The Impact of Ocean Acidification on the Role of Lipids in Coral Biomineralisation

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Introduction

Globally, an estimated 1 billion people benefit from coral reefs both directly and indirectly, providing food sources, protecting shorelines and attracting tourism, making this habitat extremely vital to humans as well as marine life[1]. The coral skeletons that build coral reefs are composite materials that are made up of the mineral aragonite and an organic matrix[2]. This matrix is composed of proteins, polysaccharides and lipids, but little is known about the role of lipids in coral skeleton formation[3]. Biomolecules are important for controlling aragonite morphology. They affect the rate at which the skeleton is produced, its shape and properties. Under ocean acidification, the biomolecule concentration of coral skeletons increases[4]. My research project aims to determine how lipids affect aragonite formation and structure over a range of different concentrations.

The main two lipids that I am investigating are palmitic acid (PA) and phosphatidylcholine (PC). Previous data shows that soy lecithin slows down the aragonite precipitation rate significantly. This lecithin contained 94% phosphatidylcholine and 6% other lipids. From this, it could be that phosphatidylcholine is the lipid to slow down the precipitation rate, considering the proportion of phosphatidylcholine present in soy lecithin. My role was to investigate whether this hypothesis was true. It is known that ocean acidification slows down aragonite precipitation rate, so if phosphatidylcholine slows precipitation rate further, this could have a detrimental effect on the growth of coral reefs in the future. This research is therefore very important for our coral reefs.

To test this, I did experiments at two different saturation states, at $\Omega=13$ and at $\Omega=11$. Saturation state is a measure of the availability of ions that are required to build the aragonite material. A high saturation state means high availability and vice versa. Under ocean acidification, the availability of these ions decreases. I did experiments at the saturation states that occur at the coral calcification site in the present day (Ω=13), and which are predicted to occur under ocean acidification (Ω=11).

I have precipitated aragonite and explored how the presence of different concentrations of the lipids affected the aragonite precipitation rate. To compare the aragonite morphologies at different concentrations of biomolecules, I used a Scanning Electron Microscope (SEM). I used Raman spectroscopy to explore how the lipids affect the structure of the aragonite crystals.

Method

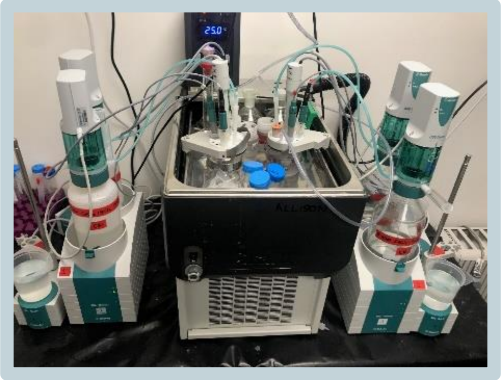


FIGURE 1: THE ARAGONITE PRECIPITATION SETUP

In this research project, I precipitated aragonite *in vitro* from modified seawater, adjusting the pH and saturation state (Ω_{ar}) to reflect conditions that may occur at the coral calcification site in the present day and under ocean acidification. A specialised apparatus was used to maintain the pH and composition of the solution at a stable level (Figure 1). I then measured the dissolved inorganic carbon (DIC) of the modified seawater using a DIC analyser at both the start and end of the precipitation process to estimate Ω_{ar}. Ensuring Ω_{ar} are consistent throughout the experiments means that I can control for any fluctuations in the carbonate chemistry that could affect the results' reproducibility and accuracy. This ensures that any observed changes in aragonite precipitation are due to the experimental conditions, instead of variations in the carbonate system, leading to more reliable data.

Twenty-five aragonite samples were precipitated, and data was collected on several samples through Raman Spectroscopy and Scanning Electron Microscopy (SEM).

Results

Raman Spectroscopy

Raman Spectroscopy looks at the disorder around the carbonate group in the calcium carbonate molecule. Disorder can be caused by the incorporation of biomolecules into the aragonite, which causes the calcium and carbonate ions to become slightly distorted out of position. The full width half maxima (FWHM) of the Raman spectrum υ_1 band in aragonite is positively correlated with distortion of the aragonite unit cell, as determined by X-ray diffraction[5]. In this project, I am looking at whether the biomolecule is affecting the structure of aragonite, which would be shown in a change in FWHM. The υ_1 Raman band at 1085cm^{-1} reflects the vibration of the planar carbonate ions under a layer of calcium ions on top. The conditions to which the precipitation takes place can affect the disorder in the aragonite lattice, and I've tested whether these biomolecules affect that disorder.

I used a one-way ANOVA followed by Tukey's pairwise comparison to test for differences in the Raman U_1 band FWHM between the aragonite precipitated in the ethanol control and aragonite precipitated with lipids. For the results to be significantly different, p value ≤ 0.05. Highlighted values indicate a difference between the control and another sample.

TABLE 1: ANOVA TO COMPARE RAMAN υ_1 FWHM BETWEEN ETHANOL CONTROL, PHOSPHATIDYLCHOLINE AND PALMITIC ACID AT $\Omega=13$

	P Values		
	1 m M PA	110 uM EC	120 uM EC
Ethanol Control	**2.45×10^{-5}**	1.0	0.44

In my one-way ANOVA, significant differences are found as p(same) = 5.12×10^{-6}, which is smaller than 0.05. Tukey's pairwise shows a difference between the palmitic acid and ethanol control (Table 1), and therefore indicates a difference between the disorder of the aragonite with palmitic acid and the ethanol control aragonite lattice. Consequently, this shows that palmitic acid influences the aragonite structure.

TABLE 2: ANOVA TO COMPARE RAMAN υ_1 FWHM BETWEEN ETHANOL CONTROL AND PHOSPHATIDYLCHOLINE AT $\Omega=11$

	40 µM PC OA
Ethanol Control OA	**p = 0.0016**

Despite phosphatidylcholine not influencing aragonite structure at Ω=13, phosphatidylcholine does affect the aragonite structure under ocean acidification, as p value ≤ 0.05 (Table 2) This an interesting finding that should be looked at further.

Precipitation Rate

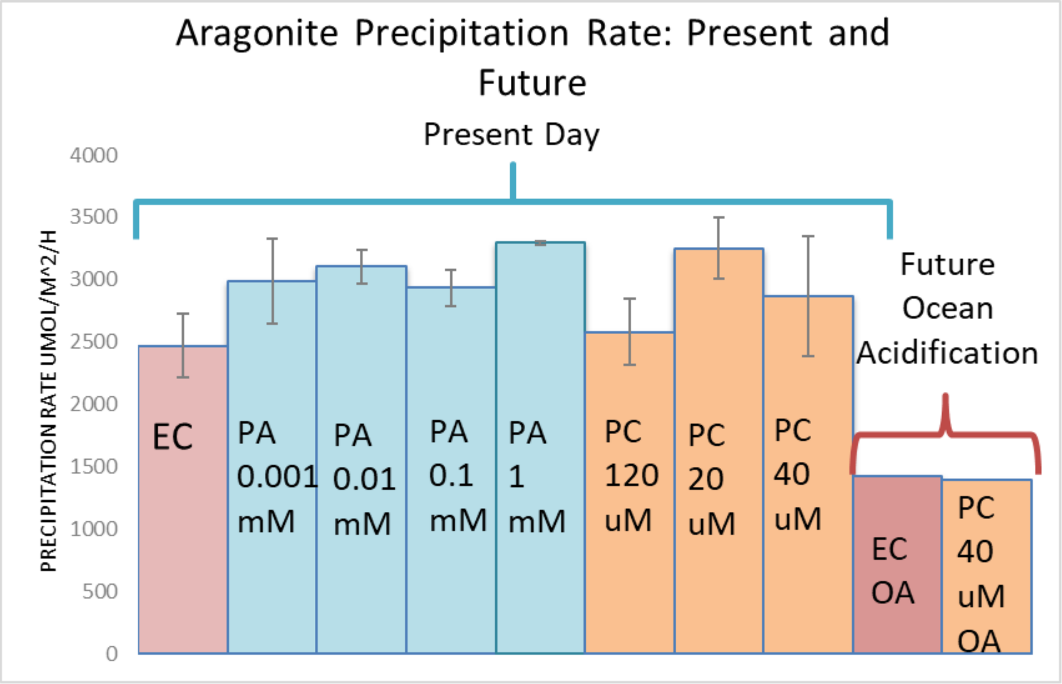


FIGURE 4: A BAR CHART SHOWING THE ARAGONITE PRECIPITATION RATE IN PRESENT DAY AND IN THE FUTURE

Figure 4 Legend: EC: Ethanol Control; PA: Palmitic Acid: PC: Phosphatidylcholine: OA: Ocean Acidification

Figure 4 shows a bar chart comparing the aragonite precipitation rates with no biomolecules (purple bars) against experiments with biomolecules. The experiments were conducted under different saturation states, one at Ω= 13, the saturation state that currently occurs at calcification sites and a lower saturation state of Ω=11, which represents ocean acidification. The bars are means of replicate experiments, and the error bars represent the standard deviations. The bar graph aims to show the difference in precipitation rate between the present day and the future ocean acidification conditions. From the bar graph, it is estimated that the aragonite precipitation during ocean acidification will be slower than the precipitation in our current ocean conditions. This is a worrying result, as aragonite is a vital building block of coral reefs. The slower it is precipitated, the slower the growth of the reef and the less quickly the coral would be able to react if there was to be a coral bleaching event. This could potentially lead to fewer coral skeletons present in our reefs[6].

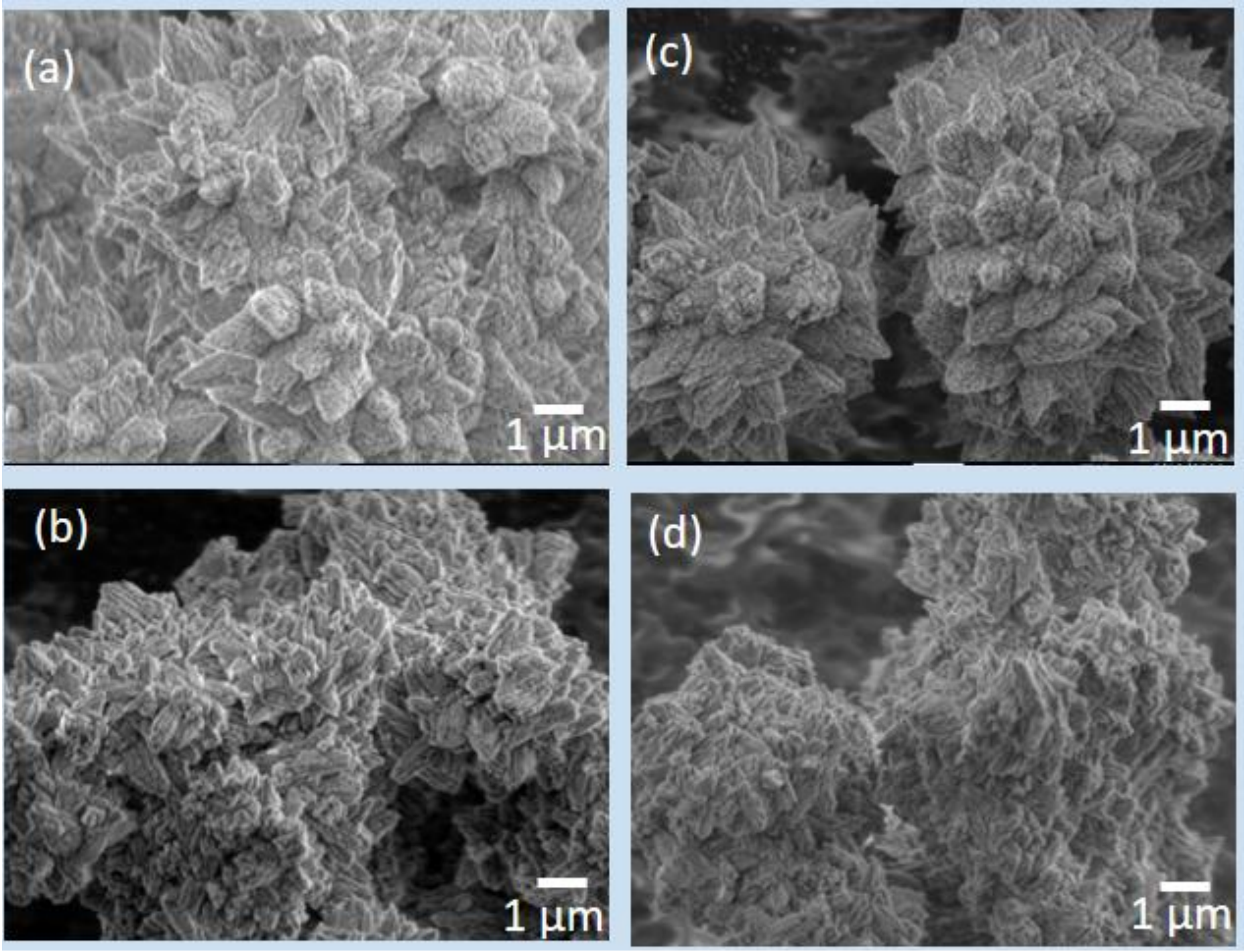
However, Figure 4 displays a hugely positive result when looking at the influence of palmitic acid on aragonite precipitation rate. Comparing the purple EC bar with the blue PA bars in Figure 4 under present day conditions, there is an increase in the precipitation rate at all concentrations of palmitic acid. Additionally, a one-way ANOVA test was done followed by Tukey's pairwise comparison, where it was found that there was a significant difference between the aragonite precipitation rates in the 1 mM palmitic acid compared to the ethanol control (Table 3). The p value highlighted in Table 3 indicates the difference. It can be said that increasing the palmitic acid concentration increases aragonite precipitation rate. This could be hugely important, because if the palmitic acid concentration causes the coral skeleton to grow quicker, this might help to offset the effect of ocean acidification.

TABLE 3: ANOVA COMPARISON OF P VALUES OF ARAGONITE PRECIPITATION RATES BETWEEN ETHANOL CONTROL AND DIFFERENT CONCENTRATIONS OF PALMITIC ACID

	p values			
	0.001 mM PA	0.01 mM PA	0.1 mM PA	1 mM PA
Ethanol Control	0.16	0.072	0.22	**0.022**

Another positive conclusion from the graph is that the phosphatidylcholine did not significantly lower the aragonite precipitation rate at ocean acidification levels, and so has no effect on the aragonite precipitation rate. Therefore, it can be assumed that phosphatidylcholine is not the biomolecule in soy lecithin that dramatically decreases the precipitation rate. But this means that the other substances present in soy lecithin must be investigated further to determine whether they are the molecules responsible for the slower aragonite precipitation rate, or whether phosphatidylcholine must be in combination with other lipids to have an effect.

Scanning Electron Microscopy – Morphologies



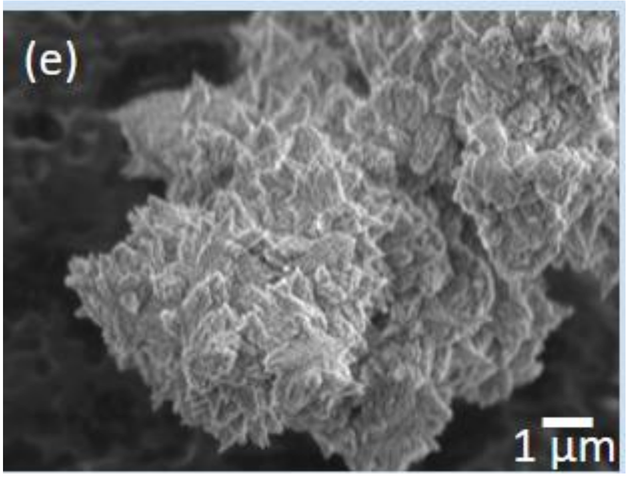


Figure 3: SEM images of aragonite samples (a) x10000 EC (b) x10000 EC OA (c) x10000 PC 110 µM (d) x10000 PC 40 µM OA (e) x10000 PA 1 mM

I imaged 6 aragonite samples using a scanning electron microscope (SEM) with my samples containing a variety of concentrations of the biomolecules palmitic acid and phosphatidylcholine, as well as an ethanol control to compare against. This was done at the University of York. SEM displays the crystal morphologies at the micron level, and I am identifying if the biomolecules cause changes in the aragonite morphology under both present and ocean acidification conditions.

The aragonite crystals formed under ocean acidification conditions at the coral calcification site (Figure 3b) are smaller than crystals formed under present day conditions (Figure 3a).

The aragonite crystals with phosphatidylcholine formed under ocean acidification conditions (Figure 3d) are smaller than the crystals formed with phosphatidylcholine under present day conditions (Figure 3c), confirming that aragonite crystals are smaller under ocean acidification.

However, when comparing the 110 μM phosphatidylcholine (c) to the ethanol control (a), the crystal morphology is very similar. Therefore, phosphatidylcholine has no effect on crystal morphology.

Looking at the effect of 1 mM palmitic acid (e) on the crystal morphology, it is found that its crystals are smaller than the ethanol control (a), showing the palmitic acid affects the crystal morphology. It was interesting to observe that the crystals are smaller, despite its aragonite growth rate being faster than the ethanol control.

Future Work

The data that I have collected from this research project can be used for future work to find out more about the role of lipids in coral biomineralisation under future ocean acidification.

There are many steps that this research can lead to. Soy lecithin (made up of predominantly phosphatidylcholine and other lipids) shows a slowing effect on aragonite precipitation. However, phosphatidylcholine alone has no effect in slowing down the aragonite precipitation rate, which shows that inhibitory effect of lecithin either reflects the presence of other lipids in the lecithin or indicates the phosphatidylcholine must be in combination with other lipids to have that inhibitory effect. Consequently, further work could test this by comparing aragonite precipitation rates with phosphatidylcholine combined with other lipids in the proportions in which they occur in lecithin, and whether there is an enhanced effect under ocean acidification.

Additionally, on a more positive outlook, the palmitic acid must be investigated further as to why it accelerates aragonite precipitation and how this beneficial property could be used to offset the effect that ocean acidification has on the aragonite precipitation rate. Furthermore, more research could be done on why palmitic acid's crystals were smaller than the ethanol control's, despite its growth being faster than the ethanol control's.

Moreover, collecting more Raman Spectroscopy data on the different concentrations of palmitic acid would be interesting to do. With the limited amount of time I had, I could only analyse six aragonite samples with the Raman Spectrometer, as it takes a long time to analyse, and so I was able to only look at the disorder of one concentration of palmitic acid. As I now know that palmitic acid affects precipitation rate and morphology as well as the highest concentration of palmitic acid affecting the disorder in the calcium carbonate structure, then perhaps the lower concentrations could also show a difference.

These findings concerning the lipids' roles on coral biomineralisation will be presented to the scientific coral community and could be the catalyst for more research this topic. Moreover, further findings could also uncover the answer about how to deal with the effects of ocean acidification in our coral reefs- a vital habitat to about 1 billion people and could ensure that we don't lose these amazing habitats to ocean acidification.

Conclusion

There are several conclusions that have been made from this research project. Palmitic acid, a very prevalent fatty acid in coral skeleton, accelerates aragonite precipitation[7]. There's a good chance that increasing palmitic acid's concentration increases aragonite precipitation rate. Future work could be to look at what happens with palmitic acid under ocean acidification to identify if that helps to offset the effect of ocean acidification.

Figure 2 shows that palmitic acid has a smaller crystal aragonite morphology despite it growing faster than phosphatidylcholine and the ethanol control samples- as usually faster precipitation rates mean larger aragonite crystals. More work could be done to identify why this occurred.

The Scanning Electron Microscope images showed that phosphatidylcholine has no effect on the crystal morphology. Aragonite morphologies under ocean acidification lead to smaller crystals.

Ocean acidification lowers aragonite precipitation rate, but phosphatidylcholine has no role in determining the aragonite precipitation rate.

High concentrations of palmitic affect the disorder of the aragonite structure, and phosphatidylcholine only affects the disorder when under ocean acidification.

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