy reserve concentrations (**g**, **h**) and tissue biomass (**i**, **k**) ± standard error (SE) of *Acropora aspera*. SE is missing for bleached/recovered subtidal corals in November 2016 as sample size was one (Table S3). Asterisks indicate significant differences between healthy and bleached/recovered corals within each environment. Significant environment (envo), health and time effects are indicated when present (Table S4). Note that the health status refers to the assessment in April 2016

bleaching resilience and recovery in this unique macrotidal environment. Although all corals were dominated by the same ITS2 sequence (C3, Table S2), DIV profiles, which can be interpreted as putative species (Hume et al. 2019a, b), and ITS2 sequence composition differed significantly between environments and coral health status (Table 1). Surprisingly, however, survival and high bleaching resilience were decoupled from tissue energy reserves which typically promote recovery from bleaching (Grottoli et al. 2014; Schoepf et al. 2015b).

Differential heat tolerance of intertidal and subtidal symbionts

The intertidal and subtidal differ strongly in terms of environmental conditions, especially daily temperature fluctuations (max. 7 °C vs. 3 °C, respectively; Fig. S1, Schoepf et al. 2015a), but also light intensities which were more moderate in the subtidal than intertidal (max. 1800 µmol m^{-2} $s^{-}1$ vs. 2400 µmol m^{-2} $s^{-}1$; Dandan et al. 2015; Schoepf et al. 2015a). Nevertheless, the symbiont communities of corals from both environments were dominated by the same ITS2 sequence (C3: 46% of reads from all samples, Fig. S3, Table S2). However, the bleaching response during the heat stress differed dramatically between intertidal and subtidal corals. Our results show a higher chlorophyll *a* per symbiont cell ratio, which is a better indicator of photodamage than chlorophyll *a* per area (Warner et al. 1996; Schoepf et al. 2015a), in bleached corals in the highly variable (intertidal) than in the more moderate (subtidal) temperature environment (Fig. 2). Bleached intertidal *A. aspera* not only lost fewer symbionts (Fig. 2e, this study) and chlorophyll *a* per area (Fig. 2c, Schoepf et al. 2020), but also fully maintained chlorophyll *a* on a per cell basis (Fig. 2a), which was in stark contrast to bleached subtidal symbionts (Fig. 2). These findings are consistent with previous work from the Kimberley (Schoepf et al. 2015a) and other reefs with strong temperature gradients (Warner et al. 1996; Palumbi et al. 2014). Our findings therefore suggest that intertidal symbiont composition was more stable than subtidal symbionts, indicating that long-term acclimatization and/or local adaptation to the strong environmental gradient may have led to differential heat tolerance among symbionts. Furthermore, it is possible that observed differences in bleaching response were linked to intraspecific symbiont- (Howells et al. 2012) and/or holobiont-derived adaptation (Parkinson et al. 2015).

Our results further suggest that bleached subtidal corals suffered from strong resource limitation at the time of peak heat stress. The loss of 88% of their symbionts and 56% of their cell-normalized chlorophyll *a* likely significantly reduced translocation of autotrophic carbon to the coral host (Grottoli et al. 2004; Hillyer et al. 2018). As a consequence, they may have been more reliant on alternative carbon sources, such as heterotrophic feeding on DOM, POM and zooplankton (e.g., Grottoli et al 2006; Goldberg et al. 2018) In contrast, bleached intertidal corals likely still received substantial photosynthetically derived nutrients from the symbionts given the high retention levels of both symbiont densities and chlorophyll *a* per cell during peak bleaching (Fig. 2a, e). This likely played an important role in the differential survival and recovery capacity of intertidal and subtidal corals.

The role of symbiont composition

Although all corals harbored *Cladocopium*, DIV profiles nevertheless differed significantly between environments and between bleached and healthy corals within each environment at the peak of the heatwave in April 2016 (Fig. 1; Table 1). In April, DIV1 (C3-C1-1345_C-C1n-1329_C-1346_C) was present in most healthy subtidal corals and in all healthy and two-thirds of bleached intertidal corals (Figs. 1, S3). Given the generally higher bleaching resilience and lower mortality of bleached intertidal corals (Schoepf et al. 2020; this study), it is possible that the symbiont/s in these corals represented by DIV1 were linked to higher heat resistance. In contrast, 78% of all bleached subtidal corals were associated with DIV2 (C3-C1-C1n-C3v-C1q; Figs. 1, S3). Since these corals bleached more severely and showed a much higher mortality compared to bleached intertidal corals (Schoepf et al. 2020; this study), DIV2 potentially represented symbiont/s which that were either more heat-sensitive or at least unable to promote the bleaching resistance and recovery of these corals.

Higher coral recovery capacity, as observed for bleached intertidal corals (Schoepf et al. 2020; this study), is sometimes linked to a temporal shift in the dominant symbiont (e.g., Silverstein et al. 2015). However, not all coral species shuffle symbionts (Goulet 2006; Stat et al. 2009; Cunning et al. 2016) or to the same degree (Cunning et al. 2018), and often this trait is completely absent. In this study, we found no evidence for symbiont shuffling based on DIV profiles over time. However, ITS2 sequence composition present at low-abundance background levels can also influence the coral's bleaching response (Berkelmans and Van Oppen 2006; LaJeunesse et al. 2009) and recovery capacity (Bay et al. 2016). Here, differences in low-abundance background symbiont types $< 1\%$ (Fig. S3) may have played a role in bleaching resilience, because symbiont community composition based on ITS2 sequences differed significantly between April and November (Fig. S4, Table 1).

Decoupling of energy reserve consumption and bleaching resilience

We found little evidence that energy-rich tissue reserves played a key role in promoting survival and rapid recovery from mass bleaching (Grottoli et al. 2006; Anthony et al. 2009). This was surprising as bleaching resistance and recovery of corals is typically associated with high levels of energy reserves (Anthony et al. 2009; Grottoli et al. 2014; Schoepf et al. 2015b) which are often catabolized during bleaching to support metabolic energy needs (Rodrigues and Grottoli 2007). However, coral species differ in

their efficiency to use energy reserves as alternative energy sources and often discriminate between different energy reserve pools (Grottoli et al. 2006; Rodrigues and Grottoli 2007; Levas et al. 2013; Schoepf et al. 2015b; Wall et al. 2019). Here, we found that this trait can also differ between populations of the same species occurring across fine-scale, strong environmental gradients (see also Kenkel et al. 2013).

In the subtidal, bleached corals mainly catabolized energy-poor carbohydrate stores during peak bleaching, but did not catabolize energy-rich lipid reserves, despite being severely bleached and likely in a state of resource limitation. Only one of these colonies survived the heat stress event (Fig. 3, S2, Table S3), which is consistent with generally high mortality of the subtidal coral community during the 2016 bleaching event (Fig. S2, Schoepf et al. 2020). In contrast, bleached intertidal corals largely maintained all three of their energy stores in April 2016 (Fig. 3), followed by rapid recovery within six months after peak bleaching. Although we observed these distinct environment-specific trends in energy reserve consumption, all bleached and recovered corals nevertheless had lower protein levels than healthy corals irrespective of environment and time (Fig. 3a, b). This suggests that bleached corals generally catabolized this energy reserve during both bleaching and recovery, even though direct comparisons of bleached and healthy corals within each environment did not reveal significant catabolization of proteins in bleached corals. Furthermore, the significant interactive effect of environment and time for protein concentrations (Table S4) appears to be driven by declining protein levels in subtidal corals throughout recovery, while this was not the case for intertidal corals. Together, this suggests that even though both intertidal and subtidal corals catabolized protein levels, subtidal corals were likely not fully meeting their energetic demand, thus leading to the observed high mortality. Even though the visibly healthy corals in this study were exposed to heat stress, it is possible that differences in energy reserves could have been more pronounced between healthy and bleached corals within each environment if the former had not experienced any heat stress.

Generally, the maintenance of lipid stores in bleached corals from both environments was surprising because it is often assumed that lipids are the primary source of nutrition during bleaching (Grottoli et al. 2004) given their high energetic value compared to protein and carbohydrates (+ 40 and + 56%, respectively) (Gnaiger and Bitterlich 1984). Furthermore, they constitute up to 40% of the total tissue biomass (Stimson 1987; Grottoli et al. 2004), serve as long-term energy reserves (Grottoli et al. 2004) and are crucial for a successful reproduction cycle (Szmant and Gassman 1990; Hughes et al. 2019) which heavily relies on lipid reserves (Ward et al. 2002). However, other studies have also shown that corals can maintain their individual energy pools irrespective of bleaching status during both experimental and natural heat stress (Levas et al. 2013; Wall et al. 2019) and only partially consumed lipid stores during recovery (Wall et al. 2019). Importantly, not only biomass quality but also quantity can affect the bleaching mortality rate of corals (Thornhill et al. 2011). Corals can experience a loss in biomass quantity during bleaching, while the quality can remain unchanged (Wall et al. 2019). In this study, tissue biomass quantity did not differ between healthy and bleached corals in each environment. It is surprising that most subtidal corals in our study not only bleached but mostly died as a consequence of the heat stress. Since this study only investigated total lipid amount, it is possible that relative changes in lipid metabolism, particularly in relation to storage vs. structural compounds, occurred (Imbs and Yakovleva 2012). The preference for the consumption of carbohydrate stores during and after heat stress, as found in bleached subtidal corals (Fig. 3c–d), has been observed in other studies (Schoepf et al. 2015b; Wall et al. 2019). Given the low energetic value of carbohydrate and protein pools (Gnaiger and Bitterlich 1984), bleached subtidal corals may have been limited in meeting their full metabolic energy needs over the duration of the bleaching event, resulting in high mortality by November 2016 (Fig. S2, Schoepf et al. 2020).

It generally remains poorly understood why some corals do not catabolize lipid or other energy reserves when bleached (Levas et al. 2013; Schoepf et al., 2013; Wall et al. 2019). Heterotrophic feeding is one potential explanation that may have enabled corals from both environments to maintain their lipid stores (Grottoli et al. 2006), and the generally highly turbid waters in the Kimberley region (Rosser and Veron 2011; Richards et al. 2015) suggest that particle feeding rates might be high in both environments. Furthermore, it is unlikely that feeding opportunities differed between intertidal and subtidal because long high-tide nights compensate for the brief aerial exposure of intertidal corals during evening low tides (V. Schoepf, pers. comm.). In addition, Wall et al. (2019) demonstrated that the maintenance of energy reserves during bleaching can be independent from feeding. In addition, it is possible that corals consumed energy reserves during early recovery (e.g., Schoepf et al. 2015a, b) which was not included in our sampling regime but that stores were replenished prior to November. Our findings highlight the complex interplay between coral nutritional pathways and tissue energy reserves in the context of bleaching resistance and recovery, and underpin the importance of investigating metabolic processes in bleached corals along strong environmental gradients.

Implications for future coral reef resilience

The fact that heat-resistant corals from the highly variable intertidal were able to fully maintain their lipid reserves during bleaching provides hope for the persistence of extreme reef environments under rapid climate change. Bleaching often has long-lasting negative implications for the reproductive cycle of corals (Szmant and Gassman 1990; Hughes et al. 2019), which heavily relies on lipid reserves (Ward et al. 2002). Intertidal corals may therefore be able to serve as brood stock for nearby reefs with high mortality and/or depleted lipid reserves and promote recruitment and recovery with heat-tolerant genotypes. Finally, natural migration of these more heat-tolerant coral larvae and the associated spread of heat-adapted alleles could ultimately enhance the persistence of coral reefs under future global warming (Morikawa and Palumbi 2019). However, while corals from extreme environments are resistant to fluctuating stressors, Schoepf et al. (2019) found that intertidal corals are highly vulnerable to future ocean warming, unless local physical parameters mediate bleaching susceptibility (Richards et al. 2019). This emphasizes the urgent need to rapidly restrict global CO_2 emissions in order to reduce further warming of the oceans.

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Author contributions VS designed the study and led the field work. MJ conducted the physiological analyses, while LT, MJ, AK and MS conducted the genetic analyses. MJ executed the data analyses and statistical analyses with input from VS, LT and MS. MJ led the writing of the paper, with all co-authors contributing to the final manuscript.

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Data availability Data will be submitted to www.pangea.de after publication.

Declarations

Conflict of interests The authors declare that no conflict of interest exists.

Ethical approval Corals were collected using exemption #2549 from the Western Australia Department of Fisheries.

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