POP2022-03 Protected Coral Reproduction

Final Report

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Executive summary

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

Within the current project, histological methods were 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 covering all 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.

This study has generated some interesting and important data on the reproductive traits of protected New Zealand deep sea corals. We have confirmed that *G. dumosa* and *E. rostrata* collected within the New Zealand region are gonochoric (single sex within a polyp), with both species having either male or female specimens (i.e., all polyps on a specimen were the same sex).

We have also confirmed that *G. dumosa* is a brooder in wild populations on the Chatham Rise, a reproductive mode whereby gametes are fertilised and develop internally into larvae before being released into the surrounding water. Stage IV oocytes were present throughout the year and the limited number of male specimens examined had mature stage IV spermiaries present in both seasons sampled (April and August). We conclude, from the limited seasonal spread of available data, that there was no evidence of reproductive periodicity in *G. dumosa* and that *G. dumosa* may have the ability to reproduce year-round when environmental conditions are favourable. Previous observations of larvae in aquaria from September to November 2020 (Beaumont et al. 2024), and with a consistent food supply, support this theory.

Although there was a limited seasonal spread of data for *E. rostrata*, there was no evidence of seasonality with mature or maturing oocytes present in all female specimens examined (sampled in April, June and August). There was no evidence of larvae nor brooding and as such, *E. rostrata* are considered broadcast spawners. Mature (stage IV) spermaries were observed in male specimens from all seasons sampled (April, June and August). We, therefore, suggest that *E. rostrata* could be a continuous or an aperiodic spawner, rather than a seasonal spawner, though further sampling would be required to confirm this.

Enallopsammia rostrata had a lower estimated fecundity than *G. dumosa* though they had a similar sized maximum oocyte diameter (although morphologically there are differences as *E. rostrata* oocytes are long and thin and *G. dumosa* are more rounded). However, *E. rostrata* is considered likely to be a broadcast spawner and *G. dumosa* a brooder. This goes against the general assumption that brooders have fewer but larger oocytes/larvae.

The inclusion of black corals (Antipatharia) and hydrocorals (Stylasteridae) in this study were as a trial only to assess the quality of histological sections that could be prepared from fixed specimen samples in order to enable clear observations of reproductive data. Our trials on the black corals *Leiopathes bullosa* and *Sibopathes* sp. showed that it will be possible to assess the reproductive state of future sections of these species. However, hydrocorals proved problematic due to their extensive calcification, with more than 95 % of the animal being comprised of hard carbonate skeletal matrix and therefore difficulty in obtaining adequate tissue for examination.

The histological analyses of the stony cup coral *Desmophyllum*, and the two gorgonian octocoral species *Paragorgia* and *Primnoa* planned for this study are being carried out by a PhD student at the University of Gothenburg but these results have been delayed, and as such they will be added to this report as an addendum when available (expected early 2025).

Specimens used within this study were historic (some dating back to 2000) and many had not been preserved with histological analyses in mind. While we were able to get some data from all specimens used, in some cases the quality of data was compromised by the quality of the histological sections. In addition, the variability observed in reproductive data between polyps and specimens within this study highlights the importance of replicate samples across multiple time points when investigating reproductive mode, seasonality and fecundity. We recommend that, where possible, deep-sea corals specimens are collected and placed into an appropriate preservative to enable further histological analyses to address knowledge gaps.

There remain questions regarding the reproduction of corals that can only be addressed by observations of live animals, such as larval behaviour, pelagic larval duration and settlement preferences.

These data and results have been communicated to relevant concurrent research projects (e.g., INT2022-04, risk assessment for protected corals) where they have been used to help evaluate scores for productivity attributes in Productivity Susceptibility Analyses (PSA). In addition, they will inform future research to support risk assessment and development of appropriate management options.

1 Background

The specific objectives of this project (POP2022-03) were 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 built 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). Following a selection process that included identifying species of high and medium risk in a pilot risk assessment (Clark et al. 2014), and there being adequate samples available for each species in the NIWA Invertebrate Collection (NIC), five candidate species were identified for this targeted reproduction study. These were the stony cup coral *Desmophyllum dianthus* (Order Scleractinia), the stony reef-forming corals *Goniocorella dumosa* and *Enallopsammia rostrata* (Order Scleractinia), and the gorgonian octocorals *Primnoa notialis* and *Paragorgia arborea* (Order Scleralcyonacea previously known as Alcyonacea) (Figure 2-1 A-E). In addition, suitable specimens of black coral (Order Antipatharia) and hydrocoral (Order Anthoathecata, Family Stylasteridae) samples were selected to trial histological analyses and attempt to obtain reproductive information. This was to ensure all New Zealand protected coral groups were investigated in the study (Figure 2-1 F - G).

1.1 Existing knowledge

Goniocorella dumosa was previously thought to be a seasonal gonochoristic broadcast spawner with fertilisation occurring in April/May, coinciding with the end of the Austral summer biomass accumulation (Burgess and Babcock 2005). It is important to note that this conclusion was based on the histology on specimens from a single collection date (April 2001). Subsequently, opportunistic observations of *G. dumosa* larvae, in aquaria, (Beaumont et al. 2024) showed this species to be a brooder, with larval release observed in the Austral spring (between September and November, the experiment ended in December 2020).

Previous histological analyses of *Enallopsammia rostrata* in both New Zealand (Burgess and Babcock 2005) and the South West Atlantic (Brazil, Pires et al. 2014), have shown this species to be an aperiodic broadcast spawner. As with their *G. dumosa* study, Burgess and Babcock (2005) drew their conclusions from specimens collected from a single timepoint (April 2001). However, Pires et al. (2014) collected and analysed specimens at 13 time points during a 12 month period.

Histological work on *Desmophyllum dianthus* from Chilian fjords (<50 m) has shown them to be periodic broadcast spawners, spawning in the Austral winter (Feehan 2016).

For all these early studies, questions remained as to the reproductive mode and timing of the corals in wild populations in the New Zealand region.

The Primnoidae *Primnoa notialis* is considered a gonochoric broadcast spawner (Feehan and Waller 2015). The authors studied two specimens collected in the South Pacific in November 1964, of which one specimen was male and one specimen was a female (3 polyps).

No deep-water specimens of Paragorgiidae (e.g., *Paragorgia arborea*) have been examined for reproduction (Waller et al. 2023). However, Lacharite,Metaxas (2013) studied the recruitment of

deep-water gorgonian corals in the northwest Atlantic and suggested that *P. arborea* is likely a brooder.

Here we report on new information on the reproductive ecology of *G. dumosa* and *E. rostrata* as determined by histological analyses of historical samples, together with initial results of histological trials on black corals and hydrocorals. *Desmophyllum dianthus*, *P. notialis* and *P. arborea* samples are still under investigation by PhD student Diego Moreno Moran at the University of Gothenburg in Sweden. These results will be added to this report when available (expected early 2025).

2 Methods

2.1 Specimen selection

In order to select specimens for use in this study, specimens held within the NIWA Invertebrate Collection (NIC) and identified as potential candidates for histology (See Appendix A) were assessed to check for adequate live tissue at the time of collection. Specimens that were not previously fixed in formalin were post-fixed in 10 % buffered formalin. To maximise the usefulness of generated data, specimen selection was restricted to where the NIC held useful numbers of specimens from similar spatial locations and across seasons. For a given species a single region was selected to remove the effect of changing reproductive timing and strategy across disparate regions with differing environmental parameters. For each species we attempted to select a region that had samples in the collection from a broad range of dates across calendar years so the timings of gonad maturation and spawning could be better characterised. The lack of significant specimens across all regions, together with limited funding, meant only a single region per species could be studied in this project.

Specimens selected for analysis are given in Appendix B. These include 25 branching reef-forming scleractinian specimens - 12 *Goniocorella dumosa* and 13 *Enallopsammia rostrata*), 18 scleractinian cup corals (*Desmophyllum dianthus*), 32 scleralcyonacean (gorgonian) octocorals (14 *Primnoa notialis* and 18 *Paragorgia arborea*). In addition, 2 Antipatharia specimens (1 *Leiopathes bullosa* and 1 *Sibopathes* sp.), and 2 stylasterid specimens (1 *Stylaster eguchii* and 1 *Errina* sp.) were included to assess the feasibility of histological analyses on these groups. Example images of the selected coral species are given in Figure 2-1.

Specimens were photographed and scanned with a Shining 3D^{tm} EinScan Pro HD 3D scanner to enable counts of polyps per colony.

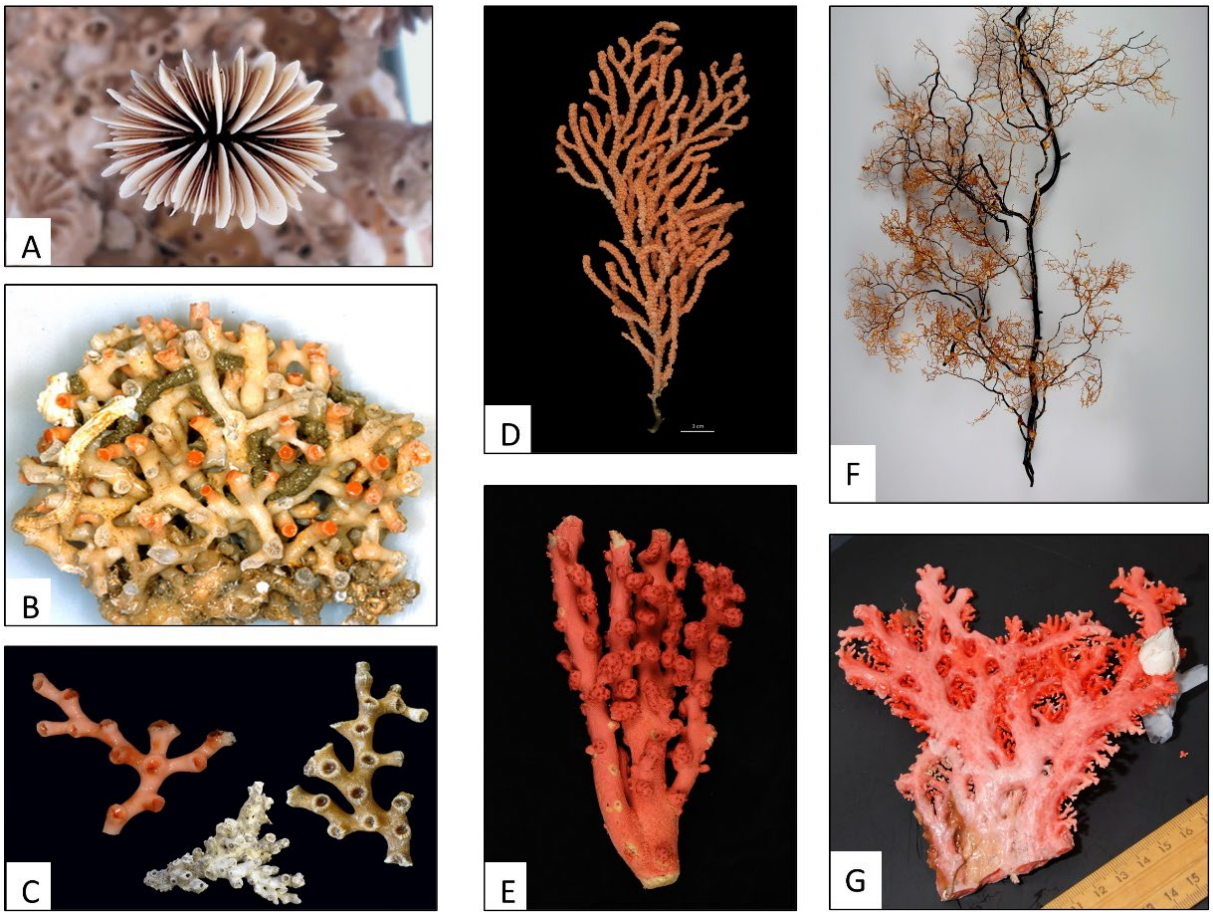

Figure 2-1: Example specimen photographs of coral groups selected for the reproductive study.
A) *Desmophyllum dianthus*; B) *Goniocorella dumosa*; C) *Enallopsammia rostrata*; D) *Primnoa notialis*; E) *Paragorgia arborea*; F) *Leiopathes* sp.; G) *Errina* sp. (NIWA images).

2.2 Histological preparation

Polyps 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.

Trials were conducted to assess the feasibility of preparing adequate quality histological sections from both formalin-fixed and ethanol fixed specimens, the latter with post-fix in formalin prior to processing. For these initial trials polyps from samples with calcified skeletons (scleractinians and stylasterids) 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. The decalcified coral specimens were processed overnight in an automated tissue processing machine. A small number of sections from each polyp were stained and mounted onto slides to assess histology quality. Where possible, reproductive data were captured from these sections.

The second round of histology involved specimens from the NIC that had not been fixed in Formalin. These specimens had either been frozen at capture and then fixed in ethanol when taken into the NIC or fixed from fresh in 80-100% ethanol. The decalcification reagent used for these specimens was

based on the commercially available decalcifying reagent Osteomol™. This is a hydrochloric acid based decalcifying reagent that also contains formalin, thus post-fixes the tissue as it is removing the carbonate skeleton. This is a moderately aggressive decalcifying reagent which speeds up the tissue processing time. Specimens were embedded in paraffin wax in a longitudinal orientation. For budgeting efficiency, multiple polyps were embedded into each cassette.

An initial four-micron thick section was taken from midway through the polyp to verify whether the coral specimen was male or female. If male, no further sections were required. If a specimen was female, then the remaining block was sectioned at every 100 microns (with each section being four microns thick). Every second section (so one section every 200 microns) was mounted onto a slide, stained and cover-slipped. A distance of 200 µm between sections was chosen to enable counts and measurements of most oocytes of stages III, IV and V using previously recorded oocyte sizes (mean diameter of stage III oocytes was 269 µm, Tracey et al. 2021).

Adhesive slides were used to collect the cut sections as decalcified tissue and lipid rich oocytes have 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 to optimise optical clarity and maximise archival storage.

2.3 Determining maturity of reproductive tissue and polyp fecundity

Histological sections were photographed with a Nikon SMZ25 stereomicroscope at 20 x (overview) and at 60 x magnification and a Nikon Ni Eclipse compound microscope at 40 – 1000x magnification. Polyp images were assessed for quality (staining and intactness) and sexed.

For female specimens, oocytes were identified, staged (as per Table 2-1), counted, and measured (using Fiji ImageJ, Schindelin et al. 2012), or the Nikon imaging software NIS-Elements™. Stage I, II and III oocytes were only measured where a nucleus was present. Stage IV oocytes and stage V larvae were measured if the oocyte/larvae appeared to present as a representative cross section roughly through the mid-plane of the oocyte/larvae. Atretic (degenerating) oocytes were not counted or measured but were used to help discern between male and female polyps. Examples of oocyte stages of *G. dumosa*, taken from Tracey et al. (2021) are given in Appendix C.

Male specimens were assessed for maturity of spermaries as per Table 2-1. Sections were classified according to the most advanced spermiary observed in the histological section. Male specimens only had a single histological section analysed per polyp, levels were not taken through the polyp. The number of reproductive propagules per polyp was not estimated for male specimens.

Oocyte counts were recorded from sections taken at 200 µm for a half of each polyp. Therefore, fecundity of female polyps was estimated by doubling counts of mature and maturing oocytes (stages III, IV and V). Due to the small size of stage I and II oocytes (<200 um), the counts of these oocytes were quadrupled to acknowledge that some of these immature oocytes would have been missed between sections. We present estimates of total fecundity (using counts of all stages of oocytes present) for comparisons with results presented in Burgess and Babcock (2005); as well as estimates of annual fecundity using counts of mature and maturing oocytes (stages III, IV and V only). Annual fecundity is a measure of the reproductive potential of a polyp in any given year. Our estimates should be considered as minimum values as many sections had suspected missing oocytes or poorly resolved areas leading to unidentified oocytes.

Table 2-1: Developmental stages of oocytes and spermatocytes (adapted from Burgess 2002).

Stage	Oocytes/Larvae	Spermaries
I	Oogonia: Enlarged interstitial cells, with large nuclei in mesoglea of mesenteries	Small clusters of interstitial cells
II	Immature Oocytes (previtellogenic): Accumulation of small amount of cytoplasm around nuclei	Small spermatocytes with small nuclei, number of cells within a spermiary much larger
III	Oocytes undergoing Vitellogenesis: variable size, main period of vitellogenesis	Spermatocytes with little cytoplasm, developed flagella not evident, lumen usually present
IV	Vitellogenic Oocytes: full sized with indented nucleus migrating to edge of oocyte, large vitellogenin bodies fill the cytoplasm, cortical granular layer may be seen	Spermatozoa with fully developed flagella, ready to spawn
V	Brooding larvae of various stages of development	

3 Results: Scleractinia (stony branching corals)

Two species of reef-forming Scleractinia were investigated within this study (*Goniocorella dumosa and Enallopsammia rostrata)* and one species of solitary cup coral *(Desmophyllum dianthus).*

3.1 *Goniocorella dumosa* (GDU)

All *G. dumosa* specimens included within this study were collected from the Chatham Rise in water depths ranging from 241 m to 640 m. The collection date, number of polyps analysed, and sex of polyps/specimens are summarised in Table 3-1.

Of the 12 specimens, 8 were female, three were male and 1 was unsexed/immature. Specimens had either male or female polyps (Table 3-1), not both, confirming this species is gonochoric. The quality of histological slides varied between specimens. For example, sections from specimen GDU_112065 were poorly stained and the identification of anything but the largest oocytes was difficult (see Figure 3-1 A). Other specimens were better stained but were friable and/or had "voids" where it was likely that oocyte tissue was missing/had floated away (e.g., Figure 3-1 B). Some polyps (e.g., GDU_140346) resulted in sections which were mostly intact with clearly visible oocytes (e.g., Figure 3-1 C).

As an example of a female specimen, GDU_148101 (Figure 3-2) was 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 3-3). Even the high lipid containing mature oocytes adhered well to the slide.

Table 3-1: Summary of polyps analysed from each *G. dumosa* specimen. M/F/U = Male/Female/Unsexed (or immature). Ordered by collection month. 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.

Species	NIC number	Collection date	Year	Depth (m)	Preservation method	No. of Polyps analysed	M/F/U	Comment
GDU	88266	1 January	2004	440	EtOH	10	U	Poor histological sections
GDU	112065	5 January	2004	241	EtOH	11	F	Friable sections with poor staining
GDU	141768	20 January	2020	379	EtOH	16	F	
GDU	102639	11 April	2015	570	EtOH	3	F	
GDU	102472	11 April	2015	497	EtOH	2	M	Analysed as part of histology trials
GDU	102566	11 April	2015	622	EtOH	10	M	
GDU	140313	21 June	2019	396	F,EtOH	18	F	
GDU	140326	21 June	2019	387	F,EtOH	22	F	
GDU	140346	22 June	2019	461	F,EtOH	17	F	
GDU	148101	16 August	2020	486	F,EtOH	2	F	Analysed as part of histology trials
GDU	148157	19 August	2020	640	F,EtOH	6	M	Analysed as part of histology trials
GDU	27578	31 December	2006	409	EtOH	12	F	
Total polyps						**129**		

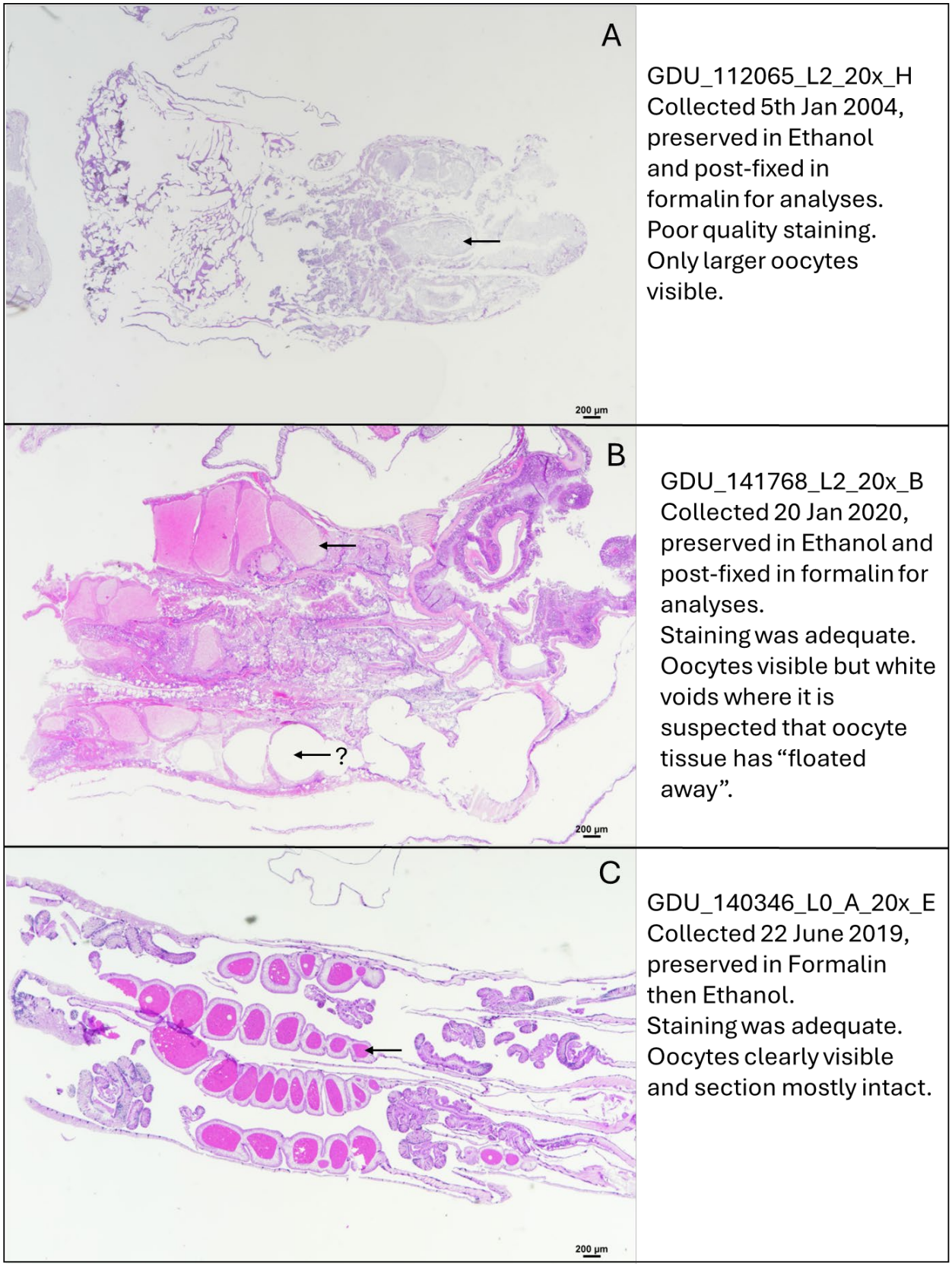

Figure 3-1: Variable quality of histology sections from available specimens. Example oocytes indicated by a black arrow. Scale bars are 200 µm. A) Staining is poor and only larger oocytes (stage IV) are visible; B) Staining is adequate and oocytes (stages III and IV) are visible but there are white voids where it is suspected that oocyte tissue is missing; C) Staining is adequate and oocytes (stages II, III and IV) are visible and mostly intact.

Figure 3-2: Fragment of *G. dumosa* specimen NIWA148101. A terminal and sub-terminal polyp were clipped from the matrix for tissue processing.

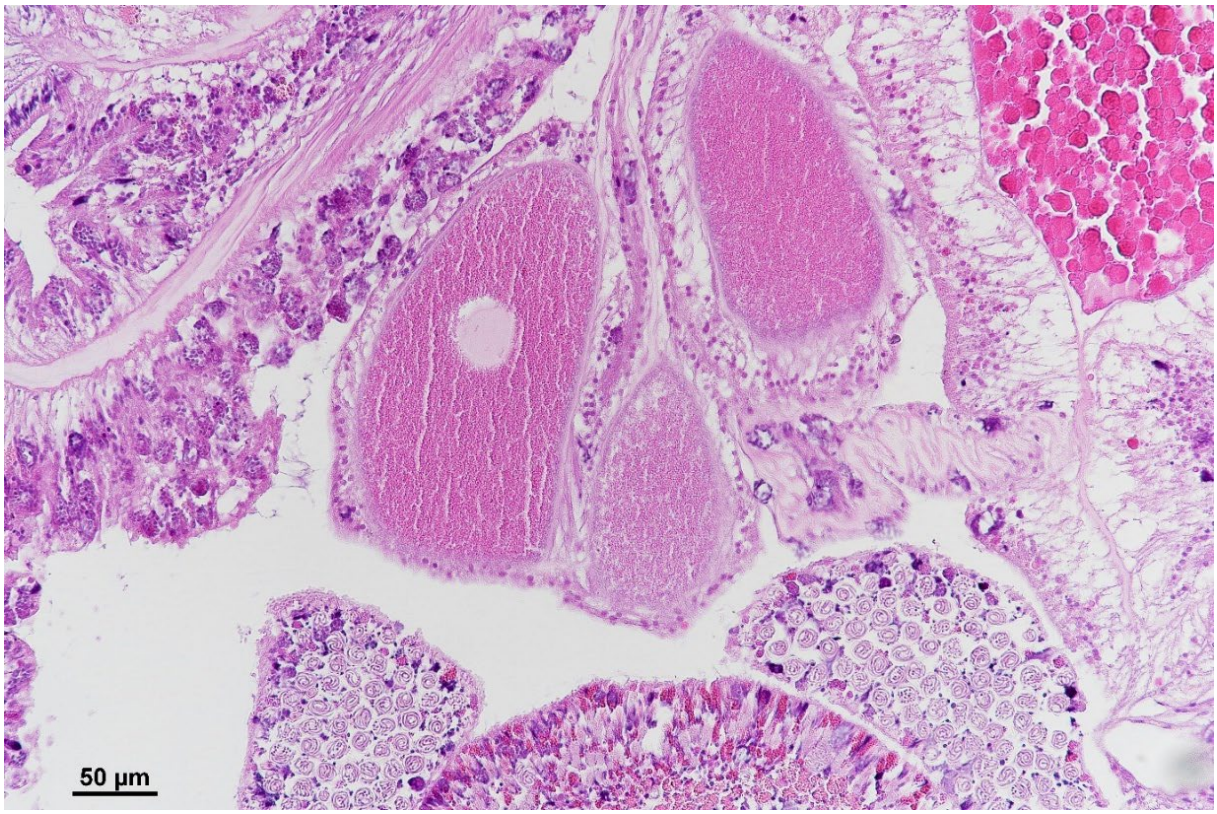

Figure 3-3: Longitudinal section through a terminal polyp of *G. dumosa* specimen NIWA148101. Specimen is a female, maturing oocytes are evident in the centre of the section, a partial mature (stage IV) oocyte can be seen in the top right of the image. 200x magnification. Scale bar is 50 µm.

Specimen GDU_102472 (Figure 3-4) is an example of a male specimen which was originally fixed in ethanol then post-fixed in 10 % neutral buffered formalin prior to tissue processing. The dark purple stained spermiaries containing mature spermatozoa are evident in the centre of the section (Figure 3-5, Figure 3-6).

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 3-3) 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 allowed 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 3-4: Fragment of *G. dumosa* specimen NIWA102472. A terminal and sub-terminal polyp were clipped from the matrix for tissue processing.

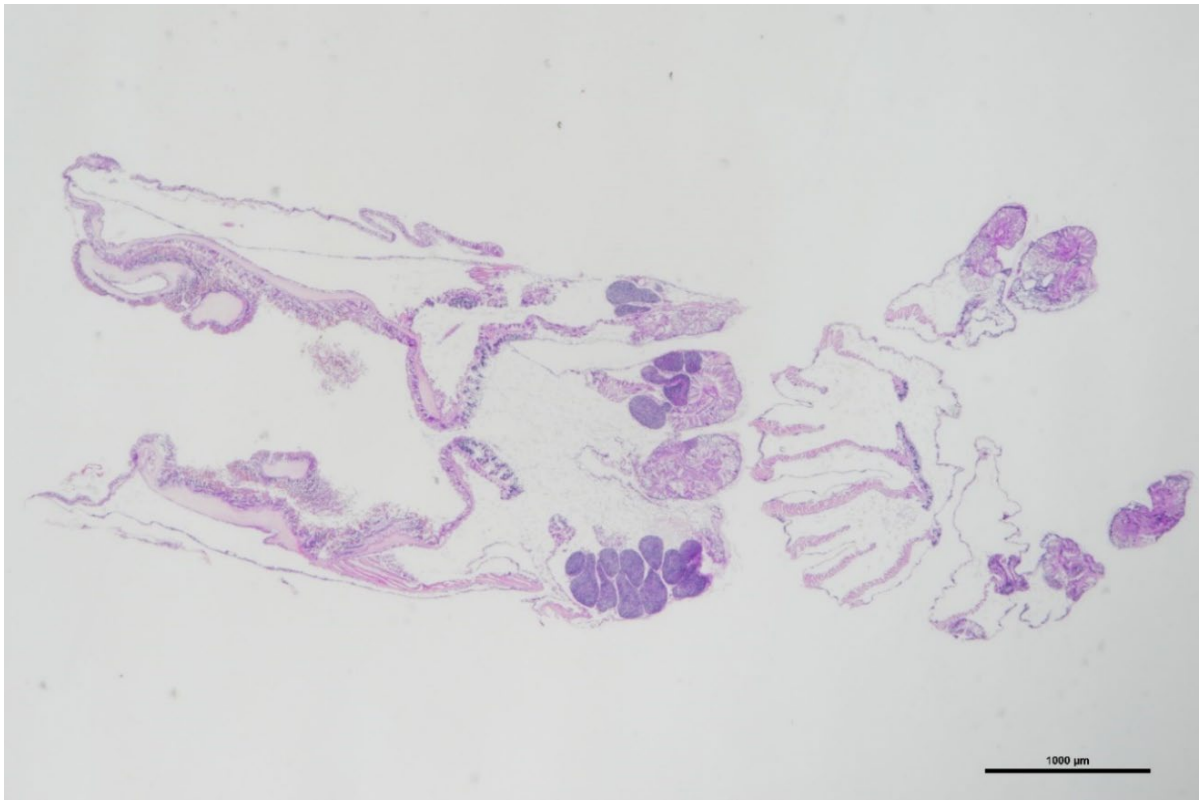

Figure 3-5: Longitudinal section through a terminal polyp of *G. dumosa* specimen NIWA102472. Specimen is a male, the dark purple stained spermiaries containing mature spermatozoa are evident in the centre of the section. 20 x magnification. Scale bar is 1000 µm.

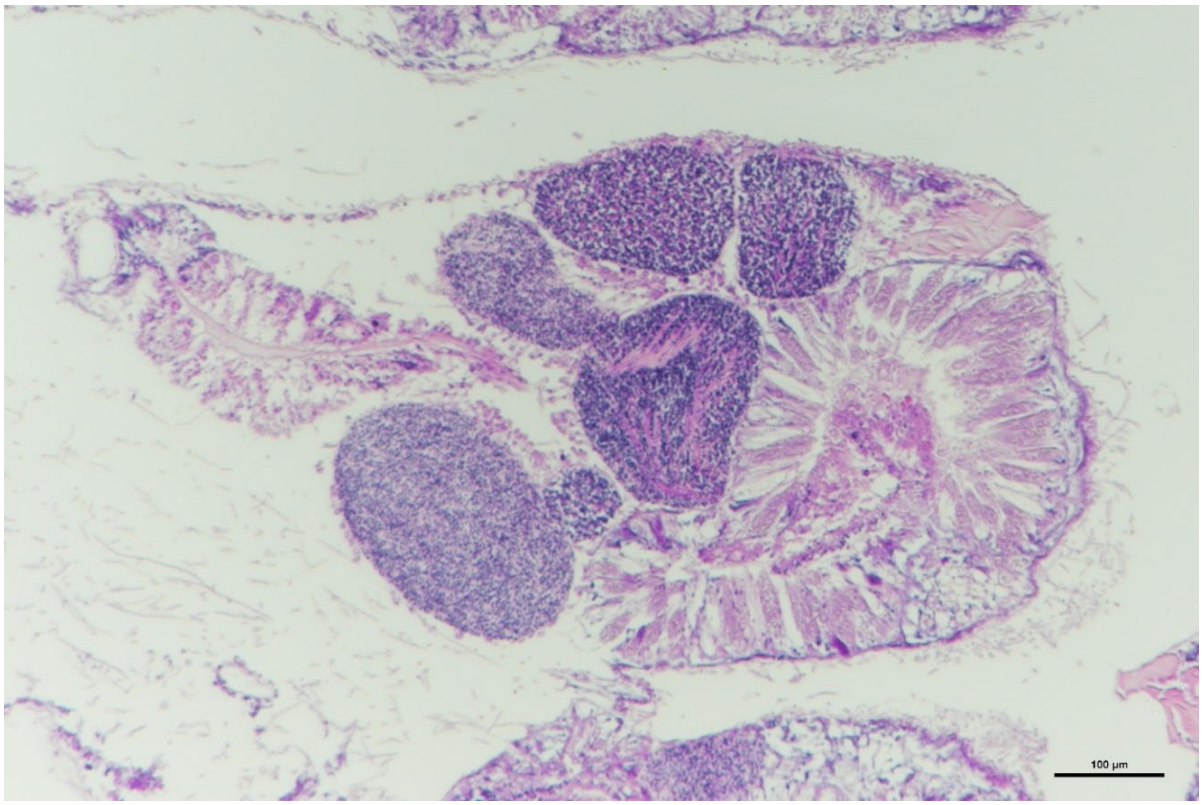

Figure 3-6: Longitudinal section through a terminal polyp of *G. dumosa* specimen NIWA102472. Specimen is male, the dark purple stained spermiaries containing mature spermatozoa are evident in the centre of the section. 132 x magnification. Scale bar is 100 µm.

3.1.1 Female reproductive data (seasonality, mode and oocyte size)

In total 1084 oocytes were recorded within the 101 Female polyps analysed. Of these, 19 were stage I, 130 were stage II, 401 were stage III, 469 were stage IV, 7 were stage V and 38 were un-staged.

While the seasonal spread of data was restricted by availability of samples, stage IV oocytes were present in all specimens across all seasons sampled, except for GDU_140313 which collected on the 21 June 2019 (Figure 3-8,Table 3-2) which had a maximum oocyte maturity of stage III. However, two other specimens collected on the 21st and 22nd June of the same year had stage IV oocytes present showing between-specimen variation and the importance of multiple specimens (where available).

The presence of stage V larvae within these specimens confirms that G. dumosa is a brooder. Stage V larvae were only observed in specimen GDU_141768, collected on the 20th January 2020 (e.g., Figure 3-7). This specimen also had the largest stage IV oocytes, similar in size to the stage V larvae (Figure 3-8). However, large stage IV oocytes were also observed in samples collected in April, June and December. The maximum, minimum and mean recorded size of measured oocytes is given in Table 3-3.

Table 3-2: Maximum observed maturity of oocytes within female *G. dumosa* specimens.

Species	NIC number	Collection date	Polyps analysed	Max. oocyte stage	Comment
GDU	112065	5 January	11	IV mature	
GDU	141768	20 January	16	V mature	
GDU	102639	11 April	3	IV mature	
GDU	140313	21 June	18	III maturing	
GDU	140326	21 June	22	IV mature	
GDU	140346	22 June	17	IV mature	
GDU	148101	16 August	2	IV mature	Histology trials (smaller section of polyp analysed)
GDU	27578	31 December	12	IV mature	
Total polyps			**101**		

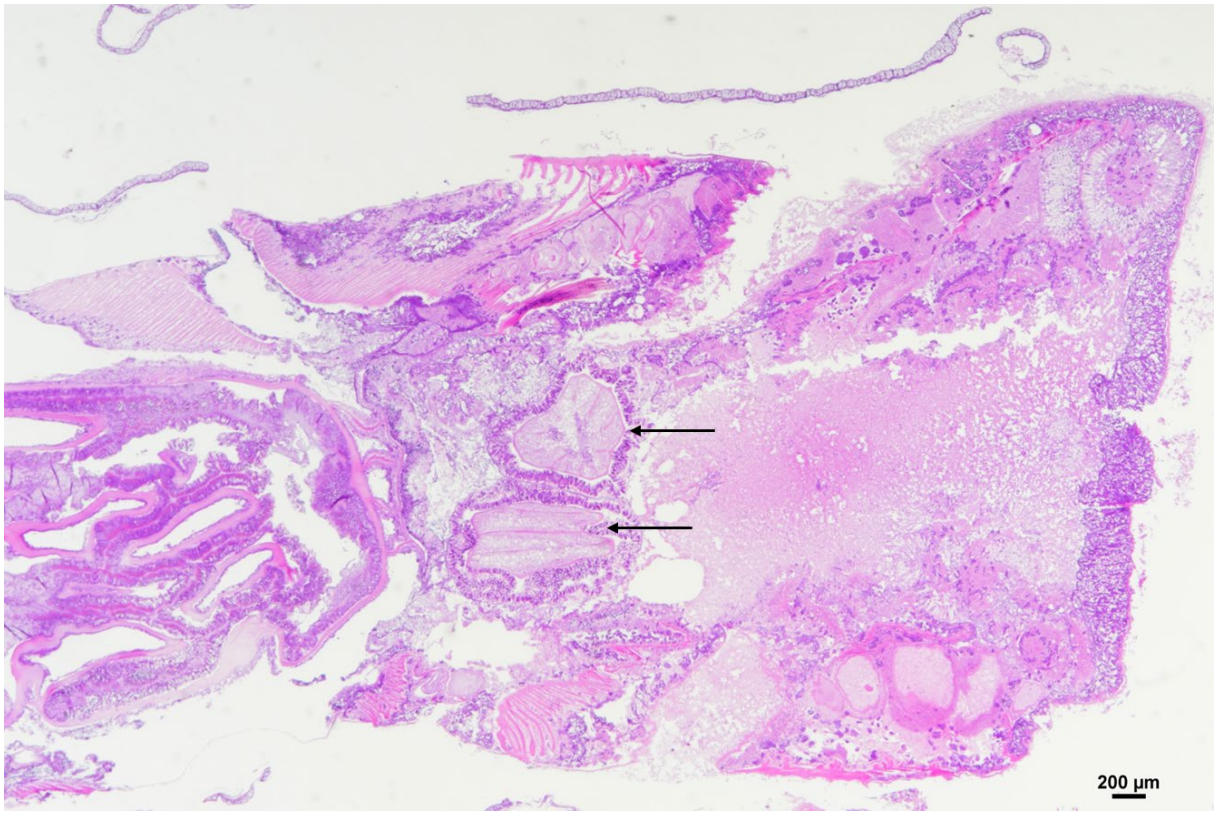

Figure 3-7: Specimen GDU141768 polyp E with two stage V larvae visible in the centre. Stage V larvae indicated with black arrows. Less mature oocytes are visible at centre top and bottom right of the image. Scale bar is 200 microns.

Table 3-3: Summary measurements of *G. dumosa* oocyte stages. Note that for stages I, II and III only oocytes with a visible nucleus were measured. Stage IV Oocytes and stage V larvae were measured if they were intact and were not obviously tangentially sliced.

Oocyte stage	Count	Max diameter (µm)	Min diameter (µm)	Mean diameter (µm) ± SD
I	13	38	8	24 ± 5
II	71	258	16	69 ± 33
III	67	355	47	181 ± 54
IV	306	1151	72	465 ± 145
V	7	1142	527	785 ± 112

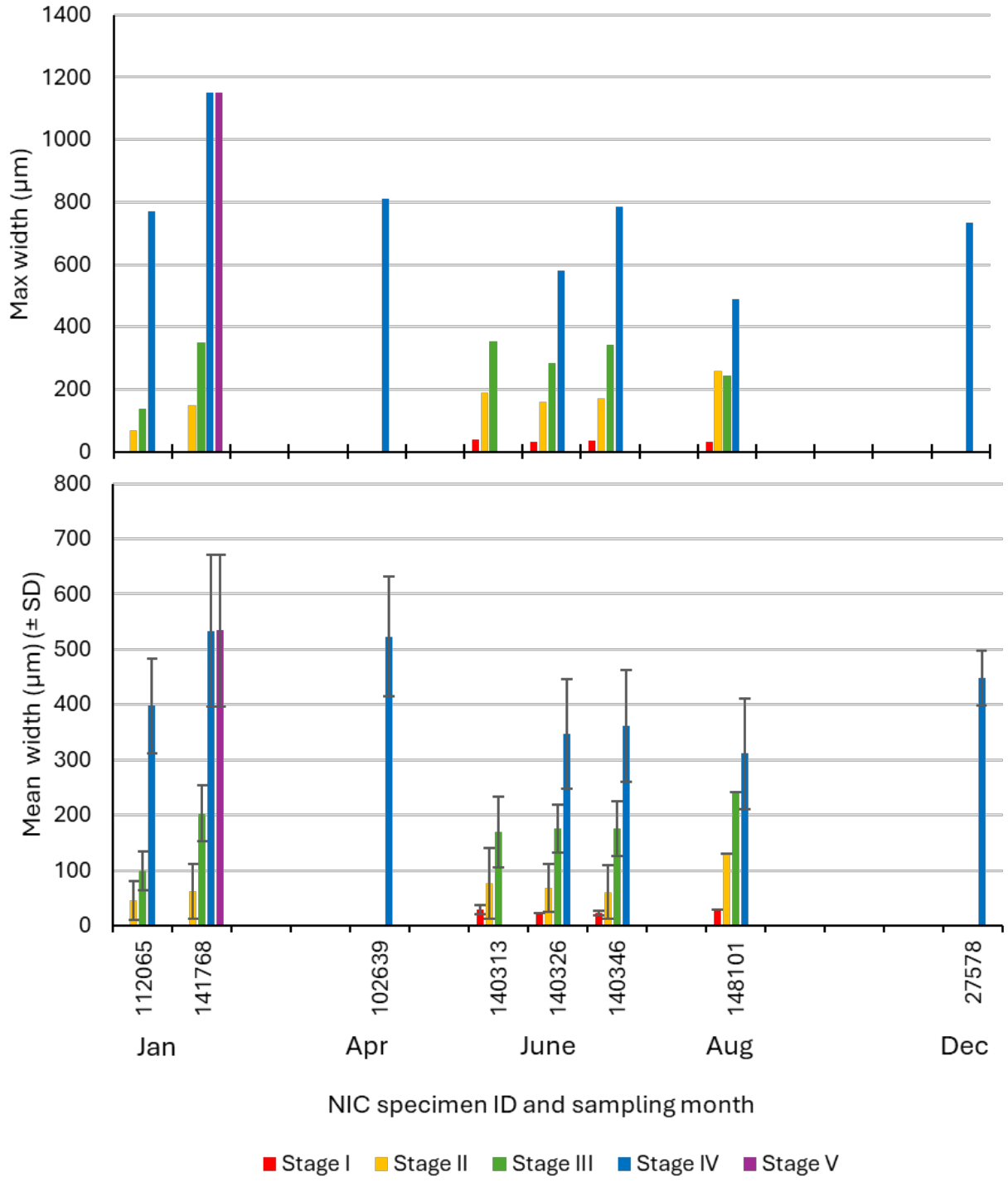

Figure 3-8: Maximum and mean size of observed *G. dumosa* oocytes within each specimen.

Fecundity estimates

The total observed oocyte count for each (half) polyp and frequency of oocyte stage is shown in Figure 3-9 (raw data available on request). Only polyps that were orientated correctly to obtain latitudinal sections were included here. The highest recorded abundance of oocytes was from specimens 141768 (20 Jan 2020) and 140346 (22 June 2019), though there was significant variability between polyps from within these specimens. It is important to note that the variability between specimens may reflect the quality of histological sections as well as true variability between seasons. Specimen 112065 was collected in January 2004 and resulted in very poor histological sections which meant that only the largest oocytes were able to be collected. The relatively low abundance may reflect this.

Most of the data presented here were from polyps collected in January and June, with three polyps sampled in April and four in December. Stage IV oocytes were dominant in most of the January and April samples; one polyp was immature with no oocytes. There was no compelling evidence for seasonal variations in fecundity.

The mean, maximum and minimum number of oocytes per ½ polyp, and estimated oocytes per full polyp, for each specimen is presented in Table 3-4. Maximum oocyte counts within the half polyps analysed ranged from 4 – 78 and mean values from 1.5 ± 1.91 to 32 ± 20.54 oocytes per half polyp.

Estimates of fecundity per full polyp showed a maximum range between 8 and 172 oocytes per polyp (Table 3-4), with mean values ranging between 3.5 ± 4.12 to 70.22 ± 45.59 oocytes per polyp.

A maximum of two stage V larvae were observed in any half polyp (specimen GDU_141768 polyp E) (Appendix C). From this, the estimated number present within a full polyp would be four larvae.

Using the fecundity estimates within Table 3-4, and measurement and polyp counts from 3D imaging (e.g., Figure 3-10), we can estimate that the mean reproductive potential of the specimens analysed. For example, the 3D scans have shown a colony fragment 58 mm by 45 mm by 49 mm can contain up to 49 polyps (e.g., specimen GDU_141768, Table 3-5). Couple this with the mean estimation of fecundity per polyp (Stages III, IV and V) implies this colony fragment of *G. dumosa* coral could produce 2826 ± 1830 viable larvae in a given year, assuming all stage III oocytes and up are going to mature in that given year. In reality the number will be less as not all oocytes will get fertilised, mature and liberate as functioning larvae. A number of these oocytes will inevitably become non-viable and will be resorbed by the coral polyp through the process of atresis.

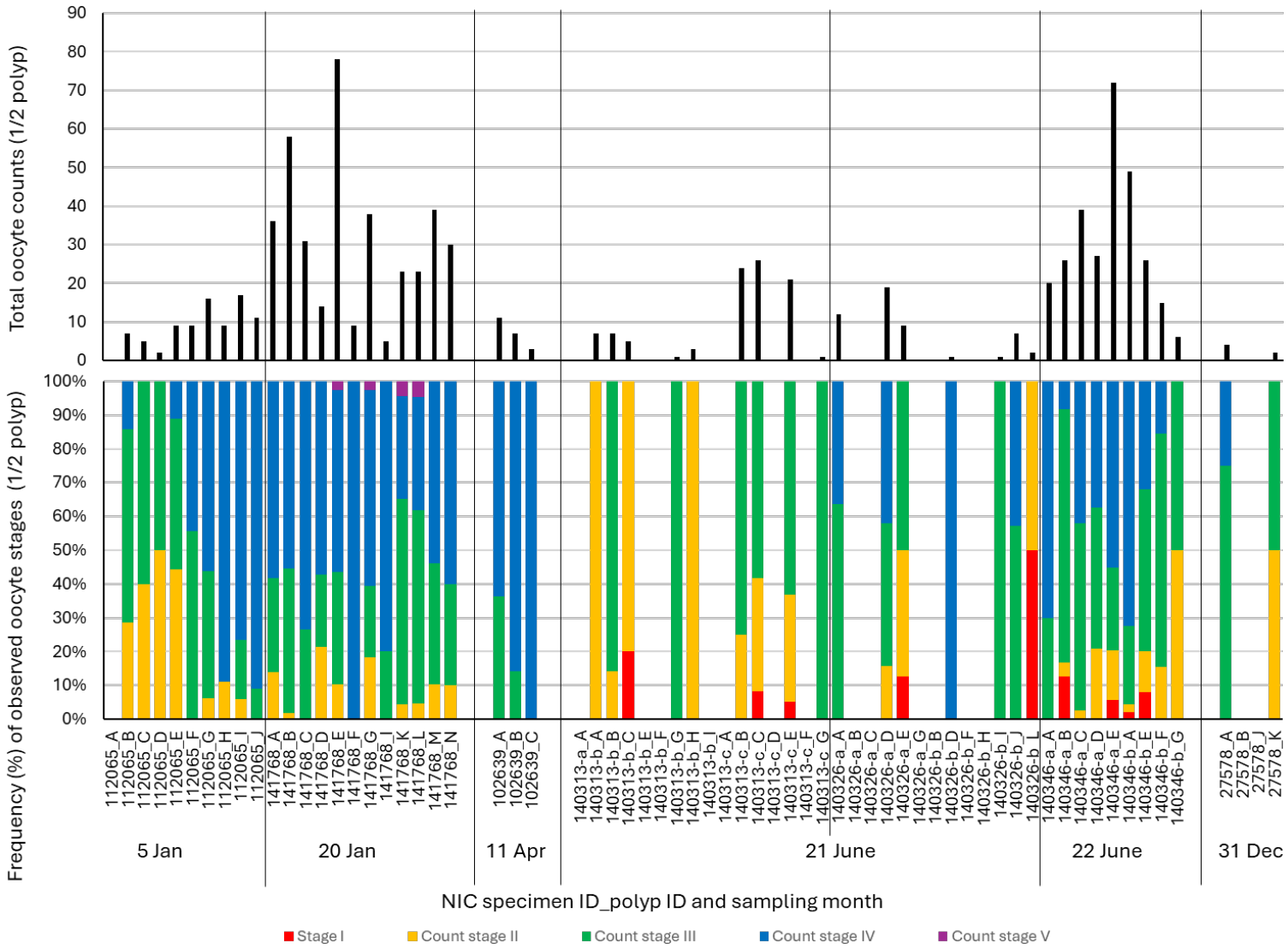

Figure 3-9: Total observed oocytes per ½ polyp and frequency of oocyte stage for *Goniocorella dumosa*. Note that the variation in oocyte counts and frequency of oocyte stages may reflect the quality of the histological sections as well as seasonal variation or variation between specimens/polyps. Only polyps that were orientated correctly to obtain latitudinal sections were included here. None of the specimens collected in August (GDU_148101) were suitable for use in fecundity estimates. Vertical lines show breaks between specimens and collection dates.

Table 3-4: Fecundity estimate for *G. dumosa* specimens. Observed oocyte counts per half-polyp and estimated fecundity within a full *G. dumosa* polyp (all oocytes and just stages III and above). Sections were taken at every 200 µm within half of each polyp. Therefore, total fecundity was estimated by quadrupling counts of stage I and II oocytes and by doubling oocytes within stages III, IV and V.

Metric	Specimen	No of polyps	Maximum oocytes per polyp	Minimum oocytes per polyp	Mean ± SD oocytes per polyp
Raw counts (1/2 polyp):	27578	4	4	0	1.5 ± 1.91
All oocytes	102639	3	11	3	7 ± 4
	112065	10	17	0	8.5 ± 5.42
	141768	12	78	5	32 ± 20.54
	140313	16	26	0	5.94 ± 9.18
	140326	13	19	0	3.92 ± 6.06
	140346	9	72	6	31.11 ± 19.80
	ALL specimens	**67**	**78**	**0**	**13.76 ± 17.3**
Estimated total fecundity (full polyp):	27578	4	8	0	3.5 ± 4.12
All oocytes	102639	3	22	6	14 ± 8
	112065	10	36	0	19.4 ± 11.2
	141768	12	172	10	69.5 ± 44.88
	140313	16	72	0	16.63 ± 24.47
	140326	13	44	0	9.23 ± 13.99
	140346	9	172	18	70.22 ± 45.59
	ALL specimens	**67**	**172**	**0**	**31.37 ± 38.48**
Estimated annual fecundity (full polyp):	27578	4	8	0	2.5 ± 3.79
Stage III, IV and V only	102639	3	22	6	14 ± 8
	112065	10	32	0	14.6 ± 11.04
	141768	12	140	10	57.67 ± 37.35
	140313	16	36	0	6.5 ± 11.92
	140326	13	32	0	6.15 ± 10.34
	140346	9	110	6	51.11 ± 33.41
	ALL specimens	**67**	**140**	**0**	**22.89 ± 30.44**

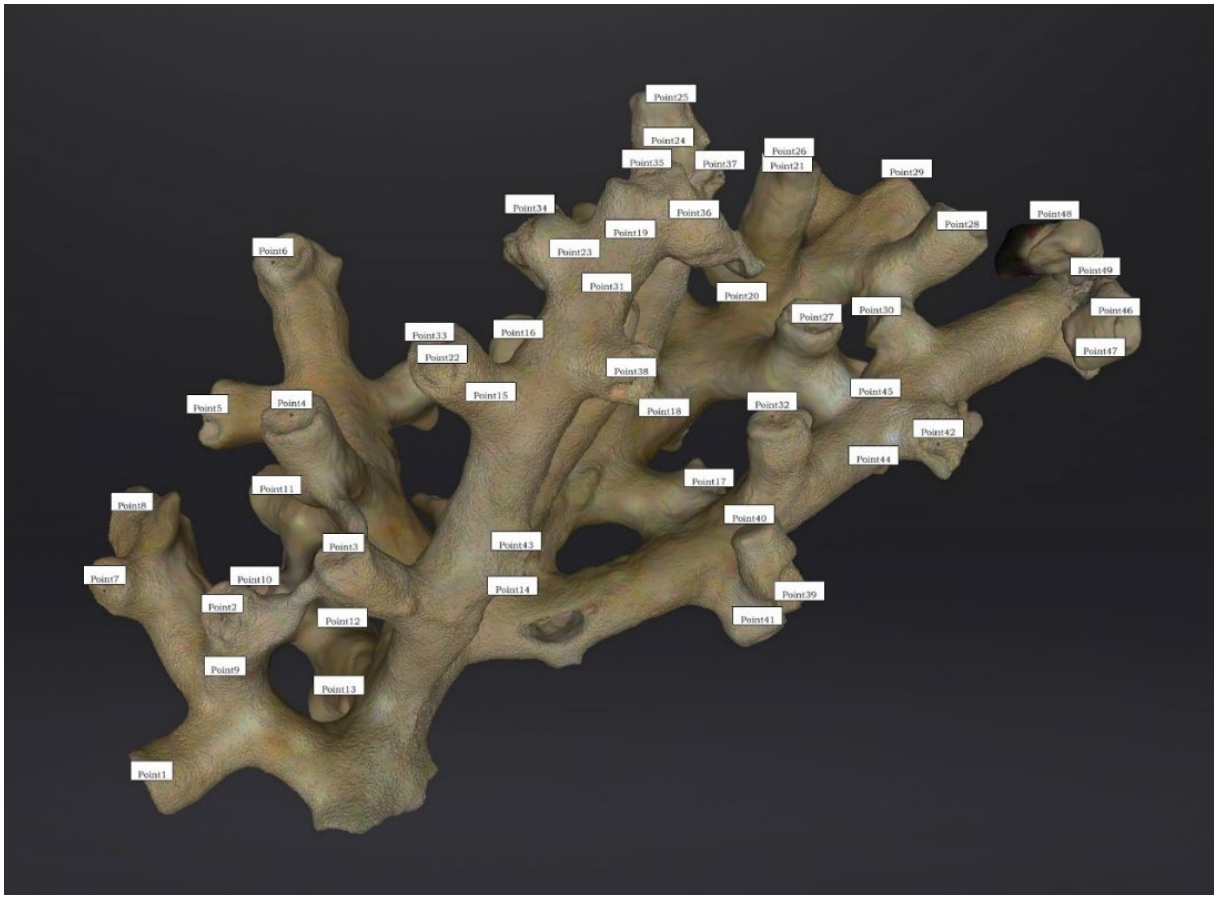

Figure 3-10: Example of a 3D image of a GDU specimen and polyp counts. *G. dumosa* specimen NIWA-141768. The 3D model was rotated to identify and mark all individual polyps on the 3D reconstruction. Sixteen polyps were analysed from this specimen.

Table 3-5: Estimated potential fecundity per specimen fragment.

	Fragment dimensions				Oocytes per polyp (mean ± SD)		Estimated oocytes per fragment	
Specimen	Length (mm)	Width (mm)	Height (mm)	Polyps per fragment	All oocytes	Stage III and up only	All oocytes	Stage III and up only
27578	66.01	53	65.93	105	3.5 ± 4.12	2.5 ± 3.79	**368 ± 433**	**263 ± 398**
102639	58.34	37.35	36.52	32	14 ± 8	14 ± 8	**448 ±256**	**448 ± 256**
112065	72.27	75.65	87.18	219	19.4 ± 11.2	14.6 ± 11.04	**4249 ± 2452**	**3197± 2417**
140313	33.14	30.58	43.2	34	16.63 ± 24.47	6.5 ± 11.92	**565 ± 832**	**221±405**
140326	25.89	19.13	26.1	16	9.23 ± 13.99	6.15 ± 10.34	**148 ± 234**	**98 ± 165**
140346	45.38	45.27	40.9	38	70.22 ± 45.59	51.11 ± 33.41	**2668 ±1732**	**1942 ±1270**
141768	58.43	44.9	48.58	49	69.5 ± 44.88	57.67 ± 37.35	**3406 ± 2199**	**2826 ± 1830**

3.1.2 Male reproductive data

Three specimens were confirmed to be male: GDU_102472, GDU_102566, and GDU_148157. Mature spermiaries were observed in specimens from both April and August (Table 3-6).

Table 3-6: Maximum observed maturity of spermiaries within male *G. dumosa* specimens.

Species	NIC number	Collection date	Polyps analysed	Max stage
GDU	102472	11 April 2015	2	Stage III Maturing
GDU	102566	11 April 2015	10	Stage IV Mature
GDU	148157	19 August 2020	6	Stage IV Mature
Total polyps			**18**	

3.2 *Enallopsammia rostrata* (ERO)

The *E. rostrata* specimens used within this study are summarised in Table 3-7. Specimen ERO_43171 (used in initial histology trials) was collected from the northern Bay of Plenty. The rest of the specimens were collected from the Chatham Rise.

Of the 13 specimens, five were female, six were male and two were unsexed/immature. Specimens had either male or female polyps which confirms this species is gonochoric. Note, however there was a single polyp on a male specimen which was possibly an immature female. This polyp had a very different growth form from the rest of the polyps in the colony and most likely represents a newly settled colony on an existing living host colony, the morphology of this polyp strongly suggests it is a different species from the host colony, therefore should not be included in this analysis.

An example of a female *E. rostrata* specimen, ERO_53483) was initially fixed in ethanol and post-fixed in formalin prior to tissue processing. This specimen had a very robust calcified skeleton so required extensive decalcification. The section shows oocytes present in the mesenteries towards the left of the section (Figure 3-11). Shrinkage of oocytes is apparent from long term storage in ethanol (Figure 3-12).

Table 3-7: Summary of polyps analysed from each *E. rostrata* specimen. M/F/U = Male/Female/Unsexed (immature?). Ordered by collection month. 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

Species	NIC number	Collection date	Year	Depth (m)	Location	Preservation method	No. of Polyps analysed	M/F/U	Comment
ERO	102305	4 April	2015	918	Chatham Rise	EtOH	12	U	
ERO	102568	11 April	2015	622	Chatham Rise	EtOH	10	F	
ERO	102631	11 April	2015	570	Chatham Rise	EtOH	12	M	
ERO	43171	17 April	2002	1366	Northern Bay of Plenty	EtOH	2	M	Analysed as part of histology trials
ERO	53483	22 June	2009	820	Chatham Rise	EtOH	9	F	
ERO	53554	25 June	2009	613	Chatham Rise	EtOH	10	F	A few polyps exhibited old well degenerated atretic oocytes. No other female reproductive data available from this specimen
ERO	54027	27 June	2009	760	Chatham Rise	EtOH	8	F	
ERO	54169	27 June	2009	716	Chatham Rise	EtOH	8	M	
ERO	53486	22 June	2009	820	Chatham Rise	EtOH	6	M	
ERO	53719	26 June	2009	641	Chatham Rise	EtOH	11	M	
ERO	148158	19 August	2020	640	Chatham Rise	F, EtOH	2	F	Analysed as part of histology trials
ERO	148159	19 August	2020	640	Chatham Rise	F, EtOH	3	M	Analysed as part of histology trials
ERO	81272	16 December	2000	621	Chatham Rise	EtOH	9	U	Possibly resting male but hard to identify immature spermiaries
Total polyps							**103**		

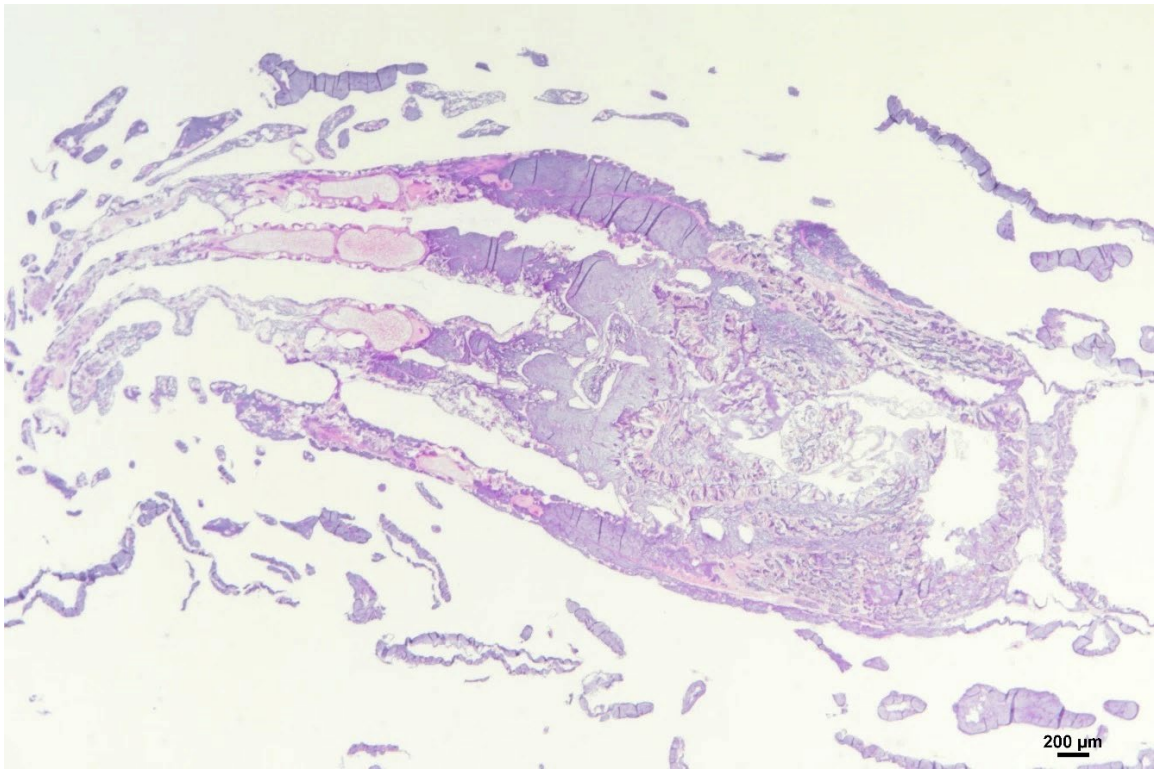

Figure 3-11: Example image of a female ERO polyp. Specimen ERO_53483. Oocytes (pink) are visible in mesenteries towards the left of the image. The base of the polyp is to the right. Scale bar is 200 microns.

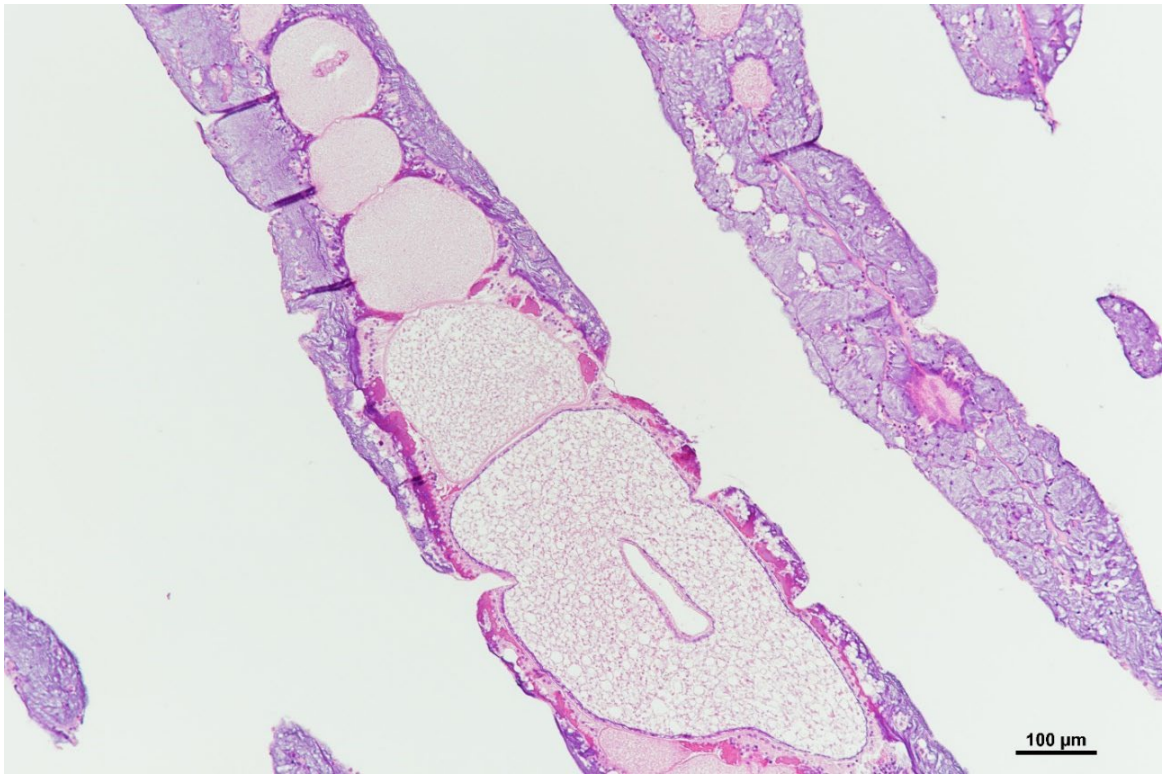

Figure 3-12: Example image of oocytes within a female ERO polyp, 100x magnification. Specimen ERO_102568. Image shows in the central mesentery three stage III oocytes in the top half of the mesentery, the uppermost is sectioned through the nucleus, and two Stage IV oocytes in the lower half of the mesentery. The structure in the centre of the Lower Stage IV oocyte is an invagination of the oocyte wall, an artefact of the excessive shrinkage of the oocyte resulting from long term ethanol fixation. Scale bar is 100 microns.

An example of a male *E. rostrata* specimen, ERO_148159 (Figure 3-13) 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 (Figure 3-14,Figure 3-15). 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 3-13: Fragment of *Enallopsammia rostrata* specimen NIWA148159. A terminal and a sub-terminal polyp were clipped from the matrix for tissue processing.

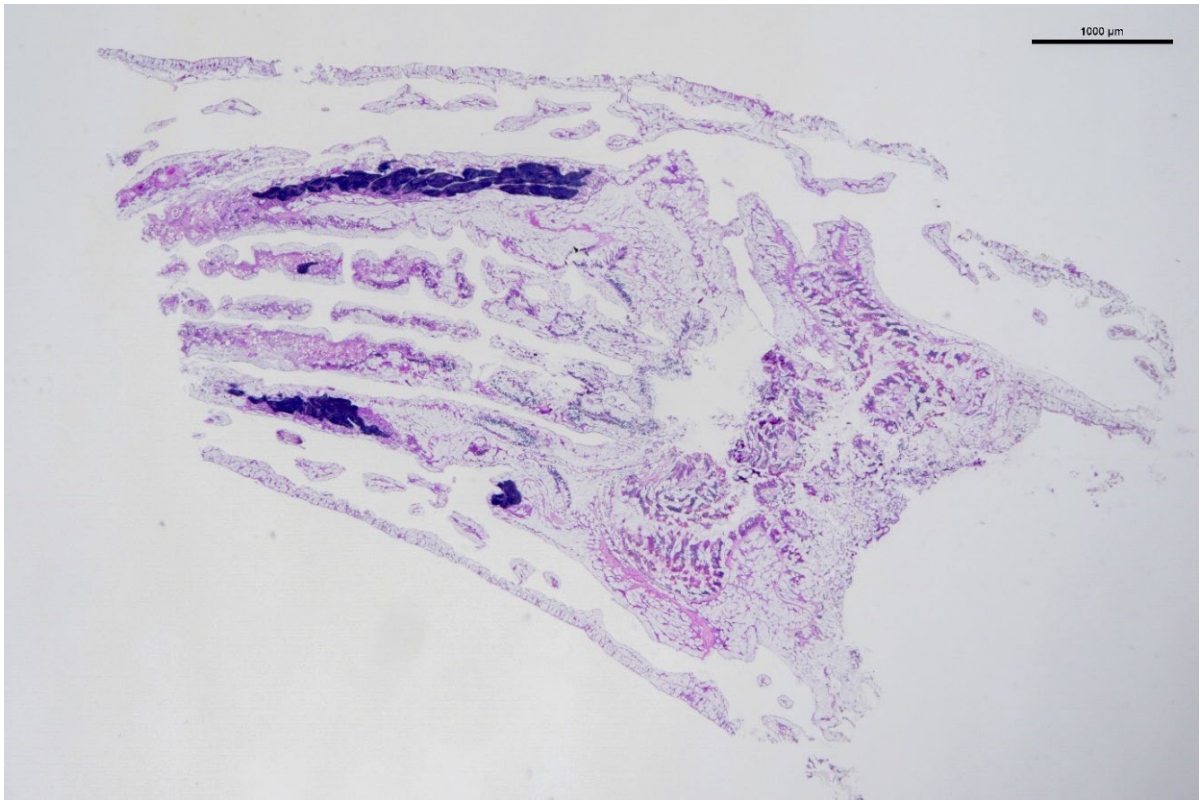

Figure 3-14: Longitudinal section through a terminal polyp of *Enallopsammia rostrata* specimen NIWA148159. Specimen is a male, the dark purple stained spermiaries containing mature spermatozoa are evident in the centre left of the section. 17x magnification. Scale bar is 1000 µm.

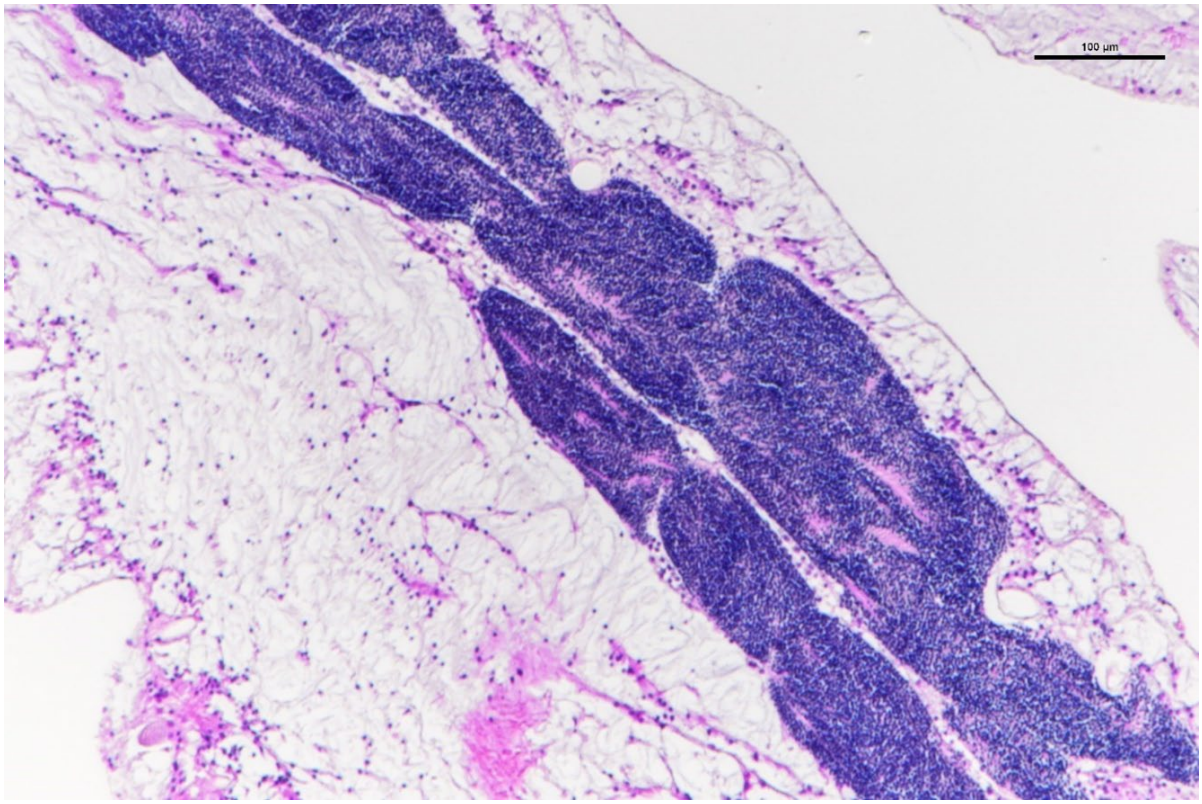

Figure 3-15: Longitudinal section through a terminal polyp of *Enallopsammia rostrata* specimen NIWA148159. Specimen is a male, the dark purple stained spermiaries containing mature spermatozoa are evident in the centre left of the section. 150x magnification. Scale bar is 100 µm.

3.2.1 Female reproductive data

In total, 345 oocytes were recorded within the 29 female polyps analysed. Of these, 28 were stage II (immature), 172 were stage III (maturing) and 143 were stage IV (mature).

Mature (stage IV) oocytes were present in all female specimens sampled in April and June (Figure 3-16, Table 3-8), with a maximum maturity of stage III (maturing) oocytes in August. Stage II (immature) and stage III (maturing) oocytes were present in all samples. Note that the August sample (ERO 148158) was from the initial trials and only a small number of histology sections were analysed (i.e., not a complete half polyp as for other specimens). The maximum, minimum and mean recorded size of measured oocytes is given in Table 3-9.

Table 3-8: Maximum observed maturity of oocytes within female E. *rostrata* specimens. Ordered by collection day/month. Note that ERO_148158 was sampled as part of histology trials and less histology sections were analysed per polyp (not a complete half polyp).

Species	NIC number	Collection date	No. of Polyps analysed	Max F stage	
ERO	102568	11 April	10	IV mature	
ERO	53483	22 June	9	IV mature	
ERO	54027	27 June	8	IV mature	
ER0	148158	19 August	2	III maturing	Initial trials only
Total polyps			**29**		

Table 3-9: Summary measurements of *E. rostrata* oocyte stages. Note that for stages II and III, only oocytes with a visible nucleus were measured. Stage IVs were measured if they were intact and not tangentially sliced. Oocytes, particularly when more mature, were markedly elongate, hence we have presented mean values as the mean (of maximum and minimum) and the mean of the maximum measurements.

Oocyte stage	Count	Max diameter (µm)	Min diameter (µm)	Mean diameter (µm) ± SD	Mean maximum diameter (µm) ± SD
II	19	147	16	70.47 ± 19.22	88.84 ± 23.10
III	60	366	27	153.31 ± 50.52	191.78 ± 66.07
IV	50	1088	82	333.49 ± 128.13	494.1 ± 216.41

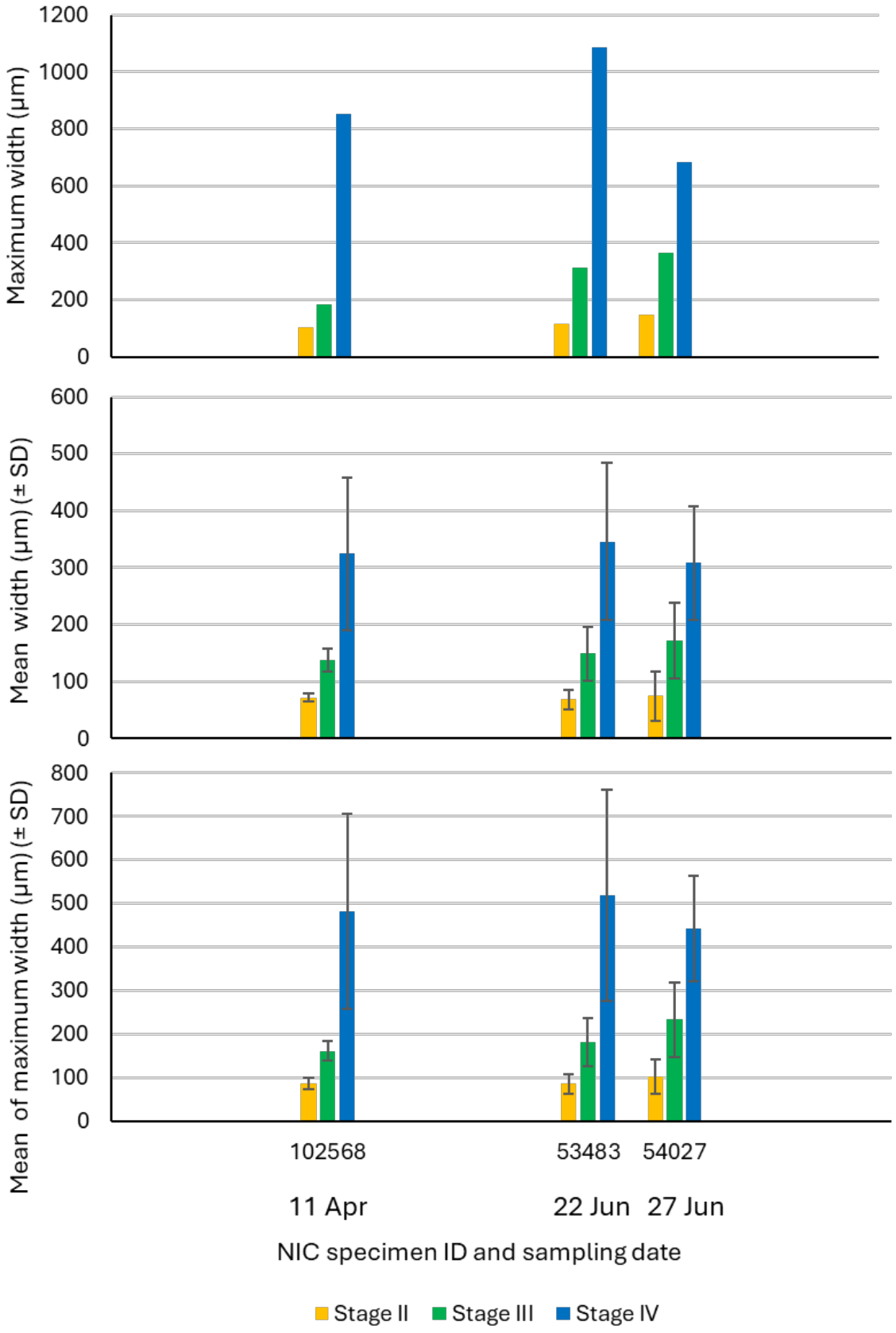

Figure 3-16: Maximum and mean size of observed *E. rostrata* oocytes within each specimen. *E. rostrata* oocytes become markedly elongate as they mature, oriented along the central axis of narrow mesenteries. We have presented both the mean (of the maximum and minimum measurements) and a mean of the maximum width of oocytes. No size data were available from specimen ERO_18158.

Fecundity estimates

The total observed oocyte count for each (half) polyp and frequency of oocyte stage is shown in Figure 3-17. Note only specimens suitable for fecundity estimates are included (specimen ERO_148158, with a maximum observed oocyte stage III, was excluded). The highest recorded oocyte abundance was in specimen ERO_53483 collected on 22 June. However, this plot shows the variability between polyps within a specimen and between specimens. Specimen ERO_54027 was collected just 5 days after ERO53483 in the same year (2009) and had lower oocyte abundance.

Stage II, III and IV oocytes were present in all specimens (Figure 3-17), though there was variability in the frequency of oocyte stages between polyps.

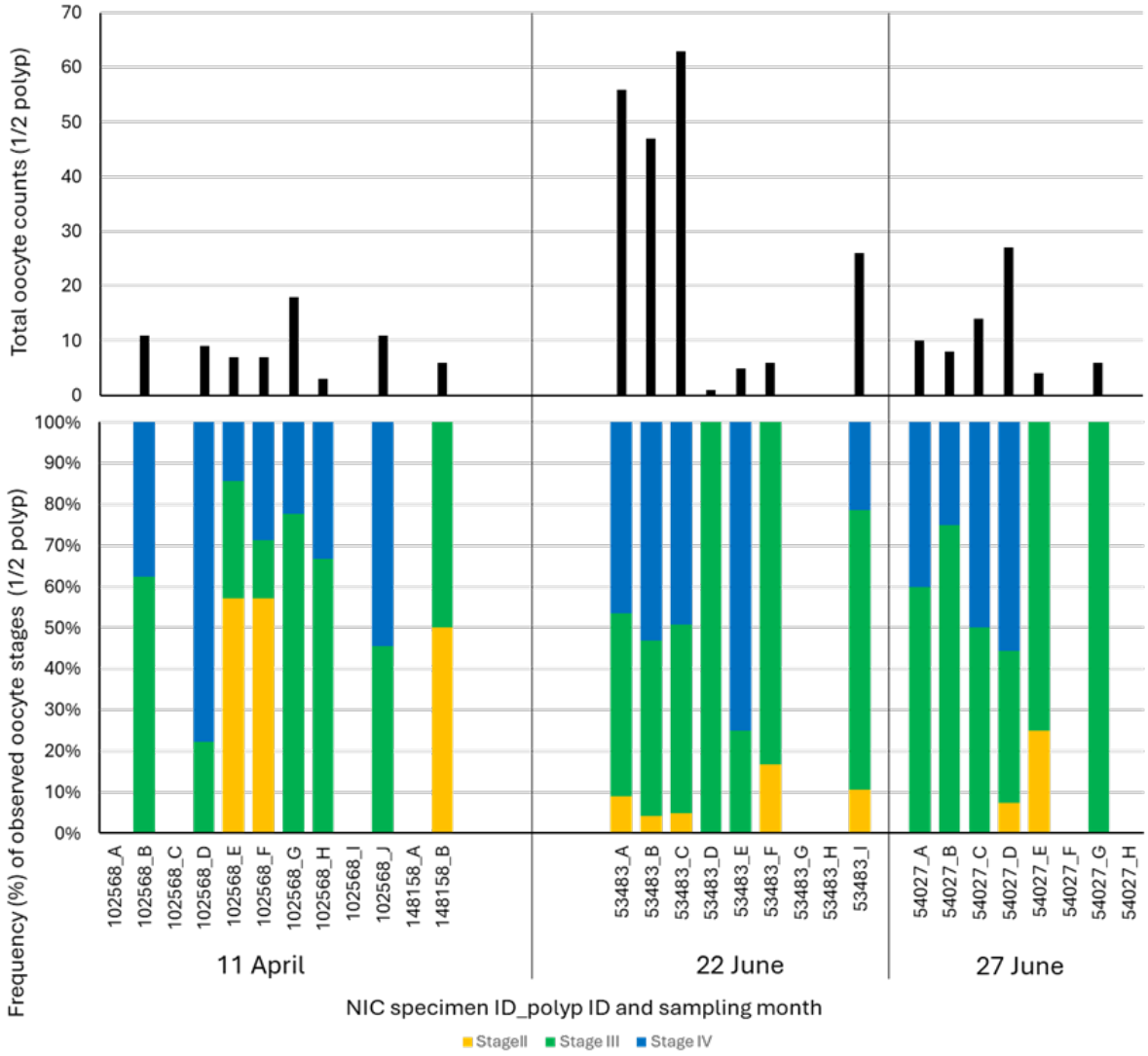

Figure 3-17: Total observed oocytes per 1/2 polyp and frequency of oocyte stage. Vertical lines show breaks between specimens and collection dates.

The mean, maximum and minimum number of oocytes per ½ polyp, and estimated oocytes per full polyp, for each specimen, is presented in Table 3-10. Maximum oocyte counts within the half polyps ranged from 6 to 63, with mean values ranging from 3 ± 4.24 to 22.67 ± 26.05. Across all GDU specimens, the mean number of oocytes per polyp was 11.9 ± 16.77.

Estimates of fecundity per full polyp (all oocytes) showed a maximum range between 18 and 128 oocytes, with mean values between 9 ±12.73 and 48 ± 54.83, with a mean oocyte count per polyp across all GDU specimens of 25.59 ± 35.34 (Table 3-10). We have also presented an estimated fecundity using only oocytes of stage III and up. This is, perhaps, a better estimate of the potential fecundity of a polyp over a single reproductive season. Estimates here suggested a maximum range of 6 to 116 and a mean range of 3 ± 4.24 to 42 ± 48.41. The mean number of oocytes (stage III and above) estimated per polyps across all GDU specimens was 21.72 ± 31.51.

Table 3-10: Fecundity estimate for *E. rostrata* specimens. Observed oocyte counts per half-polyp and estimated fecundity within a full *E. rostrata* polyp. Sections were taken at every 200 µm within half of each polyp. Therefore, fecundity was estimated by quadrupling counts of stage II and by doubling counts of oocytes within stages III and IV. These should be considered minimum estimates.

Metric	Specimen	No. of polyps	Maximum oocytes per polyp	Minimum oocytes per polyp	Mean ± SD
Raw counts (1/2 polyp):	102568	10	18	0	6.6 ± 5.95
All oocytes	148158	2	6	0	3 ± 4.24
	53483	9	63	0	22.67 ± 26.05
	54027	8	27	0	8.63 ± 8.83
	All specimens	**29**	**63**	**0**	**11.90 ± 16.77**
Estimated fecundity (full polyp):	102568	10	36	0	14.2 ± 12.24
All oocytes	148158	2	18	0	9 ± 12.73
	53483	9	128	0	48.22 ± 54.83
	54027	8	58	0	18.5 ± 19.09
	All specimens	**29**	**128**	**0**	**25.59 ± 35.34**
Estimated fecundity (full polyp):	102568	10	36	0	11 ± 11.82
Stage III and IV only	148158	2	6	0	3 ± 4.24
	53483	9	116	0	42 ± 48.41
	54027	8	50	0	17 ± 17.10
	All specimens	**29**	**116**	**0**	**21.72 ± 31.51**

Using the fecundity estimates in Table 3-10, and measurements and polyp counts from 3D imaging (e.g., Figure 3-18), we can estimate that the mean reproductive potential of specimens analysed. For example, the 3D scans have shown a colony fragment 55 mm by 27 mm by 42 mm could contain up to 17 polyps (e.g. specimen ERO_53483, Table 3-11). Coupled with the mean estimation of fecundity per polyp (stages III, IV and V) it can be implied that this fragment of *E. rostrata* coral could produce 714 ± 823 viable larvae in a given year, assuming all stage III oocytes and up are going to mature in that given year. In reality the number will be less as not all oocytes will mature, be spawned and fertilized, and then develop as viable larvae. A number of these propagules will inevitably become non-viable and will be resorbed by the coral polyp through the process of atresis.

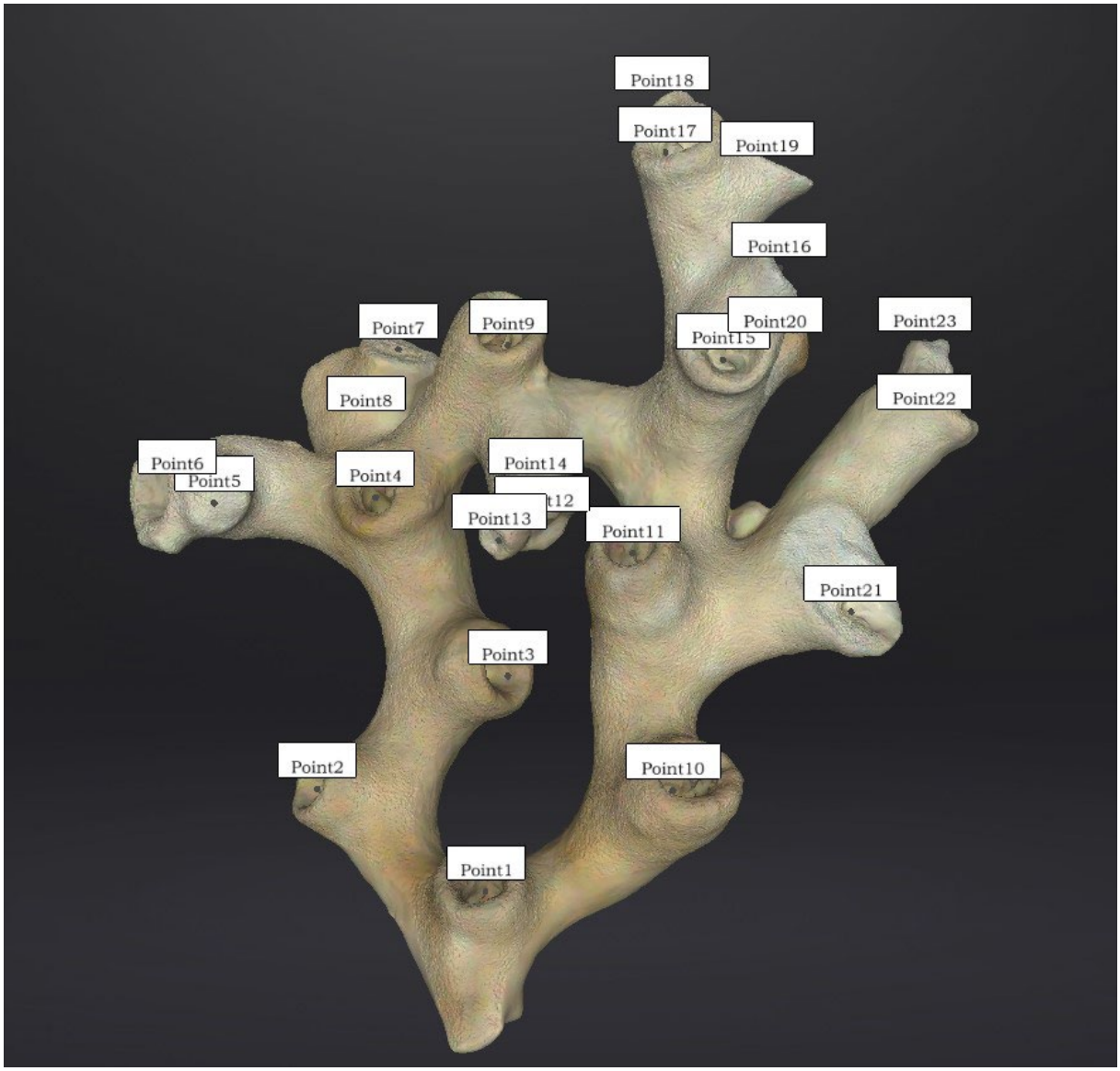

Figure 3-18: Example of a 3D image of an ERO specimen and polyp counts. *E. rostrata* specimen NIWA-53486. The 3D model was rotated to identify and mark individual polyps on the 3D reconstruction. Six polyps were analysed from this specimen.

Table 3-11: Estimated potential fecundity per specimen fragment.

	Fragment dimensions				Oocytes per polyp (mean ± SD)		Estimated oocytes per fragment	
Specimen	Length (mm)	Width (mm)	Height (mm)	Polyps per fragment	All oocytes	Stage III and IV only	Mean oocytes (all)	Mean oocytes (stage III and IV)
102568	62.11	27.4	41.49	25	14.2 ± 12.24	11.00 ± 11.82	**355 ± 306**	**275 ± 296**
53483	55.3	27.41	41.55	17	48.22 ± 54.83	42 ± 48.41	**820 ± 932**	**714 ± 823**
54027	46.4	25.65	36.35	15	18.5 ± 19.09	18.5 ± 19.09	**289 ± 286**	**255 ± 257**

3.2.2 Male reproductive data

The maximum observed maturity of polyps/specimens ranged from immature to mature stage IV spermiaries (Table 3-12). However, Stage IV mature spermiaries were observed in all seasons sampled (April, June, August).

Table 3-12: Maximum observed maturity of spermiaries within male E. *rostrata* specimens. Ordered by collection day/month.

Species	NIC number	Collection date	No. of Polyps analysed	Max M stage
ERO	102631	11 April	12	IV mature
ERO	43171	17 April	9	IV mature
ERO	53486	22 June	6	III maturing
ERO	53719	26 June	11	IV mature
ERO	54169	27 June	8	III maturing
ERO	148159	19 August	13	IV mature
Total polyps			**67**	

3.3 *Desmophyllum dianthus* (solitary cup coral)

Table 3-13 shows the specimens that were processed in New Zealand and shipped to the University of Gothenburg (Sweden) for analysis. Specimens were selected, imaged, 3D scanned and decalcified for histology prior to shipping. The results of this work will be included in this report once the analyses have been completed.

Table 3-13: ***Desmophyllum dianthus*** **samples for histological analyses.** F = fixed in formalin, EtOH is ethanol and Alcohol is unknown alcohol. Where the preservation method is "F, EtOH", the specimen has been first preserved in formalin then transferred into ethanol. * indicates where samples were potentially compromised/damaged during freighting to Sweden.

Catalogue number	Station ID	Date	Latitude	Longitude	Depth	Pres Type
88048 *	S248	19/02/1980	-44.6017	167.82001	30	Alcohol
149740	S261	22/02/1980	-45.3517	166.9883	32	Ethanol - previously unknown
104980	Z16074	2/02/1993	-45.349	167.056	15	F, EtOH
148125	TAN2009/57	16/08/2020	-44.159	-174.554	486	EtOH
140347 *	TAN1903/110	22/06/2019	-43.360667	179.74233	461	EtOH
127181	SO254/76 ROV13 BIOBOX1	19/02/2017	-45.026531	171.90355	678.3	EtOH
102485	TAN1503/116	11/04/2015	-44.159667	-174.55483	497	EtOH
88004	S181	31/10/1979	-43.445	173.5	392	Alcohol
88005	Q343	14/11/1979	-44.13	175.7967	500	Alcohol
54050	TAN0905/113	27/06/2009	-44.1495	-174.75683	519	EtOH
53534	TAN0905/95	25/06/2009	-44.136	-174.72117	613	EtOH
47034	X486	4/07/1994	-42.777	-179.9138	910	EtOH
25068 *	TAN0604/10	28/05/2006	-42.7653	-179.9282	1005	EtOH
4012 *	Z10698	16/04/2001	-42.78617	-179.9853	993	None
88075	TAN0104/153	18/04/2001	-42.7325	-179.8985	1076	F, EtOH
25591	S30	18/09/1978	-50.6833	167.67999	265	Alcohol
53838	TAN0905/105	26/06/2009	-44.157333	-174.55417	485	EtOH
54285	TAN0905/119	28/06/2009	-44.158167	-174.555	487	EtOH

4 Results: 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.

4.1 *Leiopathes bullosa* (NIWA53045)

The sections through the *Leiopathes bullosa* specimen (NIWA53045), preserved in Ethanol and post-fixed in formalin prior to histological analyses, took clean slices through the skeletal matrix and adjacent polyps producing well-stained and complete tissue sections (Figure 4-1, Figure 4-2). This specimen is likely to be male. The round organelles to the right of the image in Figure 4-2, 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.

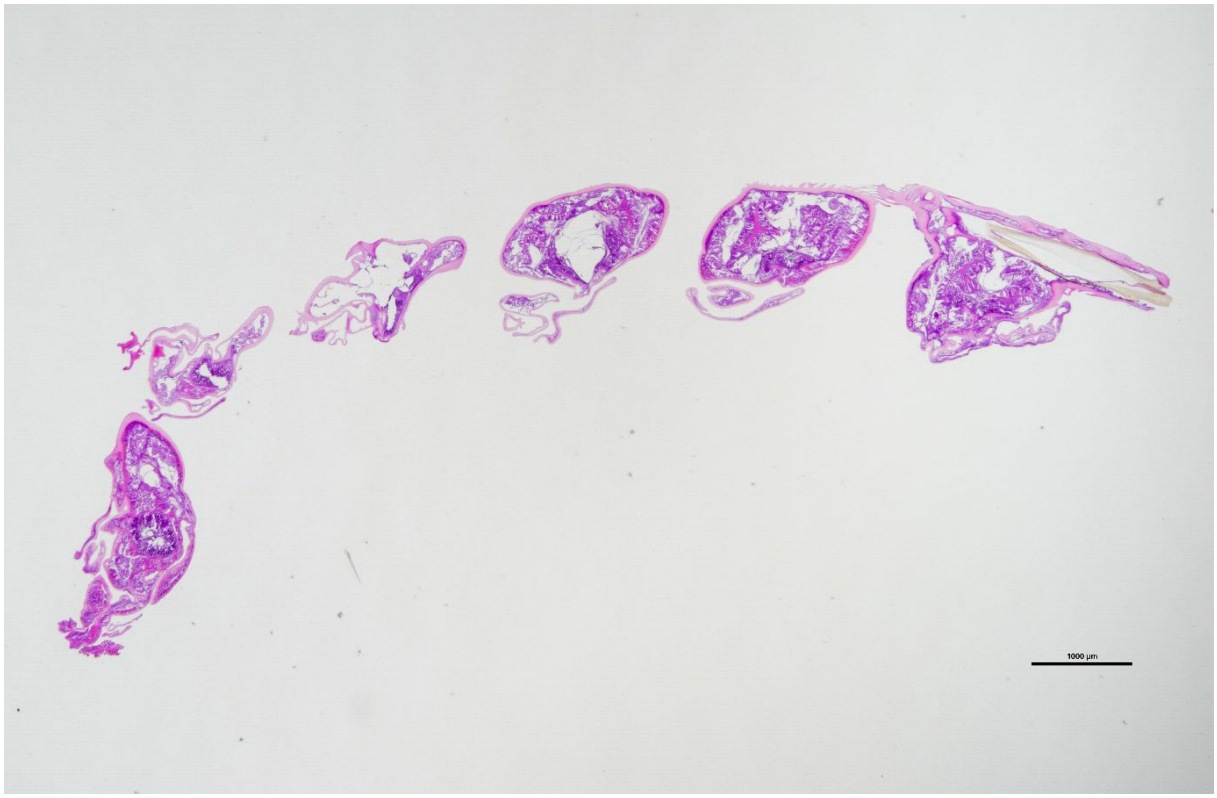

Figure 4-1: Longitudinal section through terminal polyps of *Leiopathes bullosa* specimen NIWA53045. Specimen is likely a male. 12x magnification. Scale bar is 1000 µm.

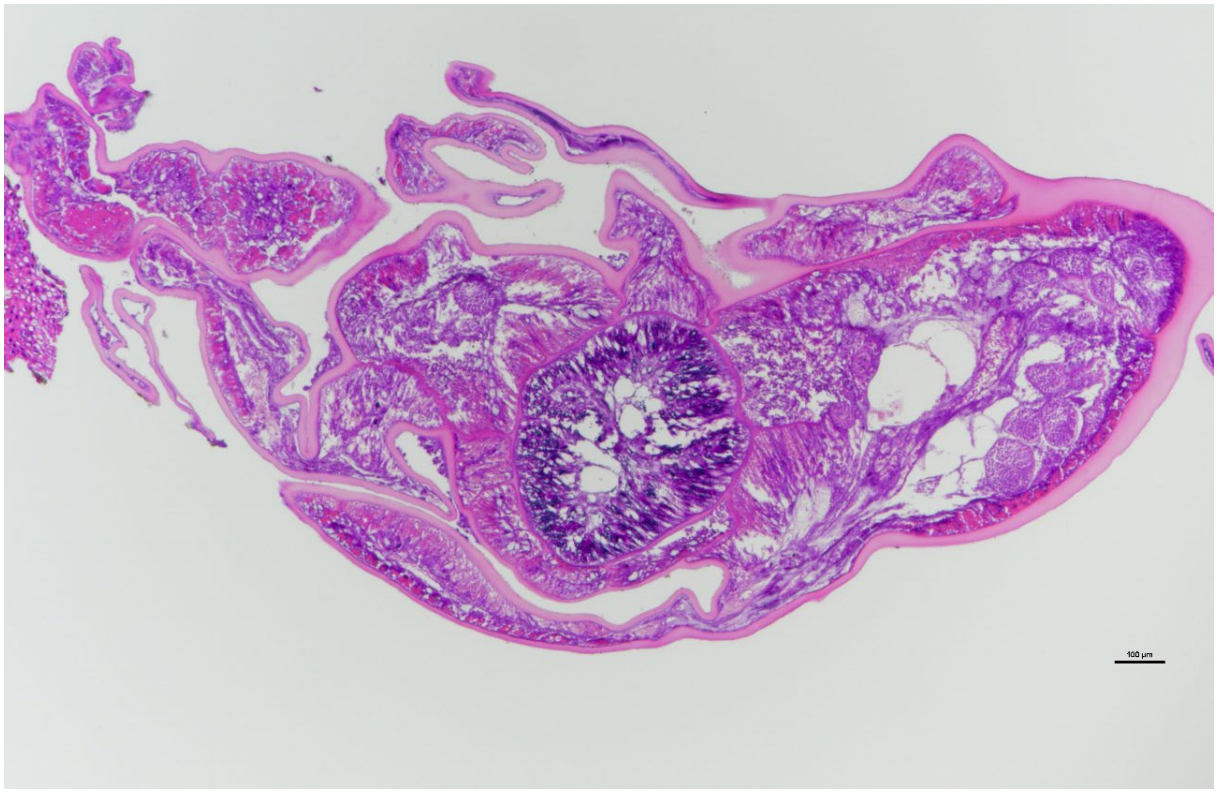

Figure 4-2: Longitudinal section through a terminal polyp of *Leiopathes bullosa* specimen NIWA53045. Specimen is likely a male. The round organs to the right of the image embedded in the mesenteries proximal to the light pink stained connective tissue are likely early stage male spermiaries. 60x magnification. Scale bar is 100 µm.

4.2 *Sibopathes* sp. (NIWA2071)

This *Sibopathes sp*. (specimen NIWA2071) was from a sample collected in 2004. It was initially fixed in Formalin prior to transfer 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 4-3). 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.

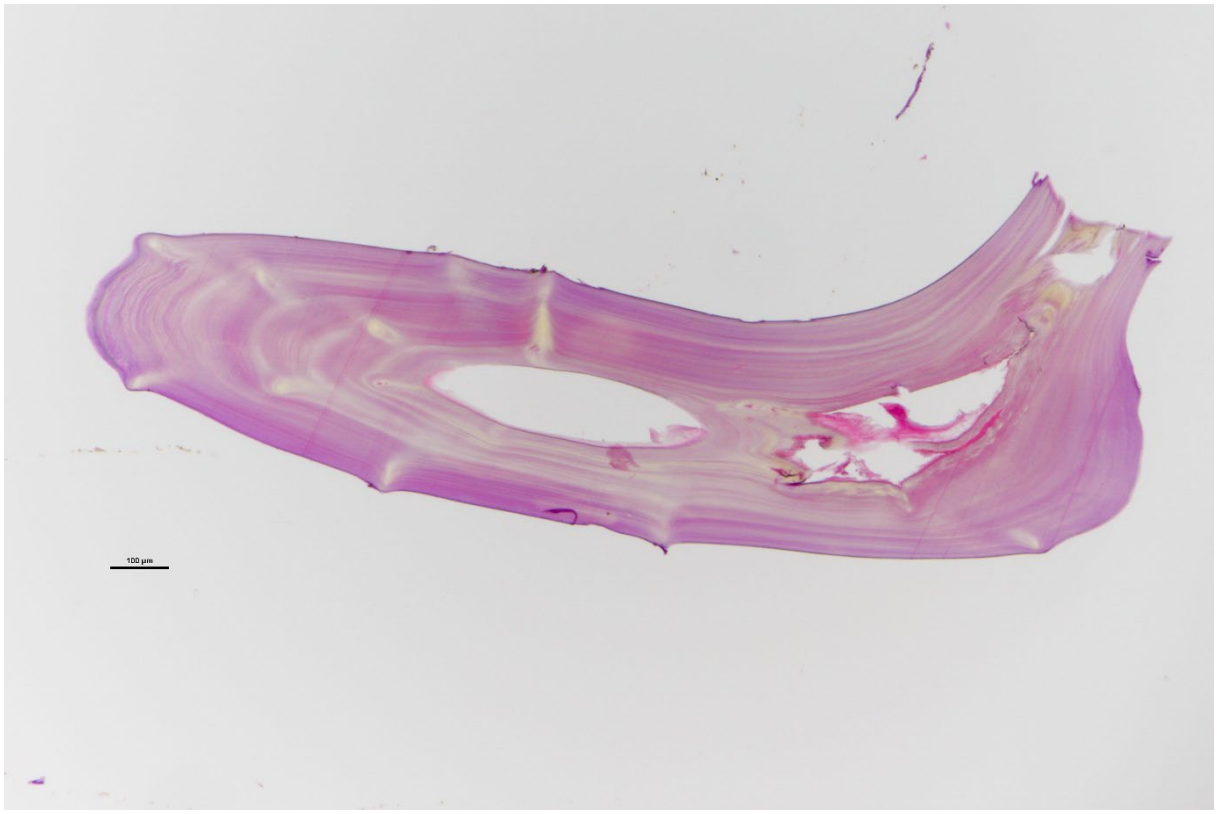

Figure 4-3: Longitudinal section through a *Sibopathes* sp. specimen NIWA2071. Specimen comprised skeletal matrix only. 60x magnification. Scale bar is 100 µm.

5 Results: Scleralcyonacea (Gorgonian octocorals)

5.1 *Paragorgia arborea*

Table 5-1 shows the specimens that were processed in New Zealand and shipped to the University of Gothenburg (Sweden) for analysis. Specimens were selected, sub-sampled, imaged and 3D scanned prior to shipping. The results of this work will be included in this report once the analyses have been completed.

Table 5-1: *Paragorgia arborea* samples for histological analyses. F = fixed in formalin, EtOH is ethanol and Alcohol is unknown alcohol.

Catalog_Number	Station_ID	Date	Latitude	Longitude	Depth	Pres_Type
3309	Z11009	08/06/2002	-33.926667	167.90667	955	None
17970	Z10956	01/11/2001	-44.73533	-177.18633	753	Alcohol
28155	Z10987	23/01/2002	-33.926667	167.91833	1225	Alcohol
28158	Z10907	01/11/2001	-44.735333	-177.18333	753	Alcohol
28161	Z10920	01/11/2001	-44.735333	-176.81367	753	Alcohol
41725	TRIP2699/89	13/10/2008	-44.205	-174.48167	739	EtOH
41829	TRIP2324/76	27/11/2006	-47.253333	178.33	987	EtOH
41999	TRIP2551/50	14/12/2007	-44.495	-174.78833	1288	Isopropanol - orig formalin
46315	TRIP2571/65	02/03/2008	-47.558333	177.86	888	EtOH
46317	TRIP2494/13	02/09/2007	-47.581667	177.78	931	EtOH
46318	TRIP2551/254	07/01/2008	-44.721667	-177.045	794	EtOH
47198	TRIP2653/70	21/07/2008	-47.505	177.86333	905	EtOH
47201	TRIP2653/71	22/07/2008	-47.326667	178.21	889	EtOH
66268	TRIP2970/52	23/11/2009	-48.515	175.27667	798	EtOH
66269	TRIP2920/46	21/09/2009	-47.241667	178.70167	820	EtOH
66273	TRIP3028/73	01/01/2010	-47.296667	177.3	577	EtOH
66276	TRIP2744/15	22/12/2008	-44.628333	-177.62833	1019	EtOH
66277	TRIP2920/36	19/09/2009	-44.735	173.06167	882	EtOH

5.2 *Primnoa notialis*

Table 5-2 shows the specimens that were processed in New Zealand and shipped to the University of Gothenburg (Sweden) for analysis. The results of this work will be included in this report once the analyses have been completed.

Table 5-2: ***Primnoa notialis*** **samples for histological analyses.** F = fixed in formalin, EtOH is ethanol and Alcohol is unknown alcohol. Where the preservation method is "F, EtOH", the specimen has been first preserved in formalin then transferred into ethanol.

Catalog Number	Station ID	Date	Latitude	Longitude	Depth	Pres Type
9700	TAN0307/81	02/05/2003	-49.799099	-175.306	1180	Alcohol
40666	TAN0803/88	15/04/2008	-55.381833	158.43033	501	EtOH
40897	TAN0803/98	16/04/2008	-56.246333	158.50567	676	EtOH
40905	TAN0803/98	16/04/2008	-56.246333	158.50567	676	EtOH
41743	Z9585	29/11/1998	-48.558333	164.95667	1061	EtOH
44168	TRIP2416/54	28/04/2007	-47.47	177.02	720	EtOH
44612	TRIP2506/45	03/10/2007	-47.301667	172.44333	1110	EtOH
61920	TRIP3065/214	09/03/2010	-45.031667	175.495	1070	F, EtOH
66124	TRIP2718/300	19/12/2008	-46.773333	172.05167	722	EtOH
106532	TRIP4815/20	11/10/2016	-47.266667	178.85	911	EtOH
106594	TRIP4837/5	18/01/2017	-51.585	161.3	1364	EtOH
106595	TRIP4837/5	18/01/2017	-51.585	161.3	1364	EtOH
114362	TAN0307/46	23/04/2003	-49.665001	178.90666	524	EtOH
156601	TRIP5851/92	24/12/2019	-50.1	165.8	1395	EtOH

6 Results: Stylasteridae Hydrocorals

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

6.1 *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.

6.2 *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 6-1 and Figure 6-2 show that this specimen was a male, with maturing and mature spermatozoa evident within the dark-purple stained spermiaries.

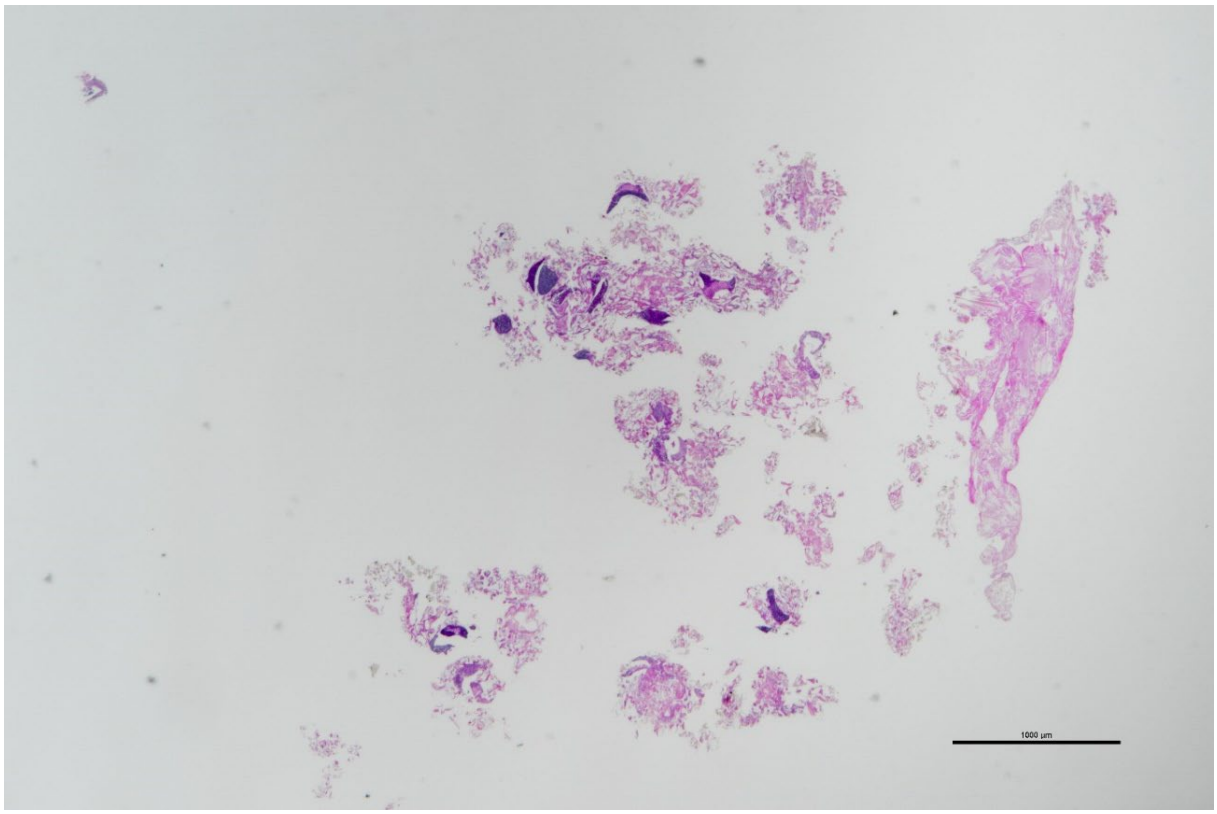

Figure 6-1: Section through a terminal branch tip of a *Stylaster eguchii* specimen NIWA91243. Specimen is a male. 20x magnification. Scale bar is 1000 µm.

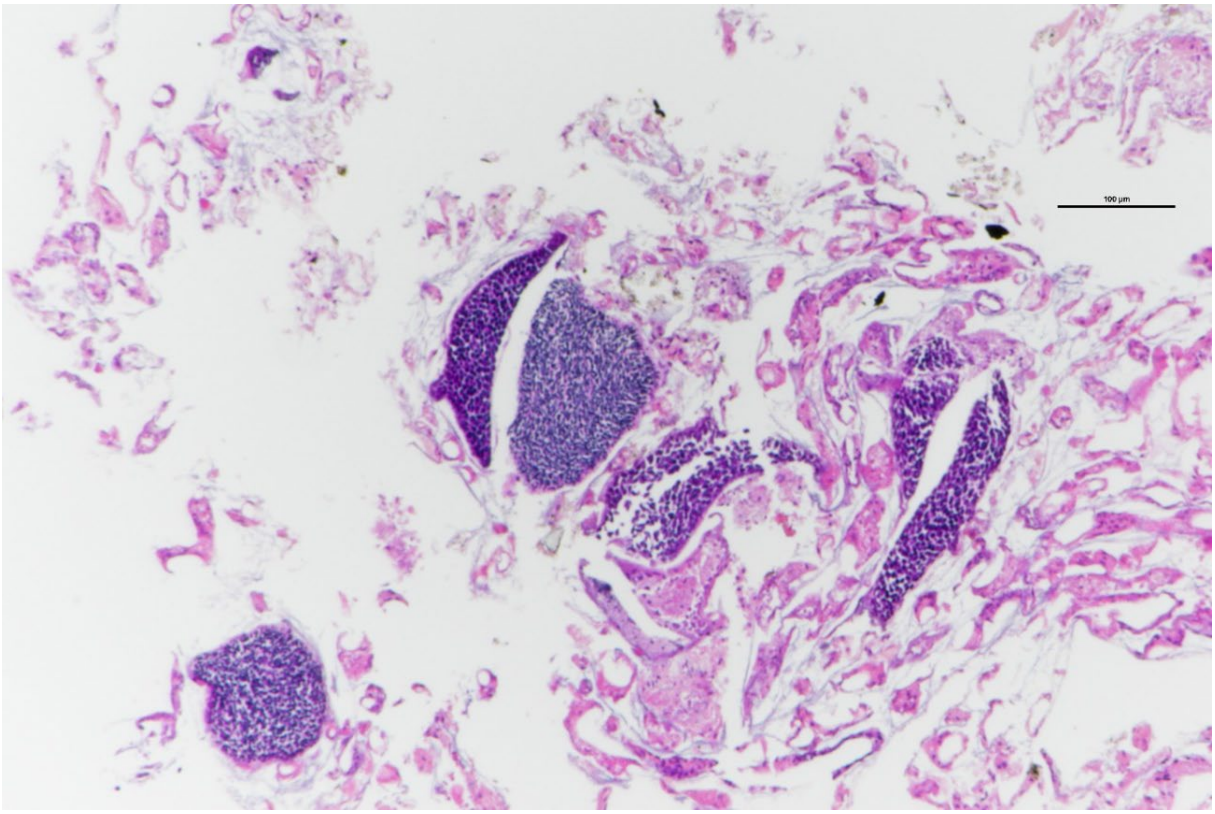

Figure 6-2: Section through a male ampullae of a *Stylaster eguchii* specimen NIWA91243. Dark purple stained spermiaries containing maturing and mature spermatozoa are evident in the centre and lower left of the section. Ampullae are the reproductive bodies of stylasterid corals, occurring as raised hemispheres on the surface of branches or as spherical inclusions within the branches depending on the species. 140x magnification. Scale bar is 100 µm.

7 Summary

This study has generated some interesting and important data on the reproductive traits of protected New Zealand deep sea corals. These data have been communicated to relevant concurrent research projects (e.g., INT2022-04, risk assessment for protected corals) and will inform future research.

7.1 Scleractinia (stony branching corals)

Goniocorella dumosa (GDU)

This study has confirmed this species is a brooder in wild populations within the New Zealand region, with mature stage V larvae observed in samples collected on 20 January 2020 from the Chatham Rise. In addition, stage IV oocytes were present in all seasons sampled (January, April, June, August and December). The limited number of male specimens examined also had mature stage IV spermiaries present in both seasons sampled (April and August). The lack of widespread seasonal data from male specimens restricts our ability to determine seasonality of male gamete release and timing of fertilisation.

We conclude, from the limited seasonal spread of available data, that there was no evidence of reproductive periodicity in *G. dumosa* and that *G. dumosa* may have the ability to reproduce year-round when environmental conditions are favourable. Observations of larvae from September to November 2020 (Beaumont et al. 2024), in aquaria with a consistent food supply, perhaps supports this theory.

We estimated total fecundity per polyp of up to 172 oocytes with a mean across all polyps of 31.37 ± 38.48. The estimate of annual fecundity was a maximum of 140 oocytes with a mean of 22.89 ± 30.44. The high variability observed within polyps and within specimens resulted in high standard deviations of the mean. These fecundity estimates could indicate the reproductive potential of a polyp, however, it is worth noting that we estimated up to 4 larvae were present within a polyp and previous observations of live animals showed up to 10 larvae within a single polyp. We have no data on the percentage of oocytes that reach maturity, are fertilized, develop into mature larvae and survive post larval release.

Our fecundity estimate is lower than that of Burgess and Babcock (2005) who suggested *G. dumosa* had a fecundity of approximately 480 ± 216 oocytes per polyp. This could be due to the difficulty of counting the immature oocytes within our samples (due to old ethanol fixed specimens and/or spacing between histology slices). Burgess and Babcock observed a maximum oocyte diameter of 135 µm compared to our maximum recorded diameter of 1142 µm indicating the specimens collected in the single timepoint of their study were less mature than many of the specimens presented in this work and thus our reduced oocyte count could also be a function of oocyte maturity.

Goniocorella dumosa is gonochoric, with polyps being either male or female. In addition, specimens had either male or female polyps, and not both. We have no knowledge of fertilisation processes or the dispersal or competence period of male gametes, but for fertilisation to occur both male and female specimens will need to be present. As such, the density of colonies in a population may influence reproductive success.

We noted high variability between both polyps and specimens. This again highlights the importance of replication and analysing more than a single time point when investigating reproductive ecology/biology and perhaps explains how Burgess and Babcock (2005) had concluded this species was a seasonal broadcast spawner from their specimens collected in April 2001.

Enallopsammia rostrata (ERO)

Just five of the thirteen *E. rostrata* specimens studied were female, and only three specimens provided detailed reproductive data. However, despite limited samples and seasonal spread, we have shown that *E. rostrata* had mature (stage IV) oocytes in specimens collected in both April and June, and maturing (stage III) oocytes in August. It is important to note that the August sample was from the initial histology trials with only a limited number of sections analysed rather than a complete half-polyp. Stage II and III oocytes were present in all specimens. There was no evidence of seasonality within the small number of female samples examined. There was also no evidence of stage V larvae, or brooding, within the specimens examined.

Six specimens were male and mature (stage IV) spermaries were observed in all seasons sampled (April, June and August). We suggest it is possible that *E. rostrata* could be a continuous or aperiodic spawner, rather than a seasonal spawner, though further sampling would be required to confirm this. This would be in agreement with the conclusions of Pires et al. (2014) on their SW Atlantic specimens.

We recorded a maximum diameter of an *E. rostrata* oocyte as 1088 µm which is very similar to that of Pires et al. (2014) who recorded a maximum diameter of 1095 µm. Burgess and Babcock (2005), however, recorded a maximum oocyte diameter of just 400 µm in their specimens from April 2001.

We estimated a total fecundity per polyp of up to 128 oocytes with a mean across all polyps of 25.59 ± 35.34. the estimated annual fecundity was up to 116 oocytes per polyp with a mean of 21.72 ± 31.51. Our estimate was lower than that of Burgess and Babcock (2005) with 144 ± 96 oocytes per polyp.

Enallopsammia rostrata is gonochoric, with polyps being either male or female and colonies are of a single sex. As for *G. dumosa*, we have no knowledge of fertilisation processes or the dispersal or competence period of male gametes, but suggest that the density of colonies within a population may influence reproductive success.

Comparisons between GDU and ERO

The tables below (Table 7-1 and Table 7-2) provide a comparison between the reproductive traits of *G. dumosa* and *E. rostrata*. It is interesting that *E. rostrata* and *G. dumosa* have a similar sized maximum oocyte diameter (though *E. rostrata* oocytes are long and thin and *G. dumosa* are more rounded) and that *E. rostrata* has a lower fecundity than *G. dumosa* given *E. rostrata* is considered likely to be a broadcast spawner and *G. dumosa* is a brooder (Table 7-1). This goes against the general assumption that brooders have fewer but larger oocytes/larvae.

There was no evidence of reproductive periodicity in either *G. dumosa* or *E. rostrata*, with mature oocytes/spermiaries observed in all seasons sampled (Table 7-2).

Table 7-1: Comparison of reproductive data for *G. dumosa* and *E. rostrata*.

	GDU	ERO
No. of specimens examined	12	13
No. of Male (M)/Female (F)/Unsexed (U) specimens	3M, 8F, 1U	6M, 5F, 2U
Maximum oocyte stage	V (larvae)	IV (mature)
Maximum oocyte diameter (µm)	1142	1088
Reproductive mode	Brooding	Broadcast (assumed)
Maximum estimated oocytes per polyp	172	128
Estimated total polyp fecundity (mean ± SD)	31.37 ± 38.48	25.59 ± 35.34
Estimated annual polyp fecundity (mean ± SD)	22.89 ± 30.44	21.72 ± 31.51

Table 7-2: Maximum observed maturity of *G. dumosa* and *E. rostrata* by month. An "-" indicates no data were available, an * indicates this observation was made from histology trials and incomplete data. Stage V oocytes are mature larvae. Stage IV oocytes and spermiaries are mature. Stage III oocytes and spermiaries are maturing.

	GDU		ERO	
Month	Female oocytes	Male spermiaries	Female oocytes	Male spermiaries
January	V	-	-	-
February	-	-	-	-
March	-	-	-	-
April	IV	IV	IV	IV
May	-	-	-	-
June	IV	-	IV	IV
July	-	-	-	-
August	IV	IV	III*	IV
September	-	-	-	-
October	-	-	-	-
November	-	-	-	-
December	IV	-	-	-

7.2 Black corals

The inclusion of black corals (Antipatharia) to this study was as a trial to assess the quality of histological sections that can be prepared from specimens in order to enable clear observations of reproductive data. Our trials on *Leiopathes bullosa* and *Sibopathes* sp. showed that it will be possible to assess the reproductive state of future sections of these species.

7.3 Hydrocorals

As with the black corals, the inclusion of hydrocorals (Stylasteridae) to this study was to determine an appropriate methodology for decalcifying and producing high quality histological sections for observations of reproductive state.

Histological specimens of hydrocorals proved problematic due to their extensive calcification, with more than 95 % of the animal being comprised of hard carbonate skeletal matrix. We were not able, within this project, to find a method that produced good results for *Errina* sp. though we believe the next steps would be to try embedding the sectioned coral fragments in an agarose gel prior to the decalcification step. This will help to entrain the small amounts of non-calcified tissue in a matrix helping to prevent it from becoming lost from the cassette during the histological processing. However, we were able to get useful histological sections from *Stylaster eguchii*.

7.4 Data limitations

Specimens used within this study were historic (some dating back to 2000) and many had not been preserved with histological analyses in mind. While we were able to get some data from all specimens used, in some cases the quality of data was compromised by the quality of the histological sections.

A distance of 200 µm between sections was chosen to enable counts and measurements of most oocytes of stages III, IV and V using previously recorded oocyte sizes (Tracey et al. 2021). We note that other published studies (e.g., Burgess and Babcock 2005; Pires et al. 2014) took sections every 4-8 µm, however, by reducing the distance between sections to 200 µm and the number of slides per polyp, we were able to extend the analysis to more specimens and polyps which enabled a better understanding of the seasonality and variability between specimens and polyps. The downside of this approach is that we will have underestimated the abundance of immature oocytes (stages I and II) due to their small size (e.g., 69 ± 35 µm and 117 ± 46.92 µm respectively for G. dumosa, (Tracey et al. 2021)).

In addition, oocyte measurements for *G. dumosa* in this study were smaller than that recorded by Tracey et al. (2021). For example, their mean measurement of a stage III oocyte was 269± 87.14 µm whereas the mean value for a stage III oocyte in this study was 181 ± 54 µm. This size difference is likely to have been caused by excessive shrinkage due to the preservation methods and time since collection and perhaps resulted in missing more stage III oocytes than expected. In any future histological work on historical specimens, we would perhaps take sections every 150 µm instead of every 200 µm to confidently capture all stage III oocytes.

It is worth noting that the histological quality of some specimens made the identification of small, immature, oocytes very difficult if not impossible, and so putting more resources into a less polyps may not have increased the quality of data produced. It does, however, mean that estimates of fecundity are only approximate but we were able to confidently assess the number of mature oocytes/larvae and the reproductive maturity and seasonality of polyps and specimens.

7.5 Relevance of results to ecological risk assessments

The project has generated data and results that are important both for ecological risk assessment and to inform the development of appropriate management strategies.

There are three key elements that support, and will further, risk assessments:

7.5.1 Reproductive mode

The identification of brooders versus broadcast spawners indicates whether larvae are likely to settle close to the adult population or be widely distributed. Ecological theory has generally regarded broadcast spawners as less vulnerable because their progeny are dispersed beyond the area of

impact if the adult population is subject to damage (and distant populations of mature adults can be source populations for impacted sites). However, how this attribute is treated in risk assessments is tricky, as both modes can be regarded as good or bad depending on the local environment.

Broadcast spawners disperse their larvae and hence spread risk over a larger area, but also expose their larvae to more areas of unsuitable habitat where success of settlement may be low. Brooders potentially have limited dispersal capacity to colonise new grounds (such as spatially-separated seamounts) but there is a greater chance of local settlement and recruitment (establishment and growth) in the vicinity of existing adult populations where conditions should be favourable. The distinction between brooders and broadcast spawners is very important for assessing management strategies, as protecting high density areas of adult brooders would be more important than where the species is a broadcast spawner.

The confirmation that *G. dumosa* is a brooder in wild populations, together with the short period between larval release and settlement (from 2 days, Beaumont et al. 2024), suggests that this species likely has limited recovery potential compared with broadcast spawners and, therefore, is more vulnerable to disturbance than previously thought. We found no evidence of planula larvae within the small number of female *E. rostrata* specimens examined and so this species is still assumed to be a broadcast spawner. Further work is required to confirm this.

7.5.2 Fecundity

This has rarely been considered in invertebrate risk assessments due to lack of data. There is also uncertainty whether larger larvae should be more successful than smaller larvae in settlement phases where they grow more quickly and predation risk is reduced. However, these data, together with information on mature oocyte size, can directly affect risk assessment. Species releasing similar sized oocytes but having different fecundities mean lower risk would be assigned to the more fecund species due to potentially higher productivity.

We have shown *E. rostrata* to have similar oocyte size (maximum diameter) and polyp fecundity to *G. dumosa* (a brooder) and to have very low fecundity compared to other broadcasting species. For example, our data show *E. rostrata* to have an average total fecundity of 26 oocytes per polyp which is very low compared to *Lophelia pertusa* with an average total fecundity of > 2000 oocytes per polyp (Waller and Tyler 2005). *Enallopsammia rostrata* and *G. dumosa* are, therefore, considered to have relatively low fecundity which could increase their assigned risk.

7.5.3 Timing of spawning

Results on seasonality of spawning is important as highly seasonal spawners would have higher risk than a species that has multiple or continuous spawning potential over the year. The latter strategy means there is a greater chance of some spawning events occurring when conditions are favourable, even though it may be less productive than a seasonal spawner during a good season when a greater number of larvae are spawned at the right time (e.g. during favourable conditions).

We found no evidence of reproductive periodicity in either *G. dumosa* or *E. rostrata*, with mature oocytes/spermiaries observed in all seasons sampled.

The influence these three elements have on risk assessment is closely tied to knowledge on the environmental cues for settlement, establishment and recruitment. Our knowledge of the latter is poor, and so how the differing reproductive characteristics transfer into risk and management is uncertain, but determining them is an important step in our understanding.

7.6 Recommendations

While we have advanced knowledge of protected New Zealand deep-sea coral reproduction, many questions and data gaps remain.

Questions that could be addressed by further histological analyses include seasonality and reproductive mode where this is not yet known. The variability observed in reproductive data between polyps and specimens within this study highlights the importance of replicate samples across multiple time points when investigating reproductive mode, seasonality and fecundity. We recommend that, where possible, when deep-sea corals are collected that specimens be placed into an appropriate preservative to enable future histological analyses to address remaining knowledge gaps.

Increased knowledge of environmental cues for settlement and recruitment are necessary to be able to improve how we interpret the reproductive parameters in a risk assessment context. At present the best we can do is assume an even distribution of suitable habitat but know this isn't the case in the real world. Time series of surveys (e.g., Graveyard seamount series of 5 surveys 2001-2020) are one approach to provide such information but these are specific to *Solenosmilia variabilis* and *Madrepora oculata*. However, further experimental laboratory studies could be a practical way to advance our knowledge more rapidly about settlement cues, as well as larval behaviour and pelagic larval duration which are important aspects in determining dispersal potential. Such studies would build on knowledge of spawning time and fecundity from histological work and be incorporated into carefully defined laboratory experiments.

Analyses should be broadened to include more of the key coral groups that are able to be identified from imagery and hence used in abundance-based habitat-suitability models as applied in INT2022-04. For example, the stony corals *S. variabilis* and *M. oculata* are key seamount species and should be examined. Black corals also appear to have variable longevity and growth rates with region and species, and so the feasibility work carried out here should be continued.

An improved understanding of the variability within higher taxonomic categories is especially important if risk assessment starts being carried out at smaller spatial scales, where the taxa will differ between such areas. For example, gorgonian octocorals are a diverse group and often combined at order or family level for risk assessment (e.g., Primnoidae, Paragorgiidae). These are significant density taxa in some areas and inclusion of several more species is important to better understand the range of reproductive variability within these groups.

8 Acknowledgements

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

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 Alcohol 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

Catalogue Number	Order	Family	Genus	Species	Station ID	Date	Start latitude	Start longitude	Start depth	End depth	Count	Preservation method
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	13/4/2011	-46.467167	166.609333	184	182	1	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

Catalogue Number	Order	Family	Genus	Species	Station ID	Date	Start latitude	Start longitude	Start depth	End depth	Count	Preservation method
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*	TAN0905/7	14/06/2009	-42.6263333	-179.9391667	1174	1300	1	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

Catalogue Number	Order	Family	Genus	Species	Station ID	Date	Start latitude	Start longitude	Start depth	End depth	Count	Preservation method
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	Alcohol
88225	Scleractinia	Caryophylliidae	*Goniocorella*	*dumosa*	Q341	14/11/1979	-44.1183	176.32	264		10	Alcohol
91198	Scleractinia	Caryophylliidae	*Goniocorella*	*dumosa*	TAN0308/147	3/06/2003	-34.302334	168.38634	850	825	1	Alcohol
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

Catalogue Number	Order	Family	Genus	Species	Station ID	Date	Start latitude	Start longitude	Start depth	End depth	Count	Preservation method
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	Alcohol
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

Appendix B Specimens selected for histological analysis

Specimens included in the histological analyses. 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. Samples marked with a "*" did not travel well to Sweden and may not give good data.

Catalog Number	Order	Family	Genus	Species	Station_ID	Date	Latitude	Longitude	Depth (m)	Pres_Type
27578	Scleractinia	Caryophylliidae	*Gonicorella*	*dumosa*	TAN0701/14	31/12/2006	-43.35766667	179.5828333	409	EtOH
88266	Scleractinia	Caryophylliidae	*Gonicorella*	*dumosa*	TAN0401/23	1/01/2004	-43.6378326	-178.6425018	440	Alcohol
102566	Scleractinia	Caryophylliidae	*Gonicorella*	*dumosa*	TAN1503/120	11/04/2015	-44.1358333	-174.7195	622	EtOH
102639	Scleractinia	Caryophylliidae	*Gonicorella*	*dumosa*	TAN1503/122	11/04/2015	-44.1476667	-174.7481667	570	EtOH
112065	Scleractinia	Caryophylliidae	*Gonicorella*	*dumosa*	TAN0401/48	5/01/2004	-43.8721657	-175.4550018	241	EtOH
140313	Scleractinia	Caryophylliidae	*Gonicorella*	*dumosa*	TAN1903/106	21/06/2019	-43.3676667	179.4513333	396	F, EtOH
140326	Scleractinia	Caryophylliidae	*Gonicorella*	*dumosa*	TAN1903/108	21/06/2019	-43.3681667	179.4508333	387	F, EtOH
140346	Scleractinia	Caryophylliidae	*Gonicorella*	*dumosa*	TAN1903/110	22/06/2019	-43.3606667	179.7423333	461	F, EtOH
141768	Scleractinia	Caryophylliidae	*Gonicorella*	*dumosa*	TAN2001/71	20/01/2020	-43.811	-179.731	379	EtOH
148101	Scleractinia	Caryophylliidae	*Gonicorella*	*dumosa*	TAN2009/57	16/08/2020	-44.159	-174.554	486	EtOH
148157	Scleractinia	Caryophylliidae	*Gonicorella*	*dumosa*	TAN2009/80	19/08/2020	-44.136166	-174.7211666	640	EtOH
102472	Scleractinia	Caryophylliidae	*Gonicorella*	*dumosa*	TAN1503/116	11/04/2015	-44.1596667	-174.5548333	497	EtOH
88048*	Scleractinia	Caryophylliidae	*Desmophyllum*	*dianthus*	S248	19/02/1980	-44.60169983	167.8200073	30	Alcohol
149740*	Scleractinia	Caryophylliidae	*Desmophyllum*	*dianthus*	S261	22/02/1980	-45.35169983	166.9882965	32	EtOH
104980	Scleractinia	Caryophylliidae	*Desmophyllum*	*dianthus*	Z16074	02/02/1993	-45.349	167.056	15	F, EtOH
148125	Scleractinia	Caryophylliidae	*Desmophyllum*	*dianthus*	TAN2009/57	16/08/2020	-44.159	-174.554	486	EtOH
140347*	Scleractinia	Caryophylliidae	*Desmophyllum*	*dianthus*	TAN1903/110	22/06/2019	-43.3606667	179.7423333	461	EtOH
127181	Scleractinia	Caryophylliidae	*Desmophyllum*	*dianthus*	SO254/76ROV13_BIOBOX1	19/02/2017	-45.0265312	171.9035492	678.3	EtOH
102485	Scleractinia	Caryophylliidae	*Desmophyllum*	*dianthus*	TAN1503/116	11/04/2015	-44.1596667	-174.5548333	497	EtOH
88004	Scleractinia	Caryophylliidae	*Desmophyllum*	*dianthus*	S181	31/10/1979	-43.44499969	173.5	392	Alcohol
88005	Scleractinia	Caryophylliidae	*Desmophyllum*	*dianthus*	Q343	14/11/1979	-44.13	175.7967	500	Alcohol

Catalog Number	Order	Family	Genus	Species	Station_ID	Date	Latitude	Longitude	Depth (m)	Pres_Type
54050	Scleractinia	Caryophylliidae	*Desmophyllum*	*dianthus*	TAN0905/113	27/06/2009	-44.1495	-174.7568333	519	EtOH
53534	Scleractinia	Caryophylliidae	*Desmophyllum*	*dianthus*	TAN0905/95	25/06/2009	-44.136	-174.7211667	613	EtOH
47034	Scleractinia	Caryophylliidae	*Desmophyllum*	*dianthus*	X486	04/07/1994	-42.777	-179.9138	910	EtOH
25068*	Scleractinia	Caryophylliidae	*Desmophyllum*	*dianthus*	TAN0604/10	28/05/2006	-42.7653	-179.9282	1005	EtOH
4012*	Scleractinia	Caryophylliidae	*Desmophyllum*	*dianthus*	Z10698	16/04/2001	-42.78617	-179.9853	993	None
88075	Scleractinia	Caryophylliidae	*Desmophyllum*	*dianthus*	TAN0104/153	18/04/2001	-42.7325	-179.8985	1076	F, EtOH
25591	Scleractinia	Caryophylliidae	*Desmophyllum*	*dianthus*	S30	18/09/1978	-50.68330002	167.6799927	265	Alcohol
53838	Scleractinia	Caryophylliidae	*Desmophyllum*	*dianthus*	TAN0905/105	26/06/2009	-44.15733333	-174.5541667	485	EtOH
54285	Scleractinia	Caryophylliidae	*Desmophyllum*	*dianthus*	TAN0905/119	28/06/2009	-44.15816667	-174.555	487	EtOH
148158	Scleractinia	Dendrophylliidae	*Enallopsammia*	*rostrata*	TAN2009/80	19/08/2020	-44.136166	-174.7211666	640	F, EtOH
148159	Scleractinia	Dendrophylliidae	*Enallopsammia*	*rostrata*	TAN2009/80	19/08/2020	-44.136166	-174.7211666	640	F, EtOH
43171	Scleractinia	Dendrophylliidae	*Enallopsammia*	*rostrata*	TAN0205/37	17/04/2002	-32.59116667	-179.643	1366	EtOH
53483	Scleractinia	Dendrophylliidae	*Enallopsammia*	*rostrata*	TAN0905/71	22/06/2009	-42.73616667	-179.6901667	820	EtOH
53486	Scleractinia	Dendrophylliidae	*Enallopsammia*	*rostrata*	TAN0905/71	22/06/2009	-42.73616667	-179.6901667	820	
53554	Scleractinia	Dendrophylliidae	*Enallopsammia*	*rostrata*	TAN0905/95	25/06/2009	-44.136	-174.7211667	613	
53719	Scleractinia	Dendrophylliidae	*Enallopsammia*	*rostrata*	TAN0905/99	26/06/2009	-44.13966667	-174.7196667	641	
54027	Scleractinia	Dendrophylliidae	*Enallopsammia*	*rostrata*	TAN0905/112	27/06/2009	-44.14283333	-174.7248333	760	
54169	Scleractinia	Dendrophylliidae	*Enallopsammia*	*rostrata*	TAN0905/116	27/06/2009	-44.175	-174.5521667	716	
81272	Scleractinia	Dendrophylliidae	*Enallopsammia*	*rostrata*	Z10759	16/12/2000	-44.675	175.8217	621	
102305	Scleractinia	Dendrophylliidae	*Enallopsammia*	*rostrata*	TAN1503/56	3/04/2015	-42.79	-179.9873333	918	
102568	Scleractinia	Dendrophylliidae	*Enallopsammia*	*rostrata*	TAN1503/120	11/04/2015	-44.1358333	-174.7195	622	
102631	Scleractinia	Dendrophylliidae	*Enallopsammia*	*rostrata*	TAN1503/122	11/04/2015	-44.1476667	-174.7481667	570	
3309	Alcyonacea	Paragorgiidae	*Paragorgia*	*arborea*	Z11009	08/06/2002	-33.9266667	167.9066667	955	None
17970	Alcyonacea	Paragorgiidae	*Paragorgia*	*arborea*	Z10956	01/11/2001	-44.73533	-177.186333	753	Alcohol
28155	Alcyonacea	Paragorgiidae	*Paragorgia*	*arborea*	Z10987	23/01/2002	-33.9266667	167.9183333	1225	Alcohol
28158	Alcyonacea	Paragorgiidae	*Paragorgia*	*arborea*	Z10907	01/11/2001	-44.7353333	-177.183333	753	Alcohol

Catalog Number	Order	Family	Genus	Species	Station_ID	Date	Latitude	Longitude	Depth (m)	Pres_Type
28161	Alcyonacea	Paragorgiidae	*Paragorgia*	*arborea*	Z10920	01/11/2001	-44.7353333	-176.813667	753	Alcohol
41725	Alcyonacea	Paragorgiidae	*Paragorgia*	*arborea*	TRIP2699/89	13/10/2008	-44.205	-174.481667	739	EtOH
41829	Alcyonacea	Paragorgiidae	*Paragorgia*	*arborea*	TRIP2324/76	27/11/2006	-47.253333	178.33	987	EtOH
41999	Alcyonacea	Paragorgiidae	*Paragorgia*	*arborea*	TRIP2551/50	14/12/2007	-44.495	-174.788333	1288	F, Isopropanol
46315	Alcyonacea	Paragorgiidae	*Paragorgia*	*arborea*	TRIP2571/65	02/03/2008	-47.5583333	177.86	888	EtOH
46317	Alcyonacea	Paragorgiidae	*Paragorgia*	*arborea*	TRIP2494/13	02/09/2007	-47.5816667	177.78	931	EtOH
46318	Alcyonacea	Paragorgiidae	*Paragorgia*	*arborea*	TRIP2551/254	07/01/2008	-44.7216667	-177.045	794	EtOH
47198	Alcyonacea	Paragorgiidae	*Paragorgia*	*arborea*	TRIP2653/70	21/07/2008	-47.505	177.863333	905	EtOH
47201	Alcyonacea	Paragorgiidae	*Paragorgia*	*arborea*	TRIP2653/71	22/07/2008	-47.326667	178.21	889	EtOH
66268	Alcyonacea	Paragorgiidae	*Paragorgia*	*arborea*	TRIP2970/52	23/11/2009	-48.515	175.276667	798	EtOH
66269	Alcyonacea	Paragorgiidae	*Paragorgia*	*arborea*	TRIP2920/46	21/09/2009	-47.241667	178.701667	820	EtOH
66273	Alcyonacea	Paragorgiidae	*Paragorgia*	*arborea*	TRIP3028/73	01/01/2010	-47.296667	177.3	577	EtOH
66276	Alcyonacea	Paragorgiidae	*Paragorgia*	*arborea*	TRIP2744/15	22/12/2008	-44.628333	-177.628333	1019	EtOH
66277	Alcyonacea	Paragorgiidae	*Paragorgia*	*arborea*	TRIP2920/36	19/09/2009	-44.735	173.061667	882	EtOH
9700	Alcyonacea	Primnoidae	*Primnoa*	*notialis*	TAN0307/81	02/05/2003	-49.799099	-175.306	1180	Alcohol
40666	Alcyonacea	Primnoidae	*Primnoa*	*notialis*	TAN0803/88	15/04/2008	-55.3818333	158.4303333	501	EtOH
40897	Alcyonacea	Primnoidae	*Primnoa*	*notialis*	TAN0803/98	16/04/2008	-56.2463333	158.5056667	676	EtOH
40905	Alcyonacea	Primnoidae	*Primnoa*	*notialis*	TAN0803/98	16/04/2008	-56.2463333	158.5056667	676	EtOH
41743	Alcyonacea	Primnoidae	*Primnoa*	*notialis*	Z9585	29/11/1998	-48.5583333	164.9566667	1061	EtOH
44168	Alcyonacea	Primnoidae	*Primnoa*	*notialis*	TRIP2416/54	28/04/2007	-47.47	177.02	720	EtOH
44612	Alcyonacea	Primnoidae	*Primnoa*	*notialis*	TRIP2506/45	03/10/2007	-47.3016667	172.4433333	1110	EtOH
61920	Alcyonacea	Primnoidae	*Primnoa*	*notialis*	TRIP3065/214	09/03/2010	-45.031667	175.495	1070	F, EtOH
66124	Alcyonacea	Primnoidae	*Primnoa*	*notialis*	TRIP2718/300	19/12/2008	-46.773333	172.051667	722	EtOH
106532	Alcyonacea	Primnoidae	*Primnoa*	*notialis*	TRIP4815/20	11/10/2016	-47.266667	178.85	911	EtOH
106594	Alcyonacea	Primnoidae	*Primnoa*	*notialis*	TRIP4837/5	18/01/2017	-51.585	161.3	1364	EtOH
106595	Alcyonacea	Primnoidae	*Primnoa*	*notialis*	TRIP4837/5	18/01/2017	-51.585	161.3	1364	EtOH

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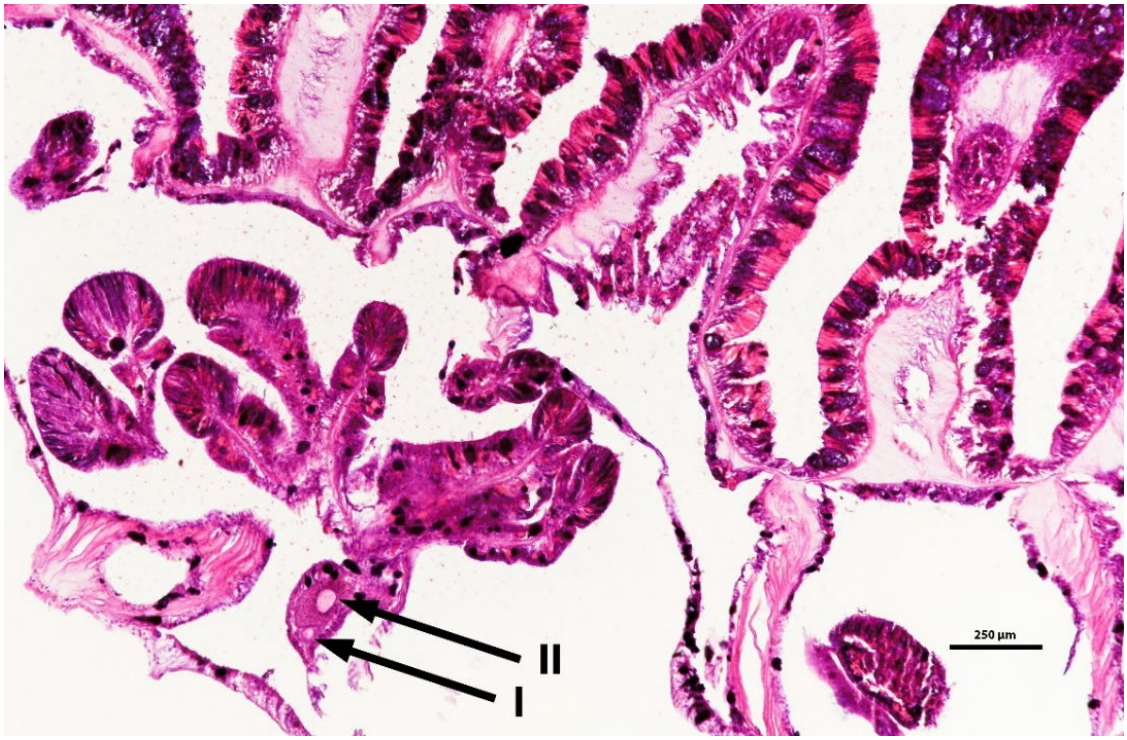
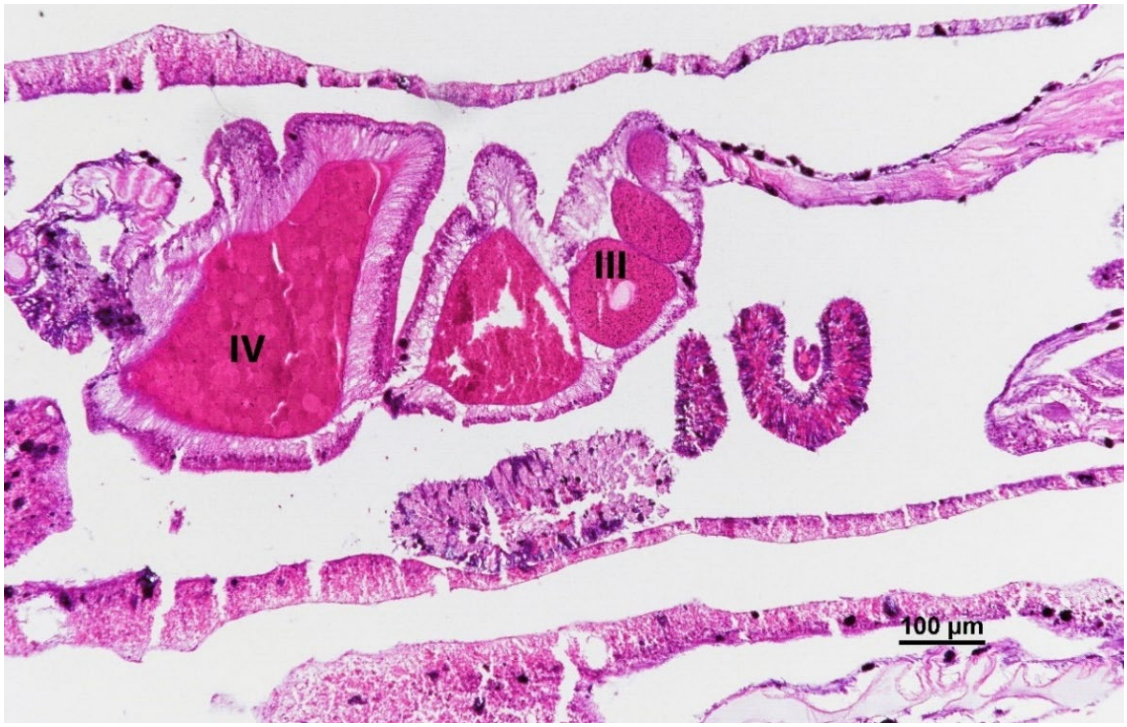
114362	Alcyonacea	Primnoidae	*Primnoa*	*notialis*	TAN0307/46	23/04/2003	-49.6650009	178.906662	524	EtOH
156601	Alcyonacea	Primnoidae	*Primnoa*	*notialis*	TRIP5851/92	24/12/2019	-50.1	165.8	1395	EtOH
53045	Antipatharia	Leiopathidae	*Leiopathes*	*bullosa*	TAN0905/7	14/06/2009	-42.62633333	-179.9391667	1174	EtOH
2071	Antipatharia	Cladopathidae	*Sibopathes*		KAH0204/7	14/04/2002	-34.11916733	174.1524963	800	F, EtOH
91243	Anthoathecata	Stylasteridae	*Stylaster*	*eguchii*	TAN1106/3	13/04/2011	-46.467167	166.609333	184	EtOH
77555	Anthoathecata	Stylasteridae	*Errina*		D18	22/04/1963	-52.51670074	160.5166931	128	F, EtOH

Appendix C Example images of G. dumosa oocyte stages

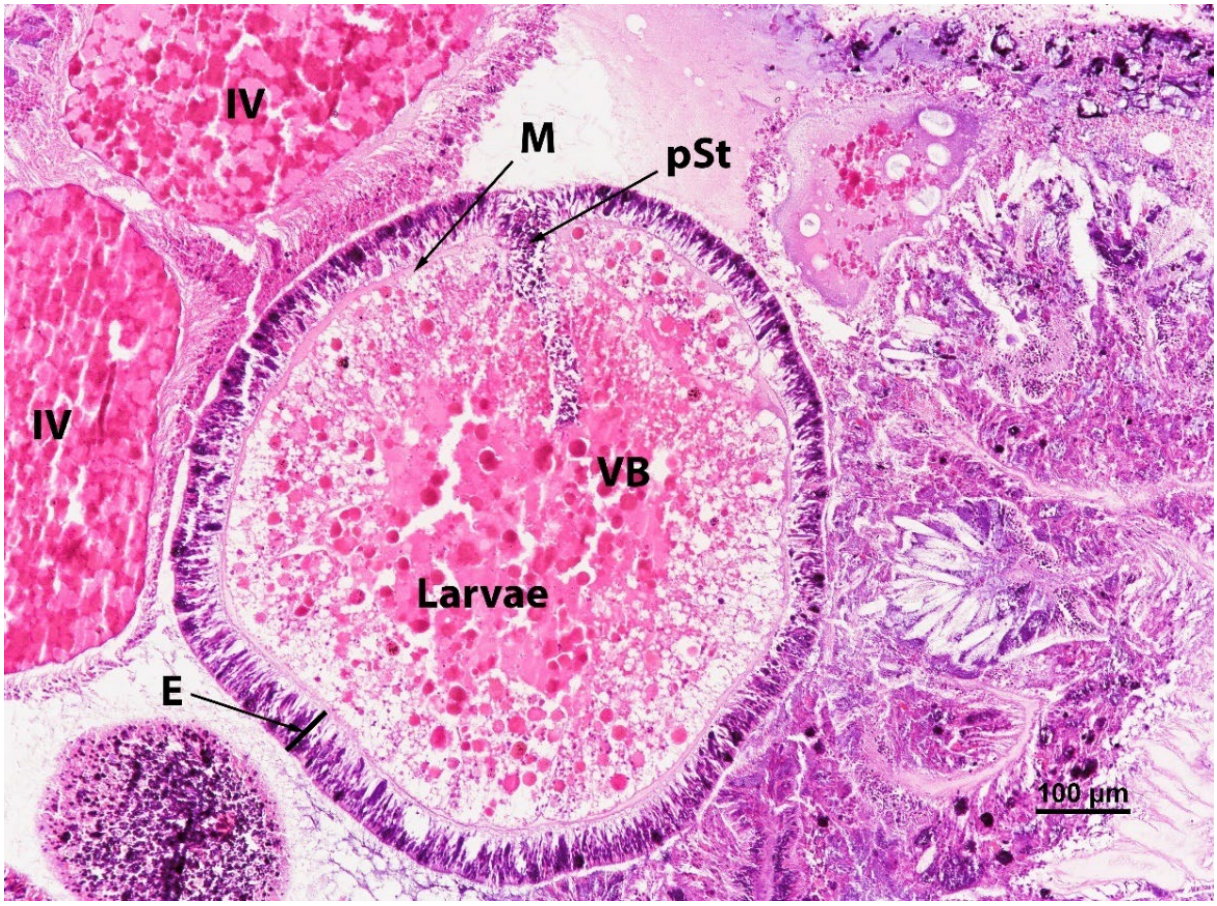
Adapted from (Tracey et al. 2021)



Stage I and II Oocyte. I Oocyte, displaying an enlarged interstitial cell with a large nucleus in the mesentery. A Stage II Oocyte sits adjacent to this, exhibiting accumulating cytoplasm. Scale bar 250 µm.



Stage III Oocytes. Stage III Oocytes displaying brightly pink staining vitellogenic bodies in the cytoplasm. The section cut has only gone through one of the four stage III oocytes nuclei. Stage IV Oocyte exhibiting mature globular vitellogenic bodies in the cytoplasm, this oocyte is a tangential section and has not intersected the nucleus. Scale bar 100 µm



Stage IV and V Oocytes. Stage IV Oocytes displaying mature globular vitellogenic bodies. The planula larva (stage V) visible in this image is obviously multicellular, already showing a high degree of cellular differentiation. Ectodermal layer (E) is well defined, sitting on a thin light pink staining mesogleal layer (M). The infolding ectoderm at the top of the larvae will form the stomodaeum (pSt), the future mouth. This end will be the oral pole. Vitellogenic bodies (VB) inside the larvae are still abundant but are being actively consumed (reducing in size and number). Scale bar 100 µm.