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# Fragile Cold-Water Coral Reefs: 3D Analysis of *Lophelia pertusa*'s Aragonite Crystal Structure

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*MASTS Marine Biogeochemistry Forum Small Grant Round March 2021*

**Background**: The deep sea remains a vastly underexplored area of our planet, considered to be a major biological and ecological research frontier [1]. Once thought to consist of barren abyssal plains, the deep sea can support biodiversity at levels rivalling coastal environements[2]. One such diverse habitat in the deep sea is that of cold-water coral (CWC) reefs. Ranging from depths of ~50-4000 meters, scleractinian (skeleton-forming) species of CWC can form massive reef structures[3]. Expansive reefs are found across the world's ocean, with the largest being the ~100 km$^2$ Røst Reef in Northern Norway[4]. Such CWC reefs provide habitats for ~1300 species globally, categorizing them as biodiversity hotspots[3]. Foundation species such as the well-studied scleractinian cosmopolitan species, *Lophelia pertusa*, create hard substrate and niches to act, for example, as spawning grounds for certain species of recreationally valuable sharks [5] and skates [6], and host invertebrates and commercially important fish [7,8]. This biodiversity supports ecosystem services such as biomimetics [9], pharmaceutical development [10] , carbon storage [11], and fisheries[12] (a complete review of CWC ecosystem services can be found in [13]). At the mid-Atlantic ridge, the highest yields of deep sea fish occur on or near CWC reefs [14] and 90% of total North Atlantic commercial fish tonnage is attributed to fish species associated with CWC reef habitats [12].

Not all occurrences of CWC create deep sea habitats as among several thousand species, there are ~615 skeleton-building scleractinian corals, with just a small percentage of those creating massive reef framework [15]. 3D structural complexity of framework building CWC creates biodiversity hotspots by providing crucial deep-sea habitats [16,17]. However, these ecosystem builders are vulnerable to OA, one of the consequences of climate change[17,18]. Aragonite saturation states (ΩArag) are predicted to fall from 4.1 to 2.2 by 2100[19], bringing many reefs close to the Aragonite Saturation Horizon (ASH) (The depth where aragonite becomes undersaturated, restricting coral growth). CWC are threatened by OA as the decreased availability of carbonate ions (caused by the increase of CO2) can cause aragonite dissolution[20]. Currently, only 5% of CWC live below the ASH, yet that number is predicted to increase to 70% by the end of the decade as the ASH gets rapidly shallower [20].

To understand OA consequences, studies tested CWC calcification rates under decreased pH conditions, finding no significant differences between control and treatments in long-term experiments[18,21–24]. However, differences in corallite (polyp skeletal cup) biomineralization, aragonite crystal shape and molecular bonding organization in low pH conditions did occur [18,25]. While previous research has found smaller crystals in *Lophelia pertusa* collected below the ASH, the actual size of the crystals and setup is unknown as they were too small to be measured by Electron Backscatter Diffraction[25]. As biomaterial properties are determined at the nanostructural level[26] by crystal set-up and nanoporosity, it is crucial to examine CWC aragonite crystal formation to understand OA and the shoaling ASH's effects on skeletal growth, fragility, and load-bearing capacity and what this implies for our future reefs.

**Motivation:** CWC have never been 3D imaged in high enough resolution and large enough volumes to visualize centers of calcification (skeletal-tissue interface) or crystal sizes and alignments. It is important to have 3D visualization of living specimen's skeletal-tissue interface to understand skeletal growth mechanisms. Biomineralization and crystal formation in CWC occurs within centers of calcification, the weakest area of coral skeleton[27], suggested to be where OA-induced structural failure would occur[28]. By 3D imaging centers of calcification by serial section facilitated through combining focused ion beam milling with scanning electron microscope (FIB/SEM) this interface as well as organic (tissue) and inorganic (skeletal) phases can be visualized. CWC reefs in California already experience future ocean conditions as natural upwelling decreases pH and ASH shoaling is rapid[29]. Quantifying differences between CWC growing in aragonite undersaturated and saturated waters allows reef future predictions. By comparing crystals in skeletons of both morphotypes and from different localities with known environmental parameters, we can predict drivers of growth form and crystal size, both of which are important with regard to the structural integrity of the corals.

**Research Question and Objectives:** How is CWC skeleton built and what is the 3D structure of its aragonite crystals?
(1) 3D image tissue-skeleton interface and aragonite crystals for the first time.
(2) Determine if crystal size and alignment in *L. pertusa* is stable across pH and $\Omega$Arag levels and between morphotypes.

**Methods:** *Samples:* Three existing *L. pertusa* samples per site (Mingulay Reef Complex, Rockall Bank, Southern California Bight, & Oslo Fjord) for a total of 12 samples will be selected to examine skeletal-tissue interfaces, crystal alignment/orientation/porosity, and draw comparisons between.

*Microscopy:* Serial Surface View (SSV)[30] on a dual-beam FIB/SEM (ZEISS Crossbeam 350) will 3D image CWC aragonite crystals. FIB/SEM allows internal microstructure imaging[31] and is an ideal method for imaging larger volumes of $100^3$ μm at a spatial resolution of 10 nm[17]. FIB milling will be used to create SSVs, where ~10nm layers are removed one at a time and subsequently imaged via SEM[15], allowing *L. pertusa* crystallite microstructures (<5 μm)[28] to be imaged in 3D. Each sample takes 4 hours to image, totalling 48 hours of FIB/SEM time.

**Results:** This work is ongoing and has expanded due to additional funding. Thanks to this grant, one coral was imaged as a part of a pilot study to understand the best FIB/SEM methods for desired results. Two sessions were completed on a test sample collected from Pisces, Rockall Bank, Scotland. As a new user, these were assisted sessions and incurred additional costs. SEM images from this microscopy time are the main outputs from this grant (Figures 1 & 2). From this pilot study we now have a good way forward to continue with the remaining samples. Samples should be sputtered/gold coated for future analysis for best SEM imaging. Furthermore, milling pillars seems promising though the exact power used to FIB is yet to be determined to create the ideal slices for the aragonite material.

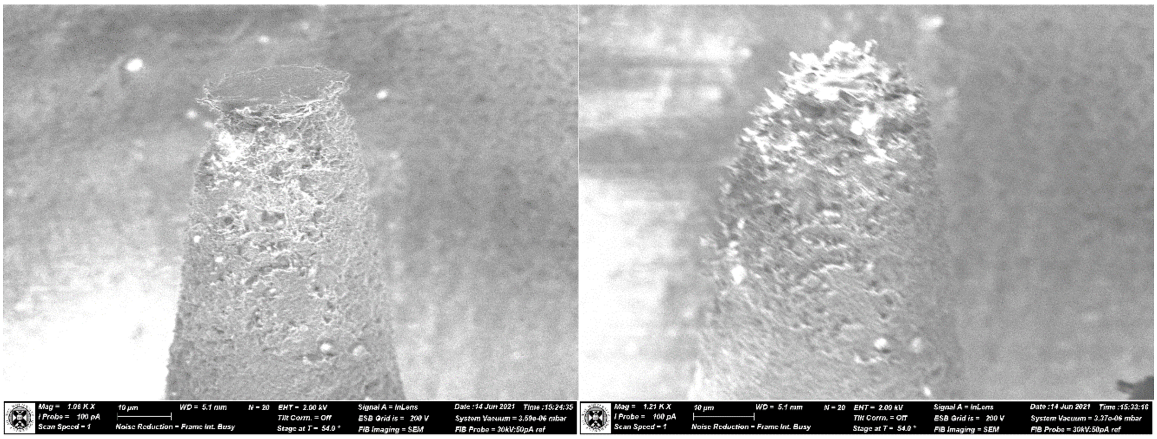

**Figure 1.** A micropillar on the CWC surface viewed in SEM before and after milling away the top layer. This was done to investigate the best way to SSV the aragonite crystals for 3D image reconstruction.

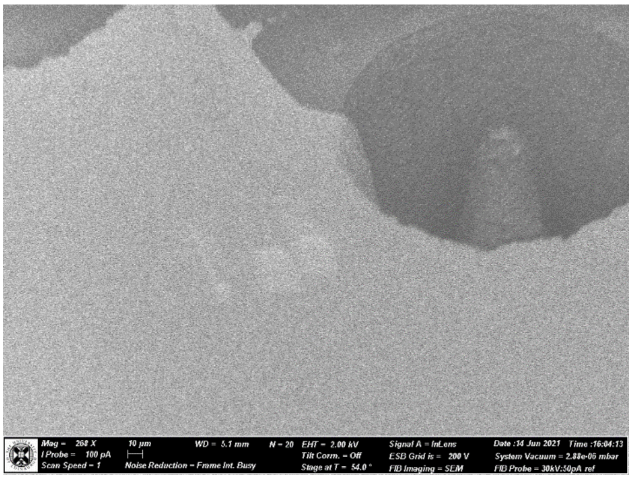

**Figure 2.** A zoomed-out image of the micropillar with three test FIB-SEM mills completed to the left of the micropillar. Note the zig-zag pattern showing the sample drifted during milling. This is a current outcome that continues to be troubleshooted.

**Next Steps:** I received the 2021 ZEISS-GSL scholarship for innovative microscopy. Through the scholarship and an existing SAGES grant, I will build upon the pilot study done here to further image CWC, beginning in October. Possible methods include more FIB/SEM, synchrotron imaging, and the new ZEISS product CrystalCT. Having the option to FIB/SEM with built-in EBSD capabilities would also be a step forward. MASTS will be updated once the study has been completed and be thanked for funding in any resulting presentations and publications.

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