Proceedings of the 12th International Coral Reef Symposium, Cairns, Australia, 9-13 July 2012
9A Coral bleaching and climate change

Working with Nature to Identify Coral Reefs with Increased Environmental Tolerance

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Abstract. Understanding what drives tolerance among coral species is key to deriving focused and effective management plans for the future. Corals have survived for millions of years and have witnessed great changes in the earth's climate. This study compares coral species across growth environments looking at architectural differences within the skeletal structure and discusses how this may impact upon their tolerance to stress events. Results identify that variation occurs in the density of coral skeleton, density of aragonite and porosity of the skeleton. Symbiont population densities were found to be variable among coral species, but no significant differences were found across light environments. This study suggests that the micro-density of aragonite, laid down to form the coral skeleton, can vary from the previously assumed density of pure aragonite (2.94g cm^{-3}). Massive corals were found to have greater variability within these values and to be significantly lower that aragonite deposited in the skeletons of branching corals. These differences in skeletal architecture may hold the key in discovering the fundamental variables driving coral tolerance differences. Coral skeleton density may alter the relationship at the skeletal-tissue interface, therefore influence bleaching severity. The ability to identify susceptibility of corals to stress via proxies such as skeletal architecture will enable direction of management to areas most at need and those most likely to become refugia in the future.

Key words: Growth History, Skeletal Density, Bleaching, Tolerance, Plasticity.

Introduction

Generally seen as organisms restricted to a narrow environmental range, it has become increasingly apparent that scleractinian corals have a wider distribution than previously reported (Kleypas *et al*., 1999). Marginal habitats are those which exist on the boundaries of those originally deemed to be 'optimal' for coral growth. However, with predicted future environmental change, these marginal habitats will become increasingly important as ecological refugia, providing shelter and maintaining genetic diversity for coral reefs in the future. It is therefore fundamental to explore both the genetic and physiological changes imposed upon scleractinian corals in order to understand environmental tolerance and drivers of resilience.

The boundaries of scleractinian coral occurrence and the extent of their marginality were well documented by Kleypas *et al.* (1999), which identified that corals can exist within a temperature range of 16-34.4°C, salinity range of 23.3-41.8 PSU and widely variable light range. However, later studies have shown that the success and tolerance of corals is not uniform across environmental gradients (Castillo & Helmuth, 2005; Ulstrup *et al.*, 2011). This study aims to investigate whether environmental tolerance it is driven by genetics, or if phylogenetic changes, attributed to the growth environment, define success.

Corals have been described to fall into two categories; species which appear to suffer catastrophic bleaching mortality "Type 1" and those which demonstrate sub-lethal bleaching response's "Type 2" (Suggett & Smith, 2010). Type 1 corals are generally represented by branching corals who exhibit rapid coral tissue loss and reduced tolerance thresholds to stress (Marshall & Baird 2000). Type 2 corals can be described as those more resilient species such as those in the massive and submassive families which tend to demonstrate a slower decline in coral health during bleaching episodes (van Woesik *et al*., 2011). Here we analyze the skeletal ultra-structure of corals from these two categories in an attempt to identify differences driving the bleaching response.

Material and Methods

Coral fragments were collected, in May 2011, from two growth environments in the Seychelles: (1) a high light reef slope off the Northern Bay of Praislin Island, and (2) a low light turbid fringing reef off the south side of Curiesue Island. A total of 9 coral species 6 branching acroporids, 1 branching pocillioporid and 2 massive coral species were collected. Fragments (3-5cm in length), were collected using diagonal edge

cutters for branching species and hammer and chisel for massive species.

Symbiont density

Symbiont densities were measured in control corals using the WaterPik method (Johannes & Wiebe, 1970). A small area of tissue of a known surface area was removed using a WaterPik (WP450) using as little seawater as possible, extractions aimed to achieve full tissue removal in under 10ml of seawater. This slurry was then evenly mixed and a small volume pipetted into a Haemocytometer (Neubauer) for cell counts. To calculate cell densities, six replicate counts were made for each tissue extraction. The number of cells was then normalized to surface area of skeleton from which tissue was extracted, in order to calculate number of cells found to cells per cm^{-2} of tissue.

Skeletal densities

Skeletal characteristics such as bulk density, micro density and porosity were calculated using the buoyant weight technique based on Archimedes principles (Jokiel *et al.*, 1978). These calculations were then updated following Bucher et al., (1999). The calculation of buoyant weight allows the measurement of coral mass without the sacrifice of the specimen. The following equation was then applied:

$$B_W = W_W\,(1 - D_W / D_A) \qquad (1)$$

Where B_W = Buoyant Mass; W_W = Wet Mass; D_W = Density of Medium; D_A = Density of Aragonite (approximately 2.93 g/cc).

Buoyant weight methodologies were performed using a precision balance where measurements could be performed both above and below the scale aided by a metal hook from and platform. The scale was suspended above a known volume of distilled water where temperature and salinity were controlled as to ensure accurate determination of medium density.

The principles of the buoyant weight technique were used to measure surface area of the coral skeleton. By identifying the mass of paraffin wax needed to coat objects of know surface area, we can determine reliable estimates of coral colony surface area. There are varying methodologies to this technique utilizing either a single wax layer (Veal et al., 2010) or a double wax coating (Vytopil & Willis, 2001; Naumann *et al.*, 2009). Due to the size of the corallites on *Acropora* species and the need to use uniform methodologies across species the double wax coating was used here. A number of objects of varying shape size and material were first weighed and then dipped in molten paraffin wax maintained at a constant temperature of 75ºC. Items were spun to ensure even coverage and to prevent dripping. Once the wax layer was set the objects were re-weighed and again dipped in to the wax. Once the second coating of wax was dry corals were again re-weighed and the mass of the second coating of wax determined. Bleached coral skeletons were encased in paraffin wax in the same way as the calibration objects. Once the mass of the second coating of wax was determined it was used to determine likely surface area by comparing it to the calibration curve produced from objects of known surface area.

In order to take measurements of the density of skeletal material and the volume of enclosed spaces it is important to combine both these methodologies as described by Bucher et al. (1999). The initial wet and dry mass of coral samples was measured. After wet mass was determined using the buoyant weight technique, coral fragments were oven dried at 60°C for 24 hours to obtain dry mass. The paraffin wax also served as a water tight seal needed in order to calculate skeletal density calculations. Equation (2) shows the calculations for Bulk Density, (3) for Micro Density and (4) for Porosity

$$\text{Bulk Density} = \text{Dry} / (\text{Dry}_{wax} - B_{Wwax}) \times D_W \qquad (2)$$

$$\text{Micro Density} = \text{Dry} / (\text{Dry} - B_W) \times D_W) \qquad (3)$$

$$\text{Porosity} = 100 \times \frac{(\text{Dry}_{wax} - B_{Wwax}) \times D_W - \text{Dry}}{(\text{Dry} - B_W) \times D_W} \qquad (4)$$

Where; Dry = Dry Mass, Dry_{wax} = Dry Mass with wax coat, B_W = Buoyant Mass, B_{Wwax} = Buoyant Mass with wax coat, D_W = Density of medium.

Results

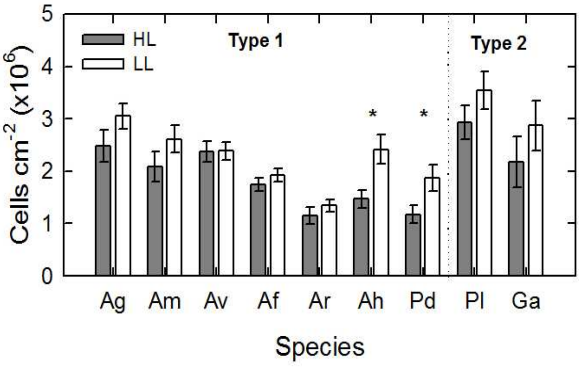

Figure 1: Comparison of symbiont densities across coral species from high and low light growth environments. Type 1 species are *Acropora gemnifera* (Ag), *Acropora microphtalamus* (Am), *Acropora valiensis* (Av), *Acropora formosa* (Af), *Acropora robusta* (Ar) *Acropora horrida* (Ah), and *Pocillopora damicornis* (Pd);

Type 2 species are *Porites lutea* (Pl) and *Goneastrea aspera* (Ga). Over the coral community no significant differences were identified between high and low light conditions using a dependent t-test. However some significant differences (*) were found between individual species as identified using one way t-tests. *A. gemnifera; A. microphthalamus; A. robusta; P. damicornis; P.lutea* (mean ± SE, n = 6) *A. valiensis; A. formosa G. aspera* (mean ± SE, n = 3).

Symbiont Density

Cell counts of tissue samples in corals were analyzed in order to identify differences in symbiont densities between differing growth light environments. Significant differences were identified between two of the seven branching species compared (Fig. 1), however there were no significant differences within the remaining type 1 or type 2 corals. Results indicated that symbiont densities were variable across coral species but that this relationship was not necessarily correlated to light intensity of the growth environment.

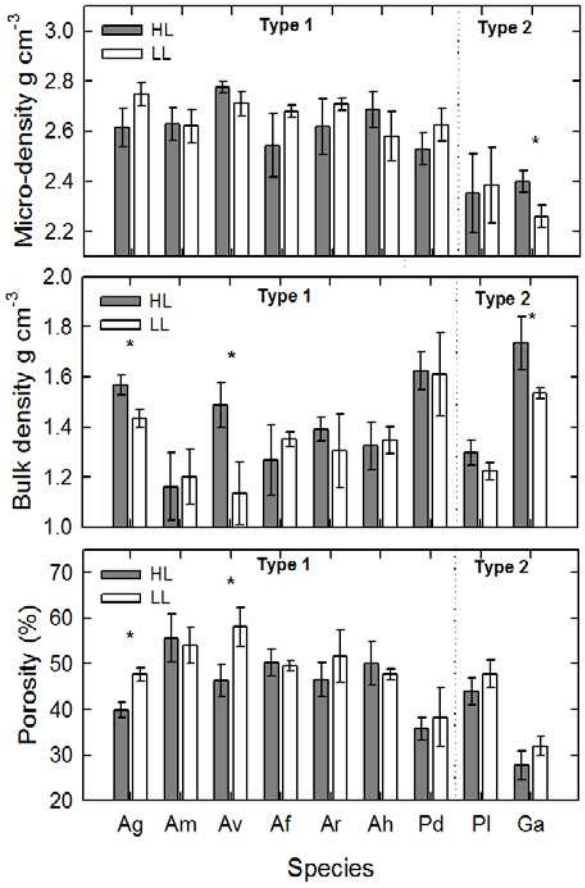

Figure 2: Comparison of skeletal density characteristics across coral species from high and low light growth environments. Type 1 species are *Acropora gemnifera* (Ag), *Acropora microphtalamus* (Am), *Acropora valiensis* (Av), *Acropora formosa* (Af), *Acropora robusta* (Ar) *Acropora horrida* (Ah), and *Pocillopora damicornis* (Pd); Type 2 species are *Porites lutea* (Pl) and *Goneastrea aspera* (Ga). No statistical differences were found between high and low light environments of type 1 and type 2 corals however some significant differences (*) were found between individual species as identified using one way t-tests. Significant differences were also identified between the combined average micro-density and porosity measurements of type 1 and type 2 coral species. *A. gemnifera; A. microphthalamus; A. robusta; P. damicornis; P.lutea* (mean ± SE, n = 6) *A. valiensis; A. formosa G. aspera* (mean ± SE, n = 3).

Skeletal Densities

The relationship between growth light environment and skeletal characteristics was explored to identify whether the growth environment contributes toward physiological changes within the coral structure.

Results showed that overall no significant differences existed between coral species in high and low light environments (Fig. 2). However, some significant differences were found between individual coral species but with no general trend.

The micro-density of aragonite in coral skeletons was found to differ significantly between type 1 corals compared to type 2 corals ($t_{(88)} = 6.90$, $P < 0.001$). Type 2 corals were also found to be significantly more porous than type 1 species ($t_{(88)} = 3.84$, $P < 0.001$).

Discussion

The environment can exert a profound influence upon the genotype of coral species. Here we show how zooxanthellae populations are less impacted upon by light environment, but rather how the architecture of the coral skeleton has greater plasticity than previously thought.

Symbiont Density

It has previously been reported that symbiont densities decrease with depth and, therefore, with light intensity (Dunstan, 1982). However Drew (1972) reported that symbiont densities remained relatively constant in coral colonies regardless of depth and light intensity at around 1.45 million cells per cm^2. This has been further reinforced in the literature where it is argued that unlike symbiont density, chlorophyll per symbiont cell concentration is dynamic and variable (Leletkin, 2000). Our results show that although symbiont densities vary across coral species, within species there is no consistent difference between high and low light environments.

Skeletal Densities

The density of aragonite has long been assumed to be constant at the equivalent of pure aragonite at 2.94 g cm^{-3} (Dodge et al., 1984; Hughes, 1987; Bosscher, 1993). However, more recent studies have identified that micro-density of the coral skeleton is more variable than previously thought among acroporid coral species ranging from 2.781 to 2.873 g cm^{-3} (Bucher et al., 2008). In this study we show how this

variability is even more pronounced in non-acroporid coral species with branching acroporid species ranging from 2.232 to 2.835 g cm^{-3} whilst massive coral species range from 1.787 to 2.815 g cm^{-3}.

Massive coral species are generally slower growing than branching coral species. It is therefore a fair hypothesis that massive corals have the potential to invest more energy in a denser skeleton than branching corals which initial lay down a low density skeleton before later infilling to reinforce strength (Hughes, 1987; Shiari *et al.*, 2008). Nevertheless, our results do not support this hypothesis and instead identify that massive corals produce a lower micro-density aragonite skeleton. The effect of differing densities of aragonite is unknown; however, Chamberlain (1978) found that the resistance of branching coral skeletons to pressure was far greater than corals of a massive morphology. The reasoning for this was assumed to be due to the increased probability of breakages in branching species. It may be that the increased micro-density of the coral skeleton is responsible for this increase in skeletal strength, although some areas of experimental error may occur within acroporid species. Indeed, fragments were taken close to the growing tip which have been shown to have a greater variability in density (Bucher *et al.*, 2008) due to progressive infilling of the coral skeleton with time (Gladfelter, 1982). Part of the variation observed in this study therefore may be an artifact of these within colony differences.

Bulk density of the coral skeleton relates to the density of skeletal material laid down within the coral colony. No significant differences were found between light environments of type 1 and type 2 corals. Bulk-density and porosity are inversely linked due to porosity related to the volume of air spaces compared to volume of skeleton. However, porosity measurements have clear significant differences with branching type 1 coral species being much more porous than massive type 2 corals.

Although no differences were found in this study between high and low light growth environments, variability within skeletal architecture has been found, identifying the potential for forced variation. The light environment in this study was characterised via average light environment at a set depth and utilising factors such as turbidity as indicators of lower light environments. However the light environment exists at a micro scale within each colony and wide variation can occur among communities. Coral branches facing directly into the sun's incidental angle will experience a different light environment than those occurring at the side of the coral or a more acute angle to the sun's rays. It is important that these micro-habitats are characterised at a colony level and when conducting field experiments that this is accounted for especially during light environment comparisons.

The skeletal architecture may be an important factor with respect to the coral host tissue-skeletal attachment. Changes in density may alter desmoidial binding sites and attachment strength. This may lead to variation during tissue loss and bleaching. Differences in bleaching severity and mechanisms between type 1 and type 2 corals have previously been suggested and described (Suggett & Smith, 2010), however the drivers for these are currently unknown. Recent research has begun to explore these desmoidial attachment processes, nevertheless, these have yet to be related to the bleaching response and the potential impacts have yet to be described.

Dynamic Management

The issue of 'paper parks' have plagued coral reef conservation efforts. Too many parks designated without enforcement leads to ineffective management and a bias in protected area reporting. There is a need to approach management from a new angle, carefully choosing areas and ensuring justification. The ability to determine coral susceptibility via proxies such as the density of coral skeletal components would allow for the identification of those corals with the greatest and the least tolerance to stress and environmental change. Protection must be ensured not only those species more at risk, but that also to those that are most likely going to serve as dominant future reef components thus to preserve the gene pool.

Conclusion

This study detects that variation between coral skeletal characteristics does exist, although was unable to relate this variation to the environment. However, it is likely that if these architectural changes are able to be influenced, the environment will have a large role to play within this. What is more important is the relationship of the corals structural architecture to the stress response. Significant differences were identified in both the skeletal microdenstiy and porosity of type and type 2 corals. If these differences can be linked to bleaching susceptibility we will gain greater insight in to the drivers of variation relating to environmental tolerance. This could potentially be used to identify habitats with increased tolerance relating to skeletal characteristics.

Acknowledgement

We gratefully acknowledge The Mitsubishi Corporation and the Natural Environmental Research Council (NERC) for financially supporting this research. Also, extreme thanks to The Earthwatch Institute (UK), Seychelles Center for Marine Research and Technology (SCMRT), Seychelles Bureau of Standards, Nature

Protection Trust of the Seychelles (NPTS), the Island Development Company (IDC) of the Seychelles and the Government of the Seychelles Republic for providing essential field logistical and administrative support, without which this work would not have been possible.

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