POP2022-03 Protected Coral Reproduction

Milestone 3 - Year 1 Progress Update

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Climate, Freshwater & Ocean Science

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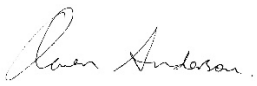	Reviewed by:	Owen Anderson
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Executive summary

This report summarises progress towards Milestone 3: “Year 1 Progress report” of a 2-year deep-sea protected coral reproduction study (POP2022-03) for the Conservation Services Programme, Department of Conservation. It should be noted that since the contract was not signed until February 2023, this Year-1 progress report is a summary of the first five months of the project rather than a full 12 months.

This project builds on a previous literature review of the reproductive and larval process of New Zealand protected deep-sea corals. In that review, the following species were identified as suitable reproduction study species: the stony cup coral *Desmophyllum dianthus* (Order Scleractinia), the stony reef-forming corals *Goniocorella dumosa* and *Enallopsammia rostrata* (Order Scleractinia), and the alcyonacean gorgonian octocorals *Primnoa notialis* and *Paragorgia arborea*.

Within the current project, histological methods are being used to understand reproductive strategies for the above species, together with some additional histological samples of black corals (Antipatharia) and hydrocorals (Stylasteridae) to attempt to obtain reproductive information for each of the New Zealand protected coral groups.

The specific objectives of this project (POP2022-03) are to:

1. Address knowledge gaps in reproductive strategies for protected coral species in the New Zealand region.
2. Use available life history and reproductive data to inform relative productivity/vulnerability parameters for relevant concurrent and future research.

Histological methods are described and illustrated examples of histological sections shown for all protected coral groups. The figures help describe the reproductive state from the available material and are helpful first steps toward understanding appropriate sample preparation methodologies. Future research steps are presented.

1 Background

The specific objectives of this project (POP2022-03) are to:

1. Address knowledge gaps in reproductive strategies for protected coral species in the New Zealand region.
2. Use available life history and reproductive data to inform relative productivity/vulnerability parameters for relevant concurrent and future research.

This project builds on a previous literature review of the reproductive and larval process of New Zealand protected deep-sea corals conducted as part of DOC project BCBC2020-01 (Tracey et al., 2021). From that review, five candidate species were identified for a targeted reproduction study: the stony cup coral *Desmophyllum dianthus* (Order Scleractinia), the stony reef-forming corals *Goniocorella dumosa* and *Enallopsammia rostrata* (Order Scleractinia), and the alcyonacean gorgonian octocorals *Primnoa notialis* and *Paragorgia arborea* (Figure 2-1 A-E). These species were identified as suitable study species because they were identified as being at high and medium risk in a pilot coral risk assessment (Clark et al. 2014), and because there were adequate samples available for each species in the NIWA Invertebrate Collection (NIC) to carry out such a study. Additionally, suitable NIC specimens will be identified for black coral (Order Antipatharia) and hydrocoral (Family Stylasteridae) species to trial some histological analyses and attempt to obtain reproductive information for each of the New Zealand protected coral groups (Figure 2-1 F - G).

Here we report on progress towards Milestone 3: “Year 1 Progress report”. Note that the contract was signed in February 2023, so this year 1 progress report is a summary of the first five months of the project rather than a full 12 months.

2 Sampling for histological pilot testing

The specimens held within the NIC and identified as potential candidates for histology are given in Appendix A. Following an assessment of polyp condition, a subset of 10 specimens were selected for initial pilot histology analyses (Table 2-1) including 6 scleractinian specimens (3 *Goniocorella dumosa* and 3 *Enallopsammia rostrata*), 2 antipatharian specimens (1 *Leiopathes bullosa* and 1 *Sibopathes* sp.), and 2 stylasterid specimens (1 *Stylaster eguchii* and 1 *Errina* sp.). The alcyonacean gorgonian octocorals (*Primnoa notialis* and *Paragorgia arborea*) were not included in these initial trials due to time constraints and an initial focus on the methodology required for the highly calcified scleractinian corals.

Specimens were deemed suitable for the histological pilot testing if there appeared to be adequate live tissue at the time of collection. Specimens that were not previously fixed in formalin were post-fixed in 10 % buffered formalin. Tip and branch polyp sections were clipped from each specimen and placed in labelled cassettes for histology. All samples were processed at the Gillies McIndoe Research Institute in Wellington, with the methods detailed below.

Polyp samples with calcified skeletons (Orders Scleractinia and Anthoathecata (stylasterid hydrocorals)) were decalcified with either ethylene diamine tetra-acetic acid (EDTA) or formic acid. EDTA is a relatively gentle decalcifying agent, which helps to limit degradation of soft tissues, whereas formic acid is a moderately aggressive decalcifying agent. The decalcifying solution was changed every day and samples were monitored until the decalcification was complete. No decalcification was required on the Antipatharia samples.

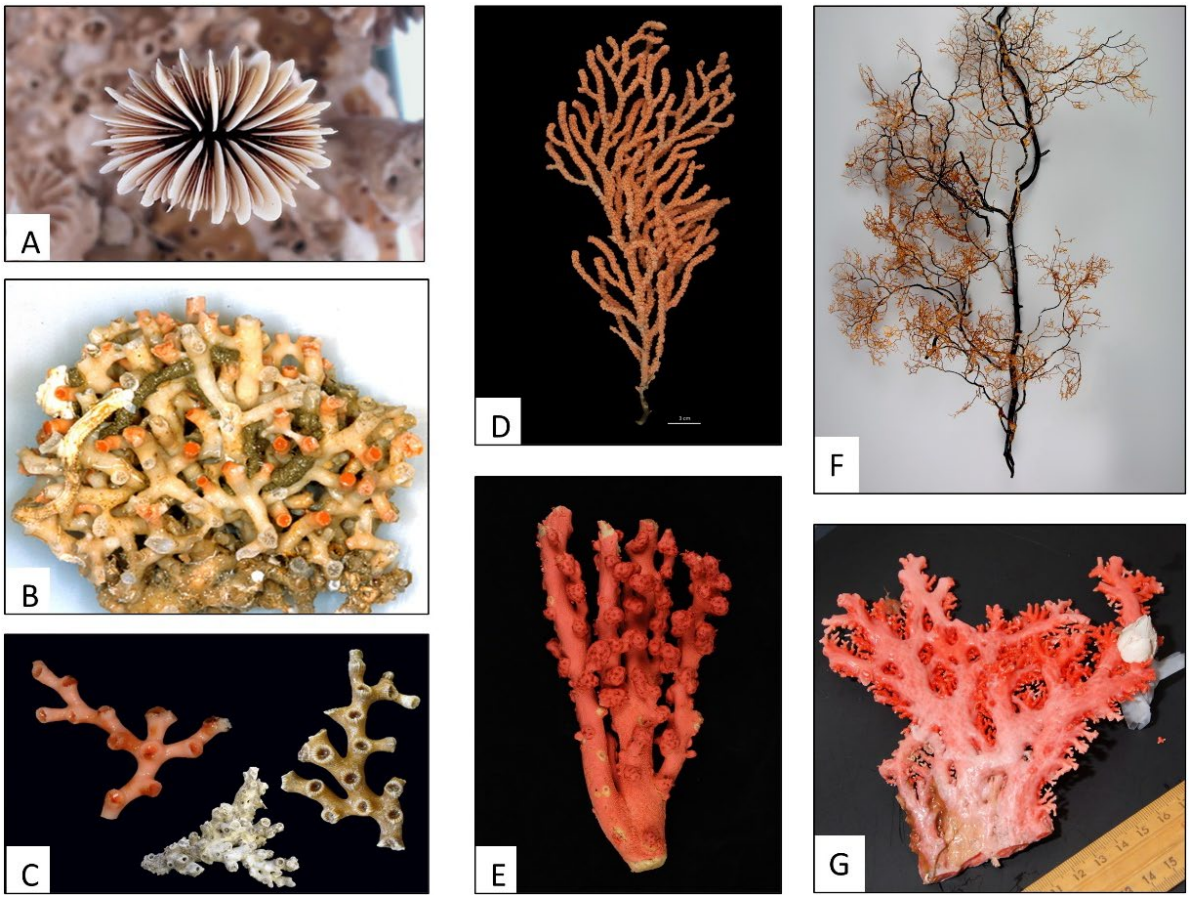

Figure 2-1: Example specimen photographs of corals selected for the reproductive study. A) *Desmophyllum dianthus*; B) *Goniocorella dumosa*; C) *Enallopsammia rostrata*; D) *Primnoa notialis*; E) *Paragorgia arborea*; F) *Leiopathes* sp.; G) *Errina* sp. (NIWA images).

The decalcified coral specimens were processed overnight in an automated tissue processing machine. Specimens were embedded in paraffin wax in a longitudinal orientation. Sections were cut at 4 microns thickness and levels were taken through the polyp to ensure that the region of the polyp containing the gonad tissue would appear in at least one of the sections. Adhesive slides were used to collect the cut sections as decalcified tissue has a tendency to float off clean glass slides during staining. Sections were dried at room temperature and stained with Haematoxylin and Eosin in an automated slide staining machine. Sections were cover-slipped.

Table 2-1: Specimens included in the initial histology pilot testing trials. F = fixed in formalin, EtOH is ethanol. Where the preservation method is "F, EtOH", the specimen has been first preserved in formalin then transferred into ethanol

Catalogue Number	Class	Order	Family	Genus	Species	Original Preservation Method	Remarks
148158	Anthozoa	Scleractinia	Dendrophylliidae	*Enallopsammia*	*rostrata*	F, EtOH	
148157	Anthozoa	Scleractinia	Caryophylliidae	*Goniocorella*	*dumosa*	F, EtOH	
148101	Anthozoa	Scleractinia	Caryophylliidae	*Goniocorella*	*dumosa*	F, EtOH	
148159	Anthozoa	Scleractinia	Dendrophylliidae	*Enallopsammia*	*rostrata*	F, EtOH	
102472	Anthozoa	Scleractinia	Caryophylliidae	*Goniocorella*	*dumosa*	EtOH	Post-fixed
43171	Anthozoa	Scleractinia	Dendrophylliidae	*Enallopsammia*	*rostrata*	EtOH	Post-fixed
53045	Anthozoa	Antipatharia	Leiopathidae	*Leiopathes*	*bullosa*	EtOH	
2071	Anthozoa	Antipatharia	Cladopathidae	*Sibopathes*		F, EtOH	
91243	Hydrozoa	Anthoathecata	Stylasteridae	*Stylaster*	*eguchii*	EtOH	Post-fixed
77555	Hydrozoa	Anthoathecata	Stylasteridae	*Errina*		F, EtOH	

3 Preliminary histological analyses

There was a long delay in obtaining access to a suitable histological preparation facility (caused by COVID-19 and earthquake strength issues of buildings). However, access to experienced technicians and a high-quality laboratory have been negotiated with the Gillies McIndoe Research Institute in Wellington. While we have not been able to complete the analysis of the pilot histology samples, as was originally envisioned, below we present some example images with brief descriptions.

The following polyp sections were examined under a microscope and photographs were taken to identify reproductive structures.

3.1 Scleractinia (stony corals)

The objective of the histology trials for scleractinian specimens was to determine an appropriate methodology for decalcifying and producing high quality histological sections for observations of reproductive state.

3.1.1 *Goniocorella dumosa* (NIWA Catalogue numbers: 148101 and 148157)

Specimen NIWA148101 (Figure 3-1) was a female *Goniocorella dumosa*. This specimen had been initially fixed in formalin and then transferred to ethanol for long term storage in the NIC. The tissue is well preserved. Sections cut nicely following the decalcification procedure. The section stained evenly and clearly showed the various stages of oocyte in the tissue (Figure 3-2). Even the high lipid containing mature oocytes adhered well to the slide.

Figure 3-1: Fragment of *G. dumosa* specimen NIWA148101. A terminal and sub-terminal polyp were clipped from the matrix for tissue processing.

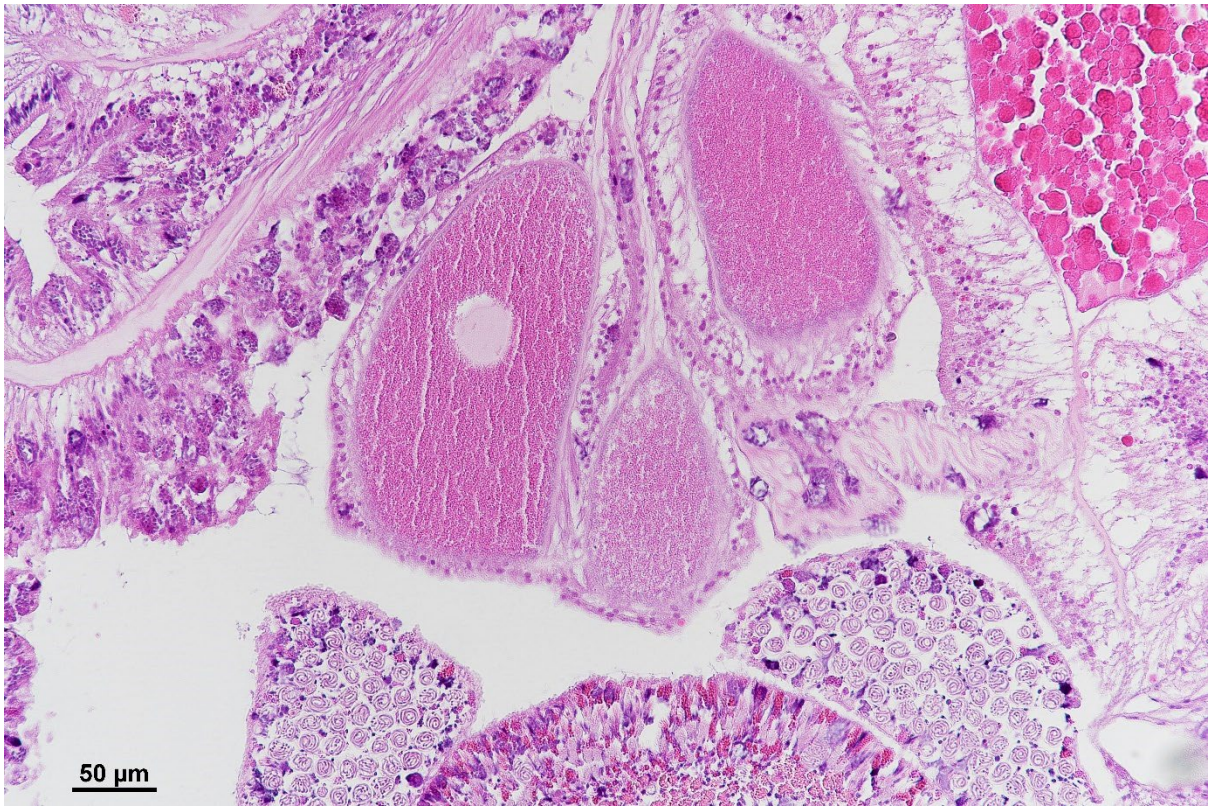

Figure 3-2: Longitudinal section through a terminal polyp of *G. dumosa* specimen NIWA148101. Specimen is a female, maturing oocytes are evident in the centre of the section, a partial mature oocyte can be seen in the top right of the image. 200x magnification. Scale bar is 50 µm.

Specimen NIWA102472 (Figure 3-3) was originally fixed in ethanol then post-fixed in 10 % neutral buffered formalin prior to tissue processing. This specimen is a male (Figure 3-4,Figure 3-5) and the dark purple stained spermiaries containing mature spermatozoa are evident in the centre of the section.

The staining is not quite so vibrant as was observed in specimens of this species that had been initially fixed in formalin (e.g., Figure 3-2) and the preservation of the intracellular organelles is also not as good in this section compared to specimens initially fixed in formalin. However, the quality of the initially ethanol-fixed specimens was adequate to allow accurate characterisation of the reproductive state and will allow accurate morphometric and meristic data to be collected from the histological sections. This will allow us to access a much wider pool of specimens held within the NIC and better sample spatially and temporally across the New Zealand region to ensure a more robust characterisation of the reproductive biology of the coral.

Figure 3-3: Fragment of *G. dumosa* specimen NIWA102472. A terminal and sub-terminal polyp were clipped from the matrix for tissue processing.

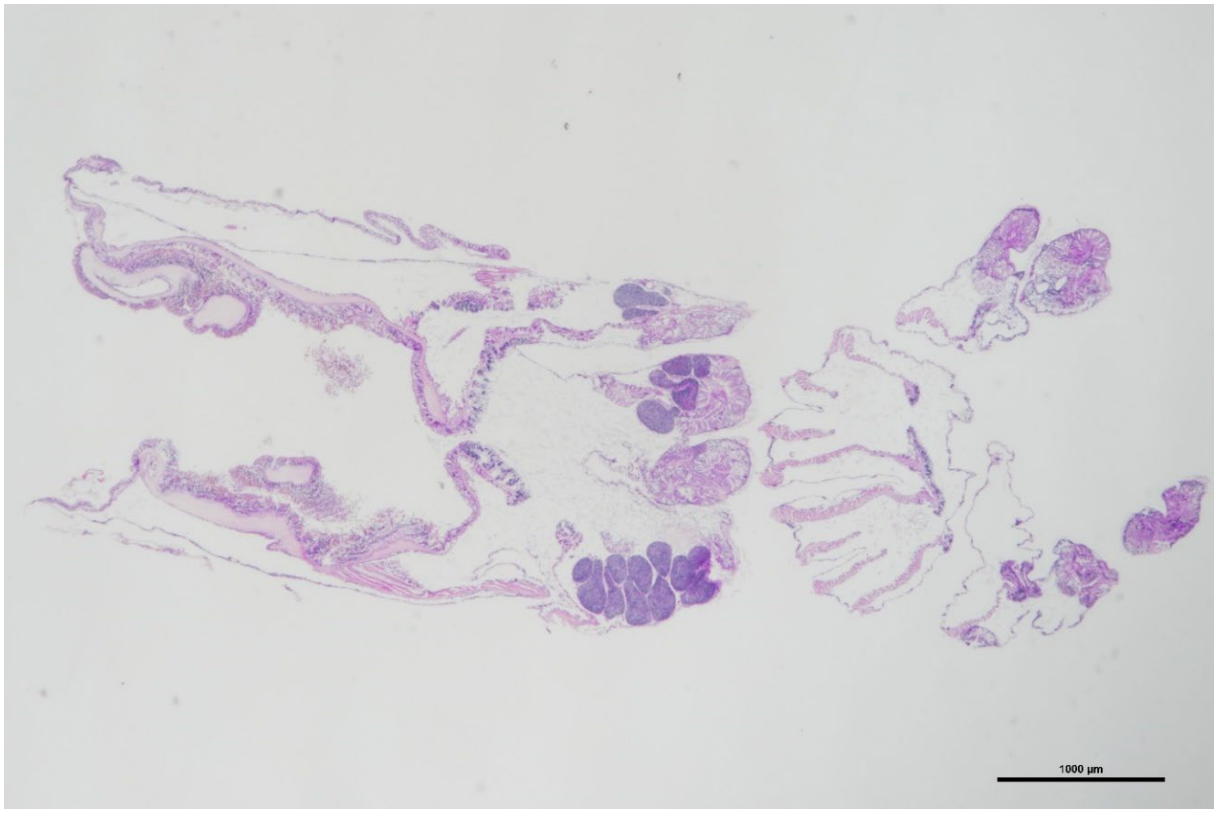

Figure 3-4: Longitudinal section through a terminal polyp of *G. dumosa* specimen NIWA102472. Specimen is a male, the dark purple stained spermiaries containing mature spermatozoa are evident in the centre of the section. 20 x magnification. Scale bar is 1000 µm.

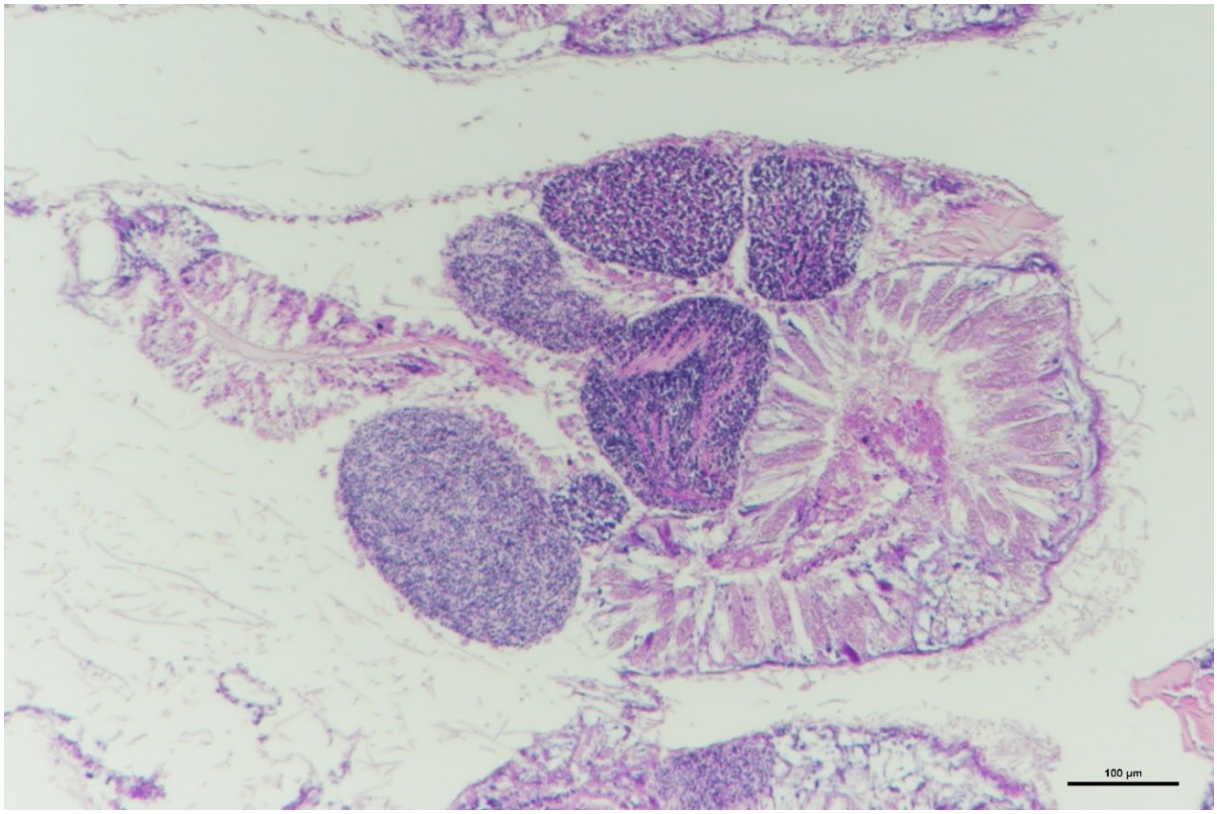

Figure 3-5: Longitudinal section through a terminal polyp of *G. dumosa* specimen NIWA102472. Specimen is male, the dark purple stained spermiaries containing mature spermatozoa are evident in the centre of the section. 132 x magnification. Scale bar is 100 µm.

3.1.2 Enallopsammia rostrata (NIWA148159)

NIWA148159 (Figure 3-6) was a male *Enallopsammia rostrata* (Figure 3-7,Figure 3-8). This specimen was initially fixed in formalin and then transferred to ethanol for long term storage in the NIC. This specimen had a very robust calcified skeleton so required extensive decalcification. Sections were cut cleanly, presenting high-quality stained sections with good preservation of the tissue organelles and intra-cellular structure. The sections showed spermiaries embedded in the mesenteries of the polyp. The spermiaries contain mature spermatozoa, the pink regions in the spermiaries are where bundles of spermatocyte tails have aligned in the lumen of the spermiaries.

Figure 3-6: Fragment of *Enallopsammia rostrata* specimen NIWA148159. A terminal and a sub-terminal polyp were clipped from the matrix for tissue processing.

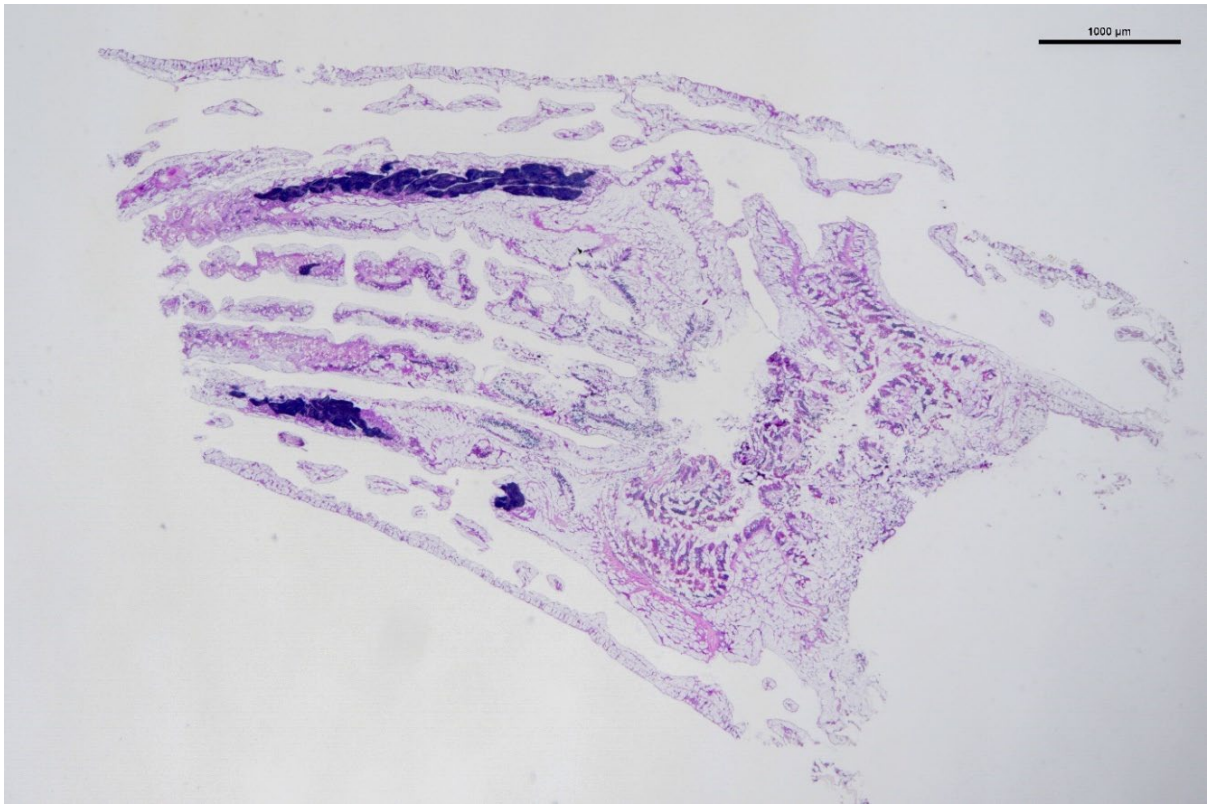

Figure 3-7: Longitudinal section through a terminal polyp of *Enallopsammia rostrata* specimen NIWA148159. Specimen is a male, the dark purple stained spermiaries containing mature spermatozoa are evident in the centre left of the section. 17x magnification. Scale bar is 1000 µm.

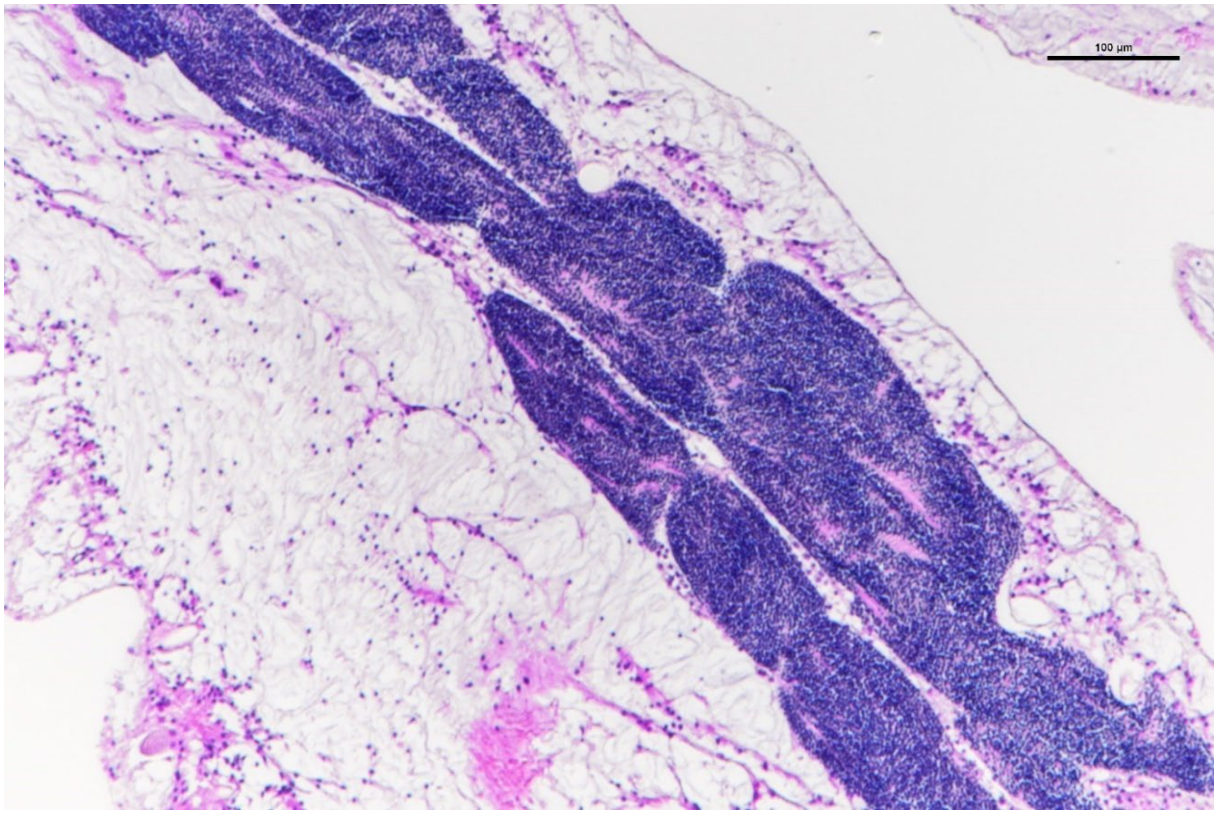

Figure 3-8: Longitudinal section through a terminal polyp of *Enallopsammia rostrata* specimen NIWA148159. Specimen is a male, the dark purple stained spermiaries containing mature spermatozoa are evident in the centre left of the section. 150x magnification. Scale bar is 100 µm.

3.2 Antipatharia (Black corals)

The objective of the histology trial of antipatharian samples was to assess the quality of histological sections that can be prepared from Antipatharia samples to enable clear observations of reproductive state.

Black coral skeleton is comprised of a keratin-like matrix. While it is not calcified, this matrix can be very dense and hard. Trials were done to see if histological sections could be cleanly taken from small specimens clipped from the terminal ends of black coral branches. In this region of the coral the skeleton matrix is generally quite thin and delicate compared to further down the branches.

3.2.1 Leiopathes bullosa (NIWA53045)

The sections through the *Leiopathes bullosa* specimen (NIWA53045) took clean slices through the skeletal matrix and adjacent polyps producing well-stained and complete tissue sections (Figure 3-9, Figure 3-10). This specimen is likely to be male. The round organelles to the right of the image in Figure 3-10, embedded in the mesenteries proximal to the light pink stained connective tissue, are most likely early-stage male spermiaries. This trial indicates that the reproductive state of future sections of *L. bullosa* should be able to be reliably and accurately assessed.

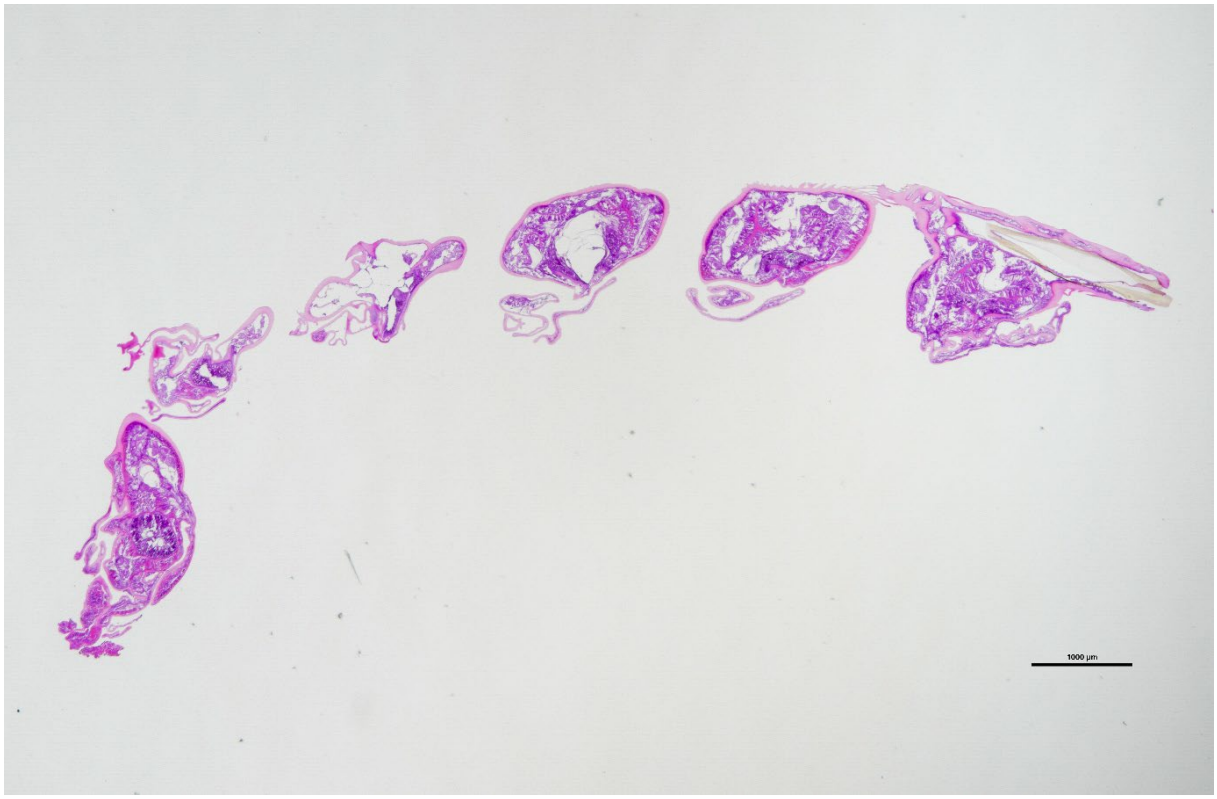

Figure 3-9: Longitudinal section through terminal polyps of *Leiopathes bullosa* specimen NIWA53045. Specimen is likely a male. 12x magnification. Scale bar is 1000 µm.

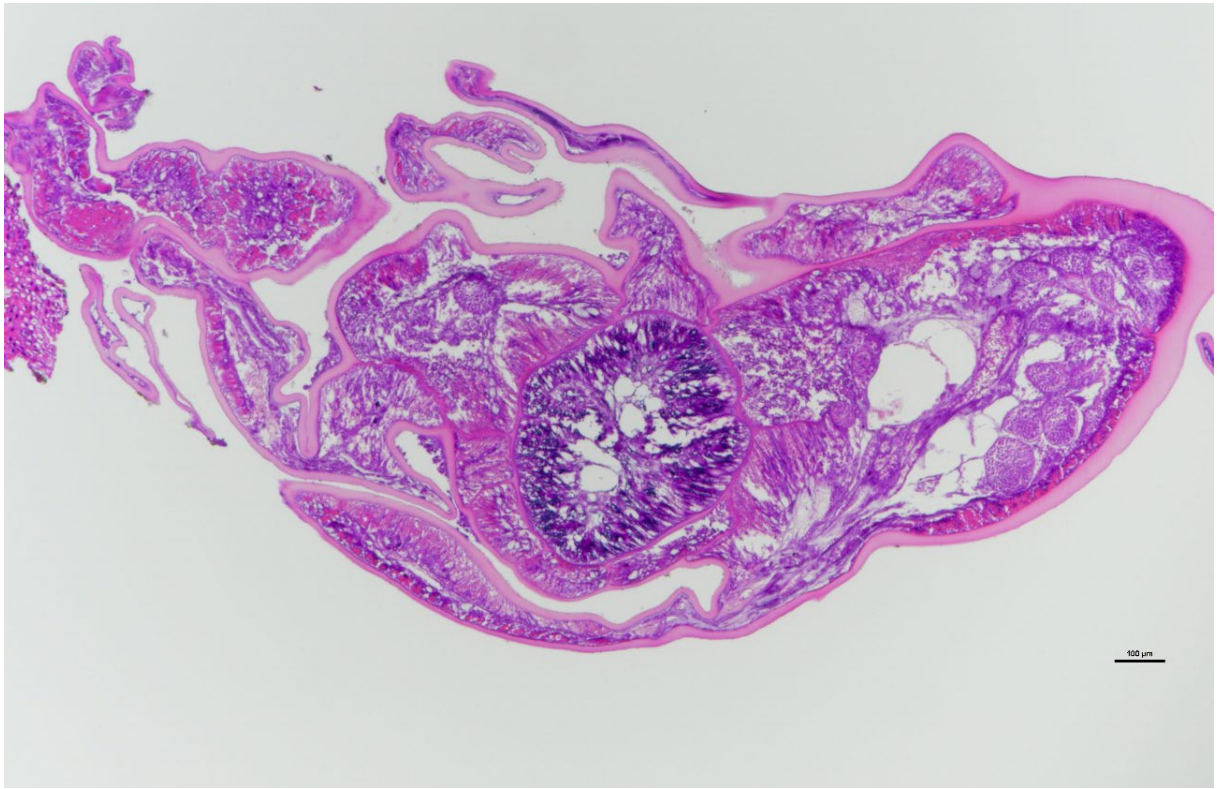

Figure 3-10: Longitudinal section through a terminal polyp of *Leiopathes bullosa* specimen NIWA53045. Specimen is likely a male. The round organs to the right of the image embedded in the mesenteries proximal to the light pink stained connective tissue are likely early stage male spermiaries. 60x magnification. Scale bar is 100 µm.

3.2.2 *Sibopathes* sp. (NIWA2071)

This *Sibopathes sp*. (specimen NIWA2071) was from a sample collected in 2004. It was initially fixed in Formalin prior to transferal to ethanol for long term storage in the NIC. The material the sample was removed from appeared to comprise only the keratin skeleton, and did not appear to have any soft tissue associated with the coral skeleton. The specimen was included, so that the prepared histological section could be checked for the presence of soft tissue, as there are only limited formalin-fixed specimen of black coral available within the NIC.

The keratin skeletal fragment did section and stain well showing that standard histological sections can be prepared from keratinised black coral tissue, however, histology confirmed the absence of soft (or reproductive) tissue (Figure 3-11). Numerous bases of lateral spines can be seen starting to grow out of the main branch skeleton. This black coral specimen may have been dead at capture, resulting in a specimen with no adherent soft tissue on the skeletal matrix.

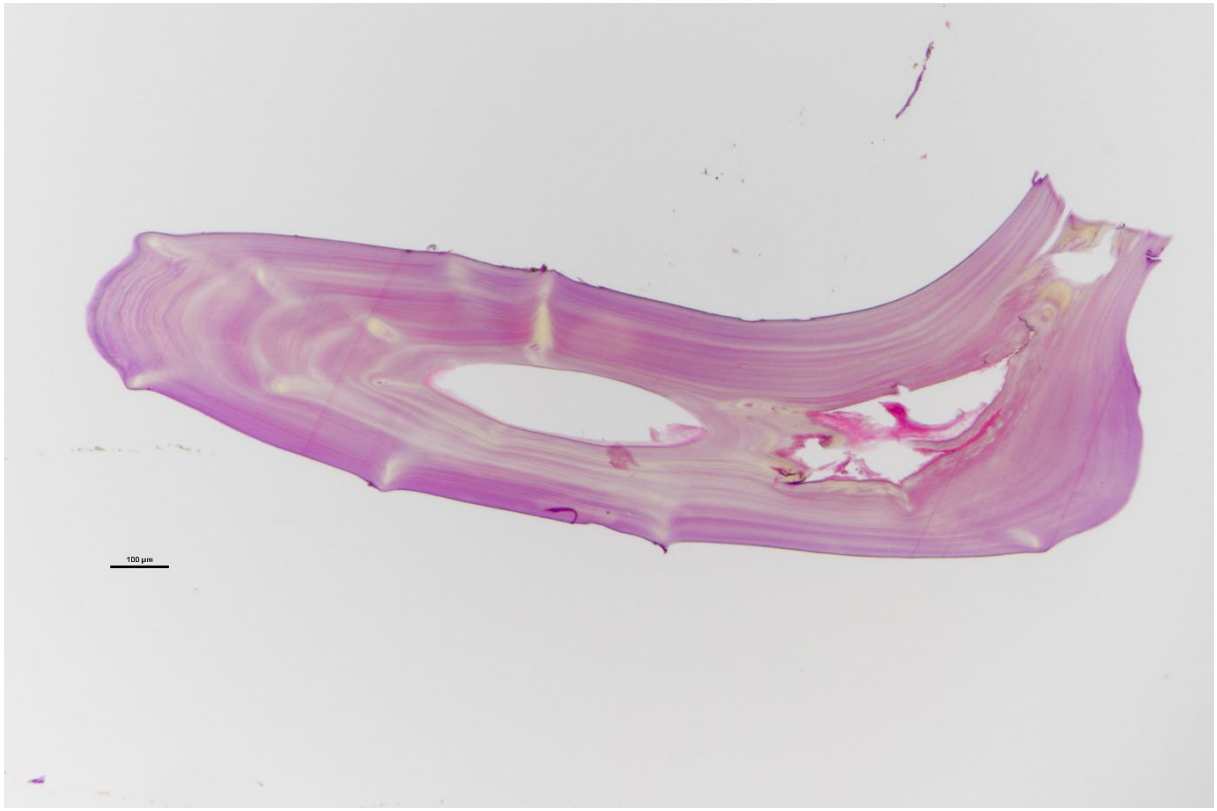

Figure 3-11: Longitudinal section through a *Sibopathes* sp. specimen NIWA2071. Specimen comprised skeletal matrix only. 60x magnification. Scale bar is 100 µm.

3.3 Stylasteridae Hydrocorals

The objective of the histology trials on stylasterid hydrocoral samples was to determine an appropriate methodology for decalcifying and producing high quality histological sections for observations of reproductive state.

3.3.1 *Errina* sp. (NIWA77555)

Stylasterid hydrocorals are extensively calcified, with more than 95 % of the animal being comprised of hard carbonate skeletal matrix. As a result, once the calcified matrix has been dissolved during the decalcification procedure, there is very little organic material remaining to hold the structural integrity of the specimen together. In this case, the *Errina* specimen (NIWA77555) dissolved so

completely that any remaining micro-tissue fragments remaining were flushed from the cassette during the tissue processing, so no histological slides could be produced.

3.3.2 Stylaster eguchii (NIWA91243)

The *Stylaster eguchii* specimen (NIWA91243) was ethanol fixed and so was post fixed in 10 % neutral buffered formalin prior to tissue processing. As with *Errina* sp. (NIWA77555), the extensive calcification of this species meant that the decalcified tissue retained very little of its structural integrity. However, in this specimen some soft tissue remained, and histological sections were able to be prepared. The images in Figure 3-12 and Figure 3-13 show that this specimen was a male, with maturing and mature spermatozoa evident within the dark-purple stained spermiaries.

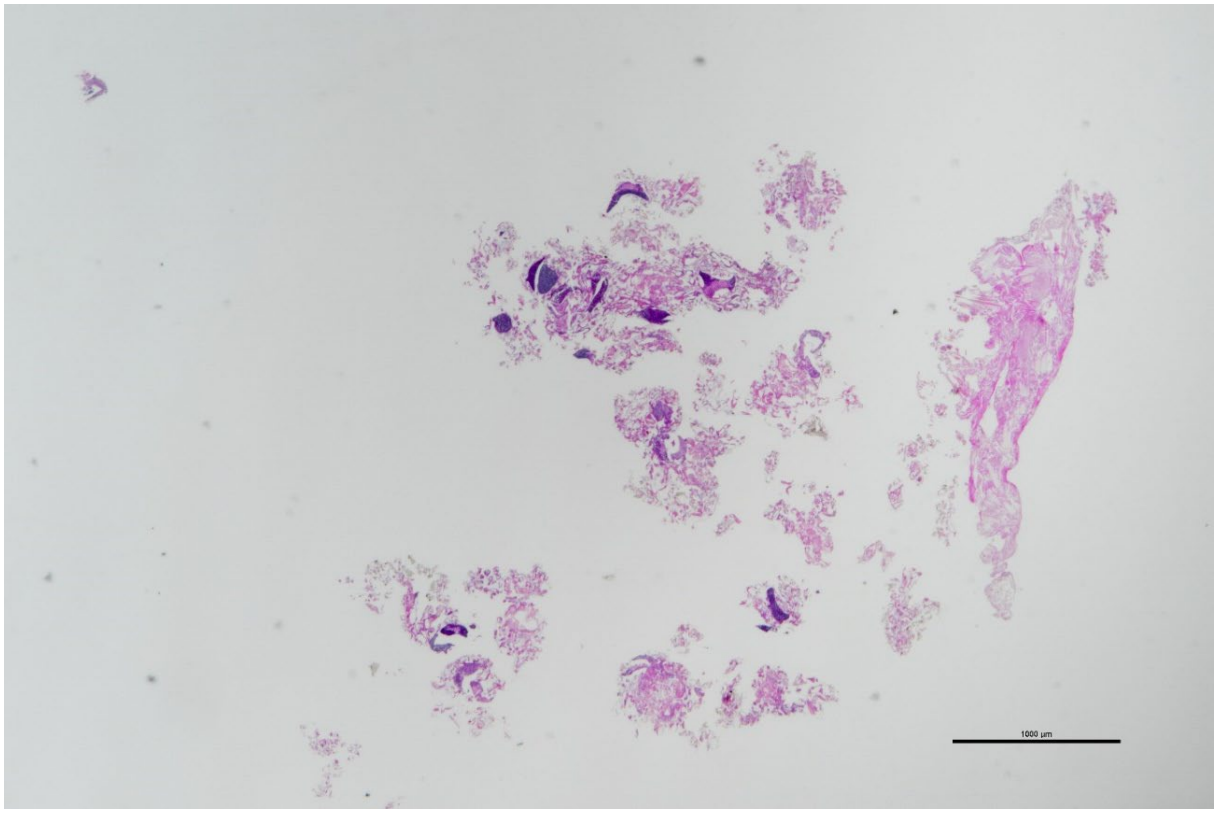

Figure 3-12: Section through a terminal branch tip of a *Stylaster eguchii* specimen NIWA91243. Specimen is a male. 20x magnification. Scale bar is 1000 µm.

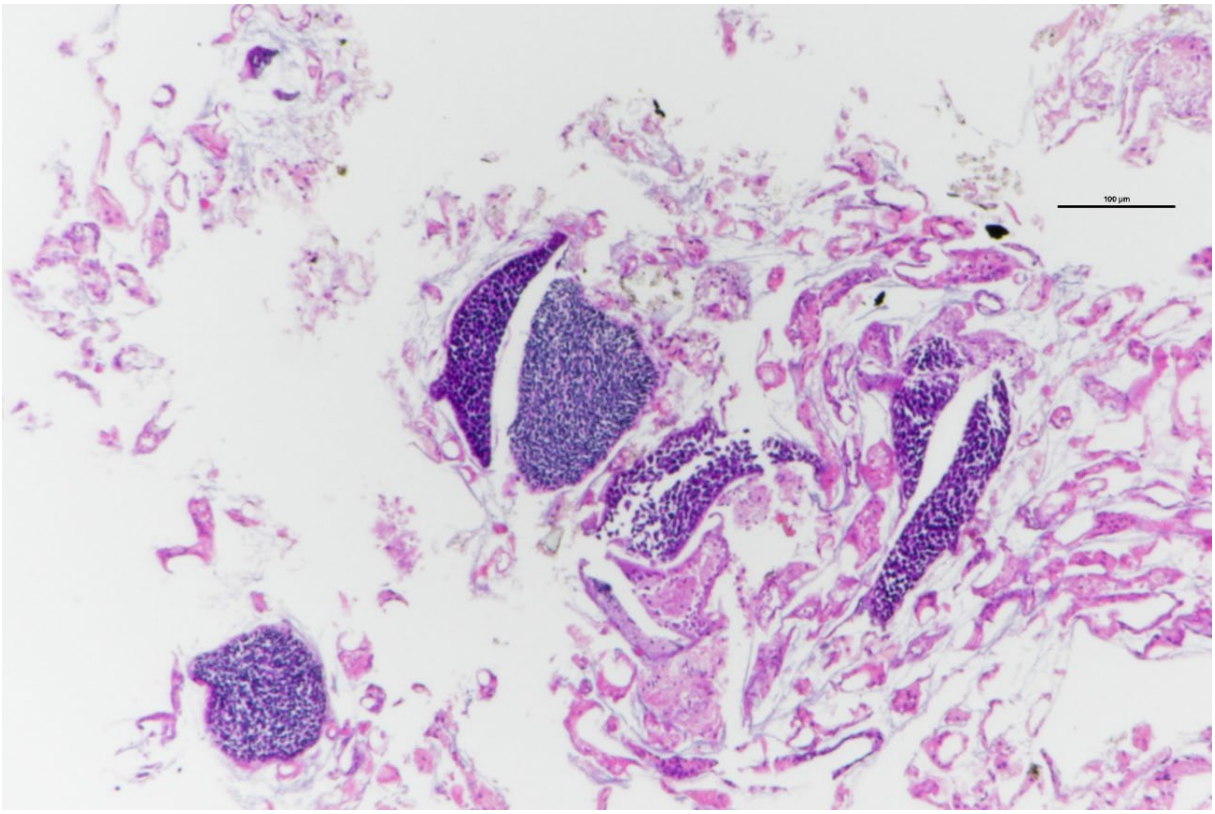

Figure 3-13: Section through a male ampullae of a *Stylaster eguchii* specimen NIWA91243. Dark purple stained spermiaries containing maturing and mature spermatozoa are evident in the centre and lower left of the section. Ampullae are the reproductive bodies of stylasterid corals, occurring as raised hemispheres on the surface of branches or as spherical inclusions within the branches depending on the species. 140x magnification. Scale bar is 100 µm.

4 Proposed next steps (2023–24)

4.1 Objective 1

The aim of Objective 1 is to address knowledge gaps in reproductive strategies for protected coral species in the New Zealand region. Histological sections taken through individual polyps of coral, which contain the reproductive tissue or gonads, can be analysed to observe the sex, chronology of reproductive tissue maturation and the degree or state of maturation and fertilisation present with in the polyps tissues.

4.1.1 Scleractinia (Stony corals)

Branching stony corals

1. Detailed review of the pilot histological sections to determine how many histology slides are required per polyp to enable reproductive mode and fecundity to be determined for both *E. rostrata* and *G. dumosa*.
2. Note that more histological analyses may be required as many of the pilot specimens appear to be male polyps.
3. Determine how many polyps can be analysed within the scope/budget of this project.
4. Review the polyp condition of available specimens of *E. rostrata* and *G. dumosa* in the NIC, including those only fixed in ethanol, to prioritise specimens for histological analyses.
5. Sub-sample the identified priority specimens for histology.
6. Assess the sex and reproductive state of specimens using histological sections. The size range of oocytes will be measured and, if possible, the fecundity of polyps will be estimated.

Solitary stony corals

1. Run pilot studies to assess the method and the number of histology sections required per polyp for reproductive studies.
2. Determine how many polyps can be analysed within the scope of this project.
3. Review the available specimens and polyp condition of the cup coral *Desmophyllum dianthus* held within the NIC to prioritise samples for histology.
4. Assess the sex and reproductive state of specimens using histological sections. The size range of oocytes will be measured and, if possible, the fecundity of polyps will be estimated.

4.1.2 Alcyonacea (Gorgonian octocorals)

1. Run pilot studies to assess the method and the number of histology sections required per polyp for reproductive studies.
2. Determine how many polyps can be analysed within the scope of this project.

3. Review the available specimens and polyp condition of the octocorals *Primnoa notialis* and *Paragorgia arborea* held within the NIC, to prioritise specimens for histological analysis.

4. Subsample identified priority specimens for histology.

5. Assess the sex and reproductive state of specimens from histology sections. The size range of oocytes will be measured and, if possible, the fecundity of polyps will be estimated.

4.1.3 Antipatharia (Black corals)

1. Review the holdings of ethanol-fixed black corals in the NIC and prioritise specimens for analysis.

2. Determine how many specimens can be analysed within the scope of this project.

3. Sub-sample identified priority specimens for histology.

4. Assess the reproductive state of specimens.

4.1.4 Stylasteridae (Hydrocorals)

1. Run more trials of decalcification procedures on hydrocoral specimens.

2. Trial using the commercial Merck reagent 'Osteosoft'. OSTEOSOFT® is optimized to gently decalcify sensitive tissues containing calcium. It is a chelating reagent based on complex-forming agents, it replaces the Calcium ions in the carbonate matrix preserving the integrity of the tissue and fine structures.

3. If a reliable method is determined for producing acceptable quality histological sections of hydrocorals, then we will review the specimens held within the NIC and take some subsamples for histological analysis.

4. Assess the reproductive state of histological specimens.

4.2 Objective 2

The aim of Objective 2 is to use available life history and reproductive data to inform relative productivity/vulnerability parameters for relevant concurrent and future research.

This project is running concurrently with a coral risk assessment project (INT2022-04), and we will be liaising closely with the NIWA team on the risk assessment work to ensure our data collection aligns with the needs of their work and to keep them updated on our results.

5 Analyses and report writing

A written summary of results will be provided, along with a full technical report. The technical report will include (but not be limited to) results by taxon for: coral fecundity, reproductive seasonality, reproductive mode (brooding vs. broadcasting) gonochorism and sex ratios, gross morphology and gamete morphology (and correlates with reproductive traits), and reproductive output at individual and (if applicable) local population level. The report will consider results in light of parallel risk assessment research and if possible, suggest how taxon-specific traits be considered in coral conservation management. The results and accompanying report will be presented to and reviewed by the CSP Technical Working Group and made available online.

6 Acknowledgements

We thank the Department of Conservation — Te Papa Atawhai, particularly the CSP programme, for their ongoing support of this research, the Gillies McIndoe Research Institute histology lab team for processing our coral samples, and Owen Anderson for internally reviewing this report.

7 Glossary of abbreviations and terms

Alc unk	unknown alcohol
Ampullae	The reproductive bodies of stylasterid corals, occurring as raised hemispheres on the surface of branches or as spherical inclusions within the branches depending on the species
DOC	Department of Conservation
EDTA	Ethylene Diamine Tetra-Acetic acid
EtOH	Ethanol
F	Formalin
Gonochorism	Describes coral species in which polyps and/or colonies are either male or female
Lumen	The inner open space or cavity of a tubular organ, such as the gastro-vascular cavity of a coral polyp
Mesentery	Internal longitudinal partition of tissue providing structural support and increasing surface area, which is important in nurtition and fertility of anthozoans. Multiple mesenteries are arranged radially wihtin the gastrovascular cavity of the polp (between septa in scleractinian corals) and are attached to the oral disk.
NIC	NIWA Invertebrate Collection
Oocyte	Female reproductive cell
Spermatozoa	Mature motile male sex cell that contains the genetic information to be transmitted by the male, final stage in spermatogenesis
Spermiaries	An organ in which spermatozoa are produced

8 References

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Tracey, D., Goode, S., Waller, R., Marriott, P., Beaumont, J., Moss, G., Cummings, V., Mobilia, V. (2021) Protected coral reproduction: Literature review, recommended study species, and description of spawning event for *Goniocorella dumosa*. NIWA Client Report No:2021097WN. Project: BCBC2020-01- DOC19305 Prepared for Department of Conservation. 63 p.

Appendix A NIC-held samples identified as potential candidates for histology

Table A-1: Catalogue number, taxonomic information, species name, sampling information (Station ID, collection date, and position), count, and preservation method for NIC-held samples identified as potential candidates for histology. F = fixed in formalin, EtOH is ethanol and Alc unk is unknown alcohol. Where the preservation method is "F, EtOH", the specimen has been first preserved in formalin then transferred into ethanol.

Catalogue Number	Order	Family	Genus	Species	Station ID	Date	Start latitude	Start longitude	Start depth	End depth	Count	Preservation method
46377	Alcyonacea	Paragorgiidae	*Paragorgia*	*arborea*	TRIP2571/53	29/02/2008	-50	176.06	952	1118	1	F, EtOH
66274	Alcyonacea	Paragorgiidae	*Paragorgia*	*arborea*	TRIP3028/136	10/01/2010	-44.453333	-178.60167	735		1	F, EtOH
61920	Alcyonacea	Primnoidae	*Primnoa*	*notialis*	TRIP3065/214	9/03/2010	-45.031667	175.495	1070	1100	1	F, EtOH
61980	Alcyonacea	Primnoidae	*Primnoa*	*notialis*	TRIP3077/127	31/03/2010	-48.816667	175.38333	769	767	1	F, EtOH
1356	Anthoathecata	Stylasteridae	*Adelopora*	*moseleyi*	P842	28/11/1979	-32.573299	156.2883	285		2	F, EtOH
3037	Anthoathecata	Stylasteridae	*Conopora*	*laevis*	KAH0204/7	14/04/2002	-34.119167	174.1525	800	670	1	F, EtOH
90609	Anthoathecata	Stylasteridae	*Conopora*	*verrucosa*	TAN0104/289	19/04/2001	-42.764832	-179.98599	800	757	1	F, EtOH
3039	Anthoathecata	Stylasteridae	*Conopora*	*verrucosa*	KAH0204/29	17/04/2002	-34.163166	173.96249	790	782	1	F, EtOH
1311	Anthoathecata	Stylasteridae	*Crypthelia*	*robusta*	P9	25/01/1977	-32.672501	167.4608	406		1	F, EtOH
3044	Anthoathecata	Stylasteridae	*Crypthelia*		KAH0204/29	17/04/2002	-34.163166	173.96249	790	782	1	F, EtOH
79955	Anthoathecata	Stylasteridae	*Errina*	*fissurata*	TAN0402/64	13/02/2004	-72.33033	170.49133	312	312	1	F, EtOH
79946	Anthoathecata	Stylasteridae	*Errina*	*fissurata*	TAN0402/77	14/02/2004	-72.116669	172.71317	499	499	1	F, EtOH
79948	Anthoathecata	Stylasteridae	*Errina*	*fissurata*	TAN0402/73	14/02/2004	-72.083336	173.14183	536	536	1	F, EtOH
79943	Anthoathecata	Stylasteridae	*Errina*	*fissurata*	TAN0402/71	13/02/2004	-72.063835	173.26334	630	630	1	F, EtOH
79953	Anthoathecata	Stylasteridae	*Errina*	*fissurata*	TAN0402/69	13/02/2004	-72.059669	173.353	750	750	1	F, EtOH
79952	Anthoathecata	Stylasteridae	*Errina*	*fissurata*	TAN0402/142	26/02/2004	-72.018333	170.80817	302	302	1	F, EtOH
79947	Anthoathecata	Stylasteridae	*Errina*	*fissurata*	TAN0402/139	26/02/2004	-72.014	170.77583	236	236	1	F, EtOH
79944	Anthoathecata	Stylasteridae	*Errina*	*fissurata*	TAN0402/151	26/02/2004	-71.997169	172.12399	512	512	1	F, EtOH
79949	Anthoathecata	Stylasteridae	*Errina*	*fissurata*	TAN0402/156	26/02/2004	-71.992668	172.207	675	675	1	F, EtOH
79954	Anthoathecata	Stylasteridae	*Errina*	*fissurata*	TAN0402/34	10/02/2004	-71.768501	171.10117	235	235	1	F, EtOH
79942	Anthoathecata	Stylasteridae	*Errina*	*fissurata*	TAN0402/162	26/02/2004	-71.475334	171.99716	738	738	1	F, EtOH
79950	Anthoathecata	Stylasteridae	*Errina*	*fissurata*	TAN0402/111	18/02/2004	-71.304497	170.618	357	357	1	F, EtOH

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79951	Anthoathecata	Stylasteridae	*Errina*	*fissurata*	TAN0402/205	29/02/2004	-71.16317	171.04767	1014	1014	1	F, EtOH
79945	Anthoathecata	Stylasteridae	*Errina*	*fissurata*	TAN0402/204	29/02/2004	-71.154335	171.18649	1138	1138	1	F, EtOH
79956	Anthoathecata	Stylasteridae	*Errina*	*laterorifa*	TAN0402/117	18/02/2004	-71.309166	170.57317	322	322	1	F, EtOH
90711	Anthoathecata	Stylasteridae	*Errina*		Z10645	11/02/2001	-71.862667	171.129	198		1	F, EtOH
77555	Anthoathecata	Stylasteridae	*Errina*		D18	22/04/1963	-52.516701	160.51669	128		1	F, EtOH
1546	Anthoathecata	Stylasteridae	*Lepidotheca*	*chauliostylus*	U582	5/02/1988	-31.8617	172.4333	790		1	F, EtOH
3069	Anthoathecata	Stylasteridae	*Lepidotheca*	*fascicularis*	KAH0204/29	17/04/2002	-34.163166	173.96249	790	782	1	F, EtOH
3070	Anthoathecata	Stylasteridae	*Lepidotheca*		KAH0204/40	18/04/2002	-34.164166	173.964	820	805	1	F, EtOH
3071	Anthoathecata	Stylasteridae	*Lepidotheca*		KAH0204/29	17/04/2002	-34.163166	173.96249	790	782	1	F, EtOH
3119	Anthoathecata	Stylasteridae	*Lepidotheca*		KAH0204/32	17/04/2002	-34.162	173.96183	810	780	1	F, EtOH
91243	Anthoathecata	Stylasteridae	*Stylaster*	*eguchii*	TAN1106/3							EtOH
73307	Anthoathecata	Stylasteridae	*Stylaster*		TAN1105/53	29/03/2011	-33.958833	171.795	108	107	10	F, EtOH
90954	Anthoathecata	Stylasteridae			B570	9/10/1962	-46.389999	169.8033	16	16	1	F, EtOH
90950	Anthoathecata	Stylasteridae			G307	26/01/1968	-44.1167	-179.2167	402		1	F, EtOH
90958	Anthoathecata	Stylasteridae			G184	18/01/1968	-44.099998	-179.4167	344		1	F, EtOH
90956	Anthoathecata	Stylasteridae			G290A	25/01/1968	-43.6667	179.0167	368		1	F, EtOH
90949	Anthoathecata	Stylasteridae			G173	17/01/1968	-43.650002	-179.46671	373		1	F, EtOH
90953	Anthoathecata	Stylasteridae			G239	22/01/1968	-43.650002	179.60001	410		1	F, EtOH
90952	Anthoathecata	Stylasteridae			G380	6/02/1968	-43.55	-177.9	366		1	F, EtOH
90955	Anthoathecata	Stylasteridae			G259A	23/01/1968	-43.55	179.3667	410		1	F, EtOH
90951	Anthoathecata	Stylasteridae			G233	22/01/1968	-43.533298	179.60001	412		1	F, EtOH
90957	Anthoathecata	Stylasteridae			G382	6/02/1968	-43.45	-177.95	402		1	F, EtOH
90959	Anthoathecata	Stylasteridae			B692	30/10/1962	-40.936699	173.81329	29	29	1	F, EtOH
127391	Anthoathecata	Stylasteridae			TAN0107/323	24/05/2001	-36.145667	178.20167	924	712	2	F, EtOH
127405	Anthoathecata	Stylasteridae			TAN0107/234	24/05/2001	-36.1345	178.20117	1140	698	1	F, EtOH
72543	Anthoathecata	Stylasteridae			TAN1104/59	11/03/2011	-35.3595	178.5105	1270	1410	1	Formalin
3124	Anthoathecata	Stylasteridae			KAH0204/40	18/04/2002	-34.164166	173.964	820	805	1	F, EtOH

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3134	Anthoathecata	Stylasteridae			KAH0204/29	17/04/2002	-34.163166	173.96249	790	782	1	F, EtOH
3123	Anthoathecata	Stylasteridae			KAH0204/21	16/04/2002	-34.072	174.068	630	560	1	F, EtOH
90991	Antipatharia	Antipathidae	*Cirrhipathes*	*propinqua*	TAN0107/51	19/05/2001	-35.74	178.4975	415	320	1	F, EtOH
126077	Antipatharia	Antipathidae	*Stichopathes*	*variabilis*	SO254/18ROV05_ BIOBOX15	3/02/2017	-29.28924	-178.01862	291.8		1	Formalin
39210	Antipatharia	Cladopathidae	*Cladopathes*		TAN0802/305	14/03/2008	-67.168	171.179	648	620	1	F, EtOH
2071	Antipatharia	Cladopathidae	*Sibopathes*		KAH0204/7	14/04/2002	-34.119167	174.1525	800	670	1	Formalin
53045	Antipatharia	Leiopathidae	*Leiopathes*	*bullosa*	TAN0905/71							EtOH
19974	Antipatharia	Myriopathidae	*Antipathella*	*fiordensis*	S679	7/02/1986	-45.3	167	0	28	1	F, EtOH
19975	Antipatharia	Myriopathidae	*Antipathella*	*fiordensis*	S679	7/02/1986	-45.3	167	0	28	1	F, EtOH
19977	Antipatharia	Myriopathidae	*Antipathella*	*fiordensis*	S679	7/02/1986	-45.3	167	0	28	1	F, EtOH
19979	Antipatharia	Myriopathidae	*Antipathella*	*fiordensis*	S679	7/02/1986	-45.3	167	0	28	1	F, EtOH
19980	Antipatharia	Myriopathidae	*Antipathella*	*fiordensis*	S679	7/02/1986	-45.3	167	0	28	1	F, EtOH
39209	Antipatharia	Schizopathidae	*Bathypathes*	*patula*	TAN0802/305	14/03/2008	-67.168	171.179	648	620	1	F, EtOH
123390	Antipatharia	Schizopathidae	*Bathypathes*		TAN0107/232	24/05/2001	-36.1455	178.19967	750	570	1	F, EtOH
85930	Antipatharia	Schizopathidae	*Parantipathes*		TAN0104/188	18/04/2001	-42.709332	-179.96001	959	959	1	F, EtOH
103544	Antipatharia				TAN0308/99	28/05/2003	-33.754334	167.2845	254	259	1	F, EtOH
103543	Antipatharia				TAN0308/49	20/05/2003	-29.218166	158.9975	300	300	1	F, EtOH
148161	Scleractinia	Caryophylliidae	*Desmophyllum*	*dianthus*	TAN2009/80	19/08/2020	-44.136166	-174.72117	640	622	11	Formalin
24785	Scleractinia	Caryophylliidae	*Desmophyllum*	*dianthus*	TAN0104/152	18/04/2001	-42.729833	-179.89033	1130	1000	1	F, EtOH
118260	Scleractinia	Caryophylliidae	*Desmophyllum*	*dianthus*	TAN1612/28	25/10/2016	-29.285	-177.857	499	615	1	Formalin
127327	Scleractinia	Caryophylliidae	*Desmophyllum*	*dianthus*	TAN0107/227	23/05/2001	-36.139667	178.19617	603	365	1	F, EtOH
47925	Scleractinia	Caryophylliidae	*Desmophyllum*	*dianthus*	TRIP2699/17	2/10/2008	-44.463333	-174.89	1008	1087	1	F, EtOH
127403	Scleractinia	Caryophylliidae	*Desmophyllum*	*dianthus*	TAN0107/234	24/05/2001	-36.1345	178.20117	1140	698	2	F, EtOH
104980	Scleractinia	Caryophylliidae	*Desmophyllum*	*dianthus*	Z16074	2/02/1993	-45.349	167.056	15	35	2	F, EtOH
88074	Scleractinia	Caryophylliidae	*Desmophyllum*	*dianthus*	TAN0104/47	16/04/2001	-42.792835	-179.981	950	900	4	F, EtOH
88075	Scleractinia	Caryophylliidae	*Desmophyllum*	*dianthus*	TAN0104/153	18/04/2001	-42.7325	-179.8985	1076	990	8	F, EtOH

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147900	Scleractinia	Caryophylliidae	*Goniocorella*	*dumosa*	TAN2001/81	22/01/2020	-43.531833	177.10367	279	263	1	Formalin
81281	Scleractinia	Caryophylliidae	*Goniocorella*	*dumosa*	TAN0104/116	17/04/2001	-42.798168	179.98183	1000	922	1	F, EtOH
140313	Scleractinia	Caryophylliidae	*Goniocorella*	*dumosa*	TAN1903/106	21/06/2019	-43.367667	179.45133	396	396	1	Formalin
140326	Scleractinia	Caryophylliidae	*Goniocorella*	*dumosa*	TAN1903/108	21/06/2019	-43.368167	179.45083	387	380	1	Formalin
140346	Scleractinia	Caryophylliidae	*Goniocorella*	*dumosa*	TAN1903/110	22/06/2019	-43.360667	179.74233	461	450	1	Formalin
140375	Scleractinia	Caryophylliidae	*Goniocorella*	*dumosa*	TAN1903/153	25/06/2019	-43.365333	179.4505	390	390	1	Formalin
54068	Scleractinia	Caryophylliidae	*Goniocorella*	*dumosa*	TAN0905/113	27/06/2009	-44.1495	-174.75683	519	609	30	F, EtOH
147900	Scleractinia	Caryophylliidae	*Goniocorella*	*dumosa*	TAN2001/81	22/01/2020	-43.531833	177.10367	279	263	1	F, EtOH
148101	Scleractinia	Caryophylliidae	*Goniocorella*	*dumosa*	TAN2009/57	16/08/2020	-44.159	-174.554	486	659	10	F, EtOH
148157	Scleractinia	Caryophylliidae	*Goniocorella*	*dumosa*	TAN2009/80	19/08/2020	-44.136166	-174.72117	640	622	10	F, EtOH
25361	Scleractinia	Caryophylliidae	*Goniocorella*	*dumosa*	TAN0604/108	6/06/2006	-43.532799	179.62801	375	381	1	EtOH
45326	Scleractinia	Caryophylliidae	*Goniocorella*	*dumosa*	TAN0801/16	30/12/2007	-43.4645	-179.76417	416	420	4	EtOH
16001	Scleractinia	Caryophylliidae	*Goniocorella*	*dumosa*	TAN0413/123	14/11/2004	-37.340164	177.12134	570	400	1	EtOH
25499	Scleractinia	Caryophylliidae	*Goniocorella*	*dumosa*	TAN0408/23	13/07/2004	-42.829166	-177.42183	826	824	1	EtOH
45325	Scleractinia	Caryophylliidae	*Goniocorella*	*dumosa*	TAN0801/12	30/12/2007	-43.589167	179.66867	392	396	2	EtOH
53862	Scleractinia	Caryophylliidae	*Goniocorella*	*dumosa*	TAN0905/105	26/06/2009	-44.157333	-174.55417	485	533	1	EtOH
77547	Scleractinia	Caryophylliidae	*Goniocorella*	*dumosa*	TAN0101/82	13/01/2001	-43.067167	177.68017	322	319	1	Alc unk
88225	Scleractinia	Caryophylliidae	*Goniocorella*	*dumosa*	Q341	14/11/1979	-44.1183	176.32	264		10	Alc unk
91198	Scleractinia	Caryophylliidae	*Goniocorella*	*dumosa*	TAN0308/147	3/06/2003	-34.302334	168.38634	850	825	1	Alc unk
102472	Scleractinia	Caryophylliidae	*Goniocorella*	*dumosa*	TAN1503/116	11/04/2015	-44.159667	-174.55483	497	590	20	EtOH
102566	Scleractinia	Caryophylliidae	*Goniocorella*	*dumosa*	TAN1503/120	11/04/2015	-44.135833	-174.7195	622	615	1	EtOH
141768	Scleractinia	Caryophylliidae	*Goniocorella*	*dumosa*	TAN2001/71	20/01/2020	-43.811	-179.731	379	381	1	EtOH
148046	Scleractinia	Caryophylliidae	*Goniocorella*	*dumosa*	TAN2005/130	18/06/2020	-43.368833	179.45217	394	402	1	EtOH
148048	Scleractinia	Caryophylliidae	*Goniocorella*	*dumosa*	TAN2005/132	18/06/2020	-43.372167	179.45167	395	394	1	EtOH
140375	Scleractinia	Caryophylliidae	*Goniocorella*	*dumosa*	TAN1903/153	25/06/2019	-43.365333	179.4505	390	390	1	EtOH
71137	Scleractinia	Dendrophylliidae	*Enallopsammia*	*rostrata*	TAN0104/336	20/04/2001	-42.767833	-179.92183	955	890	1	F, EtOH
148158	Scleractinia	Dendrophylliidae	*Enallopsammia*	*rostrata*	TAN2009/80	19/08/2020	-44.136166	-174.72117	640	622	10	F, EtOH

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148159	Scleractinia	Dendrophylliidae	*Enallopsammia*	*rostrata*	TAN2009/80	19/08/2020	-44.136166	-174.72117	640	622	10	F, EtOH
53483	Scleractinia	Dendrophylliidae	*Enallopsammia*	*rostrata*	TAN0905/71	22/06/2009	-42.736167	-179.69017	820	1023	200	EtOH
26935	Scleractinia	Dendrophylliidae	*Enallopsammia*	*rostrata*	TAN0616/12	4/11/2006	-40.040298	178.1445	749	787	1	EtOH
26954	Scleractinia	Dendrophylliidae	*Enallopsammia*	*rostrata*	TAN0616/6	4/11/2006	-40.038502	178.14301	730	747	2	EtOH
26964	Scleractinia	Dendrophylliidae	*Enallopsammia*	*rostrata*	TAN0616/38	6/11/2006	-39.543301	178.3365	815	819	1	EtOH
27575	Scleractinia	Dendrophylliidae	*Enallopsammia*	*rostrata*	TAN0616/12	4/11/2006	-40.040298	178.1445	749	787	1	EtOH
27571	Scleractinia	Dendrophylliidae	*Enallopsammia*	*rostrata*	TAN0616/10	4/11/2006	-40.039799	178.1425	760	700	1	EtOH
82333	Scleractinia	Dendrophylliidae	*Enallopsammia*	*rostrata*	TAN1206/39	18/04/2012	-36.452	177.8463	1030	1255	4	EtOH
88412	Scleractinia	Dendrophylliidae	*Enallopsammia*	*rostrata*	Z10192	10/07/1999	-42.778667	179.98			1	Alc unk
102565	Scleractinia	Dendrophylliidae	*Enallopsammia*	*rostrata*	TAN1503/120	11/04/2015	-44.135833	-174.7195	622	615	1	EtOH
102631	Scleractinia	Dendrophylliidae	*Enallopsammia*	*rostrata*	TAN1503/122	11/04/2015	-44.147667	-174.74817	570	600	1	EtOH
127468	Scleractinia	Dendrophylliidae	*Enallopsammia*	*rostrata*	TAN1503/56	3/04/2015	-42.79	-179.98733	918	944	1	EtOH
127521	Scleractinia	Dendrophylliidae	*Enallopsammia*	*rostrata*	TAN1611/DR-8	13/10/2016	-32.100556	179.17694	1200	1167	1	EtOH
43171	Scleractinia	Dendrophylliidae	*Enallopsammia*	*rostrata*	TAN0205/37							EtOH