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Ageing deep-sea corals

- black coral *Bathypathes patula*

Prepared for Conservation Services Programme, Department of Conservation – Te Papa Atawhai

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Executive summary

Ten individual colonies of the Antipatharian black coral *Bathypathes patula* were selected from the National Invertebrate Collection (NIC) for the purposes of this ageing study. Corals were selected based on their size, completeness of the specimen and the regional water mass they grew in. Corals from the Chatham Rise and the Bay of Plenty were selected as this work will then support other comparable ageing work on deep water corals and the water masses for these two regions are reasonably well understood.

Thin section preparations of the main-stem of these ten specimens were observed with compound microscopes. Two interpretation protocols were defined to describe the zone structure observed and counts were made of these zone structures.

Four of the specimens were also sampled for radiocarbon assay. The results of these assays will be used to independently verify if either of the zone structures observed reflect annual periodicity. If this proves to be the case then the observed zone structure will be used to generate age and growth rates estimates.

1 Background

Deep-sea corals are a highly diverse group of marine organisms several of which are characterised by their overall slow growth and extreme longevity. Due to their fragile forms, skeletal composition, and location, they are vulnerable to various anthropogenic threats with some groups expected to have little to no ability to recover. Impacts include; fishing such as bottom trawling and bottom long-lining, mineral exploration and deep-sea mining. There are also the environmental impacts predicted from climate change including sea water warming and ocean acidification. To better inform risk assessments for these deep-sea corals, a knowledge of their age and growth is key to understanding coral regeneration times following trawl disturbances or other damage.

In year one of this project a report was prepared by Tracey et al (2018) that included a literature review describing the methods to age coral species and a recommendation to obtain accurate age and growth data for a key protected coral species. The main methods applied to measure age and growth of deep-sea corals were reviewed. These were (1) direct observation e.g., in situ measurements or in-aquaria experiments of linear growth or surface extensions; polyp addition rate; estimation of calcification rates (e.g., using the buoyant weight technique), (2) enumeration of skeletal growth bands and (3) radiometric analyses. The advantages and disadvantages of each method were then discussed. Recommended next steps for coral ageing research in the New Zealand region and details of an appropriate method to apply to obtain accurate age and growth data were made in year 1.

A previously determined 'High Risk' protected coral species, specifically the Antipatharian black coral genus *Bathypathes* (Family Schizopathidae) was recommended as a study species. The analytical proposed was radiocarbon (^{14}C) dating of base and tip regions of colonies, combined with growth ring counts from around 10 basal sections for selected specimens of species *B. alternata* or *B. patula*. The micro-milling of material, and the interpretation of results were to be carried out in Year 2 of the project.

Tracey et al (2018) proposed these two methods to enable comparisons with other ongoing studies and previous work that has been undertaken in New Zealand and elsewhere in the world, and also based on the review of the ageing methods presented where the success of the chosen methods was highlighted. According to the literature, these methods have worked well for black corals in other parts of the world (e.g., see Sherwood & Edinger 2009). The micro-milling of skeletal material and preparation of basal and tip thin sections to obtain count zones had previously been carried out at NIWA using bamboo octocoral species (*Keratoisis* sp. and *Lepidisis sp.*) thin sectioning method described by Tracey *et al.* (2007)). Previous growth rate data has been obtained using ^{14}C dating from the reef-forming stony branching coral *S. variabilis* (Neil *et al.* in review).

1.1 Zone counts on skeletal sections:

Enumeration of growth bands is ideal for deep-sea corals that have a high contrast between growth bands and has proved successful for bamboo corals and black corals (Roark *et al.* 2005, Love *et al.* 2007, Rogers *et al.* 2007, Tracey *et al.* 2007, Noe *et al.* 2008). However even in these high contrast corals there are potential limitations to this method (see summary in Tracey et al 2018). The majority of studies that have successfully applied counting of growth bands to determine ageing and growth rate are for gorgonian octocorals. Ages for a variety of gorgonians have been obtained based on

radial growth rates calculated from enumeration of growth bands, and these include 400 year old *Keratoisis* bamboo corals, 100 year old *Primnoa* spp., and 60 year old *Lepidisis* spp. (Mortensen and Buhl-Mortensen 2005, Sherwood *et al.* 2005, Thresher *et al.* 2004, Tracey *et al.* 2007, Thresher *et al.* 2007, Sherwood and Edinger 2009). Black corals have also been successfully aged using growth counts, with ages from 150 years old to 480 years old for *Antipathes dendrochristos* and *Leiopathes glaberrima*, respectively (Love *et al.* 2007, Williams *et al.* 2007). In a study of the black coral *Stauropathes arctica* ring counts of 55–58 were obtained (Sherwood & Edinger 2009), with the authors noting that radiocarbon dating constrained these ages to 55 and 82 years respectively. Growth rates of black corals via growth band enumeration reveal low radial growth from 0.008–0.140 µm y^{-1} (Love *et al.* 2007, Prouty *et al.* 2011). The gorgonian bubblegum corals (e.g., *Paragorgia*) and red precious corals (e.g., *Corallium*), however, have not been aged successfully using growth band counts due to inherent complex or poorly defined banding patterns (Griffin and Druffel 1989, Andrews *et al.* 2005).

1.2 Radiocarbon dating to validate zone counts

The most common radiometric method used in deep-sea corals is dating with the radiocarbon (^{14}C) isotope. This method relies on the fact that a very small amount of natural carbon in the atmosphere is radioactive in the form of ^{14}C and this is incorporated into the coral skeleton when it forms its calcium carbonate or protein and chitin (Adkins *et al.* 2002, Tracey *et al.* 2003, Consalvey *et al.* 2006). Because the half-life of radiocarbon is known to be 5,730 years, this method can be used to reliably age specimens to 50,000 years ago; beyond that, the activity of ^{14}C becomes too tiny to detect (Coleman 1991, Sheridan 1995). During the 1950s–60s, there was rapid increase of atmospheric ^{14}C resulting from the testing of nuclear devices; this so-called "bomb carbon" can also be used as a tool to calibrate ages, providing a reference point for more recent samples (Coleman 1991, Tracey *et al.* 2003, Roark *et al.* 2009; Sherwood & Edinger, 2009). Again there are limitation with this method and these were outlined in Tracey et al (2018). Despite apparent disadvantages however, radiocarbon dating has been successfully used on a number of coral species, including stony corals, black corals and gorgonian octocorals (Druffel *et al.* 1990, Roark *et al.* 2006, Carriero-Silva *et al.* 2013, Prouty *et al.* 2016, Neil *et al.* in review).

This report describes the methodology to determine the age and growth characteristics of a key high risk New Zealand deep-sea coral species, the black coral *B. patula*. Sample selection, micro-milling of material and sectioning methods, and the interpretation of age result data are presented. Age and growth characteristics of the black coral *B. patula* are provided.

This document meets the reporting requirements for Year Two of the Conservation Services Programme (CSP), Department of Conservation (DOC) Project POP2017-07 Objective to "Develop a methodology to determine the age and growth characteristics of key high risk New Zealand deep-sea (cold-water) coral species".

2 Methods

The selection of a priority species to age was based on the risk assessment priority list and the literature review, coupled with availability of samples (location and total numbers), and complementary research under the paleoclimate Marsden project, (*Corals, currents, and phytoplankton: Reconstructing 3000 years of circulation and marine productivity in the world's largest ocean gyre,* NIW1602).

Two methods to age the coral colonies were applied:

1. radiocarbon (^{14}C) dating
2. preparation of 10 thin basal sections to obtain zone counts

The aim was to age two colonies and obtain three ^{14}C dates per colony from the base and growing tip region to obtain radial and linear growth estimates. Analytical methods followed that of Tracey *et al.* (2007) and Sherwood & Edinger (2009).

2.1 Sample selection

To help select the colonies we focused on corals from Chatham Rise, from where we have the modern ^{14}C reservoir age but also selected samples from the Bay of Plenty region to test compare data from different regions to assess the variability in the growth rates between areas with different oceanography and food availability. As both regions were also included in the Marsden Project and so there was value in comparing age data between species as well as by region.

The samples were selected from existing specimens collected by fisheries observers and researchers and held in the NIWA Invertebrate Collection (NIC). A plot of the distribution data for *B. patula* helped decide on colony sample numbers by region (Figure 2-1).

2.2 Preparation of material

Once suitable samples had been identified small (less than 10mm sections were excised from the growing tips and basal portion of the corals main stem. As many of the samples had been broken during the collection process, additional sections were taken up the main stem so that reliable estimates of linear growth rate could be generated (See Figure 2-2). The samples from the growing tips were then split into two fragments, one for radiocarbon dating the other for thin section preparation. The main stem sections were first micro-milled for radiocarbon and then the remainder of the section was used for thin section preparation.

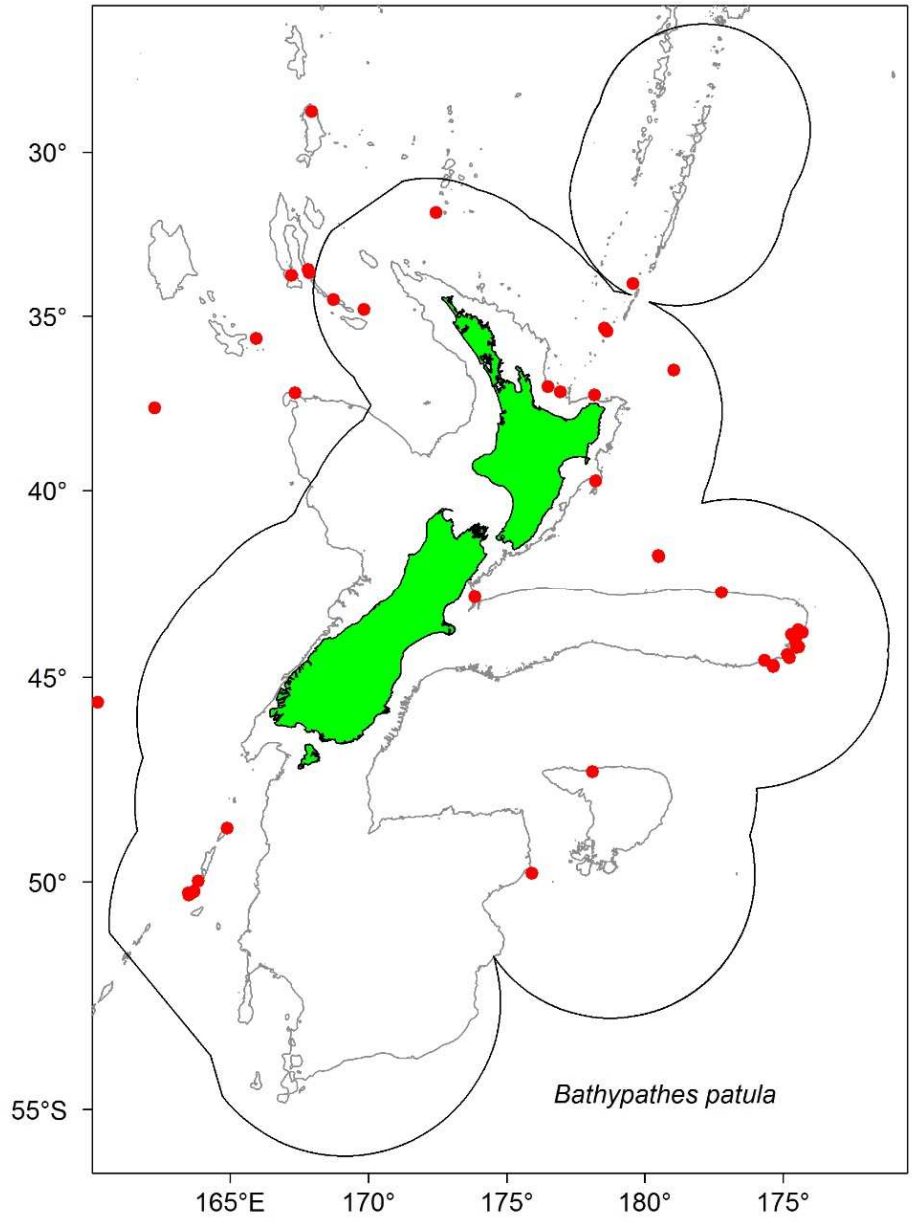

Figure 2-1: Distribution of *Bathypathes patula* for the New Zealand region.

Figure 2-2: ***B. patula*** **NIWA49468 specimen showing where samples were extracted for analysis.**

2.3 Micro-milling of material

From previous work I had noted that black corals sections are prone to drawing resin up through their porous matrix via capillary action, so sections could not be bonded with resin to a baseplate for milling as is our usual practice. Instead I manufactured a chuck to hold the section during the milling process, thus eliminating the risk of resin contaminating the radiocarbon samples.

Powdered radiocarbon samples were extracted from the edge and core of sections using a New Wave™ micromill with a 0.5 mm Brassler H2.11.006 milling burr. The edge samples comprised material from the outer 250 microns of the section, the core samples were obtained from material within a 750 micron radius of the primordium. We attempted to extract at least 1.5 mg of material per sample. The samples were then weighed and sent to Stewart Fallon at the Australian National University (ANU) Radiocarbon Dating Laboratory for analysis.

2.4 Thin sectioning method

Growth bands in deep-sea coral skeletons, which are formed repeatedly over discrete time periods, can be used to determine ages and growth rates. This method involves counting bands or zones

formed over a given distance of skeleton and is comparable to counting the rings of trees or fish otoliths.

Coral stem sections were embedded in clear two-part epoxy resin and sectioned with a diamond-wafering saw, the sections were polished and mounted on a glass microscopy slide then ground and polished until they were an optimum thickness for viewing the growth zone structure using transmitted light. The optimum thickness for sections of this species was about 250-300 microns. This is a standard technique for thin section preparation see (Andrews *et al.* 2002, Mortensen and Buhl-Mortensen 2005, Tracey *et al.* 2007).

The enumeration of periodic growth bands or increments then took place.

2.4.1 Radiocarbon dating method

Analyses were carried out at the Radiocarbon Dating Laboratory, Australian National University, Melbourne.

Prior to analyses, the samples were cleaned (e.g., acid leached) of black crusts and endolithic activity to remove any younger contaminant ^{14}C, which may alter results (Adkins *et al.* 2002; Neil *et al.* in review). The samples were prepared via acidification or combustion (e.g., conversion of skeletal carbons to CO_2) and converted to graphite (Adkins *et al.* 2002, Roark *et al.* 2006). The graphite targets are then analysed by Accelerator Mass Spectrometry (AMS)

The radiocarbon dating analyses were undertaken at the Radiocarbon Dating Laboratory, Australian National University, Melbourne. This facility was selected to date the samples as we were able to link in with the analyses being carried out at the same time by NIWA/ VUW Marsden study (Hitt in prep).

3 Results

3.1 Radiocarbon analysis

The milled samples listed in Table 3-1 have been sent to ANU for radiocarbon dating. The radiocarbon results will be interpreted once all the samples have been run.

3.2 Thin sections for zone counts

To generate zone counts thin section preparations of mainstem sections were viewed under a compound microscope with transmitted light. As an aid to zone interpretation they were also viewed using ultra-violet light following the methodology of Sherwood and Edinger (2009). For this species zone counts were made using transmitted bright field lighting. Ultraviolet illumination was a useful aid for defining the observed zone structure.

The zone structure for this species is very complex, and can be interpreted in a number of different ways. There is an initial coarse coloured banding pattern largely defined by alternating darker and lighter zones (Figure 3-1), when observed under lower power (10 – 20x). On closer examination

under higher power (100 – 200x), there is a reasonably regular fine scale banding pattern (Figure 3-2).

Zone counts were made using both interpretation protocols (Table 3-2).

Table 3-1: Samples extracted for radiocarbon dating.

	NIWA_ID	Date of collection	Sample site	Sub-site	Sample Name	Net WT (mg)	Rough age
Bathypathes sp. or *Alternata sp.*	NIWA24190	10/09/1998	A		24190-A	2.28	present
Bathypathes sp. or *Alternata sp.*	NIWA24190	10/09/1998	B	core	24190-B1	1.21	young
Bathypathes sp. or *Alternata sp.*	NIWA24190	10/09/1998	B	edge	24190-B2	1.83	present
B.patula	NIWA85940	27/03/2000	A		85940-A	1.79	present
B.patula	NIWA85940	27/03/2000	B	core	85940-B1	1.98	young
B.patula	NIWA85940	27/03/2000	B	edge	85940-B2	1.41	present
B.patula	NIWA85940	27/03/2000	C	core	85940-C1	1.20	mid
B.patula	NIWA85940	27/03/2000	C	edge	85940-C2	3.11	present
B.patula	NIWA49468	26/02/2009	A		49468-A	1.52	present
B.patula	NIWA49468	26/02/2009	B	core	49468-B1	1.87	mid
B.patula	NIWA49468	26/02/2009	B	edge	49468-B2	4.22	present
B.patula	NIWA49468	26/02/2009	D	core	49468-D1	3.09	old
B.patula	NIWA49468	26/02/2009	D	edge	49468-D2	3.59	present
B.patula	NIWA47911	08/03/2008	A		47911-A	2.35	present
B.patula	NIWA47911	08/03/2008	B		47911-B	3.30	young
B.patula	NIWA47911	08/03/2008	C		47911-C	5.74	young
B.patula	NIWA47911	08/03/2008	D		47911-D	9.21	young
B.patula	NIWA47911	08/03/2008	E		47911-E	10.00	young
B.patula	NIWA47911	08/03/2008	F	core	47911-F1	2.50	old
B.patula	NIWA47911	08/03/2008	F	edge	47911-F2	1.48	present

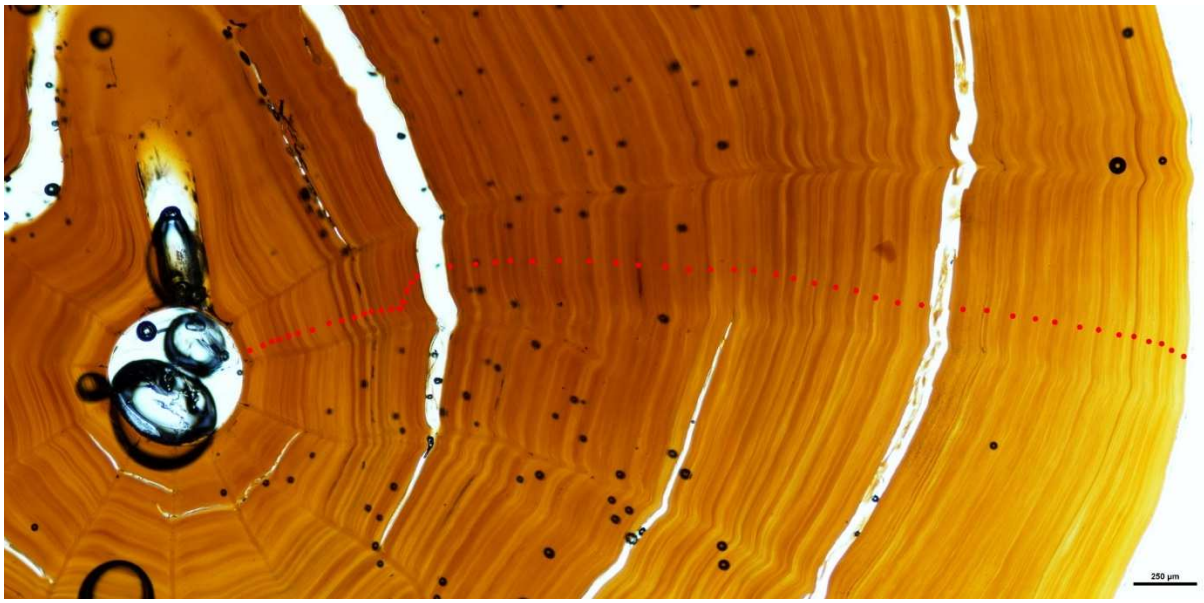

Figure 3-1: Basal section of coral NIWA47911 showing the coarse coloured banding interpretation of the observed zone structure. The specimen shows 54 zones marked with red dots.

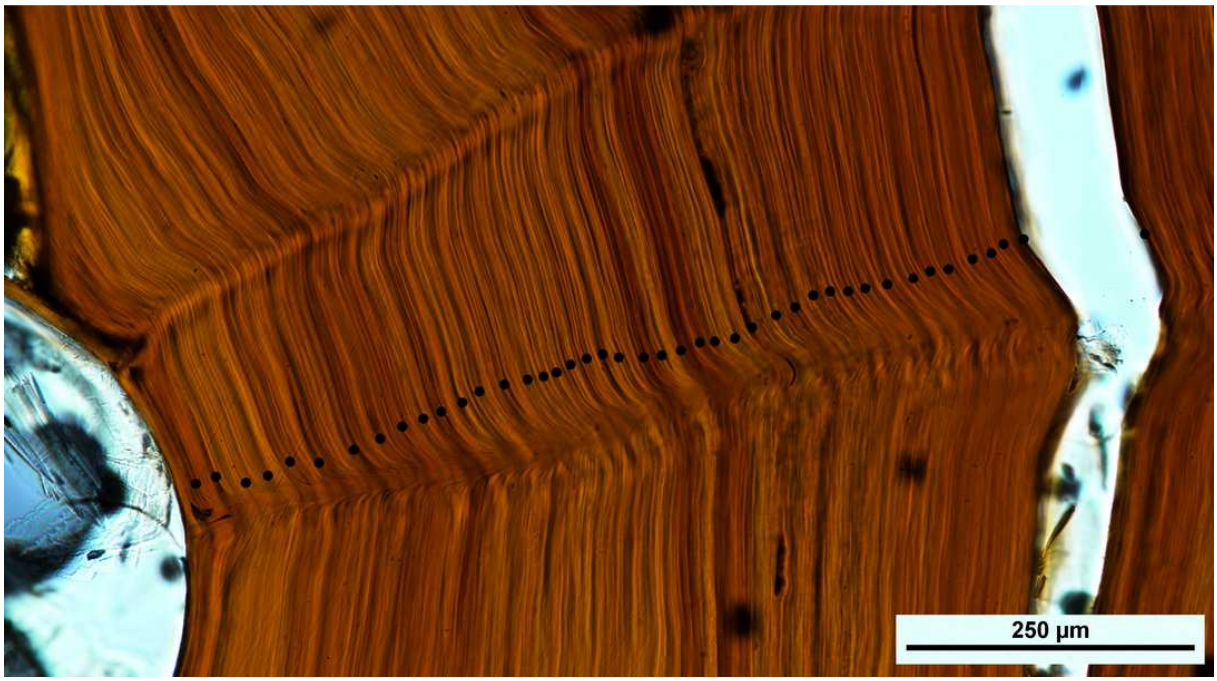

Figure 3-2: Central portion of the basal section of coral NIWA47911 showing the fine scale interpretation protocol of the observed zone structure. The specimen shows 42 zones marked with black dots across this inner region of the section; the whole section showed 211 zones.

Table 3-2: Zone counts from thin sections.

Sample Name	Sample region	colour zone counts	Fine scale Zone counts
NIWA 24190-B	Base	37	78
NIWA 85940-C	Base	54	176
NIWA 49468-A	Tip	4	11
NIWA 49468-B	Mid stem	55	370
NIWA 49468-C	Mid stem	60	374
NIWA 49468-D	Base	72	481
NIWA 47911-A	Tip	4	10
NIWA 47911-B	Near tip	4	12
NIWA 47911-C	Near tip	5	16
NIWA 47911-D	Near tip	7	19
NIWA 47911-F	Base	54	211
NIWA 66335-A	Base	94	301
NIWA 42807-A	Base	66	221
NIWA 66354-A	Base	66	221
NIWA 42812-A	Base	74	359
NIWA 66337-A	Base	104	285
NIWA 47879-A	Base	193	406

4 Summary

4.1 *Bathypathes patula* age estimates

This data presents only provisional ageing estimates. When the radiocarbon samples have been assayed and analysed then the radiocarbon results will be correlated with the provisional zone counts to determine which interpretation protocol reflect annual periodicity. If neither methodology correlates with the radiocarbon results then the thin sections will be revisited to see if there is an interpretation of the zone structure that would be consistent with the radiocarbon results.

Research on another Antipatharian species, *Antipathes dendrochristos,* also found two similar interpretations of the zone structure, in this case the coarser coloured banding structure proved to correlate with their radiocarbon and lead^{210} results.

Age estimates and growth rates will be generated for our samples when the radiocarbon assays and analysis is completed and can be used to validate the appropriate ageing methodology to apply to our samples.

In late November 2018, a visiting scientist and specialist in Antipatharian taxonomy (Jeremy Horowitz JCU) was reviewing the identifications of some of the black corals in the NIC. During this process one of the corals selected for this current ageing research was given a revised identification. The coral NIWA24190 has now been identified as *B. bifida*, *B. platycaulus*, or genus *Alternata*. Further taxonomic review of this specimen is ongoing.

4.2 Comparisons with recent NZ coral age data

The results of this project will be compared a number of age and growth studies that have recently been or are currently being undertaken for key deep-sea corals in the New Zealand region. Specifically

1. Stony branching coral *Solenosmilia variabilis* (Neil et al submitted)
2. NIWA funded project to radiocarbon date and age two key deep-sea stony branching corals *Madrepora oculata* from the Northwest Chatham Rise Graveyard Knolls, Northeast Chatham Rise Andes Knolls, and East Coast North Island region Ritchie Hills region, and *Goniocorella dumosa* from the Graveyard Knolls (NIWA unpubl. data).
3. Black corals *study on Leiopathes secunda; Antipathella fiordensis;* and two unknown *Antipatharia*) from contrasting water masses north (Bay of Plenty) and southwest of New Zealand (NIWA and Victoria University of Wellington paleoclimate Marsden funded project:
4. Preliminary results for *Bathypathes* showed the uncalibrated age ranges from the inner to outer zone of 3250 to 1173 ^{14}C years — approximately 2000 years old (sample 35104); and from 1960 to 506 ^{14}C years — approximately 1500 years old (sample 64334), (Neil H, Sinclair D, Hitt N unpubl. data).

The combination of all of this age and growth research provides the region with a significantly improved dataset of age data for key high risk New Zealand deep-sea coral species.

5 Acknowledgements

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