ASSG29 Title: The role of lipids in the resilience of corals to bleaching

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1. Introduction

Coral bleaching occurs with increasing frequency and threatens the future of coral reefs[1,2]. Bleaching occurs when corals expel their photosynthetic symbionts (zooxanthellae) in response to stressors[3,4], such as temperature, light and nutrients[5,6,7]. The resilience of corals to bleaching varies between coral species[8,9] and along water depth gradients[10,11,12,13] and is influenced by the composition of lipids in the coral tissues[14,15,16]. Lipids fulfil a range of functions in corals, acting as energy reserves, forming cell membranes, and providing signalling between cells[17]. At present it is unclear how lipids influence susceptibility to bleaching. Previous research conducted on the reefs adjacent to the CRIOBE research lab in Moorea (17°30'S, 149°50'W), French Polynesia indicates that shallow waters corals are more susceptible to bleaching than their deeper water counterparts[10,13], where *Acropora* species are more susceptible to bleaching than *Pocillopora* species at this site[8,9,12,18]. Accordingly, the aim of this study is to compare the lipid compositions of the coral tissues and skeletons between a bleaching susceptible (*Acropora retusa*) and a resilient coral species (*Pocillopora meandrina*) present at this site, and investigate any variations of lipids obtained over a depth gradient (5m vs. 25m).

The MASTS Aquatic Stressors Forum travel grant supported the opportunity to conduct this fieldwork, from the 20th of July 2022 till the 20th of September 2022, at the CRIOBE research station in Moorea, French Polynesia. With this report, we present a summary of the activities that were conducted throughout this time period and discuss some of the preliminary results obtained.

2. Activities

During my 8 weeks of fieldwork at the CRIOBE research station, a total of 26 *Pocillopora meandrina* and a total of 26 *Acropora retusa* coral colonies were tagged and collected at the selected site. The selected colonies of *P. meandrina* (Dana, 1846) and *A. retusa* (Dana, 1846) were collected within the outer reef of the island of Moorea, French Polynesia (17°485175'S, 149°85864'W) at the depths of 5m, 15m, and 25m. For each coral colony, 2 branches of approximately 3cm x 1cm in surface dimension were collected (replicates within colony) at both the beginning and end of the study (week 1 and week 7). Of the collected coral colonies, 1 branch from each coral colony of both species from each selected site was used for tissue lipid extraction as well as skeletal lipid extraction. Whereas the remainder of the collected coral samples were placed into sodium hypochlorite solution/bleach, rinsed, and dried

down, for complete removal of the coral tissue to permit skeletal lipid extractions. In this way minimizing any contamination of the tissue and endolithic lipids, as the coral tissue and skeleton have been previously found to be different with respect to their lipid composition[19].

Fig 1. Fieldwork site location, outer reef of Moorea, French Polynesia.

The CRIOBE research station adjacent to the selected coral reef site, provided me with the necessary laboratory facilities for me to perform tissue lipid extractions directly onto the freshly collect coral colonies. As well as provided me with an office space which allowed interaction with the local staff from which I was able to gain knowledge regarding the local reef, and accommodation throughout my entire stay.

The coral species selected for this research were chosen due to their abundance at the selected site, their presence along the chosen depth gradient, and their difference in susceptibility to bleaching, with *Acropora retusa* being more susceptible to bleaching than the *Pocillopora meandrina* species.

Fig 2. Fieldwork research station in Moorea, French Polynesia

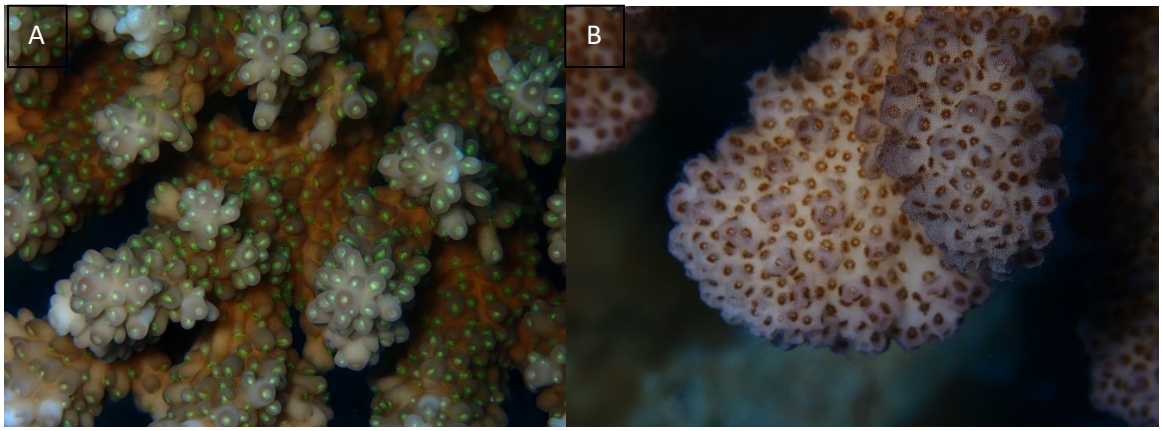


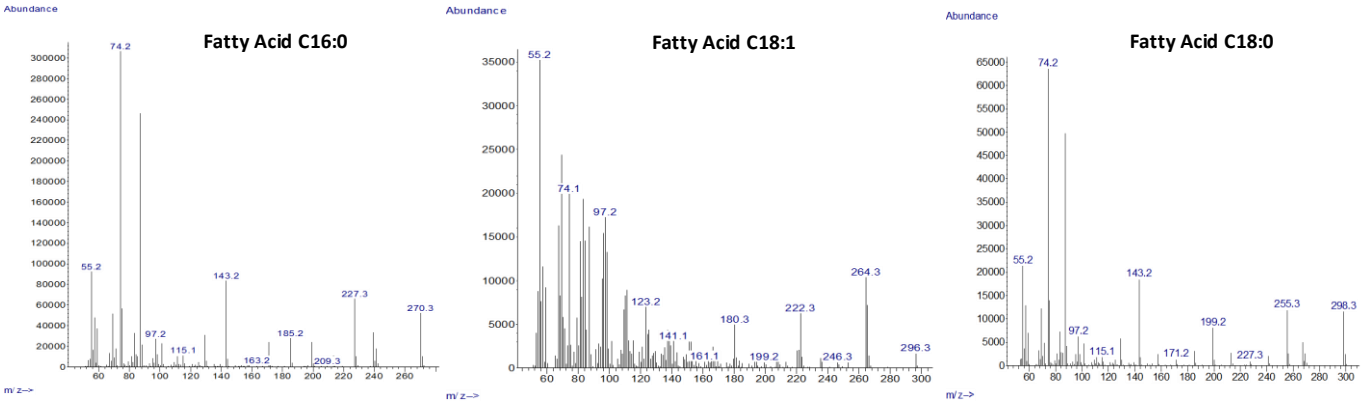
Fig 3. Images showing the studied coral species: A. *Acropora retusa* and B. *Pocillopora meandrina*, that were collected throughout fieldwork at the outer reef adjacent to the CRIOBE research station.

The coral tissue lipids were obtained at the fieldwork site by blasting off the tissue from the skeleton using a high-pressure air stream, thus allowing separation between the coral tissue and the coral skeleton. The collected tissue slurry was subsequently used to perform tissue lipid extractions following the Bligh and Dyer (1959) method, whilst the remaining coral skeleton was submerged within a sodium hypochlorite solution to remove any coral tissue remaining on the coral skeleton. To allow comparison of the lipid compositions of both the coral tissues and skeletons, between bleaching susceptible and resilient species and over a depth gradient, the collected lipid tissue extracts as well as the dried down bleached coral skeletons were transported to the University of St Andrews for analysis.

3. Preliminary Result

At this stage of the project an initial fatty acid methyl esters (FAME) analysis was conducted on a total of 20 *Pocillopora meandrina and 20 Acropora retusa* coral colonies, which were collected at both 5m and 25m, using the Gas-Chromatography Mass-Spectroscopy (GC-MS) technique. This was done to determine the total fatty acids present within the coral tissue samples and identify any differences that might occur for each coral species growing at either 5m or 25m. The main Fatty Acids (FA) detected within our coral tissue samples so far are C14:0, C16:0, C18:0, and unsaturated FA C18:1 (Fig.5), where the key variations in the lipid compositions occurring between the two depths for both *P. meandrina* and *A. retusa* are yet to be determined.

Fig 4. Mass spectra of A. methyl palmitate (C16:0), B. monounsaturated methyl octadecenoate (C18:1), and C. methyl octadecenoate (C18:0).



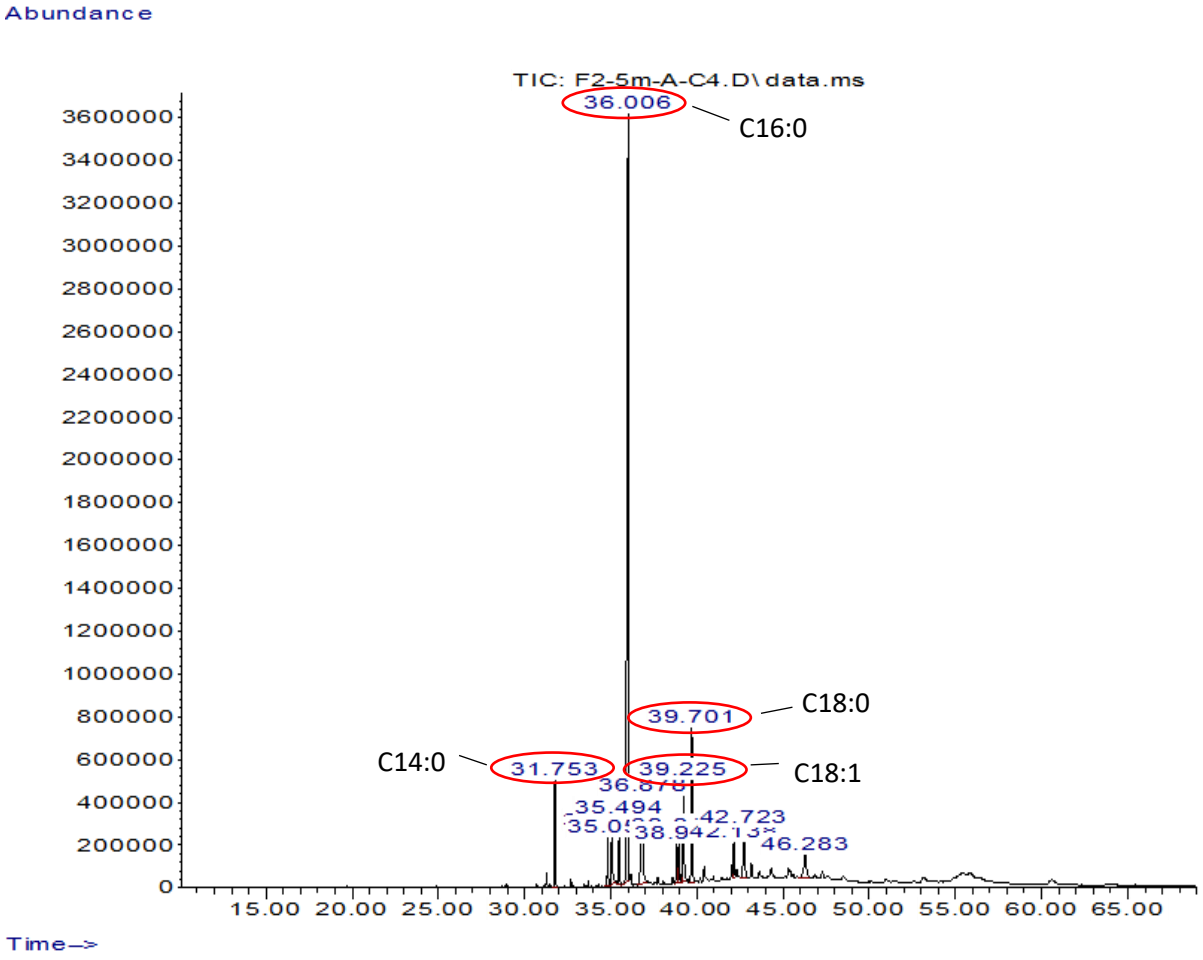


Fig 5. Representative GC-MS spectra of the total ion current chromatogram illustrating the total fatty acids present within a *A. retusa* colony collected at 5m depth within the outer reef of Moorea, French Polynesia.

Initial scanning electron microscopy (SEM) imaging was also performed on a couple of the collected coral samples to identify if there are any structural differences that may be present within the same coral species growing across the different depths of 5m and 25m of the outer reef.

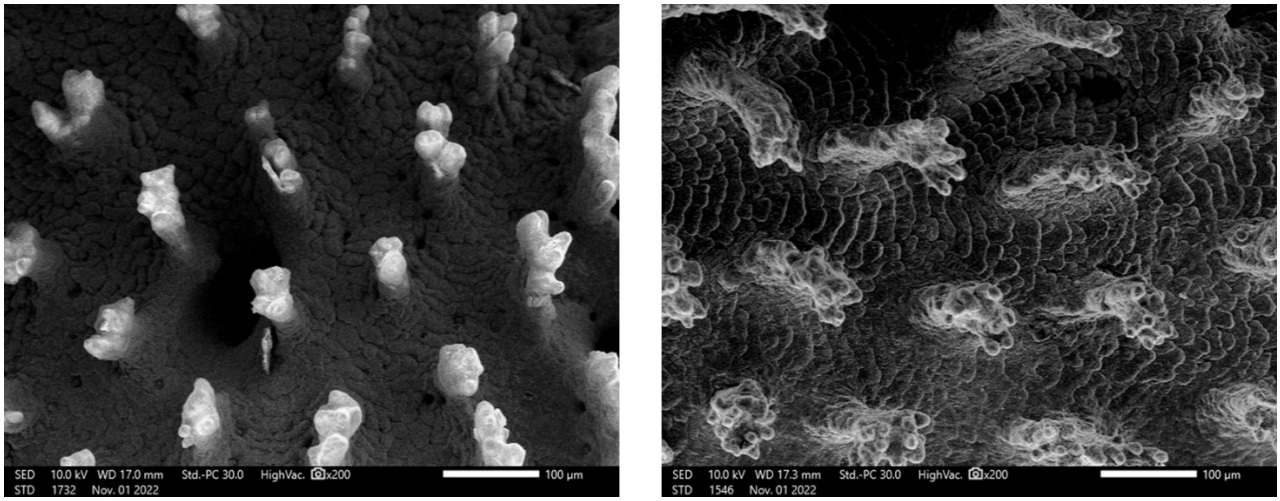


Fig 6. Scanning electron microscopy (SEM) of A. *Acropora retusa* colony at 5m; and B. *A. retusa* colony at 25m.

4. Future of the Project

To answer the aims of this project and identify how lipid compositions of the coral tissues and skeletons may vary between coral species with different bleaching susceptibility and along a depth gradient for each coral species. Different GC-MS techniques will be used on all the coral samples collect at the reef site in Moorea, French Polynesia. On top of the fatty acid methyl esters (FAME) analysis, total lipid analysis using the GC-MS technique will be conducted on all the tissue and skeletal samples, which will allow to look at the compositions of the different lipid classes and will enable us to identify changes in the individual lipid compounds across a range of lipid classes (*e.g*., sterols, wax esters, fatty acids) between coral samples. Further Electrospray Mass Spectroscopy will be used to identify the different phospholipids present within our samples. Overall, we hope that this data will indicate which lipid compounds are reduced in corals that are most susceptible to bleaching and which lipids may vary along a depth gradient. Once all spectra have been obtained, and identification of the key tissue and skeletal lipids varying across the different depths and between different species is completed. We hope to test the effects of any significant skeletal lipids present on the process of coral biomineralization by performing *in-vitro* aragonite precipitation experiments.

5. References

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