INT2022-05 Determining the resilience of Fiordland corals to fisheries impacts

Year 2 interim progress report
May 2024

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Overview of year 2:

In the last year we have undertaken four main activities: 1) the development of a Whole Genome Sequence for *Antipathella fiordensis*; 2) the collection of shallow and deep *A. fiordensis* samples from multiple sites in Fiordland for genetic analysis, and extracting DNA from these samples; 3) collecting more *A. fiordensis* abundance data from deep and shallow locations in Fiordland; and 4) securing the data for building our population model for *A. fiordensis*. As a result of these activities, we have few new data to present as year 2 has been primarily a sample and data collecting year. We expect to have results from these activities at the end of year 3.

Introduction

Antipathella fiordensis is a black coral species in the order Antipatharia, named because their chitinous skeletons are black. This species was first described as *Antipathes fiordensis* in New Zealand in 1990 from material collected in Fiordland (Grange, 1990), with a 2001 taxonomic revision of the Antipatharia placing this species in the newly created genus Antipathella (Opresko, 2001). This species has primarily been reported from the Fiordland Marine Area, although it is possible that it also occurs in deeper water around other parts of the South Island, and a likely colony has recently been reported at Kapiti Island. In Fiordland *A. fiordensis* is particularly abundant, and perhaps most importantly it occurs in very shallow water, with the distribution ranging from 4-5 m down to several 100 m (based on our recent observations). In addition to its intrinsic importance, the study of *A. fiordensis* also provides a rare opportunity to act as model for other deeper, less accessible black coral species that are likely to have similar population characteristics.

A. fiordensis is commonly found across the Fiordland Marine Area, often forming large (several m across) complex 3-dimensional tree-like structures, which have been estimated in previous research to be several hundred years old (Hitt et al. 2020). Although this species is often characterised by these massive structures, the reefs in Fiordland also support a high abundance of much smaller, younger colonies, suggesting populations may be highly dynamic. Black coral colonies are most associated with steep cliff areas, although they also occur on any rocky substrate (including boulders and cobbles).

Our current knowledge of *A. fiordensis* is based on early work focusing on its distribution and reproductive output (Grange, 1990), population genetics (Miller, 1997), relationships with mutualistic ophiuroids (Parker et al., 1997), growth/ultrastructure (Goldberg and Taylor, 1989), age (Hitt et al., 2020) and distribution in relation to salinity (Jiang et al., 2015). The 3-dimensional and fragile nature of *A. fiordensis* makes it particularly susceptible to potting, line damage and other bottom contact fisheries. In addition, the shallow and coastal nature of *A. fiordensis* in Fiordland also renders it susceptible to a range of local stressors including changes in salinity and sediment (e.g., due to changes in land use, rainfall or hydroelectric water flow) and global stressors such as ocean warming and acidification.

The specific project objectives of these services, delivered to The Department of Conservation through the Conservation Services Programme project INT2022-05 are:

1. Increase understanding of the ecology and impacts of fishing on protected corals in Fiordland, including the black coral *Antipathella fiordensis* and stylasterid corals (see NOTE 1).

2. Improve our understanding of the distribution of Fiordland black corals inside and outside of fished areas and ascertain the extent of overlap between fishing activity and coral habitat.

3. Determine patterns of genetic diversity and likely routes of connectivity within and between Fiords.

4. Use varied approaches (modelling, SCUBA and remotely operated vehicle ('ROV') surveys, pre-existing data) to inform our understanding of protected coral resilience to fishing impacts and threats in Fiordland, which can then be applied to these taxa in a wider context.

There are four main approaches to address the project objectives (agreed with the Department of Conservation) and here we report on the second progress with regards to each of these approaches. This is a three year project.

1. Fisheries impacts – compiling data from fisher surveys, observer and effort data from MPI, abundance surveys and temporal monitoring, and creation of a database of colony health status /observed fishing impacts.
2. Distribution patterns – based upon SCUBA and ROV surveys, black coral size and abundance will be determined at multiple locations in Doubtful, Dusky and Breaksea Sounds, and resulting data combined with environmental correlates.
3. Population models - Existing data (subject to availability), coupled with SCUBA and ROV surveys, and 3D photogrammetry, will be used to create population models, and will incorporate estimated recruitment, mortality and growth rates.
4. Connectivity patterns between coral populations will be determined across vertical gradients, and between fished and unfished areas using genetic approaches.

NOTE 1: That while ROV video collected during this project will contain some information on the distribution of stylasterids (since they generally occur below 30 m), there is no provision in this contract to analyse data for these videos, and further specific survey work is outside the scope of this contract.

Progress to date and method development

Although black corals are found throughout the Fiordland region, this project is exclusively focused on Doubtful, Breaksea and Dusky Sounds (Figure 1) because: 1) resource availability

prohibits working across the entire distribution range of black corals in the fiords; 2) these three fiords are relatively easy to access; and 3) there are extensive ecological data sets available for these areas and we have existing projects here, which will be drawn upon for this project. Our research includes areas inside and outside of the Fiordland Marine Area habitat protection zone, and the specific areas we have visited to date are shown in Figure 2.

Cruises completed to date:

We completed three research cruises in year 1 (to June 2023) and have completed a further three cruises in year 2 (to May 2024). We expect one, possibly two, further cruises where data will be collected for this project in year 3. It is important to note that due to the nature of this project, these cruises were not exclusively focused on black coral research, and the funding allocation from CSP only supports one research trip during each year of the project:

January 9th-14th 2023 – RV Southern Winds – focused on Doubtful and Thompson sounds
March 17th-22nd 2023 – MV Pembroke – focused on Dusky and Breaksea Sounds
May 17th-13th 2023 - MV Pembroke – focused on Dusky and Breaksea Sounds
October 9th-14th 2023 - RV Southern Winds – focused on Doubtful Sound
January 8th-13th 2024 - RV Southern Winds – focused on Doubtful and Thompson sounds
May 12th-18th 2024 - MV Pembroke – focused on Dusky and Breaksea Sounds (note this will only include the collection of the remaining genetic samples, no other sampling)

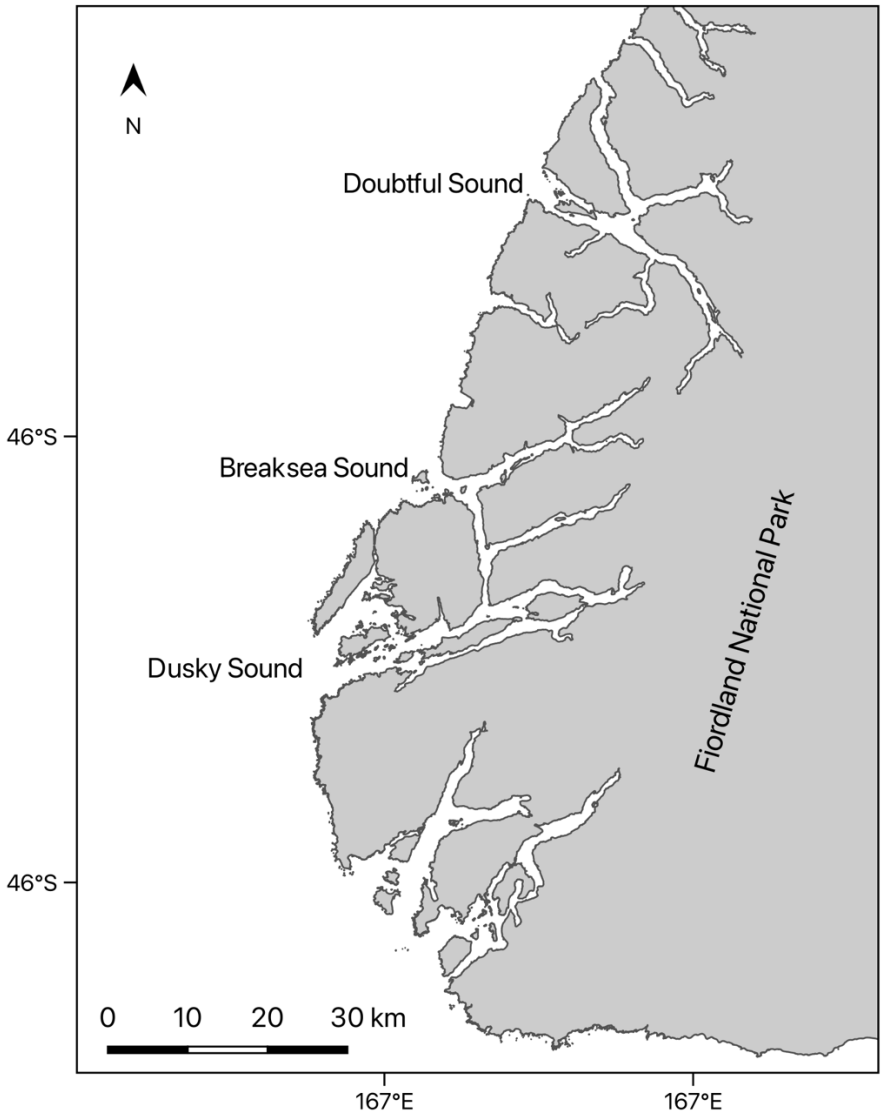

Figure 1. Location of Doubtful, Breaksea and Dusky Sounds in Fiordland

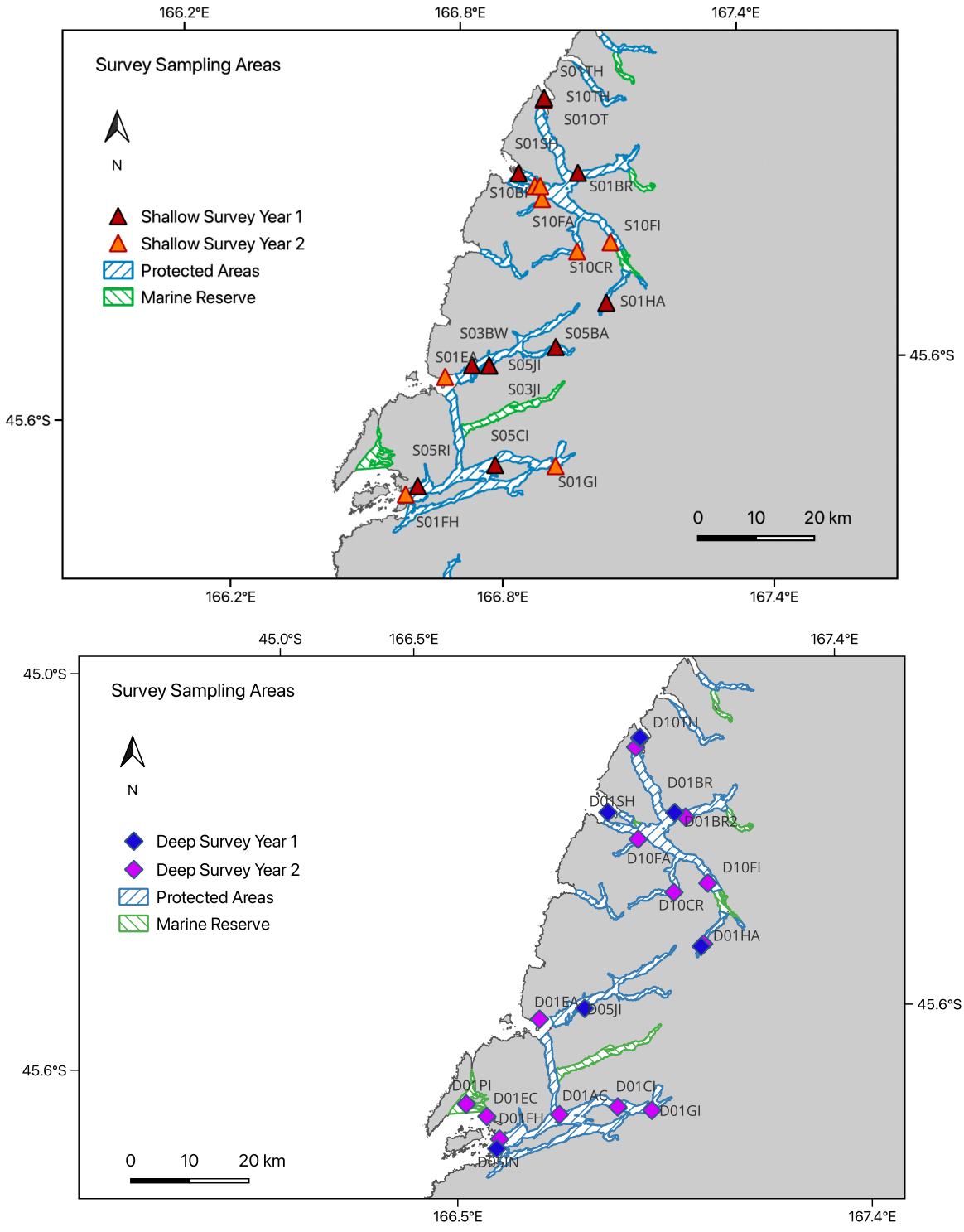

Figure 2. Locations visited during research cruises in year 1 and year 2 in Fiordland where specific black coral research was undertaken. See figures 4 and 5 for specific abundance and genetic

sampling locations. The top figure shows where shallow water surveys (15 m)or collections were made and the bottom figure the deep water (> 50 m) survey and sampling locations.

Fisheries impacts

The approaches that have been considered for this component include compiling data from fisher surveys, observer and effort data from MPI, abundance surveys and temporal monitoring, and the creation of a database of colony health status/observed fishing impacts/presence of pots.

So far we have only been able to collect information on 'loose' fishing pots and other gear, which has been collected as we have conducted our abundance surveys and during the collection of genetic samples. We have recorded the location, depth, condition and any other data/information about the pots/lines we observed. We are stilling waiting on catch data from MPI/DOC. Catch data will be based upon effort reported to FNZ. For potting, (e.g. for crayfish) effort has historically been reported at the statistical area level (area 927 for Doubtful Sound and area 928 for Breaksea and Dusky sounds). However, in 2019, the electronic reporting system was introduced which provides a much finer resolution in spatial reporting scale; therefore analysis of fishing effort overlap with coral distribution in year three may include multiple levels of temporal and spatial resolution. We are not expecting to conduct any fisher surveys.

Distribution patterns

We are collecting abundance data based on shallow (15-18 m) and deep (>50 m) surveys. We have used 3 x 20 m transects (2 m either side of the tape) for shallow observations using SCUBA, with size (width and height) measured for each coral observed in the transects. For deeper surveys, we have used a combination of our Boxfish Remotely Operated Vehicle and our Chasing ROV with a Gopro Hero 12 mounted. Where possible, we drive the ROV along approximately 60 m of reef (based on using the ROV lasers). We have been trailing photogrammetry to reconstruct the reefs from the ROV footage to estimate abundance and size of corals encountered. At the end of year two, we have conducted surveys at 20 shallow and 20 deep locations in the fiords. It is important to note that ROV surveys in Fiordland are not straightforward we anticipate continuing to collect deep water data through the three years of the project.

Population models

We plan to use pre-existing data collected by The Cawthron Institute and NIWA as part of the long-term Meridian Energy monitoring programme and our own data. We requested access to monitoring photographs taken between 2008 and 2021 (2008, 2009, 2010, 2011, 2013, 2014, 2016, 2019) at 13 sites across Doubtful Sound and these data have now been secured. A map will be provided of these sites in the final report. These photographs are taken off the same area of reef. We will specifically extract information on settlement and mortality rates for black corals as we will be able to track these events through time from the pictures. We will use this data to parameterise a size structured model, and specifically we will reassess all the photographic data

collected as part of the Meridian monitoring programme to estimate recruitment and mortality rates. This will be used, combined with our abundance data, to parameterise our models. We are developing a number of photogrammetry-based approaches to accurately measure black coral abundance and are currently monitoring a number of corals in Doubtful Sound. We hope to be able to collect our final data in January 2025. By combining 3D photogrammetry of specific corals, with the 3D characterisation of the local reef where they live (50 m2), we no longer need to tag specific corals as we can relocate them from the 3D maps. It should be noted that the data provided by Cawthron and NIWA will not be provided with the final report, since we do not own this data. Should this data be required by DOC then a specific request will need to be made.

We plan to build models to represent the population dynamics of black corals from different locations in Doubtful Sound (where the Meridian data is available). We will then use these models to model changes to black coral populations under different recruitment/mortality/impact scenarios. This work is planned for year 3.

Connectivity patterns between coral populations

A previous study on *A. fiordensis* detected genetic structure (i.e., distinct black coral populations) among populations at scales of 10-15 km (Miller, 1997), although the data showed greater differentiation within fiords compared to between fiords. Miller (1997) proposed this unusual pattern was the result of a population that has not yet reached equilibrium due to a combination of the effects of recent colonisation, asexual reproduction, and the potential longevity of individual coral genotypes. However, this earlier study was conducted using allozyme genetic markers, which are no longer used due to difficulties in isolating the impacts of selection on genetic patterns and limited ability in some cases to resolve population structure. As genetic connectivity can be used to inform population resilience by identifying source and sink populations, and isolated populations, this project component will investigate the population genetic structure of *A. fiordensis* using Genome-Wide SNPs. Single nucleotide polymorphisms (SNPs) represent the most abundant type of variation in DNA sequences among individuals in a population (Bossart and Pashley Prowell, 1998; Vignal et al., 2002). They can occur in coding and non-coding regions and therefore it is possible to use them to investigate not only genetic structure (using neutral SNPs) but also adaptive variation in populations (using SNPs under selection) (Morin et al., 2004).

Our aim is to reconstruct the Whole Genome Sequence (WGS) for *A. fiordensis* and subsequently identify a panel of SNP markers, which will be used to assess the levels of connectivity and gene flow within and across fiords and between different depths. We will also use these markers (outlier SNPs) to detect any evidence for local adaptation of populations and how environmental gradients in the fiords or possibly fisheries impacts are driving the genetic structure (Hoey et al., 2016; Holland et al., 2020). Understanding vertical connectivity is also important as it will provide information on whether deeper black coral populations can potentially replenish shallower populations (or vice versa) if they are impacted by some external stressor.

The plan was to collect *Antipathella fiordensis* samples across Doubtful, Dusky and Breaksea Sounds to assess within and between fiord connectivity. Sampling locations were to be distributed along the fiords from the inner part to the most outward part of the fiords and separated at different spatial scales from 1 km to 100s km. We had planned (depending on the final sequencing depth required) to characterise a total of 10 sampling sites across the fiords. Shallow populations were to be sampled at all sites, while both shallow (between 0-20 m) and deep populations (70-100 m) were to be sampled at two sites to assess vertical connectivity.

Small colony fragments have been collected from 20-25 individuals of *A. fiordensis* along a transect of 50 m. Specimens from shallower populations have been collected by SCUBA diving while deep colonies have been collected using a ROV. Upon collection, the specimens were preserved in DESS and stored at 4°C. We have trialed several different DNA extraction kits to get very high-quality DNA for WGS. Samples will be sequenced on the Illumina platform in May 2024. Sequences will then be compared against the reference genome and between individuals of different populations to identify genomic variants (e.g., SNPs) throughout the genome.

Results and discussion

Fisheries impacts

We have found 'loose' pots both within commercially and non-commercially fished areas, and the locations of these pots after two years of surveys are shown in Figure 3. These were mostly found below 25 m, and in all but one case had more than 2-3 m of rope attached. It is not possible to determine when these pots were placed on the seafloor, although the pots observed in the inner waters of the fiords are likely to have been in place since at least 2005 when commercial fishing ceased in these areas and these were heavily encrusted.

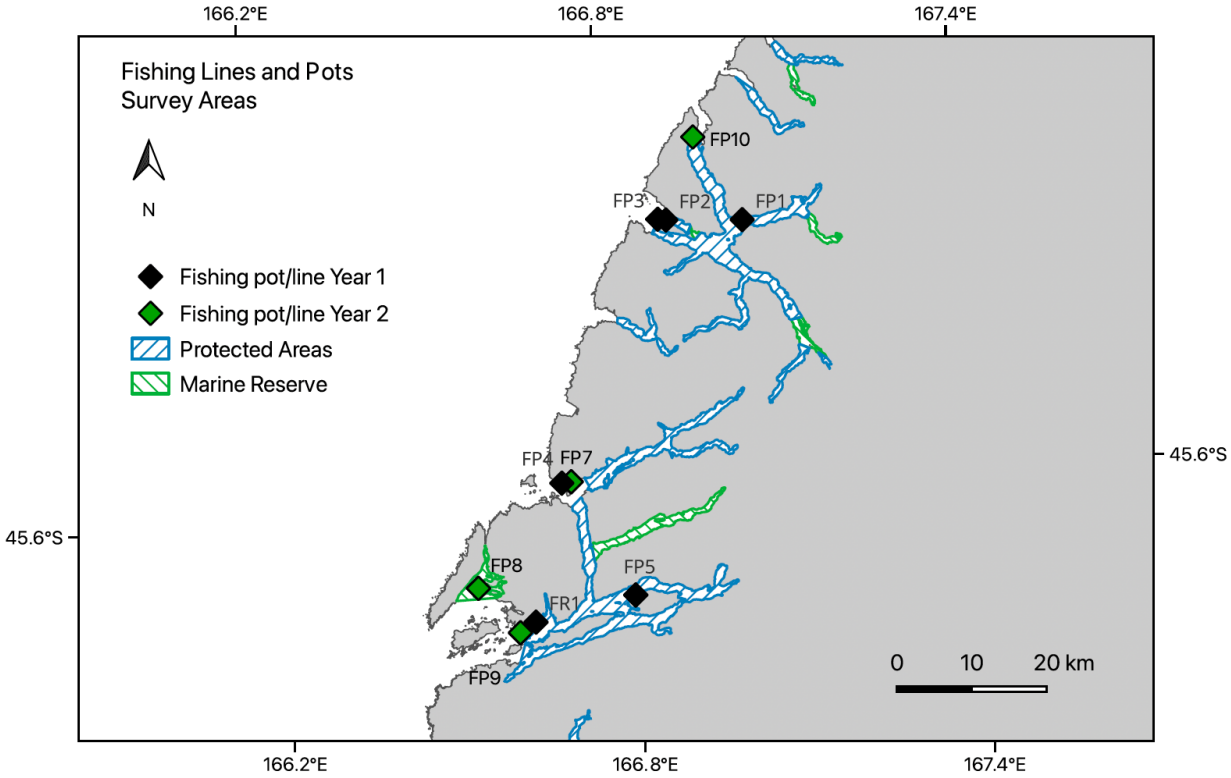

Figure 3. Locations of 'loose' cray pots found during SCUBA and ROV surveys in Year 1 and Year 2 of the project based on visiting around 30 locations (30% of dives have found pots).

Distribution patterns

We have collected abundance data from 20 shallow water sites so far, an increase from 9 sites in year 1 (Figure 4) and 20 deep water sites, although no data has yet been collected from deeper video footage. The general trend we have been observing is an increase in coral abundance (corals per 2 x 15 m transect) from the inner to the outer parts of the fiords. However, initial indications from our size data suggests those corals in the inner parts of the fiords are much larger than those in the outer areas. Our initial observations suggest that recruitment rates are greater towards the outer parts of the fiords as we see many very small corals. This might reflect the more extreme environmental conditions towards the outer fiords. We hope to compare our data with early data collected by Ken Grange where possible to see if it possible to see any temporal trends.

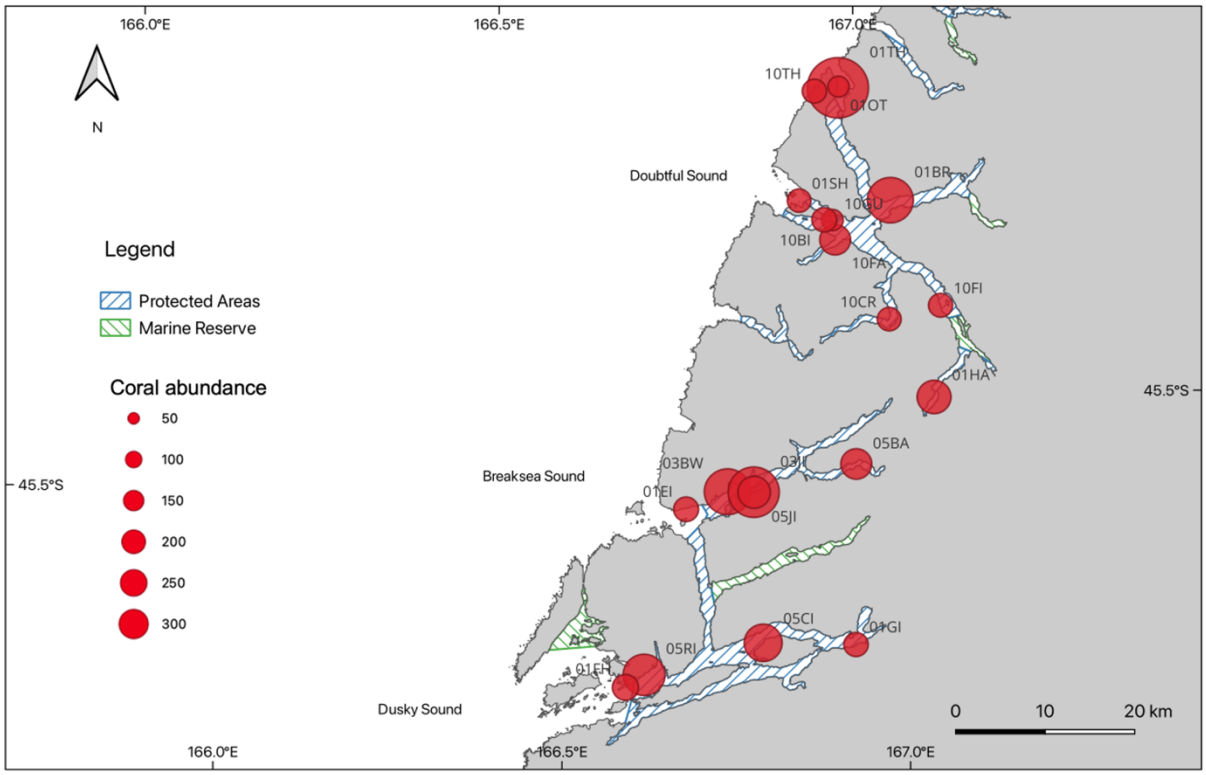

Figure 4. The total abundance of black corals at 15 m depth at 20 sites in Doubtful, Dusky and Breaksea Sounds, based on 3 x 15 m transects at each location. Data is combined across the three transects and presented as a total abundance.

Population models

Progress has been made in conceptual model development, and we have now received data from the Cawthron Institute and NIWA. Data is currently being collected from the photographs.

An Matrix Population Model (MPM) will be built by using existing data collected by the Cawthron and NIWA datasets. Specifically, we have obtained data from the following reports 'Biological monitoring field sampling Subtidal Photo-quadrats analysis' (conducted by Cawthron at 8 sites in Doubtful Sound) and the 'Black Coral and Indicator Species Monitoring Program' (lead by NIWA in 7 sites in Doubtful Sound). From these two data sets which have used permanent photo-quadrats and permanent photo transects, we will extract *A. fiordensis* population demographic data to parametrize a size structured population model. Currently we are reassessing all photographic data from 2006-2019 collected by Cawthron Institute to estimate recruitment and mortality rates of black coral colonies present in the permanent photoquadrats. This will be used, in combination with the NIWA dataset and with the abundance data we are collecting during fieldwork. We also expect to include our data collected from photogrammetry for growth rates, which will be incorporated into the model

Connectivity patterns between coral populations

We have collected shallow water coral samples from 10 shallow populations and 2 deeper water population samples with the ROV (See Figure 5) for genetic work. At each shallow location, small fragments were randomly collected from 20-30 colonies that were taller than 10 cm along a 50 m transect. One 10 samples were collected from the two deep water populations. Following collection, black coral fragments were preserved in DESS and stored at 4°C.

Two live colonies were also collected and taken to the Coastal Ecology Laboratory at VUW to perform total genomic DNA extraction on fresh tissues to create our reference genome. These two live colony samples were taken to the Institute of Environmental Science and Research (ESR) for sequencing. After library preparation (kit12/14 libraries) and final quality checks, sequencing was carried out using Oxford Nanopore Technology (ONT) on the sequencing device gridION. Raw data received from the ONT sequencing have been gone under quality checks and trimming and have been assembled to a fully sequenced draft reference genome. Genome assembly was carried out following a bioinformatic pipeline that includes reads quality assessment and filtering, de novo assembly and polishing.

To assess the completeness of expected genome content of the genome assembly we have then run BUSCO (Benchmarking Universal Single-Copy Orthologue) (Figure 6). BUSCO provides genome assembly metrics about scaffold lengths and values and will identify matches to sets of genes that are present as single-copy orthologs in a given taxonomic group, i.e., genes that are evolving under “single-copy control”, and thus expected to be present as single-copy genes in our sequenced species. Based on the genome assembly and the known taxonomic domain of origin, BUSCO provide a summary scores output, which contains the classification of the identified BUSCO markers into categories of Complete (C), Complete and single-copy (S), Complete and duplicated (D), Fragmented (F), and Missing (M). BUSCO as percentages and counts, and additional information such as the dataset used and the versions of the dependencies. Our initial genome assembly draft measured 482.7 Mb with a BUSCO completeness of 99.6% (45.9% duplicated, 0.4% fragmented; Figure 7). We are currently a running polishing analysis and conducting some further sequencing with the Bragato Institute to increase the quality of the final assembly further.

Now we almost have a complete reference genome (Figure 6), we have begun extracting DNA from all the proceed population samples, with a deadline of end of May 2024, which is when samples will be taken to the Australian Genomic Research Facility in Melbourne for sequencing. Not all samples collected from each site will be sequenced, only the 20 with the highest quality DNA. Screening of DNA will be undertaken by generating ~1 million reads per sample and mapping these against the reference genome, generating an average of 3x coverage across the genome. Variant calling will generate a panel of SNPs likely using the PALEOMIX pipeline and we will conduct a subsequent population genetic analysis. We will only be sequencing samples from eight shallow populations and two deep populations.

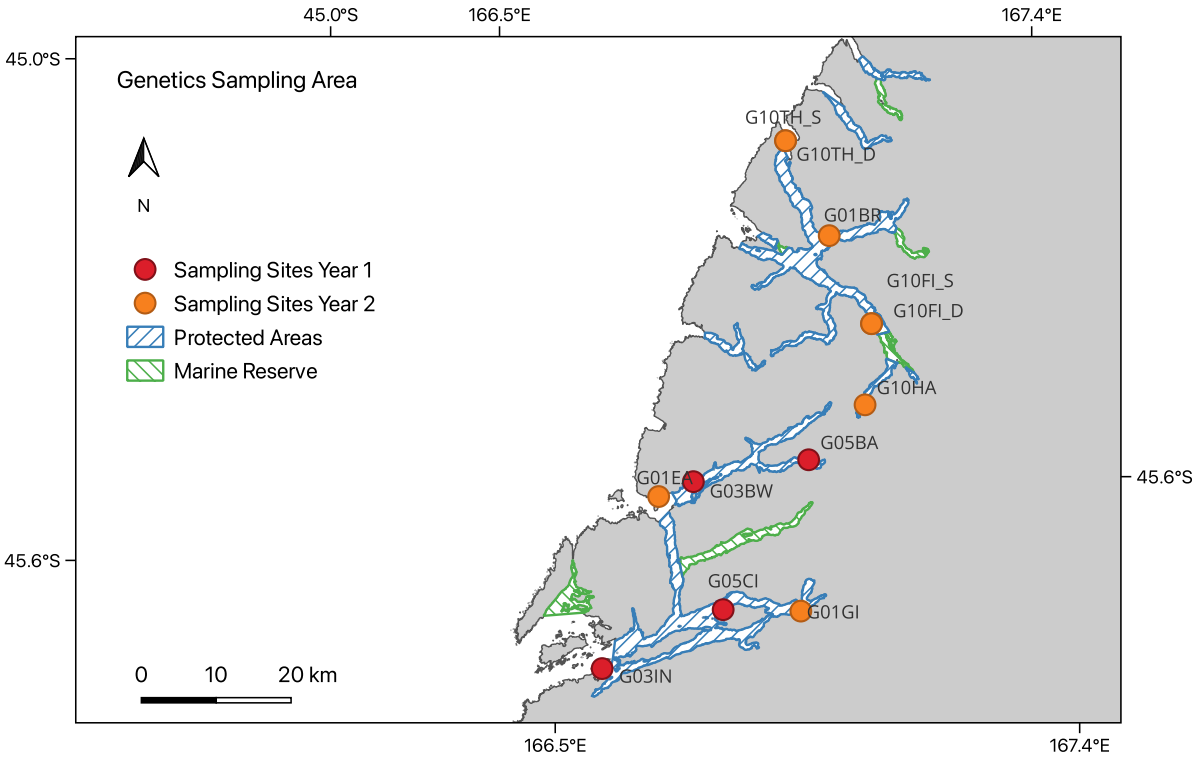

Figure 5. Locations where black coral populations (n=10) have been sampled to date for genetic analysis. This map shows also shallow (S, n=10) and deep genetic sampling (D, n=2).

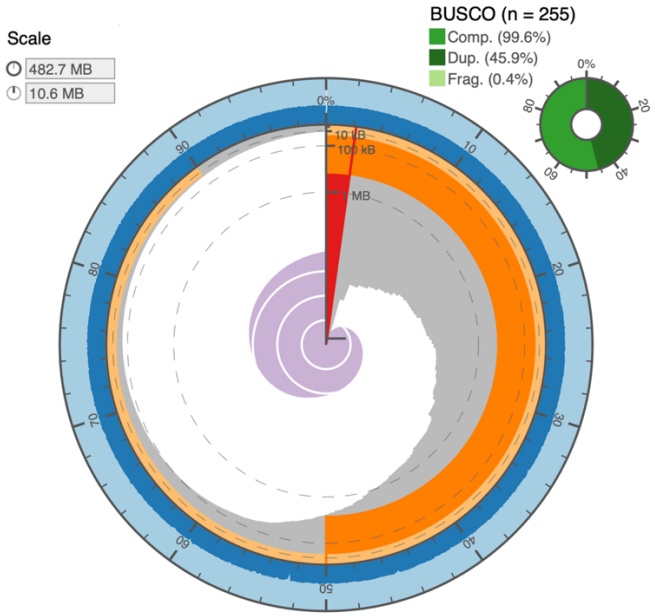

Figure 6. Circular genome plot. The circular genome plot displays the assembly and size of the genome, along with the completeness statistics (BUSCO). The plot's inner radius represents the length of the longest scaffold in the assembly, which is about 10MbT while the red segment within the plot indicates the percentage of the assembly that is in the longest scaffold. The radial axis originates at the circumference and indicates the scaffold length. Grey segments are plotted from the circumference, and the length of the segment at a given percentage shows the cumulative percentage of the assembly that is in scaffolds of at least that length. The dark and light orange arcs that connect to the radial axis indicate the N50 (the longest) and N90 (the shortest) scaffold lengths, respectively. These count for approximately 400kB and 24kB. The total number of scaffolds within a given percentage of the genome is plotted in purple, originating at the plot's center, which is approximately 6000.Successive orders of magnitude from 10 scaffolds onwards are represented by white scale lines. The fill color of the circumferential axis indicates the percentage base composition of the assembly: AT = light blue (around 40%); GC = dark blue (around 60%); N = grey. The completeness of BUSCO genes is shown in the smaller plot in the upper right corner and are represented by the mid, light, and dark green colors, respectively while 255 represent the eukaryotic lineage BUSCO selects to input the dataset. Results indicate a high score level of completeness (99.6%), however, with moderate score level of duplicate regions (45.9%), and fragmentation (0.4%). In very general terms, and in today's standards N50 > 1M and assembly size covering >95% of the expected size are considered a good assembly.

Future plans

We have one, possibly two, further research cruises planned as part of this project, where will conduct more deep and shallow water abundance surveys, and continue to measure black coral growth. Our main plan for the next year is to complete the population genetics analyses and build our population model. We will also develop a plan for using the fisheries data when the data is received from DOC/MPI (this can't be progressed until the data is available and an approach determined to assess overlap of effort reported at multiple spatial scales with coral distribution).

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