MASTS Visiting Fellowship Final Report

Prof Jonathan Erez (Visiting Fellow)

Home Institute: The Institute of Earth Sciences, The Hebrew University, Edmond J. Safra campus, Givat Ram, Jerusalem, 91904, Israel.

Dr Nicola Allison (Host)

School of Earth and Environmental Sciences, University of St. Andrews, North St., St. Andrews, Fife, KY16 9AL, U.K.

Identifying the source of skeletal carbon for tropical reef building corals

The aim of the fellowship was to identify the source of dissolved inorganic carbon (DIC) used in the precipitation of coral skeletons. The DIC at the coral calcification site is probably sourced from seawater (transported paracellularly) and from molecular CO_2 (which readily diffuses from the coral tissue into the calcification site). Corals actively increase the pH of the fluid used for calcification, decreasing the proportion of fluid DIC present as CO_2 and creating a diffusion gradient favouring the transport of molecular CO_2 from the overlying coral tissue into the calcification site. This serves to increase the DIC concentration at the calcification site substantially above seawater values.

The oxygen ($\delta^{18}O$) and carbon ($\delta^{13}C$) isotopic compositions of coral skeletons are tools for investigating the sources of DIC used in calcification in more detail assuming that skeletal $\delta^{18}O$ and $\delta^{13}C$ reflect that of aqueous DIC at the calcification site. Aqueous CO_2, carbonate and bicarbonate have unique $\delta^{18}O$ and $\delta^{13}C$ isotopic signatures[1]. Furthermore the carbonate and bicarbonate formed from molecular CO_2 are depleted significantly in ^{18}O compared to carbonate and bicarbonate in isotopic equilibrium with seawater[2]. Carbonate and bicarbonate formed from molecular CO_2 will gradually exchange oxygen isotopes with water to approach isotopic equilibrium but this process takes many hours and it is unlikely that isotopic equilibrium is fully reached before the DIC in the calcification site is utilised in skeletal precipitation[2].

Coral aragonite $\delta^{18}O$ suggests that skeletal DIC is predominantly sourced from seawater[3]. It is not currently possible to use skeletal $\delta^{13}C$ to verify models of DIC origin as the $\delta^{13}C$ of the DIC in the calcification site is also modified by the activity of the zooxanthellae (photosynthetic algae) in the overlying coral tissue. Photosynthesis preferentially fixes ^{12}C ([4]) and coral skeletal $\delta^{13}C$ is positively correlated with light availability (and presumably photosynthetic rate) in both field[5] and cultured corals[6]. For this reason skeletal carbon isotopes have not been used to infer skeletal DIC sources. However recent research demonstrates that coral photosynthesis can be switched off by changing the spectrum of light used for culturing. Specifically, corals maintained typical (normal) calcification rates when switched from normal illumination to blue light (400-520 nm) for short periods (~1 day) but stop photosynthesizing immediately[7]. This phenomenon offers a route to halt coral photosynthesis and analyse skeletal $\delta^{13}C$ and $\delta^{18}O$ in tandem to explore skeletal DIC source.

Activities and achievements

We planned to culture corals under blue light (to maintain calcification whilst stopping photosynthesis) and analyse the deposited skeleton for $\delta^{13}C$ and $\delta^{18}O$ to identify the likely source of skeletal DIC. However our preliminary experiments indicated that the branching *Pocillopora damicornis* corals maintained at the University of St. Andrews did not perform as expected when cultured under blue light. Rather than halting photosynthesis whilst maintaining calcification, blue light exposure increased photosynthesis and decreased calcification. The reason for this is unclear but coral colonies can exhibit higher photosynthetic rates under blue light if they are chromatically adapted to deeper waters[8].

To investigate the response of the *P. damicornis* genotype in our aquaria to lighting we performed a series of experiments. We cultured the corals under different light spectra whilst making multiple measurements of photosynthesis, respiration and calcification. All culturing work was conducted on a single *P. damicornis* genotype i.e. all colonies were sourced from the same parent colony. This was divided into smaller colonies (typically 3 cm in all dimensions), attached to plastic bases using epoxy adhesive and cultured in the St. Andrews culture system for >6 months before use (Figure 1a). All corals were maintained under Maxspect Razor R420 10000K LED lights providing ~ 280 µmol photons m^{-2} s^{-1} at coral height under the normal light regime (Figure 1c) on a 12 h light:12 h dark cycle.

To test the impact of lighting on the corals, the same 3 coral colonies were repeatedly exposed to different lighting regimes. During the experiments individual colonies were housed in 220 ml cylindrical acrylic chambers with water movement maintained by magnetic stirrers at the chamber bases (Figure 1). The coral chambers were contained in a water bath at 26 ± 0.2 ^{o}C. Seawater was circulated to the chambers using diaphragm pumps providing a flow rate of ~2.8 ml min^{-1}. Experiments typically lasted for 48 hours. At the start of the experiment corals were moved into the chambers and left to recover from handling under the normal light regime. After 24 hours the lighting was changed to either normal or blue lighting regimes (Figure 1) of varying intensities. Changes in the dissolved oxygen and total alkalinity concentrations of seawater inputs and outputs to the chambers were used to estimate net photosynthesis/respiration and calcification rates respectively. After each experiment the coral colonies were returned to the main aquaria.

Figure 1. Experimental set-up. a) *P. damicornis* colony housed in acrylic experimental chamber, b) multiple chambers exposed to the blue light regime and c) spectra of Maxspect Razor LED lights used in this study. Relative intensity is shown on a linear scale. LED channel settings were 100% A:40% B (normal light regime) and 0% A:100% B (blue light regime).

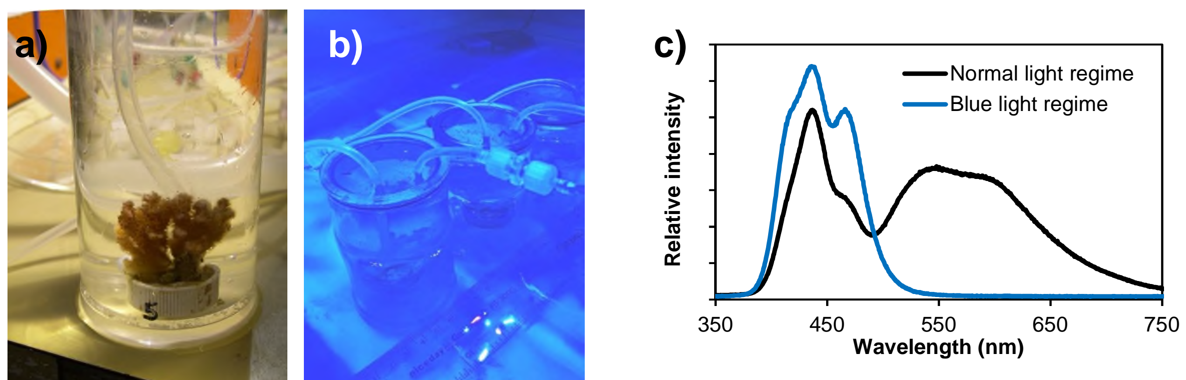
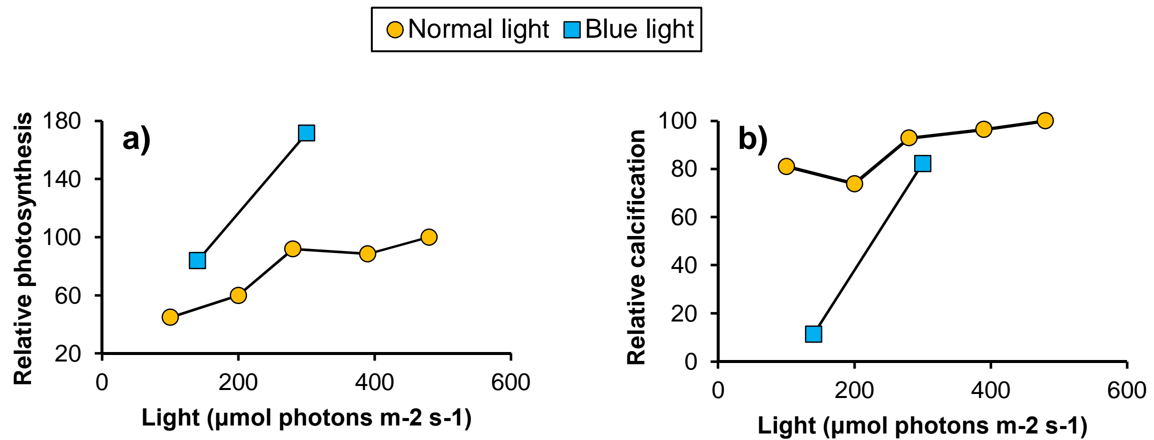


Figure 2. Relative coral a) photosynthesis and b) calcification rates under different lighting regimes and intensities. Rates are calculated as a percentage of the rate observed under a normal lighting regime of ~450 μmol m^{-2} s^{-1}. Points are means of 3 colonies.



Culturing corals under the normal lighting regime indicated that this *P. damicornis* genotype was light saturated at ~280 μmol photons m^{-2} s^{-1} i.e. increasing light intensity above this did not significantly increase photosynthesis (Figure 2a). Photosynthesis rates were significantly reduced at 200 and 100 μmol m^{-2} s^{-1} (paired t test, p=0.05). When exposed to the blue light regime the coral photosynthesis rates were higher compared to the normal light regime at comparable light intensity (Figure 2a), significantly so at a light intensity of ~300 μmol photons m^{-2} s^{-1}. Calcification rates were lower in corals exposed to the blue light compared to the normal light regime, significantly so at ~140 μmol m^{-2} s^{-1}. This result was unexpected as previous work has shown that coral photosynthesis stops within an hour of exposure to blue light[7].

Net photosynthesis and calcification are significantly positively correlated in the corals cultured in the normal lighting regime (Figure 3). Net photosynthesis and calcification are also positively correlated in the blue lighting regime but calcification rates are significantly reduced compared to the normal light regime corals for comparable photosynthesis rates.

We were unable to switch off photosynthesis while maintaining calcification in this coral. However we observed significantly reduced calcification rates under blue light when photosynthesis rates were maintained at the levels observed under the normal lighting regime. Similarly we observed significantly increased photosynthesis rates under blue light when calcification rates were maintained at the levels observed under the normal lighting regime (Figure 3).

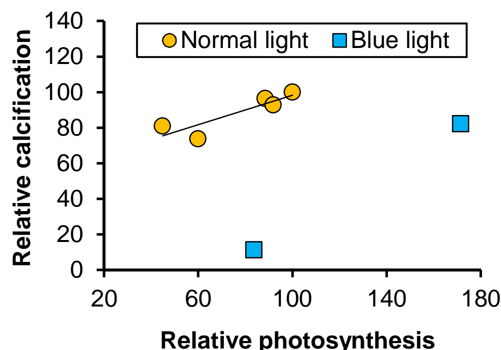


Figure 3. Relationships between relative coral photosynthesis and calcification rates under different lighting regimes and intensities. All rates are calculated as a percentage of the rate observed under a normal lighting regime of ~450 µmol m^{-2} s^{-1}. Points are means of 3 colonies.

Our preliminary data demonstrate that we cannot use the original experimental design to switch off photosynthesis. However our observations indicate that it should still be possible to ultimately use skeletal $\delta^{13}C$ to explore skeletal DIC source. We are currently culturing further corals over a wider range of blue light intensities to induce variability in coral photosynthetic rates. We will select light intensities which lead to comparable coral calcification rates but different photosynthetic rates under normal and blue lighting regimes. We will compare the skeletal $\delta^{13}C$ of corals cultured under these light intensities/regimes to identify the effect of photosynthesis on skeletal $\delta^{13}C$. We will correct skeletal $\delta^{13}C$ for this effect and then use corrected $\delta^{13}C$ in combination with $\delta^{18}O$ and $\delta^{11}B$ (an indicator of calcification site pH) to explore the origin of the coral skeleton DIC.

Biomineralisation symposium and networking

A further aim of the fellowship was to hold a one day symposium on biomineralisation to celebrate Jonathans visit and further networking opportunities. The University of St. Andrews hosted the symposium, ‘Biomineralisation: the interface of earth and life’, on 2 March 2016. Jonathan was the keynote speaker at the symposium and other invited speakers attended from the National Oceanography Centre, Southampton and the Universities of Cambridge, Glasgow and St. Andrews. The event was free and attracted ~40 delegates including undergraduates, postgraduates and staff from multiple institutions in Scotland (Universities of Edinburgh, Glasgow, Heriott-Watt and Stirling, Scottish Association of Marine Science) and England (Universities of Newcastle and York). Delegates networked with Jonathan during the symposium and afterwards at a meal. During his two fellowship visits Jonathan also had meetings with James Rae, Rosanna Greenop and Will Gray (all University of St. Andrews) and with Kirsty Crocket (SAMS). Jonathan also visited the University of Edinburgh to give a seminar. He was hosted by Professor Kate Darling in the School of Geosciences and he met with Kate and other staff during his visit to discuss collaborative research ideas.

References

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