

MASTS Small Grant Report Summary (SG34)

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Project title: High-resolution probing of the microbiota living associated with the cold-water coral *Lophelia pertusa* in the North Atlantic

Background and aims of this project

On 17th of May 2012, the *RRS James Cook* left port from Glasgow on a 35-day scientific expedition into the North Atlantic. Dubbed the “Changing Oceans Cruise”, it combined a team of scientists, technicians and crew, including MASTS-funded PhD students. Led by Prof. Murray Roberts from Heriot-Watt University, this expedition aimed to examine the potential impact of changes in the temperature and chemistry of the oceans on cold-water coral reefs – specifically, *Lophelia pertusa* – and the animals and microbes living associated with the coral. Little is known about the diversity of the bacterial community living associated with *Lophelia*, and even less is known about the function(s) that the different types of bacteria might exert upon the coral.

During this cruise, two members of the scientific team, Dr. Anne Cotton (University of Hull) and Dr. Geoffrey Cook (U.S. Fish & Wildlife Service, Alaska), successfully obtained and preserved a unique collection of *Lophelia* polyps from the various locations visited during this expedition into the North Atlantic. The samples are unique because they were taken using a state-of-the-art remotely-operated sampler that was designed specifically for obtaining deep-sea coral samples with minimal disturbance to their structural integrity and associated microbiota. The coral polyps were preserved immediately after sampling for subsequent microbiological and molecular investigation, including CARD-FISH (Catalyzed Reporter Deposition Fluorescence *in situ* Hybridization), which is a state-of-the-art method that has become one of the most widely used tools in microbial ecology. It utilizes fluorescently-labeled oligonucleotide probes that target the ribosomal RNA (rRNA) of targeted cells. If the target organism is present in a sample, it will appear as a bright object against a darkened background when viewed using an epifluorescence microscope, thus allowing for its direct observation and quantification. CARD-FISH is an *in situ* amplification method utilizing horseradish peroxidase which enhances bacterial cell detection over standard FISH protocols that can be several orders of magnitude less sensitive. The value of this method is in its ability to allow the observer to visualize the cells being targeted by the probe(s) while *in situ*.

This cruise was part of the Benthic Consortium of the UK Ocean Acidification research Programme (UKOA), a project funded and supported by the Natural Environmental Research Council (NERC), the Department for Environment, Food and Rural Affairs (DEFRA), the Department of Energy and Climate Change (DECC) and Heriot-Watt University. In this project, the laboratory of Dr. Tony Gutierrez at Heriot-Watt University employed CARD-FISH to investigate the communities of

bacteria found living associated with *Lophelia* from samples obtained at the different locations sampled in the North Atlantic.

How money was spent and activities completed

This MASTS Small Grant provided partial funding to offset some of the consumables costs associated with performing CARD-FISH to analyse the microbiota associated with the various coral polyp samples obtained during the 2012 Changing Oceans Cruise on *RRS James Cook*.

Work completed so far included:

- Fixation and storage of coral polyps for subsequent CARD-FISH analysis.
- Embedding and sectioning of individual coral polyp samples and optimizing these protocols for subsequent hybridization with CARD-FISH probes targeting several major bacterial groups.
- Optimization of CARD-FISH for visualization of targeted bacterial cells against coral soft tissue.

Outputs and/or expectations and future plans

This work is still ongoing and in collaboration with scientists from the United States and England whose research on *Lophelia* strongly relates to this project. To-date, our CARD-FISH results have revealed information on the anatomical localization of bacteria found associated (i.e. in physically attachment) with the soft tissue of *Lophelia*. We used a combination of probes that target different groups of the Domain Bacteria to investigate their association along saggital thin sections of *Lophelia* polyps after they had been decalcified. As pyrosequencing information becomes available from our US and UK collaborators – which will provide a highly resolved dataset on the diversity and structure of the bacterial community associated with *Lophelia* – this will help guide our selection for designing new probes, such that might target novel bacterial taxa. Coupling CARD-FISH with other techniques (e.g. RAMAN microspectroscopy) will help in our efforts to better understand the relationship(s) that exist between bacteria and cold-water corals.