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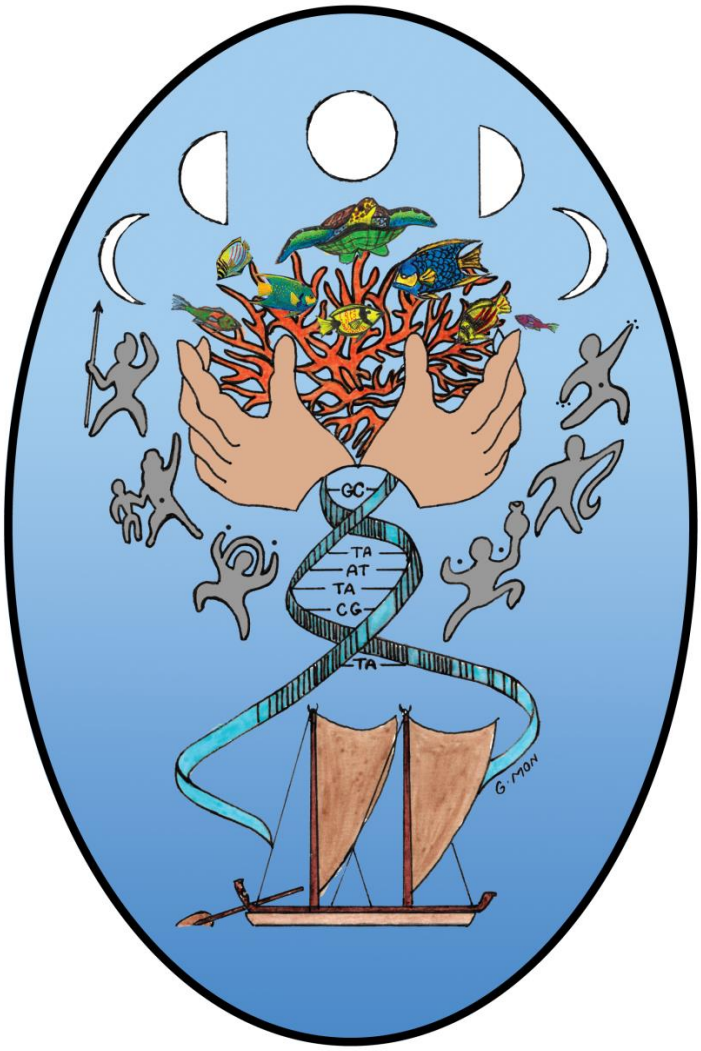

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Coast-reef scale physiological responses of *Acropora muricata* harboring *Symbiodinium* clade A

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Abstract Coral bleaching events are increasing worldwide as a result of climate change. Coral show differential bleaching susceptibilities depending on host and/or symbiont responses to changing environmental factors. Coral responses have been shown to vary over broad geographical scales with thermally-disparate environments. Of these, physiological characteristics such as *Symbiodinium* density and size, chlorophyll *a* content and estimated productivity along a coast-reef scale (<1 km) remain to be thoroughly understood. In this study, we examined these characteristics seasonally in *Acropora muricata* colonies that harbor *Symbiodinium* clade A along a coast-reef scale in Belle Mare lagoon, Mauritius. The studied reef and coast habitats are characterized by contrasting thermal and light conditions as well as bleaching histories, with bleaching occurring only at the reef site in 2009. We observed higher symbiont density in winter for coast colonies but similar symbiont cell size compared to reef colonies. These two parameters showed seasonal variation. Chlorophyll *a* content was 30% lower in reef than coast colonies, irrespective of sampling season and was significantly influenced by site. Estimated productivity was influenced by both site and season, with summer samples of coast colonies displaying 56% higher values than reef colony samples. This significant difference was maintained but less pronounced (18%) in winter samples. Our data suggest that *Symbiodinium* clade A may show physiological acclimatization along a coast-reef scale possibly as a consequence of difference in temperature and light conditions and nutrient concentrations at these two sites.

Keywords: thermally variable environment, physiological characteristics, *Symbiodinium* density, *Symbiodinium* size, chlorophyll *a*, estimated productivity

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Introduction

Anthropogenic activities compounded by climate change-driven disturbances such as elevated sea surface temperature and ocean acidification, are escalating the frequency and severity of coral bleaching events (Hoegh-Guldberg et al. 2007). These perturbations either individually or in combination induce loss of the algal endosymbiont and/or degradation of their photosynthetic pigments, leading to bleaching (Chumun et al. 2013; Gates et al. 1992). Mass coral bleaching has been detrimental worldwide (Wilkinson 2008). Coral bleaching is variable both in space and time (Kleypas et al. 2008). Bleaching may vary over large and small spatial areas during bleaching events (Piniak and Brown, 2009; Guest et al. 2012). This suggests patchy distribution of stress or development of mechanisms, by corals, to withstand thermal stress.

Multiple environmental factors and biological traits have been identified as drivers for heterogeneous responses of corals to thermal stress. A hierarchy of susceptibility has been reported among coral taxa and *Symbiodinium* clades (Oliver and Palumbi, 2011; Jones et al. 2008). Fast growing taxa such as branching *Acropora* and *Porites* are classified as more susceptible than taxa such as *Cyphastrea, Galaxea* and *Goniopora* (McClanahan et al. 2004). A similar susceptibility pattern was observed in Belle Mare lagoon, Mauritius, where the branching coral *Acropora muricata* was found to be more susceptible to bleaching and mortality than other corals such as *Pocillopora and Galaxea* (Mattan-Moorgawa et al. 2012). Differential susceptibility to bleaching between coral populations has also been attributed to the contribution of other biological traits including enzymatic and non-enzymatic antioxidant capacities (Griffin and Bhagooli 2004; Louis et al. 2016), energy reserves recovery (Schoepf et al. 2015a), photoprotection (Krämer et al. 2012; Reynolds et al. 2008) and level of expression of genes involved in heat stress response (Barshis et al. 2013). Environment factors such as light intensity levels, magnitude of thermal stress (Schoepf et al. 2015b), thermal and bleaching histories of habitats (Guest et al. 2012) have all been implicated in susceptibility variation. Bleaching history, and not thermal history, has been recently reported to be the driver of increased thermal tolerance. Following bleaching episodes, thermal tolerance was observed to occur by the holobiont shifting to more thermotolerant *Symbiodinium* genotypes (Jones et al. 2008; Thornhill et al. 2009)

Symbiodinium diversity is reported to greatly influence coral thermotolerance. For instance, there is variability in tolerance to bleaching between and within clades. Distinct *Symbiodinium* ITS2 subclades are known to exhibit diverse photo-physiological responses to thermal stress (Oliver and Palumbi, 2011; Bhagooli, 2010; Rowan 2004). A distant ITS2 C3 type is also prevalent in corals found in warmest known natural conditions (Hume et al. 2015).

It has been suggested that not only the genotype but also the abundance of algal symbionts within coral tissues may play a significant role in resisting bleaching. Symbiont density within corals is dynamic and follows a seasonal pattern (Fagoonee, 1999; Fitt et al. 2000) but excessive algal density, as a result of excess availability of nutrients in the water, decreases the resistance of host to thermal stress (Cunning and Baker 2013). Following thermal stress, estimated productivity was in turn greatly impeded (Middlebrook et al. 2010). Decreases in *Symbiodinium* densities are linked to energy-deficient corals (Anthony et al. 2009). Such stress, if sustained, causes the host to succumb to starvation or disease as a consequence of reduced carbon flow from their endosymbionts and ultimately leads to coral death (Anthony et al. 2007). Physiological differences, such as symbiont density, size and productivity between coral colonies could be a result of local adaptations/acclimatization within *Symbiodinium* types. A potential driver of local adaptation/acclimatization might be exposure to a wide spectrum of temperature variability. Corals subjected to more variable temperature regimes have been shown to be more resistant to thermal stress than those in less variable environments (Guest et al. 2012; Oliver and Palumbi, 2011; Palumbi et al. 2014; Schoepf et al. 2015b). Acclimatization due to more variable temperature regimes can occur by tuning of gene expression. Barshis et al. (2013) reported that corals from more variable environments constitutively exhibited higher expression of host genes involved in heat shock and antioxidant enzymes responses and regulation of apoptosis, that may collectively confer increased thermotolerance. A better understanding of factors driving variable bleaching, especially over small spatial scales, is likely to enable us to better predict reef trajectories and identify likely refugia under climate change. Of these, physiological characteristics such as *Symbiodinium* density and size, chlorophyll *a* content and estimated productivity along a coast-reef scale (<1 km) remain to be thoroughly documented in natural populations.

In this study, we focused on coast and reef sites in Belle Mare lagoon, Mauritius, that are characterized by differences in daily thermal fluctuation and light intensity (Bhagooli and Taleb-

Hossenkhan, 2012; Louis et al. 2016) to investigate symbiont physiological responses of *A. muricata* colonies exposed to different light and temperature conditions along a coast-reef gradient. The two sites experienced comparable maximum temperatures, but daily temperature variation was2-fold greater at the coast site than at the reef site (Louis et al. 2016). The two sites also had different thermal histories as bleaching of *A. muricata* colonies occurred only at the reef site during the 2009 bleaching event (Bhagooli and Taleb-Hossenkhan, 2012; Bhagooli and Sheppard, 2012). Additionally, disparate nitrate and phosphate levels were recorded along this coast-reef scale with highest levels at the coast site (Sadally et al. 2012; Sadally et al. 2014), which could affect *Symbiodinium* physiology. The natural set-up at the study site may help to better understand the physiological factors involved in local adaptation/acclimatization. We hypothesized that coast colonies possessed better adaptive/acclimatization features, that make them better suited to disturbed conditions such as bleaching events. The coral colonies at these two distinct habitats would thus differ in terms of physiological characteristics such as *Symbiodinium* cell density and size, chlorophyll *a* content and estimated productivity. To test these hypotheses, this study aimed at determining, on a summer day and winter day, the *in hospite Symbiodinium* density and cell size, chlorophyll *a* content, and estimated productivity. This report provides new insight about the potential involvement of the studied physiological characteristics in local adaptation/acclimatization of the coral holobiont.

Methods

Study species and study sites

Tips of individual *A. muricata* coral (approx 5 cm long) were collected from different colonies (90-100 m apart) at a coast site (n=6) and a reef site (n=6). Samples were collected between 11h00 and 15h30 at comparable depths of 0.5–2.0 m at low tide in Belle Mare lagoon, Mauritius. Sampling was repeated twice in 2014, in summer on the 14th February and in winter on the 12th of August. The same colonies were sampled in summer and winter. The two sites were separated by 700-800 m (Fig. 1). A subset of samples were used for chlorophyll fluorescence measurements and another immediately flash-frozen in liquid nitrogen and stored in the dark for further downstream analysis. Corals were sampled with permission granted by the Ministry of Fisheries, Republic of Mauritius. The sampled *A. muricata* colonies hosted *Symbiodinium* clade A (Louis et al. 2016).

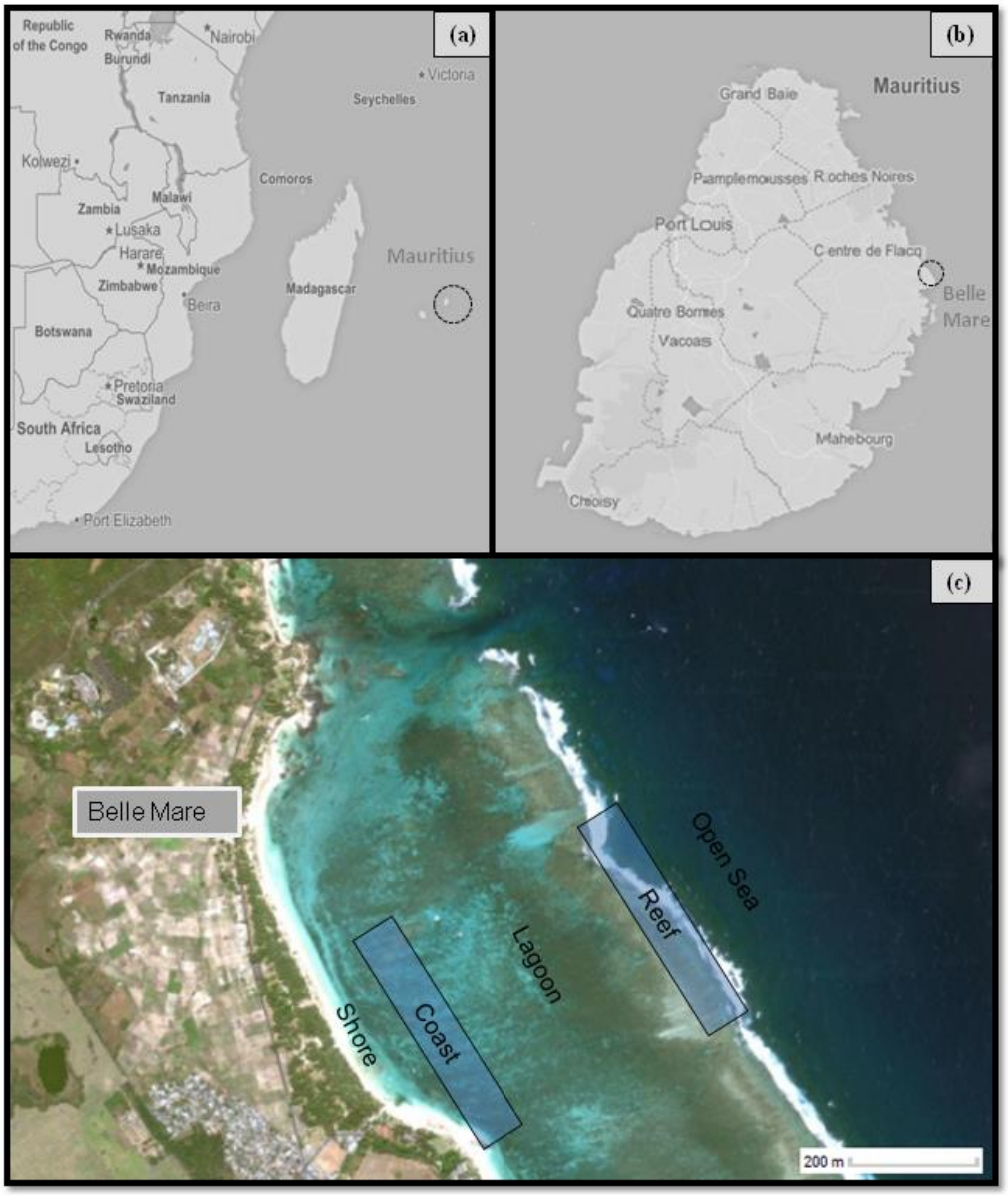

Fig. 1 (a) The island of Mauritius is found in the Western part of the Indian Ocean. (b) The study sites are located in Belle Mare lagoon, on the east coast of Mauritius. (c) The coast (20°11'33.70"S, 57°46'41.04"E) and reef (20°11'30.57"S, 57°47'2.21"E) sites (Source: Google Earth, 2016; Mapbox Studio v0.2.5)

Symbiodinium density and size determination

Tissue was removed from the coral skeleton by the water-pik technique using 0.45 µm-filtered seawater (Fitt et al. 2000). Samples were homogenized and 1 ml of each sample was taken for

Symbiodinium counts and cell size determination. *Symbiodinium* cells were enumerated from three replicate haemocytometer (Improved Newbauer) counts, under an inverted microscope (Leica Company, France). The *Symbiodinium* cell counts were normalized to total coral surface area which was determined by the aluminum foil method (Marsh 1970). *Symbiodinium* cell size was determined by measuring the diameter of coccoid cells under the inverted microscope (n=25/sample). The Image analysis software Leica Application Suite Version 4.3.0 (Leica Company, France) was used to measure the maximum diameter of intact, non-dividing *Symbiodinium* cells (Cunning and Baker 2013).

Chlorophyll *a* content determination

All the remaining tissue slurry for each colony was then filtered through Whatman GF/C glass fiber filter paper. Each filter was resuspended in 90% acetone and 10% DMSO (Iglesias-Prieto and Trench 1994). Tubes were kept in the dark at 4°C for a minimum of 24 hours to extract chlorophyll. Chlorophyll *a* content was determined using a spectrophotometric method. Absorbance was measured at three wavelengths (750, 663, 630 nm). The wavelength of 750 nm was used to control for sample turbidity, and chlorophyll *a* content was calculated based on A_{663} and A_{630} using the equation of Jeffery and Humphrey (1975).

Chlorophyll fluorescence measurements

Chlorophyll fluorescence was measured in the field with a DIVING-PAM (pulse amplitude modulation) fluorometer (Walz, Germany). Freshly collected corals nubbins were dark acclimated for 20 minutes and illuminated with actinic light of different intensities from 0 to 1325 μmol photon m^{-2} s^{-1} (PAR) in eight steps of 10 s to generate rapid light curves (RLCs). To ensure consistency of measurements, readings were uniformly taken at 1 cm from tips of corals. The relative electron transport rate (rETR; relative units) was estimated from the RLCs at each of the irradiances. Maximum rETR ($rETR_{max}$) was estimated after RLC was fitted using the Platt et al. (1980) equation.

Estimation of *Symbiodinium* productivity

Productivity can be estimated using the fluorescence parameter, $rETR_{max}$, and chlorophyll *a* content. The productivity estimation formula (Equation 1) of McMinn et al. (2005) was employed to estimate the relative maximum productivity under saturating irradiance of *Symbiodinium* from *A.muricata* colonies. Such an estimate was used for relative comparison purposes between sites and seasons.

$$\text{Estimated Productivity} = (\text{Chl } a * rETR_{max}) \quad \text{-Equation 1}$$

Statistical analyses

The Shapiro-Wilk test was used to assess normality distribution of data. *Symbiodinium* density was log transformed and *Symbiodinium* cell size, chlorophyll *a* content, and productivity data were arcsin (square-root) transformed, prior to conducting ANOVA analyses. Significant differences in means *Symbiodinium* density, chlorophyll *a* content, $rETR_{max}$ and estimated productivity of *in hospite Symbiodinium* between sites and sampling days were analyzed using one-way ANOVA and Tukey's pairwise comparison of means using SPSS (IBM version 20.0). The effects of site and season on the tested physiological parameters were determined by two-way ANOVA. P-values less than 0.05 were considered statistically significant. Correlations between physiological parameters were computed as Pearson's correlation coefficient (r) using SPSS.

Results

On the summer day, no significant difference in *Symbiodinium* density was observed between the two sites. However, on the winter day, *Symbiodinium* density was 0.1-fold significantly higher in coast compared to reef colonies (Table 1). *Symbiodinium* density was generally higher in winter (Fig. 2a). A significant main effect of season on *Symbiodinium* density was observed (Table 2 and 3). On the summer day, *Symbiodinium* cells from reef colonies were significantly larger by 0.1-fold than on the winter day (Fig. 2b). On the winter day, no significant difference was noted in symbiont cell size between coast and reef samples (Table 1). There was a significant main

effect of season on *Symbiodinium* size (Table 2 and 3). *Symbiodinium* cells were in general larger on the summer day.

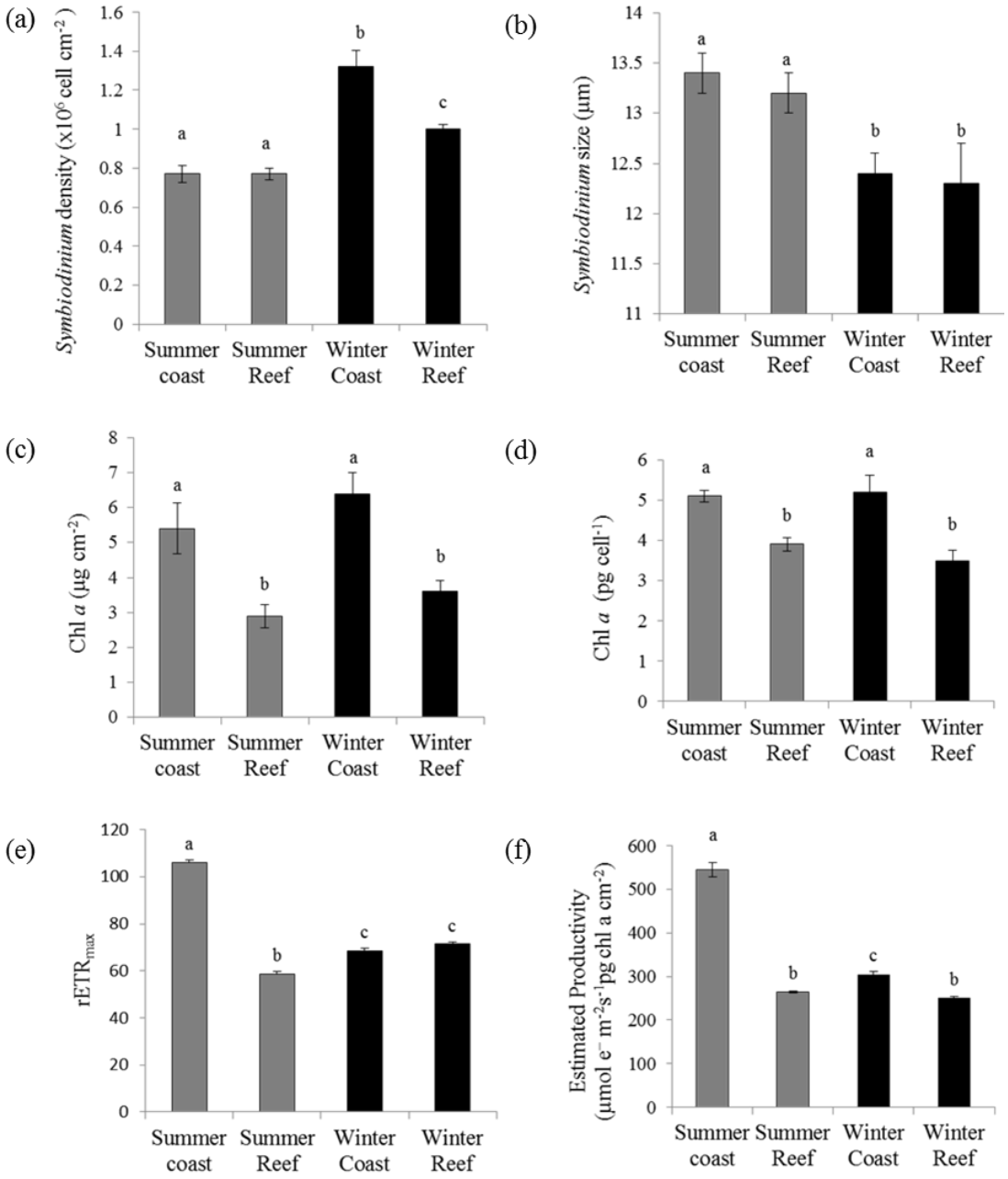

Fig. 2 (a) *Symbiodinium* cell density (b) *Symbiodinium* cell size, (c) areal chlorophyll *a* content, (d) chlorophyll *a* content per *Symbiodinium* cell, (e) $rETR_{max}$, (f) estimated productivity measured from the *A. muricata* colonies from coast and reef habitats on a summer day and a winter day. Bars represent mean ± SE. Grey bars represent the summer day while dark bars

represent the winter day samples. Letters (a, b, c) above bars represent significantly different means (n=6)

Table 1 Results of one-way ANOVA and Tukey's pairwise comparison of fold change of means of physiological parameters (*Symbiodinium* density, *Symbiodinium* size, areal and cellular chlorophyll *a* content and estimated productivity) of *Symbiodinium* from *A. muricata* colonies at Belle Mare, between the two study sites (reef and coast) during summer and winter (n = 6 per site)

Site/Season	Parameters/ Statistics	*Symb*. density	*Symb*. size	Chl *a* (µgcm^{-2})	Chl *a* (pgcell^{-1})	Estimated Productivity
Summer Coast vs Summer Reef	p-value	1.00	1.00	0.01*	0.04*	0.00***
	Fold change	0.0	0.0	-0.5	-0.2	-0.5
	Standard error	142752.7	0.1	0.1	0.8	19.5
Summer Coast vs Winter Coast	p-value	0.00 ***	0.00**	0.59	0.95	0.00**
	Fold change	0.7	-0.1	0.2	0.0	-0.4
	Standard error	151412.1	0.1	0.1	0.8	19.5
Summer Reef vs Wimter Reef	p-value	0.11	0.00***	0.67	1.00	0.61
	Fold change	0.4	-0.1	0.2	-0.1	0.4
	Standard error	49.9	0.0	0.1	0.8	19.5
Winter Coast vs Winter Reef	p-value	0.038*	0.065	0.01*	0.02*	0.00***
	Fold change	-0.2	0.0	0	-0.3	-0.20
	Standard error	151412.1	0.1	-0.5	0.8	19.5

P < 0.05 - *, *P* < 0.01 - **, *P* < 0.001 - ***

Chlorophyll *a* content per surface area of coral were respectively 0.5-fold higher for coast colonies compared to reef colonies both in summer and winter, respectively (Fig. 2c). Coast and reef samples did not vary in chlorophyll *a* content per surface area between seasons. Site showed a significant main effect on chlorophyll *a* content (Table 2 and 3). Difference in chlorophyll *a* content per cell followed the same trend (Fig. 2d). However, smaller differences were noted between sites when normalized per *Symbiodinium* cell rather than per surface area. Chlorophyll *a* content per cell was 0.2- and 0.3-fold higher for coast compared to reef colonies on the summer day and winter day, respectively (Table 1).

Table 2 Results of two-way ANOVA for effects of site and season on cell density and size, areal and cellular chlorophyll *a* content, estimated productivity of *Symbiodinium* from of *A. muricata* colonies at Belle Mare (n = 6 per site)

Dependent Variables	Source of Variation	df	MS	F	*P-value*
***Symbiodinium* cell density**	Station	1	0.02	4.23	0.06
	Season	1	0.12	30.46	0.00***
	Station x Season	1	0.02	4.03	0.06
***Symbiodinium* cell size**	Station	1	0.00	3.74	0.07
	Season	1	0.00	65.13	0.00***
	Station x Season	1	0.00	3.40	0.08
Chla (pg/cell)	Station	1	0.15	20.55	0.00***
	Season	1	0.02	0.281	0.60
	Station x Season	1	0.00	0.08	0.78
Chla (µg/cm2)	Station	1	0.41	26.20	0.00***
	Season	1	0.42	25.95	0.11
	Station x Season	1	0.00	0.022	0.88
Estimated Productivity	Station	1	0.01	23.44	0.00***
	Season	1	0.21	354.99	0.00***
	Station x Season	1	0.05	84.17	0.00***

$P < 0.05$ - *, $P < 0.01$ - **, $P < 0.001$ - ***

Estimated productivity of coast colonies was 0.5-fold higher than that of reef colonies in summer and 0.2-fold in winter. Coast colonies exhibited 0.4-fold higher productivity on the summer day compared to the winter day (Fig. 2f). Estimated productivity of reef colonies did not vary significantly between summer and winter sampling days (Table 2 and 3). Overall, coast colonies demonstrated higher estimated productivity.

A significant positive correlation (r= 0.7, p=0.02) was observed between chlorophyll *a* content per cell and chlorophyll *a* content per surface area only.

Table 3 Between- and within-groups results of one-way ANOVA for each of the physiological parameters measured

Physiological parameters	Statistical Parameter	Sum of Squares	df	Mean Square	F	p-value
***Symbiodinium* density**	Between Groups	222876.6	3	74292.2	12.4	0.000***
	Within Groups	89785.0	15	5985.7		
	Total	312661.6	18			
***Symbiodinium* size**	Between Groups	0.1	3	0.0	6.3	0.003**
	Within Groups	0.2	20	0.0		
	Total	0.3	23			
Chl a (µg cm-2)	Between Groups	36.6	3	12.2	8.6	0.002**
	Within Groups	21.4	15	1.4		
	Total	58.1	18			
Chl a (pg cell-1)	Between Groups	0.5	3	0.2	4.9	0.014*
	Within Groups	0.5	15	0.0		
	Total	1.1	18			
Estimated Productivity	Between Groups	248192.9	3	82731.0	145.2	0.000***
	Within Groups	4558.7	8	569.8		
	Total	252751.5	11			

Discussion

The influence of thermotolerant *Symbiodinium* genotypes in providing corals with resistance/resilience to bleaching is undeniable. However, this may not be the only factor conferring higher resistance to heat stress. Some local adaptation mechanisms may also be involved (Oliver and Palumbi 2011). Our results show that *Symbiodinium* cell density was not significantly different between the two sites, in summer. In contrast, coast colonies had higher *Symbiodinium* density in winter. In both seasons, when compared with the reef colonies,

Symbiodinium cells from coast colonies contained more chlorophyll *a* and that demonstrated higher estimated productivity. Coast colonies under the influence of a more thermally variable environment showed enhanced *Symbiodinium* physiological characteristics. Higher light intensity at the reef site possibly worked in combination to exacerbated reef response during summer, since differences in some physiological characteristics were more pronounced in summer. Our results in a non-bleaching period demonstrate some constitutively enhanced physiological capacities of coast colonies. A higher *Symbiodinium* density during light stress can be beneficial, as a higher number of photosynthetic cells and pigments in the host would absorb more light, thus decreasing irradiance and creating self-shading and ultimately decreasing ROS production (Wangpraseurt et al. 2012). These enhanced physiological features were possibly driven by local acclimatization, at least in part. The better physiological capacities of coast colonies might be possibly involved in helping them tolerate thermally-induced bleaching and subsequent mortality.

Consistent with previous studies, a significant seasonal effect was observed for *Symbiodinium* cell density (Fagoonee, 1999; Fitt et al. 2000). In general, higher abundance was recorded on the winter day. Differences in *Symbiodinium* cell density were observed between sites in winter only. Cunning and Baker (2013) showed that symbiont density increased in summer with respect to the host cell density, and that symbiont:host cell ratios increased the incidence of bleaching, irrespective of densities per unit surface area. Summer observations of the present study are in line with Ladrière et al. (2014) who reported that the *Symbiodinium* density of *Acropora globiceps* did not vary along such a fine scale in summer. Furthermore, higher nutrient levels recorded by Sadally et al. (2014) at the coast site might have influenced the density of *Symbiodinium* cells in that habitat (Sawall et al. 2014).

Louis et al. (2016) reported occurrence of clade A *Symbiodinium* types in *A. muricata* coast and reef colonies from Belle Mare, Mauritius. *Symbiodinium* clade A members are known be capable of alternate photosynthetic electron-transport pathways, such as cyclic electron transport, chlororespiration, and high light-induced dissociation of antenna complexes from PSII that enhance their photosynthetic capacities (Reynolds et al. 2008). These pathways increase protection of the holobiont from thermal perturbation. Therefore, it is likely that in the present case, high algal density might not lead to higher oxidative risks that result in bleaching, since these alternative pathways can cater for excess electrons and energy.

Chlorophyll *a* content per cell normally decreases in the summer and increases in the winter (Fitt et al. 2000) but no significant differences were noted between the summer day and winter day samples in the present study. However, coast colonies had significantly higher chlorophyll *a* per cell and per surface area than reef colonies on both sampling days. Lower chlorophyll *a* content and *Symbiodinium* density experienced by reef colonies may be a result of damage to the symbiont's photosynthetic apparatus as observed previously (Schoepf et al. 2015b), which in the present case may be exacerbated by higher light intensity. Corals from thermally variable environments also demonstrated higher chlorophyll retention compared to less thermally variable habitats (Palumbi et al. 2014). This could possibly explain the higher chlorophyll *a* content observed in coast colonies on the summer day.

Coast colonies showed higher estimated productivity (chl *a* x $rETR_{max}$) compared to reef colonies on both the summer and winter day. $rETR_{max}$ through photosystem II of *Symbiodinium* was positively correlated with increased carbon delivery to the host (Cantin et al. 2009). Given that corals obtain the majority of their metabolic requirements from photosynthetic carbon translocated from *Symbiodinium* (Muscatine et al. 1981), a lower estimated productivity may imply a reduced ability to meet key metabolic needs, which if sustained for long periods could lead to death. Hence, our results suggest that reef colonies are naturally more at risk to thermal stress than coast colonies. These observations may explain the higher resistance to bleaching demonstrated by coast colonies during the 2009 bleaching event. Similarly, in the Florida Keys, only inshore corals experience higher temperature ranges than offshore corals. In a simulated bleaching experiment, offshore corals exhibited reduced photosynthetic efficiency (Kenkel et al. 2013).

Nutrient enrichment is known to increase bleaching susceptibility (D'Angelo and Wiedenmann, 2014; Vega Thurber et al. 2014; Chumun et al. 2013) but in the present case coast colonies had previously been observed to be less susceptible to bleaching (Bhagooli and Taleb-Hossenkhan, 2012) despite being subjected to higher nutrient concentrations. It is possible that nutrient levels of at the coast were not sufficiently high to increase bleaching susceptibility. Rather, there is evidence that enrichment of nutrients in favorable ratios can help maintain coral metabolism and calcification during thermal stress, thereby dampening the effects of bleaching in corals (Ezzat et al. 2016).

Difference in water flow between the two sites might contribute to the physiological differences observed. High water flow is positively associated with reduction of photoinhibition and increased recovery from bleaching when assessed in laboratory (Nakamura et al. 2005, 2003) but it might not always hold true in natural conditions as reported by (McClanahan et al. 2005).

The two coral populations may also differ in heterotrophic capacity. Some corals have higher baseline heterotrophic capacity than others and this can act to buffer corals from bleaching when photosynthetic rates drop. Additionally, some species can increase feeding rates in response to bleaching conditions, which then delays the onset of bleaching and can enhance recovery (Grottoli et al. 2014; Grottoli et al. 2006). Apart from the measured physiological parameters, other factors may vary between the coral populations that are known to influence bleaching susceptibly. Total energy reserves of corals, consisting of lipids, proteins and carbohydrates, impart resilience to bleaching (Schoepf et al. 2015; Grottoli et al. 2014) and can hence also account for difference in bleaching susceptibility between the two sites. However, these aspects were not investigated in the present study but future investigations may shed light in this direction.

Studies have shown that differential susceptibility of corals to bleaching involved differential photosynthetic efficiency (Mattan-Moorgawa et al. 2012), antioxidant capacities (Griffin and Bhagooli 2004) symbiont identity, gene expression (Bellantuono et al. 2012; Barshis et al. 2013) and has been shown to be implicated in local adaptation/acclimatization but differences in physiological responses and estimated productivity of *in hospite Symbiodinium* in corals along such a fine geographical scale has received little attention. Present results, in a non-bleaching period, provide an interesting reference for forthcoming comparisons with disturbed conditions, such as bleaching events. These observations may also help to define the physiological characteristics of *Symbiodinium* from *A.muricata* within a natural bleaching refuge. We provide new insights about the potential role of productivity and the studied physiological characteristics in local adaptation/acclimatization of corals along a coast-reef scale of < 1 km. All these processes need to be deciphered if we aim to accurately predict the fate of coral reefs since the potential for reef corals to resist rising temperatures encompasses multiple interacting mechanisms.

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References

Anthony KRN, Connolly SR, Hoegh-Guldberg O (2007) Bleaching, energetics, and coral mortality risk: Effects of temperature, light, and sediment regime. Limnol Oceanogr 52:716–726

Anthony KRN, Hoogenboom MO, Maynard JA, Grottoli AG, Middlebrook R (2009) Energetics approach to predicting mortality risk from environmental stress: a case study of coral bleaching. Funct Ecol 23:539–550

Barshis DJ, Ladner JT, Oliver TA, Seneca, FO, Traylor-Knowles, N., Palumbi, SR (2013) Genomic basis for coral resilience to climate change. Proc Natl Acad Sci U. S. A. 110:1387–92

Bellantuono AJ, Granados-Cifuentes C, Miller DJ, Hoegh-Guldberg O, Rodriguez-Lanetty M (2012) Coral thermal tolerance: tuning gene expression to resist thermal stress. PloS One 7, e50685 [doi:10.1371/journal.pone.0050685]

Bhagooli R (2009) Symbiont dependent thermal bleaching susceptibility in two reef-building corals, *Stylophora pistillata* and *Platygyra ryukyuensis*. University of Mauritius Res J 15:607-626

Bhagooli R (2010) Photosystem II responses to thermal and/or light stresses of distinct endosymbiontic ITS2 *Symbiodinium* types isolated from marine animal hosts. J. Environ Res Dev 5:117–133

Bhagooli R, Sheppard CRC (2012) Prediction of recurrences of mass coral bleaching/mortality and vulnerability of reef-building corals to climate change in Mauritian and Japanese

waters. University of Mauritius Res J Special Issue: Sustainable Marine Environment 18A:105-121

Bhagooli R, Taleb-Hossenkhan N (2012) Thermal spatial heterogeneity and coral bleaching: implications for habitat refuges. Proc 12th Int Coral Reef Symp Cairns http://www.icrs2012.com/proceedings/manuscripts/ICRS2012_9D_1.pdf

Cantin NE, van Oppen MJH, Willis BL, Mieog JC, Negri AP (2009) Juvenile corals can acquire more carbon from high-performance algal symbionts. Coral Reefs 28:405–414

Chumun PK, Casareto BE, Higuchi T, Irikawa A, Bhagooli R, Ishikawa Y, Suzuki Y (2013) High nitrate levels exacerbate thermal photo-physiological stress of zooxanthellae in the reef-building coral *Pocillopora damicornis.* Eco-Engineering 25:75–83

Cunning R, Baker AC (2013) Excess algal symbionts increase the susceptibility of reef corals to bleaching. Nat Clim Change 3:259–262

D'Angelo C, Wiedenmann J (2014) Impacts of nutrient enrichment on coral reefs: new perspectives and implications for coastal management and reef survival. Curr Opin Environ Sustainability 7:82–93

Ezzat L, Towle E, Irisson JO, Langdon C, Ferrier-Pagès C (2016) The relationship between heterotrophic feeding and inorganic nutrient availability in the scleractinian coral *T. reniformis* under a short-term temperature increase. Limnol Oceanogr 61:89–102

Fagoonee I (1999) The dynamics of zooxanthellae populations: A long-term study in the field. Science 283:843–845

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

Gates RD, Bagdasarian G, Muscatine L (1992) Temperature stress causes host cell detachment in symbiotic cnidarians: implications for coral bleaching. Biol Bull 182:324–332

Griffin SP, Bhagooli R (2004) Measuring antioxidant potential in corals using the FRAP assay. J Exp Mar Biol Ecol 302:201–211

Grottoli AG, Warner ME, Levas SJ, Aschaffenburg MD, Schoepf V, McGinley M, Baumann J, Matsui Y (2014) The cumulative impact of annual coral bleaching can turn some coral species winners into losers. Global Change Biol 20:3823–383

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

Guest JR, Baird AH, Maynard JA, Muttaqin E, Edwards AJ, Campbell SJ, Yewdall K, Affendi YA, Choug LM (2012) Contrasting patterns of coral bleaching susceptibility in 2010

suggest an adaptive response to thermal stress. PloS One 7, e33353 [doi:10.1371/journal.pone.0033353]

Hoegh-Guldberg O, Mumby PJ, Hooten AJ, Steneck RS, Greenfield P, Gomez E, Harvell CD, Sale PF, Edwards AJ, Caldeira K, Knowlton N, Eakin CM, Iglesias-Prieto R, Muthiga N, Bradbury RH, Dubi A, Hatziolos ME (2007) Coral reefs under rapid climate change and ocean acidification. Science 318:1737–1742

Howells EJ, Beltran VH, Larsen NW, Bay LK, Willis BL, van Oppen MJH (2011) Coral thermal tolerance shaped by local adaptation of photosymbionts. Nat Clim Change 2:116–120

Hume BCC, D'Angelo C, Smith EG, Stevens JR, Burt J, Wiedenmann J (2015) *Symbiodinium thermophilum* sp. nov., a thermotolerant symbiotic alga prevalent in corals of the world's hottest sea, the Persian/Arabian Gulf. Scientific Reports, 5, 8562 [doi:10.1038/srep08562]

Iglesias-Prieto R, Trench RK (1994) Acclimation and adaptation to irradiance in symbiotic dinoflagellates. I. Responses of the photosynthetic unit to changes in photon flux density. Mar Ecol Prog Ser 113:163–175

Jeffrey SW, Humphrey GF (1975) New spectrophotometric equations for determining chlorophyll *a, b, c1* and *c2* in higher plants, algae and natural phytoplankton. Biochem Physiol Pflanz 167:191-194

Jones AM, Berkelmans R, van Oppen MJH, Mieog JC, Sinclair W (2008) A community change in the algal endosymbionts of a scleractinian coral following a natural bleaching event: field evidence of acclimatization. Proc R Soc Lond [Biol] 275:1359–1365

Kenkel CD, Googbody-Gringley G, Caillaud D, Davies SW, Bartels E, Matz MV (2013) Evidence for a host role in thermotolerance divergence between populations of the mustard hill coral (*Porites astreoides*) from different reef environments. Mol Ecol 22:4335–4348

Kleypas JA, Danabasoglu, G, Lough JM (2008) Potential role of the ocean thermostat in determining regional differences in coral reef bleaching events. Geophys Res Lett 35, L03613 [doi:10.1029/2007GL032257]m

Krämer WE, Caamaño-Ricken I, Richter C, Bischof K (2012) Dynamic regulation of photoprotection determines thermal tolerance of two phylotypes of *Symbiodinium* clade A at two photon fluence rates. Photochem Photobiol 88:398–413

Ladrière O, Penin L, Van Lierde E, Vidal-Dupiol J, Kayal M, Roberty S, Poulicek M, Adjeroud M (2014) Natural spatial variability of algal endosymbiont density in the coral *Acropora globiceps*: a small-scale approach along environmental gradients around Moorea (French Polynesia). J Mar Biol Assoc U. K. 94:65–74

Louis YD, Kaullysing D, Gopeechund A, Mattan-Moorgawa S, Bahorun T, Dyall SD, Bhagooli R (2016) In hospite *Symbiodinium* photophysiology and antioxidant responses in *Acropora muricata* on a coast-reef scale: implications for variable bleaching patterns. Symb 68:61 [doi:10.1007/s13199-016-0380-4]

Marsh JA (1970) Primary productivity of reef-building calcareous red algae. Ecology 51:255–265

Marshall PA, Baird AH (2000) Bleaching of corals on the Great Barrier Reef: differential susceptibilities among taxa. Coral Reefs 19:155–163

Mattan-Moorgawa S, Bhagooli R, Rughooputh S (2012) Thermal stress physiology and mortality responses in scleractinian corals of Mauritius, in: Proc 12th Int Coral Reef Symp Cairns http://www.icrs2012.com/proceedings/manuscripts/ICRS2012_9A_6.pdf

McClanahan TR, Baird AH, Marshall PA, Toscano MA (2004) Comparing bleaching and mortality responses of hard corals between southern Kenya and the Great Barrier Reef, Australia. Mar Pollut Bull 48:327–335

McClanahan TR, Maina J, Moothien-Pillay R, Baker AC (2005) Effects of geography, taxa, water flow, and temperature variation on coral bleaching intensity in Mauritius. Mar Ecol Prog Ser. 298: 131–142

McMinn A, Hirawake T, Hamaoka T, Hattori H, Fukuchi M (2005) Contribution of benthic microalgae to ice covered coastal ecosystems in northern Hokkaido, Japan. J Mar Biol Assoc U.K. 85:283–289

McPhaden MJ (2015) Playing hide and seek with El Nino. Nat Clim Change 5:791–795

Middlebrook R, Anthony KRN, Hoegh-Guldberg O, Dove S (2010) Heating rate and symbiont productivity are key factors determining thermal stress in the reef-building coral *Acropora formosa.* J Exp Biol 213:1026–1034

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

Nakamura T, Van Woesik R, Yamasaki H (2005) Photoinhibition of photosynthesis is reduced by water flow in the reef-building coral *Acropora digitifera*. Mar Ecol Prog Ser 301:109–118

Nakamura T, Yamasaki H, van Woesik R (2003) Water flow facilitates recovery from bleaching in the coral *Stylophora pistillata*. Mar Ecol Prog Ser 256:287–291

Oliver TA, Palumbi SR (2011) Do fluctuating temperature environments elevate coral thermal tolerance? Coral Reefs, 30:429–440

Palumbi SR, Barshis DJ, Traylor-Knowles N, Bay RA (2014) Mechanisms of reef coral resistance to future climate change. Science 344:895–898

Piniack GA, Brown EK (2009) Temporal variability in chlorophyll fluorescence of back-reef corals in Ofu, American Samoa. Bio Bull 216:55–67

Platt T, Gallegos CL, Harrison WG (1980) Photoinhibition of photosynthesis in natural assemblages of marine phytoplankton. J Mar Res 38(4):687-701

Reynolds JM, Bruns BU, Fitt WK, Schmidt GW (2008) Enhanced photoprotection pathways in symbiotic dinoflagellates of shallow-water corals and other cnidarians. Proc Natl Acad Sci. USA 105:13674–13678

Rowan R (2004) Coral bleaching: Thermal adaptation in reef coral symbionts. Nature 430:742–742

Sadally SB, Bhagooli R, Taleb-Hossenkhan N (2012) Micro-phytoplankton distribution and biomass at two lagoons around Mauritius Island. University of Mauritius Res. J. - Special Issue on Sustainable Marine Environment, 18A:54–55

Sadally SB, Taleb-Hossenkhan N, Bhagooli R (2014) Spatio-temporal variation in density of microphytoplankton genera in two tropical coral reefs of Mauritius. Afr. J. Mar. Sci. 36:423–438

Sampayo EM, Ridgway T, Bongaerts P, Hoegh-Guldberg O (2008) Bleaching susceptibility and mortality of corals are determined by fine-scale differences in symbiont type. Proc Natl Acad Sci 105:10444–10449

Sawall Y, Al-Sofyani A, Banguera-Hinestroza E, Voolstra CR (2014) Spatio-temporal analyses of *Symbiodinium* physiology of the coral *Pocillopora verrucosa* along large-scale nutrient and temperature gradients in the Red Sea. PloS One 9, e103179 [doi:10.1371/journal.pone.0103179]

Schoepf V, Grottoli AG, Levas SJ, Aschaffenburg MD, Baumann JH, Matsui Y, Warner ME (2015a) Annual coral bleaching and the long-term recovery capacity of coral. Proc R Soc B Biol Sci 282:20151887

Schoepf V, Stat M, Falter, JL, McCulloch MT (2015b) Limits to the thermal tolerance of corals adapted to a highly fluctuating, naturally extreme temperature environment. Sci. Rep. 5, 17639 [doi:10.1038/srep17639]

Thornhill DJ, Xiang Y, Fitt WK, Santos SR (2009) Reef endemism, host specificity and temporal stability in populations of symbiotic dinoflagellates from two ecologically dominant Caribbean corals. PloS One 4, e6262. [doi:10.1371/journal.pone.0006262]

Vega Thurber RL, Burkepile DE, Fuchs C, Shantz AA, McMinds R, Zaneveld JR (2014) Chronic nutrient enrichment increases prevalence and severity of coral disease and bleaching. Global Change Biol 20:544–554

Wangpraseurt D, Larkum AWD, Ralph P J, Kühl M (2012). Light gradients and optical microniches in coral tissues. Front Microbiol 3:316 [doi:10.3389/fmicb.2012.00316]

Wilkinson C (ed.) (2008) Status of Coral Reefs of the World: 2008. Townsville, Global Coral Reef Monitoring Network and Reef and Rainforest Research Centre