The Reproductive Biology of Deep-Sea Elasmobranchs and Batoids from Chatham Rise and the Sub-Antarctic Region of New Zealand.

By

Adèle Dutilloy

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ABSTRACT

The reproductive biology of thirteen poorly studied deep-sea elasmobranch species, on Chatham Rise and the Sub-Antarctic region of New Zealand, was assessed. The study species are all commonly caught as bycatch in commercial fisheries and include: three viviparous species (*Centroselachus crepidater, Centrophorus squamosus, Deania calcea)*, five deep-sea catsharks (*Apristurus* spp.), and five deep-sea batoid species. However, due to a lack of knowledge on their general biology, ecology, and taxonomy – the impact of fishing on these species is unknown. A species' resilience to fishing pressure depends on its biological productivity and susceptibility to capture. Accurate assessment of maturity is critical to understanding productivity and the effects of fishing pressure on fished stocks. Maturity is commonly assessed macroscopically, using a visual assessment that lacks precision and relies on subjective judgement. The wide array of macroscopic maturity assessment keys, used internationally, employ various sets of characteristics to define the same reproductive processes, which can lead to errors and inconsistencies in maturity assessment and parameter estimates (e.g. length-at-maturity), making direct comparisons between studies difficult. Objective reproductive measurements (oviducal gland size, follicle size, uterus width, follicle number and gonad weight) were used to assess the validity and quality of the macroscopic maturity staging key used in New Zealand, towards determining the onset of maturity and accurately distinguishing between macroscopic stages. The results showed that no single measurement gave a clear-cut indicator of maturity and some fish classified as 'maturing' were very likely 'mature'. Uterus width, follicle size and gonadosomatic index values were found to be the most useful attributes in determining the onset of maturity. Uterus width and follicle size were also useful in determining differences between different macroscopic stages, whilst gonadosomatic index values were useful in distinguishing between reproductive strategies. Histological observations, with a particular focus on sperm storage, were also used to inform the quality of macroscopic maturity assignment. Sperm storage was observed for the first time in *Centroselachus crepidater, Centrophorus squamosus* and *Brochiraja asperula.* This study successfully highlighted problems in the macroscopic maturity

assessment key currently used in New Zealand and proposes an improved, more objective macroscopic staging key. The improved key aims: 1) to assist in distinguishing between maturity stages, particularly between stage 2 (maturing), stage 2 (resting) and stage 6 (post-partum) females, by examining the same key reproductive structures across all macroscopic stages, and 2) to provide more representative maturity data for use in fisheries and demographic models, for more robust assessment of the impacts of fishing pressure on poorly studied deep-sea chondrichthyans.

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Table of Contents

Chapter 1 Introduction

1.1 Deep-sea

The deep-sea are the waters located beyond the upper continental shelf (deeper than approximately 200 m) with an average depth around 4200 m (Danovaro et al., 2014). Such depth leads to the presence of a range of extreme environmental factors including (but not limited to): near total darkness, low temperatures (at an average of less than 4 degrees Celsius) and high hydrostatic pressure (average of 400 atm) (Danovaro et al., 2014; Rigby and Simpfendorfer, 2015). Since sunlight is not able to penetrate water deeper than about 200 m, net photosynthetic primary production is negated, posing challenges to the deep-sea environment such as limited food availability (Danovaro et al., 2014; Rigby and Simpfendorfer, 2015). However, beneath the productive surface waters of the upper shelf, waters remain highly productive due to the presence of marine snow and the vertical migration of foraging animals (Klevjer et al., 2016; Longhurst and Glen Harrison, 1989; Turner, 2015).

Only in the 1960s was the deep-sea recognised and quantified as having high biodiversity (Danovaro et al., 2014). Technological advances (e.g. bathymetric mapping) have since been made, allowing deep-sea research to expand exponentially (Danovaro et al., 2014). In recent years, our understanding and current paradigms surrounding the deep-sea have been put into question, shedding light on the importance of diversity throughout the largest biome of the biosphere (Danovaro et al., 2014; Klevjer et al., 2016; Turner, 2015). It has been conservatively estimated (using a GIS Manifold System to analyse bathymetric data from 200 m to 6000 m) that the global deep-sea floor has an area of 434 386 264 km^2, a 20% increase on previous estimates of approximately 361 254 000 km^2 (Danovaro et al., 2014).

Despite enormous hydrostatic pressure and cold temperatures, deep-sea environments can support benthic abundances and biomasses similar to, or greater than, coastal systems, which continue to alter our understanding of productivity in the deep-sea (Danovaro et al., 2014). De Leo et al. (2010)

concluded that average megabenthic biomass in the Kaikoura Canyon, New Zealand, may exceed the highest previous estimates from depths below 500 m by 100-fold. Leduc et al. (2014) compared the productivity of the Kaikoura Canyon with Chatham Rise, New Zealand, and although they did not observe differences as pronounced as De Leo et al. (2010), they concluded nematode biomass exceeded previous estimates by at least a factor of two. They determined (with support from De Leo et al. (2010)) that the Kaikoura Canyon is the most productive, non-chemosynthetic deep-sea habitat described to date, and may contribute to deep-sea productivity in the Canyon's immediate vicinity (Leduc et al., 2014).

Although major advancements have been made in our understanding of the deep-sea, particularly with regards to invertebrates – a lack of understanding remains regarding the taxonomy, general biology and ecology of many deep-sea species, including deep-sea chondrichthyans that are commonly caught as bycatch in targeted fisheries (Danovaro et al., 2014; Kyne and Simpfendorfer, 2007). Therefore, there is a substantial need to build on our knowledge to effectively manage human use of deep-sea resources, both directly and indirectly (Danovaro et al., 2014).

1.2 Deep-sea Chondrichthyans

The 581 known deep-sea chondrichthyan species are estimated to account for 48.7 % of the global chondrichthyan fauna (approximately 1200 species), of which there are approximately 278 elasmobranchs (55.8 % of the global total), 257 batoids (29.8 % of the global total) and 46 holocephalans (93.9 % of the global total) (Cavanagh and Kyne, 2006; Kyne and Simpfendorfer, 2010, 2007; Neat et al., 2015; Priede et al., 2006; Rigby and Simpfendorfer, 2015; Verissimo et al., 2012). The majority (84.5 %) of all described deep-sea elasmobranchs are attributed to the Squaliformes and *Scyliorhinidae*, whereas 89.9 % of all described deep-sea batoids are from three families: *Arhynchobatidae, Rajidae* and *Anacanthobatidae* (Kyne and Simpfendorfer, 2007). Approximately 21 % of all known deep-sea

chondrichthyans remain taxonomically undescribed (Kyne and Simpfendorfer, 2007).

Deep-sea chondrichthyans occur at depths below 200 m, although it is believed they have not colonised waters deeper than 3000 – 4000 m (Cavanagh and Kyne, 2006; Kyne and Simpfendorfer, 2010; Neat et al., 2015; Priede et al., 2006; Rigby and Simpfendorfer, 2015; Verissimo et al., 2012). Data on the general biology and ecology of many deep-sea chondrichthyans is limited, predominantly due to difficulties in accessibility; much knowledge and understanding is derived from those species for which data is considered adequate, although recognised as incomplete (ICES, 2017a, 2017b; Kyne and Simpfendorfer, 2010, 2007; Parker and Francis, 2012; Verissimo et al., 2012). It is difficult to ascertain if estimates of a species' depth distribution are a true representation of depths at which the species is present, or an artefact of fishing depth (i.e. the depth range at which catches are taken) (ICES, 2017a, 2017b; Kyne and Simpfendorfer, 2010, 2007; Parker and Francis, 2012; Verissimo et al., 2012).

Deep-sea chondrichthyans are characterised as possessing life history traits of K-selected species: low productivity, low fecundity, late maturation, long gestation rates and high longevity (e.g. *Centrophorus squamosus*) (García et al., 2008; Kyne and Simpfendorfer, 2010; Neat et al., 2015; Parker and Francis, 2012; Rigby and Simpfendorfer, 2015; Verissimo et al., 2012). However, some uncertainty remains around how well life history characteristics are estimated as further validation in growth and age is required (ICES, 2017b). It is believed that some deep-sea chondrichthyans share similar life history characteristics to pelagic and shallow water species: relatively early maturing and fast-growing (e.g. black-mouth dogfish (*Galeus melastomus*) has a maximum age estimated at 10 years) (Moore et al., 2013; Neat et al., 2015). It is possible the deep-sea acts as a refuge where pregnant females may move deeper and become unavailable to bottom trawl gear (Parker and Francis, 2012). Data on mature (particularly pregnant) females are often lacking (Parker and Francis, 2012). Since this is a phenomenon observed worldwide, it has been hypothesised that either: 1) females become pelagic or move out of fishery survey areas (i.e. to depths below that accessible by bottom

trawls), or 2) parturition areas are outside areas that are fished (Parker and Francis, 2012).

Deep-sea chondrichthyans are thought to have long population replacement times, which may be because females represent a reproductive bottleneck in productivity, since late maturation and low fecundity lead to low lifetime production of offspring – even though offspring survival may be high (García et al., 2008; Kyne and Simpfendorfer, 2010; Simpfendorfer and Kyne, 2009; Verissimo et al., 2012). It is difficult to determine the duration and frequency of reproductive events in such species, since it is believed there is no distinct reproductive period (i.e. spawning aggregations); there are large energy requirements for egg production and the potential to exhibit long resting periods between parturition and subsequent ovulation (Figueiredo et al., 2008; ICES, 2017a, 2017b; Irvine et al., 2012; Kyne and Simpfendorfer, 2007; Parker and Francis, 2012). Resting periods have been suggested in many deep-sea squaloid species and are suggested to extend the duration of the reproductive cycle, although their duration and importance remains poorly understood (Figueiredo et al., 2008; ICES, 2017a, 2017b; Irvine et al., 2012; Kyne and Simpfendorfer, 2007; Parker and Francis, 2012). Long gestation periods (e.g. estimated at approximately 24 months in spiny dogfish (*Squalus acanthias*)) and assumed long resting periods result in diminished lifetime productivity (Irvine et al., 2012; Kyne and Simpfendorfer, 2007; Parker and Francis, 2012). The reproductive frequency and biological productivity of many deep-sea chondrichthyans are unknown (Ford et al., 2015; Kyne and Simpfendorfer, 2010; Verissimo et al., 2012). For oviparous species, estimates of fecundity are difficult as egg-laying periods and rates, are mostly unknown (Kyne and Simpfendorfer, 2007). However, irrespective of reproductive strategy, all fish go through similar key reproductive processes: 1) development and growth of gametes, 2) release of gametes, 3) cessation of gamete release and 4) preparation for subsequent reproductive events (Brown-Peterson et al., 2011).

The reproductive biology of deep-sea chondrichthyans is poorly known; combined with practical and economic difficulties in conducting deep-sea research, and the lack of general taxonomic, ecological and biological data, there is great concern

regarding the effects of human impacts on deep-sea populations (Dunn et al., 2013; ICES, 2017a; Irvine et al., 2012; Kyne and Simpfendorfer, 2010, 2007; Moura et al., 2014; Parker and Francis, 2012; Verissimo et al., 2012). It is presumed deep-sea chondrichthyans are highly vulnerable to population depletion, with low recovery rates from exploitation and fishing pressure, due to life history characteristics (low productivity, low fecundity, late maturation, long gestation periods and high longevity) (Clarke et al., 2014; Cryer et al., 2016; Dunn et al., 2013; FAO, 2014; Ford et al., 2015; García et al., 2008; Irvine et al., 2012; Kyne and Simpfendorfer, 2010; Last et al., 2009; Ministry for Primary Industries, 2013; Moura et al., 2014; Paiva et al., 2011; Parker and Francis, 2012; Rochowski et al., 2015). A species resilience to fishing pressure depends on its biological productivity and its susceptibility to capture (Gracan et al., 2013; Irvine et al., 2012; Pope et al., 2010). Since the life history traits of some deep-sea chondrichthyans may be comparable to shallow water and pelagic species, it is possible these species are expected to be at least as vulnerable to the impacts of fishing (Neat et al., 2015; Simpfendorfer and Kyne, 2009)

1.3 Fisheries and Management

Deep-sea chondrichthyans are commonly caught in considerable numbers as bycatch in trawl fisheries in the New Zealand Exclusive Economic Zone (EEZ), including those targeting hoki (*Macruronus novaezelandiae*), orange roughy (*Hoplostethus atlanticus*) and oreos (*Oreosomatidae* spp.) as well as some bottom long line fisheries targeting ling (*Genypterus blacodes*) and ribaldo (*Mora moro*) (Dunn et al., 2013; Ford et al., 2015; Moura et al., 2014; Parker and Francis, 2012; Rochowski et al., 2015). Commercial fishing mortality has led to dramatic declines in reproductive populations, as harvest is permitted *via* open access, rather than managed through the fisheries regulatory framework – the Quota Management System (QMS) (which manages individual stocks of species directly targeted, high value products, or fisheries with high catch rates) (Cryer et al., 2016; Irvine et al., 2012; Parker and Francis, 2012). In order to benefit from long term stock use, catches need to be maximised without damaging the reproductive population (Cryer et al., 2016; De Lara et al., 2007; Mace, 2001; Pope et al., 2010). Stock

assessments allow for the effect of past exploitation to be recorded and for predictions on how management decisions (e.g. quotas) affect future yields (Cryer et al., 2016; De Lara et al., 2007; Mace, 2001; Pope et al., 2010). The spawning stock biomass (the proportion of total mass of breeding-age fish) compared to the initial biomass (biomass without fishing mortality) is a crucial indicator for stock status (Cryer et al., 2016; De Lara et al., 2007; Mace, 2001; Pope et al., 2010).

Of the 113 recognised shark taxa in the New Zealand EEZ, 18 species are subject to management initiatives: 7 are considered protected species and 11 are included in the QMS (Ford et al., 2015; Ministry for Primary Industries, 2013). Those 95 species not included in management regimes are non-QMS species, with no allocated catch limits, where harvest is *via* open access (Ford et al., 2015; Irvine et al., 2012; Ministry for Primary Industries, 2013; Parker and Francis, 2012). Although reporting of non-QMS species in commercial catches is required, reporting is unreliable and catches are difficult to manage (e.g. discarding at sea, misidentification, misreporting) (Ford et al., 2015; Ministry for Primary Industries, 2013).

Programs such as the Ministry for Primary Industries' Fisheries Observer Program are used to collect data on QMS species (as well as non-QMS species), with Observers (often aboard commercial vessels) trained in fish identification (Ford et al., 2015). Fisheries surveys (e.g. the Chatham Rise trawl survey time series, for the estimation of hoki and middle depth fish abundance) are central to monitoring, as well as collecting data (Ford et al., 2015; O'Driscoll et al., 2011). Non-QMS species include all known deep-sea chondrichthyans which are largely discarded at sea, misidentified and unreported – meaning total catches are likely to be grossly underestimated (Dunn et al., 2013; Ford et al., 2015; Ministry for Primary Industries, 2013; Parker and Francis, 2012). Landings of many deep-sea species are often grouped, which may result in underestimations of catch data (ICES, 2017a, 2017b, 2013; Moura et al., 2014).

In 1999, the Food and Agriculture Organisation of the United Nations (FAO) developed the International Plan of Action for the Conservation and Management of Sharks (IPOA) which aims "*to ensure the conservation and management of*

[chondrichthyans] and their long-term sustainable use" (FAO, 2014). The IPOA recognises the importance of shark species in maintaining healthy ecosystems, the biological traits that render them susceptible to over-fishing and the potentially high human impact on chondrichthyans (FAO, 2014). The IPOA is voluntary, although the guidance principles indicate that all member states contributing to fishing mortality on a chondrichthyan stock *should* participate in stock management and conservation and *should* strive to have a National Shark Plan in place by 2001 (FAO, 2014). As a signatory of the IPOA, New Zealand first developed its National Plan of Action for the Conservation and Management of Sharks (NPOA) in 2008 (Ministry for Primary Industries, 2013). In 2013, the Ministry for Primary Industries (MPI) published the revised NPOA, with the aim *"to maintain the biodiversity and the long-term viability of all New Zealand shark populations by recognising their role in marine ecosystems, ensuring that any utilization of sharks is sustainable, and that New Zealand receives positive recognition internationally for its efforts in shark conservation and management*" (Ministry for Primary Industries, 2013).

As part of the NPOA objectives, MPI conducted a Qualitative (Level 1) Risk Assessment on a national (EEZ-wide) scale to identify the nature and extent of risks to shark populations and prioritise species-specific actions based on this risk, in an attempt to maintain mortality from fishing at or below a level that allows sustainable stock status (Ford et al., 2015; Ministry for Primary Industries, 2013). Fishing intensity and consequences on shark species were assessed and risk scores assigned using a modified Scale Intensity Consequence Analysis (SICA) approach (Ford et al., 2015). The SICA approach was chosen as the preferred assessment method due to it being the approach endorsed by Marine Stewardship Council (MSC) certification (Ford et al., 2015). Leafscale gulper shark (*Centrophorus squamosus*), longnose velvet dogfish (*Centroselachus crepidater*) and shovelnose dogfish (*Deania calcea*) were considered to be among those most at risk to the impacts of fishing. These species in this Risk Assessment ranked 4th, 5th, and 8th most at risk respectively; and have often been caught as bycatch in commercial fisheries (Ford et al., 2015). *Apristurus* spp. ranked 24th most at risk (as defined in the Risk Assessment (Ford et al., 2015)). However, it is possible that individual

species may, in fact, be at higher risk of the effects of fishing than the genus as a whole (ICES, 2017a).

Understanding a fish's reproductive biology is key to stock assessments, especially with regards to females within the population (Brown-Peterson et al., 2011; Ford et al., 2015; Gracan et al., 2013; ICES, 2017a, 2017b, 2013; Irvine et al., 2012; Kjesbu, 2009; Lowerre-Barbieri et al., 2011; Moura et al., 2014; Parker and Francis, 2012; Pope et al., 2010; Storrie et al., 2008). Maturity is key to assessing stocks; fishing pressure can affect differences in size-at-maturity and a species' resilience to fishing pressure depends on its biological productivity and susceptibility to capture (Gracan et al., 2013; ICES, 2017a, 2017b, 2013; Moura et al., 2014; Pope et al., 2010; Storrie et al., 2008). Stock boundaries can be optimised through the examination of spatial and temporal differences in life-history parameters (ICES, 2017b; McCully et al., 2012).

Length-at-maturity is a key biological parameter used in stock assessments and demographic models, since it allows for the proportion of the stock that is mature, and therefore stock productivity, to be estimated (Brown-Peterson et al., 2011; Fontoura et al., 2009; ICES, 2017a, 2017b; McCully et al., 2012; Moura et al., 2014). To sustainably manage a stock, the level of contribution of recruits to reproduction must be maintained at levels that do not reduce the reproductive capacity, or resilience of the stock to fishing, through understanding size-at-maturity and stock productivity (Cryer et al., 2016; De Lara et al., 2007; Mace, 2001; Pope et al., 2010). Therefore, accurate assessment of maturity is critical to understanding the effects of fishing pressure on fished stocks (Brown-Peterson et al., 2011; De Lara et al., 2007; ICES, 2017a, 2017b; Kjesbu, 2009; Lowerre-Barbieri et al., 2011).

1.4 Maturity

Maturity can be considered either in terms of functional maturity – whereby, *"the reproductive organs are sufficiently mature for copulation to result in the production of offspring"*, or behavioural maturity, where *"copulation behaviour is evidenced […] without necessarily resulting in the production of offspring, because*

the reproductive organs may be physiologically immature" (Tallack, 2007; Wilhelm and Nival, 1995). In fisheries science, only the former is of concern as it directly describes the reproductive capacity of the stock. As a result, the methods used to assess maturity (described below) focus on characteristics of functional maturity.

Maturity is commonly assessed macroscopically, since maturity can be assigned quickly using descriptive diagnostics in keys, through visual assessment of the reproductive organs (Gracan et al., 2013; ICES, 2017a, 2017b, 2013; Kyne and Simpfendorfer, 2007; Pope et al., 2010). Macroscopic maturity classification uses observations of prominent biological differences between individuals of the same species (or class of species) and sex, then assigning an individual to a particular stage of the reproductive cycle (Brown-Peterson et al., 2011; ICES, 2010; Lowerre-Barbieri et al., 2011). There are different scales for both males and females, with different stages representing key reproductive milestones (Brown-Peterson et al., 2011; ICES, 2017a, 2017b, 2010).

Macroscopic maturity assessment was first proposed by Hjort in 1914 for Atlantic Herring, in which numbered stages were assigned to specific reproductive stages (Brown-Peterson et al., 2011). As defined by Jones and Geen (1977) the term 'maturity stages' is meant as the degree of ripeness of the ovaries or testes as an indicator of whether a fish has reached maturity or not. The majority of maturity assessment tools have been developed based on Hjort (1914), and predominantly focus on teleost reproduction (Brown-Peterson et al., 2011; Gracan et al., 2013; ICES, 2017b). In 2002, Stehmann proposed the first chondrichthyan-specific macroscopic staging key. However, despite the Stehmann (2002) key (Table 1.4- 1; Table 1.4- 2), many macroscopic maturity diagnostics and the interpretation of reproductive processes, continue to focus on teleost reproduction (i.e. studies rarely interpret uterine development, although measurements and histology may have been carried out) (Brown-Peterson et al., 2011; Gracan et al., 2013; ICES, 2017b, 2013, 2010). The pertinent use of teleost-like reproductive criteria to assess chondrichthyan macroscopic maturity may not be suitable for universal application, since features of the female reproductive tract differ between the two

groups, resulting in confusion and inconsistent application of terminology (Brown-Peterson et al., 2011; Gracan et al., 2013; ICES, 2017b, 2013, 2010).

Macroscopic maturity assessment is relatively cheap both in terms of resources and time, although it is thought that macroscopic criteria may be too subjective to give accurate estimates (Brown-Peterson et al., 2011; Finucci et al., 2017, 2016; ICES, 2017b; Kyne and Simpfendorfer, 2007). It is a visual assessment that lacks precision and relies on subjective judgement (Brown-Peterson et al., 2011; Finucci et al., 2017, 2016; Gracan et al., 2013; ICES, 2017b; Jones and Geen, 1977; Kyne and Simpfendorfer, 2007). Biological assessment methods are subject to an array of researcher biases, including (but not limited to): species misidentification, tissue damage, differing amounts of pressure (used to measure a structure that may result in slight, but potentially significant differences in records), non-random sampling of larger individuals over others and researcher experience (Lambert et al., 2012; Petrtýl et al., 2013). Another concern of macroscopic maturity assessment is that solely considering macroscopic criteria (i.e. follicle size) may lead to the overestimation of size at first maturity, and therefore an underestimation in the overall resource (Hamlett et al., 2005; Moya et al., 2017). Macroscopic assessment assumes maturity occurs when follicles are enlarged at follicle recruitment, although (in most chondrichthyans) ova size has been shown to differ greatly at the onset of vitellogenesis, first maturity and first sperm storage (Hamlett et al., 2005; Moya et al., 2017). There is a need for more precise, objective methods for determining maturity (e.g. histology) (Brown-Peterson et al., 2011; Gracan et al., 2013; Hilge, 1977; ICES, 2017b; Jones and Geen, 1977).

Histological maturity assessment has been utilised in an attempt to better understand reproductive processes (Moya et al., 2017). Histological studies are highly valuable, as they provide more accurate means to assess gonadal development (Moya et al., 2017). However, they do not always provide information that is directly comparative to macroscopic assessment criteria, since each requires the use of different methodology (Moya et al., 2017). Although there has been much development in the production of histological maturity keys, these are not readily usable, as histology is more expensive (in time and

resources) than macroscopic staging (Brown-Peterson et al., 2011; ICES, 2017a, 2017b; Sieiro et al., 2014). Conversely, histology can provide additional developmental information, such as changes in cellular development and processes (e.g. sperm storage) which can then inform macroscopic assignment (Brown-Peterson et al., 2011; ICES, 2017a, 2017b).

Sperm storage in females was first proposed by Lo Bianco in 1909 and appears to have evolved and disappeared multiple times, as observed in insects, fish, reptiles, amphibians, birds and mammals (Holt and Lloyd, 2010; Lo Bianco, 1909). Some species (particularly mammals) store spermatozoa in the vagina, cervix, uterus or oviduct (Holt and Lloyd, 2010; Pratt Jr, 1979; Storrie et al., 2008). Birds and reptiles typically store spermatozoa in specialised sperm-storage tubules, which maintain viable spermatozoa for long periods of time (Holt and Lloyd, 2010). Fish have developed a huge array of mechanisms to store spermatozoa, including: 1) direct storage within ovarian follicles and 2) multiple copulations with immature females – in which subsequent sperm storage allows fertilisation to occur, once females reach maturity and begin ovulation (Holt and Lloyd, 2010; Pratt Jr, 1979; Storrie et al., 2008). Sperm storage was first proposed in chondrichthyans when a female ray was observed laying fertilised eggs in captivity after a long period of isolation from males (Clark, 1922; Storrie et al., 2008). Numerous studies have since reported sperm storage in chondrichthyans, including holocephalans and elasmobranchs from several families (e.g. *Alopiidae, Lamnidae, Carcharhinidae, Triakidae, Sphyrnidae* and *Callorhinidae*) (Bernal et al., 2015; Finucci et al., 2017, 2016; Hamlett et al., 2005, 1998; Moura et al., 2011; Serra-Pereira et al., 2011a; Storrie et al., 2008). In chondrichthyans, spermatozoa are stored in sperm-storage tubules in the terminal zone of the oviducal gland (Bernal et al., 2015; Finucci et al., 2017, 2016; Hamlett et al., 2005, 1998; Moura et al., 2011; Serra-Pereira et al., 2011a; Storrie et al., 2008).

Storrie et al. (2008) observed some evidence for behavioural maturity in female *Mustelus antarcticus* in Australia, where spermatozoa were observed in the uterus of one immature and several developing fish. Although sperm storage was not observed in the immature fish, this suggests behavioural maturity (mating can

occur before the reproductive organs are fully developed) (Storrie et al., 2008). Such findings could have substantial implications on our understanding of reproductive biology, the ways in which we assess populations, and stock dynamics and structure.

Sexual maturity in chondrichthyans can be assessed both macroscopically (anatomically or morphologically) or histologically, by examining external and internal reproductive characteristics, such as the oviducal gland, follicles, uteri and gonad mass (Brown-Peterson et al., 2011; ICES, 2017a, 2017b; Kyne and Simpfendorfer, 2007). However, different laboratories and different countries use various sets of characteristics to define the same reproductive processes, which can lead to inconsistencies in maturity assessment (Bromley, 2003; Brown-Peterson et al., 2011; Gracan et al., 2013; ICES, 2017a, 2017b, 2013, 2010; Kyne and Simpfendorfer, 2007; Sieiro et al., 2014; Storrie et al., 2008). Various studies have assessed the quality of microscopic versus macroscopic criteria in assessing the onset of maturity, namely follicle size at the onset of vitellogenesis (Klibansky and Scharf, 2015; Moya et al., 2017; Sieiro et al., 2014). Oddone and Amorim (2008) estimated macroscopic vitellogenic follicle size was 7 mm. However, with histology, follicles were observed to begin accumulating yolk at smaller sizes. Should the presence of yolk be the defining characteristic of maturity (over follicle size) there may be a need to redefine sexual maturity.

It has been well documented that differences observed in various life history parameters (between populations of the same species) may signal the presence of different factors acting upon local populations, including temporal and spatial differences (ICES, 2017a, 2017b; Irvine et al., 2012; Moura et al., 2014; Moya et al., 2017). Variation of reproductive characteristics (i.e. follicle size) may be explained by latitudinal differences, and the presence of protected areas may also influence life history parameters (ICES, 2017a, 2017b; Irvine et al., 2012; Moura et al., 2014; Moya et al., 2017). However, it is well recognised that criteria for maturity assessment across studies are inconsistent in terms of terminology, diagnostics and methodology – leading to difficulties in comparing reproductive results from various sources (Bromley, 2003; Brown-Peterson et al., 2011; Gracan

et al., 2013; Hilge, 1977; ICES, 2017a, 2017b; Kjesbu, 2009; Lowerre-Barbieri et al., 2011; Sieiro et al., 2014; Storrie et al., 2008). A further complication to assessing and comparing maturity is achieving representative sampling of the population. The estimation of maturity may not accurately represent the true distribution of maturity stages within a population where all stages are not equally available or vulnerable to a fishery (Brown-Peterson et al., 2011; ICES, 2017a, 2017b; Jones and Geen, 1977; Rigby and Simpfendorfer, 2015).

Although there is international consensus that maturity assessments are critical for the assessment of stocks and populations – particularly with regards to the monitoring and management of those species targeted or vulnerable to fisheries impacts – it is widely acknowledged that there is a need for improvement, consistent terminology and application of maturity scales, as well as validation of existing methodology (particularly macroscopic assessment methods) (Brown-Peterson et al., 2011; Gracan et al., 2013; Hilge, 1977; ICES, 2017a, 2017b, 2013, 2010; Kjesbu, 2009; Lowerre-Barbieri et al., 2011; Moura et al., 2014; Storrie et al., 2008). Studies focusing on reproduction note the lack of reliable data, either due to inaccessibility to adequate sample sizes, or to all maturity stages (Brown-Peterson et al., 2011; Finucci et al., 2017, 2016; Gracan et al., 2013; Hilge, 1977; ICES, 2017a, 2017b, 2013, 2010; Kjesbu, 2009; Lowerre-Barbieri et al., 2011; McCully et al., 2012; McPherson et al., 2011; Moura et al., 2014; Storrie et al., 2008). The importance of standardised macroscopic keys in determining maturity has also been well documented (Brown-Peterson et al., 2011; Finucci et al., 2017, 2016; ICES, 2017a, 2017b, 2013, 2010).

In New Zealand, the maturity staging scales for chondrichthyans were developed by Francis and Lyon (NIWA, pers. comm.) as an improvement on the 'Wanaka' key proposed by Clark and King (1989) Parker and Francis, 2012). The Francis and Lyon scale (NIWA scale; Table 1.4- 1; Table 1.4- 2) remains unpublished (Parker and Francis, 2012). One obvious problem with this key is the lack of consistency in the structures examined. For the first 3 stages ('immature', 'maturing/resting' and 'mature'), only the ovarian processes are considered key diagnostics in differentiating maturity stages (Table 1.4- 1). No consideration is given to other

reproductive processes and traits (i.e. uterus) (Table 1.4- 1). For stages 'gravid I', 'gravid II' and 'post-partum', the uterus is the key structure under examination, disregarding the ovarian processes (Table 1.4- 1). Reproduction is a continuous cycle with stage 6 (post-partum) returning to stage 2 (maturing/resting). Since data on mature females is often lacking and the defining characteristics for the respective stages are not assessed in conjunction with one another, the differentiation of stages 2 and 6 becomes problematic. This could have huge implications on our understanding of population dynamics and structure: if fish that are 'immature' are classified as 'mature', the reproductive population is overestimated (and vice versa), having a direct impact on management.

In 2010, the International Council for the Exploration of the Seas (ICES) held the first workshop on Sexual Maturity Staging of Elasmobranchs (WKMSEL) in response to the European Union's requirement to collect maturity data on elasmobranchs. Without international agreement on which maturity scales to use for chondrichthyans across laboratories, and in hopes of standardising methods, WKMSEL reviewed those keys in use and proposed two new maturity scales, one for oviparous and one for viviparous species. Although standardised keys were proposed in 2010 and re-evaluated in 2012 (Table 1.4- 1; Table 1.4- 2) and ICES recommended the need for further testing of proposed keys on a multitude of species in order to improve standardisation, few studies utilise them. Brown-Peterson et al. (2011) also proposed a new key (Table 1.4- 1; Table 1.4- 2), and conceptual reproductive model, with terminology considered to be more appropriate for describing chondrichthyan reproductive processes (e.g. the removal of the term "spawning capable" since chondrichthyans do not spawn). Many chondrichthyan maturity studies use variations of the Stehmann (2002) key (Table 1.4- 1; Table 1.4- 2).

Standardisation remains difficult and the implementation of new terminology and new macroscopic staging keys has been minimal (Brown-Peterson et al., 2011; ICES, 2017a, 2017b, 2013). The majority of macroscopic maturity keys are numeric, where the same number may represent very different reproductive processes, or a different set of characteristics work towards defining the same

reproductive process (Bromley, 2003; Brown-Peterson et al., 2011; ICES, 2017a, 2017b).

Defining existing problems with maturity assessment is necessary. However, the implications of such problems must remain in focus. If confusing terminology ultimately has no effect on the way in which we understand and interpret results, issues with inconsistent terminology may not matter in understanding a species' reproductive biology. The use of various maturity staging keys with different criteria and key diagnostics may lead to differences in the interpretation of maturity, reproductive potential and general reproductive biology (ICES, 2017a, 2017b).

1.5 Study species

This thesis focused on the reproductive biology of a wide range of poorly studied deep-sea elasmobranchs and batoids, including Squaliformes, *Apristurus* spp., and a range of Rajiformes. For this thesis, species identification was done for *Apristurus* spp. and Rajiformes.

1.5.1 *Centrophorus squamosus*

Centrophorus squamosus (Bonnaterre, 1788) (commonly known as leafscale gulper shark) have a worldwide distribution in temperate waters (Acuña-Marrero et al., 2013; Compagno, 1984; Compagno et al., 2005; Parker and Francis, 2012; Verissimo et al., 2012). They are benthopelagic, caught near or on continental slopes at depths around 145 – 2400 m (Clarke et al., 2001; Compagno, 1984; Parker and Francis, 2012; Verissimo et al., 2012). Catch depth data suggests considerable differences in sex-ratio where males are dominant at shallower depths and females in deeper waters, suggesting sexual segregation and habitat partitioning (Clarke et al., 2001). It has also been suggested that the species displays complex migratory patterns associated with reproduction (Figueiredo et al., 2008; Verissimo et al., 2012).

C. squamosus are a lecithotrophic viviparous species (Acuña-Marrero et al., 2013; Figueiredo et al., 2008; Moura et al., 2014; Verissimo et al., 2012), and are

estimated to mature at sizes between 110 cm total length (TL) and 158 cm TL, with a maximum recorded length of 164 cm TL (Clarke et al., 2001; Compagno et al., 2005; Neat et al., 2015; Parker and Francis, 2012; Verissimo et al., 2012). Length-at-maturity for *C. squamosus* has been estimated at 124 cm TL for females and 86 cm TL for males, based on Girard and Buit (1999) study from the west coast of the British Isles. However, records from New Zealand, specifically on Chatham Rise and in the Sub-Antarctic, estimated length-at-maturity for males at 98.9 cm TL and age-at-maturity at 15.4 years, and at 119.0 cm TL and 20.8 years for females (Cavanagh and Kyne, 2006; Clarke et al., 2001; Parker and Francis, 2012). This could potentially indicate slight differences in population biology with geography.

Fecundity has only been estimated in Portugal and the United Kingdom at 1 – 15 pups per litter (mean between 5 and 8 pups per litter) and size at birth at 35 – 45 cm (Figueiredo et al., 2008; Kyne and Simpfendorfer, 2007; Moura et al., 2014; Parker and Francis, 2012; Verissimo et al., 2012). Gestation period is unknown, but hypothesised as being long since that of spiny dogfish exceed 24 months (Parker and Francis, 2012). It has been suggested these fish have a continuous reproductive cycle and may be the most unproductive of chondrichthyan fishes (Kyne and Simpfendorfer, 2007).

C. squamosus are commonly taken as bycatch in mixed deep-sea trawl and longline fisheries (Clarke et al., 2001; Ford et al., 2015; Parker and Francis, 2012). Gravid females are seldom reported and commercial catches are generally comprised of mature males, some immature females and few mature females (Acuña-Marrero et al., 2013; Clarke et al., 2001; Figueiredo et al., 2008; Moura et al., 2014; Neat et al., 2015; Verissimo et al., 2012). Although the location of nursery grounds remains unknown, immature fish appear to recruit on the continental slope as they reach maturity (the site of many major fishing grounds) (Verissimo et al., 2012). *C. squamosus* are classed as Vulnerable under International Union for Conservation of Nature (ICUN) (Dunn et al., 2013).

1.5.2 *Centroselachus crepidater*

Centroselachus crepidater (Barbosa du Bocage & de Brito Capello, 1864) (commonly known as longnose velvet dogfish) have a worldwide distribution (Compagno, 1984). They are relatively common on continental and insular slopes, and can also be found near the sea floor, at depths of 230 – 1600 m (Neat et al., 2015).

C. crepidater are a lecithotrophic viviparous species (Kyne and Simpfendorfer, 2007). Estimated length-at-maturity for females is at 75.4 cm TL and age-at-maturity at 20 years, with a maximum recorded length of 130 cm TL (Kyne and Simpfendorfer, 2007). The maximum reported age is estimated at 54 years (Kyne and Simpfendorfer, 2007). Little is known on the general biology of *C. crepidater* (Moore et al., 2013; Neat et al., 2015).

No fecundity or maturity studies have been conducted on this species in New Zealand (Compagno, 1984). Fecundity has been estimated in Australia at 4 – 8 pups per litter (Last and Stevens, 1994). Fecundity is estimated at 1 – 15 pups per litter in Europe (namely the UK and Portugal) (Kyne and Simpfendorfer, 2007). Gestation has been estimated at around 1 – 2 years, and since there is evidence that *C. crepidater* exhibit a resting period between pregnancies, it is probable these fish have a reproductive cycle lasting 2 – 3 years (Kyne and Simpfendorfer, 2007). No defined breeding seasons have been reported (Moore et al., 2013; Neat et al., 2015).

C. crepidater are commonly taken as bycatch, although fish are generally discarded (Ford et al., 2015). *C. crepidater* are classed as Least Concern under IUCN (Dunn et al., 2013).

1.5.3 *Deania calcea*

Deania calcea (Lowe, 1839) (commonly known as shovelnose dogfish) have a worldwide distribution, occurring along continental slopes and outer shelves in all non-polar oceans (Dunn et al., 2013; Ford et al., 2015; Last et al., 2009; Moura et al., 2014; Paiva et al., 2011; Parker and Francis, 2012; Rochowski et al., 2015). They are commonly caught depths of 70 – 1600 m (Neat et al., 2015).

Misidentification of *D. calcea* as rough longnose dogfish (*Deania histricosa*) is known to occur (Ford et al., 2015).

Despite being abundant and widely distributed on Chatham Rise, life history characteristics of *D. calcea* are largely unknown, especially in New Zealand waters (Dunn et al., 2013; Ford et al., 2015). Deep-sea dogfish are known to segregate by size, reproductive stage and sex (Clarke et al., 2014; Irvine et al., 2012; Moura et al., 2014). However this phenomenon has not been observed for *D. calcea* in New Zealand waters (Clarke et al., 2014; Irvine et al., 2012; Moura et al., 2014).

D. calcea are lecithotrophic viviparous with an average litter size of approximately 6 pups and size at birth is around 30 cm (FAO, 2014; Ministry for Primary Industries, 2013). *D. calcea* are reported to grow to a maximum of 122 cm TL (FAO, 2014). Females are estimated to mature from 16 years at about 95 cm TL, and maximum longevity recorded is 21 years (Ministry for Primary Industries, 2013; Paiva et al., 2011). Reproductive frequency remains unknown and gravid females are caught infrequently (Ministry for Primary Industries, 2013).

D. calcea are predominantly caught as bycatch in bottom trawl fisheries targeting hoki, orange roughy and oreo. However, annual catches are likely to be grossly underestimated (Dunn et al., 2013; Parker and Francis, 2012). The diet of *D. calcea* is similar to hoki (Dunn et al., 2013). Since hoki stocks are heavily fished, decreased competition for primary prey items has resulted in a net benefit for *D. calcea*, particularly in New Zealand, and may explain stability in biomass trends (Dunn et al., 2013). However, dramatic declines are being observed in Australian waters, especially off the coast of New South Wales and in the North Atlantic (Ford et al., 2015). *D. calcea* are listed as Least Concern under IUCN (Dunn et al., 2013).

1.5.4 *Apristurus spp.*

Genus *Apristurus* (Garman, 1913) is one of the largest of all genera among elasmobranchs, with approximately 35 valid and 47 nominal species known worldwide (Iglesias et al., 2004; Moore et al., 2013; Sato et al., 2013). *Apristurus* spp. are distributed globally on continental slopes, trenches and submarine ridges,

in non-polar regions at depths of 400 – 2000 m (Iglesias et al., 2004; Moore et al., 2013; Sato et al., 2013). The genus is divided into three phenetic species groups: 1) longicephalus, 2) spongiceps and 3) brunneus – based on a combination of morphological characters – notably: snout length, spiral valve counts, cephalic lateral lines and egg casing characteristics (i.e. the presence or absence of tendrils) (Flammang et al., 2008; Iglesias et al., 2004; Sato et al., 2013).

Virtually nothing is known of the general biology of *Apristurus* spp. and a complete lack of age and growth estimates may be perpetuated by taxonomic identification difficulties, since many species have similar external morphology (Cavanagh and Kyne, 2006; Flammang et al., 2008; Human, 2011; Iglesias et al., 2004; Kyne and Simpfendorfer, 2007; Neat et al., 2015). Some *Apristurus* spp. have not yet been taxonomically described (Human, 2011; Kyne and Simpfendorfer, 2007; Sato et al., 2013). The most updated identification key for *Apristurus* spp. is in Roberts et al. (2015) and has been recognised as being imperfect (Neat et al., 2015)

Female *Apristurus* spp. tend to be larger than males, a deviation from the trend observed in the majority of chondrichthyans (Flammang et al., 2008; Kyne and Simpfendorfer, 2007). Most known species are oviparous, producing a single embryo per egg capsule (Bustamante et al., 2013; Flammang et al., 2008; Kyne and Simpfendorfer, 2007). Fecundity is difficult to estimate since egg-laying periods are mostly unknown, although it has been suggested that reproduction occurs throughout the year (Flammang et al., 2008; Kyne and Simpfendorfer, 2007). Egg case morphology has been described in 12 known species (Flammang et al., 2008; Kyne and Simpfendorfer, 2007).

Apristurus spp. are commonly caught as bycatch, although it has been suggested that *Apristurus* spp. may be more resilient to fishing efforts, due to their small size and oviparous reproductive strategy (Flammang et al., 2008; Kyne and Simpfendorfer, 2007; Neat et al., 2015). The *Apristurus* spp. studied here are listed as either Least Concern or Data Deficient under IUCN (Duffy, 2003; Huveneers and Duffy, 2015; Kyne et al., 2015; McCormack et al., 2016).

1.5.5 *Rajiformes*

Rajiformes are the only order of batoids known to be oviparous and is made up of 3 families (*Anacanthobatidae, Arhynchobatidae* and *Rajidae*), with 30 genera and an estimated 287 valid species (Chiquillo et al., 2014; Ishihara et al., 2012). Genus *Bathyraja, Dipturus* and *Amblyraja* are commonly distributed worldwide on continental shelves and slopes below 200 m, and the distribution of some *Bathyraja* spp. reach depths greater than 2900 m (Chiquillo et al., 2014; Ishihara et al., 2012; Kyne and Simpfendorfer, 2007). Both *Bathyraja* and *Amblyraja* are found in polar waters (Ishihara et al., 2012). Genus *Brochiraja* are only found in Australian and New Zealand waters on the upper-to-mid continental slope below approximately 300 m (Ishihara et al., 2012).

There is little detailed information on the biology of softnose skates, despite the high species diversity, due to taxonomic uncertainty and difficulties in sampling (Chiquillo et al., 2014; Kyne and Simpfendorfer, 2007). The majority of the available biological information is derived from hardnose skates and from the North Atlantic and North Pacific (although there is a growing body of research from New Zealand, South America and the Antarctic) (Kyne and Simpfendorfer, 2007).

Many Rajiformes exhibit year-round egg-laying, with all species producing a single embryo per egg capsule (Chiquillo et al., 2014). There is little information on fecundity, although it has been shown that temperature affects egg-laying rates, and therefore alters estimates of fecundity (Kyne and Simpfendorfer, 2007). Despite data deficiency for many species, the egg capsules, which are species specific, have been described in at least 90 species (Chiquillo et al., 2014; Ishihara et al., 2012). Egg capsules are used to determine interrelationships between species (Chiquillo et al., 2014; Ishihara et al., 2012). The Rajiformes studied here are listed as either Least Concern or Data Deficient under IUCN (Francis et al., 2009; Francis and McCormack, 2009; Francis et al., 2009; Kulka et al., 2016; Stevens, 2009).

Table 1.4- 1: Key macroscopic maturity assessment keys for females comparing the key diagnostics of each macroscopic stage criteria: the ICES (2012) key, the Brown-Peterson et al (2011) key, the NIWA key (Francis & Lyon, NIWA, pers. comm.) and the Stehmann (2002) key. Examples of studies in which these keys have been used are listed in the bottom row and are also applicable to Table 1.4- 2.

ICES (2012) Key	Brown-Peterson et al (2011) Key	NIWA Key	Stehmann (2002) Key
1 - IMMATURE *Ovaries:* small whitish, undistinguishable follicles. *Oviducal gland:* not visible - some see thickening of uteri where glad will develop. *Uteri:* thread-like, narrow.	**IMMATURE (NEVER SPAWNED)** Small ovaries, often clear, blood vessels indistinct. Only oogonia and pg oocytes present. No atresia or muscle bundles. Thin ovarian wall and little space between oocytes.	**1 – IMMATURE** *Ovaries* small and undeveloped. Oocytes not visible, or small (pin-head sized) and translucent whitish.	**A OR 1 - IMMATURE, JUVENILE** *Ovaries*: small, their internal structure gelatinous or granulated. No oocytes differentiated or all uniformly small, granular. *Uteri*: narrow, thread-like.
2 - IMMATURE (DEVELOPING) *Ovaries:* follicles different stages of development (small, med. yolky). *Oviducal gland:* distinguishable and developing. *Uteri:* enlarging.	**DEVELOPING (OVARIES BEGINNING TO DEVELOP, BUT NOT READY TO SPAWN)** Enlarging ovaries, blood vessels becoming more distinct. Pg, ca, vtg1, and vtg2 oocytes present. No evidence of pofs or vtg3 oocytes. Some atresia can be present. Early developing subphase: pg and ca oocytes only.	**2 – MATURING/RESTING** Some oocytes enlarged, up to about pea-sized or larger, and white to cream.	**B OR 2 - MATURING, ADOLESCENT** *Ovaries*: somewhat enlarged, walls more transparent. Oocytes becoming differentiated to various small sizes. *Uteri:* largely as stage A/1 but may become widened posteriorly.

ICES (2012) Key	Brown-Peterson et al (2011) Key	NIWA Key	Stehmann (2002) Key
3 - MATURE (CAPABLE TO REPRODUCE) *Ovaries:* presence large yolked follicles ready to be ovulated. *Oviducal gland:* fully developed. *Uteri:* fully developed.	**SPAWNING CAPABLE (FISH ARE DEVELOPMENTALLY AND PHYSIOLOGICALLY ABLE TO SPAWN IN THIS CYCLE)** Large ovaries, blood vessels prominent. Individual oocytes visible macroscopically. Vtg3 oocytes present or pofs present in batch spawners. Atresia of vitellogenic and/or hydrated oocytes may be present. Early stages of om can be present. Actively spawning subphase: oocytes undergoing late gvm, gvbd, hydration, or ovulation.	**3 – MATURE** Some oocytes large (greater than pea-sized) and yolky (bright yellow).	**C OR 3 - MATURE ADULT** *Ovaries:* large, well rounded. Oocytes obviously enlarged, all to about the same size, can easily be counted and measured. Oviparous - *Uteri* enlarged and widening over nearly their entire length.

ICES (2012) Key	Brown-Peterson et al (2011) Key	NIWA Key	Stehmann (2002) Key
4A - MATERNAL (EARLY PREGNANCY) *Ovaries:* not considered. *Oviducal gland:* not considered. *Uteri:* well filled and rounded with yolk content (candle shaped), embryos not observed.	**N/A**	**4 – GRAVID I** *Uteri* contain eggs or egg cases but no embryos are visible.	**D OR 4 – DEVELOPING OR ACTIVE** Viviparous – *uteri* well filled and rounded with seemingly unsegmented yolk content ("candle"). Oviparous – A distinctly large yolk-egg present in one or both Fallopian tubes. No egg capsule yet visible in shell gland, or beginning formation of egg capsule at most.

ICES (2012) Key	Brown-Peterson et al (2011) Key	NIWA Key	Stehmann (2002) Key
4B - MATERNAL (MID PREGNANCY) *Ovaries:* not considered. *Oviducal gland:* not considered. *Uteri:* well filled and rounded, embryos always visible, small with large yolk sac.	**N/A**	**N/A**	**E OR 5 – DIFFERENTIATING OR ADVANCED** Viviparous – *uteri* well filled and rounded with segmented content of large yolk balls, can easily be counted and measured. Embryos variously small, atop their huge yolk balls, larger ones with external gills filaments and unpigmented (still "candle") Oviparous - Large yolk-eggs in Fallopian tubes, or already passing through into egg capsules. Egg capsules about fully formed in one or both oviducts but still soft at upper end and located very close to Fallopian tubes.

ICES (2012) Key	Brown-Peterson et al (2011) Key	NIWA Key	Stehmann (2002) Key
4C - MATERNAL (LATE PREGNANCY) *Ovaries*: not considered. *Oviducal gland*: not considered. *Uteri*: embryos fully formed, yolk sacs reduced/absent.	**N/A**	**5 – GRAVID II** *Uteri* contain visible embryos. N/A for egg layers.	**F OR 6 – EXPECTING OR EXTRUDING** Viviparous – embryos more or less fully formed, pigmented, external gill filaments lost, yolk sacs obviously reduced. Can be counted, measured and sexed easily. Oviparous – Completed, hardened egg capsules in one or both oviducts, more or less separated from Fallopian tubes. Skate capsule surface mostly covered with dense silky fibres. Either no enlarged oocytes in Fallopian tubes, or one or two in position. If oviducts empty but still much enlarged and wide, capsules have probably just been extruded

ICES (2012) Key	Brown-Peterson et al (2011) Key	NIWA Key	Stehmann (2002) Key
5 - MATERNAL (POST-PARTUM) *Ovaries*: shrunken without follicle development and with atresic follicles. *Oviducal gland*: not considered. *Uteri*: enlarged and flaccid.	**REGRESSING (CESSATION OF SPAWNING)** Flaccid ovaries, blood vessels prominent. Atresia (any stage) and pofs present. Some ca and/or vitellogenic (vtg1, vtg2) oocytes present.	**6 – POST-PARTUM** *Uteri* flaccid and vascularised indicating recent birth.	**G OR 7 - POST-NATAL, SPENT** *Ovaries:* at resting stage, similar to stages A/1 or B/2. *Uteri* empty but still widened considerably over their full length in contrast to stages A/1 or B/2.
N/A	**REGENERATING (SEXUALLY MATURE, REPRODUCTIVELY INACTIVE)** Small ovaries, blood vessels reduced but present. Only oogonia and pg oocytes present. Muscle bundles, enlarged blood vessels, thick ovarian wall and/or gamma/delta atresia or old, degenerating pofs may be present.	**N/A**	**N/A**
(Ghasemian et al., 2015; Stephan et al., 2014)	*(Osaer et al., 2015; Paiva et al., 2011)*	*(Finucci et al., 2017, 2016; Parker and Francis, 2012)*	*(Figueiredo et al., 2008; Irvine et al., 2012; Moura et al., 2014)*

Table 1.4- 2: Key macroscopic maturity assessment keys for males comparing the key diagnostics of each macroscopic stage criteria: the ICES (2012) key, the Brown-Peterson et al (2011) key, the NIWA key (Francis & Lyon, NIWA, pers. comm.) and the Stehmann (2002) key.

ICES (2012) Key	Brown-Peterson et al (2011) Key	NIWA key	Stehmann (2002) Key
1 - IMMATURE *Claspers*: flexible, non-calcified, shorter than pelvic fins. *Testes*: small, undeveloped. *Ducts*: straight, thread-like.	**IMMATURE (NEVER SPAWNED)** Small testes, often clear and threadlike. Only sg1 present; no lumen in lobules.	**1 – IMMATURE** *Claspers* shorter than pelvic fins, soft and un-calcified, unable or difficult to splay open.	**A OR 1 – IMMATURE, JUVENILE** *Claspers* undeveloped as small, flexible sticks being shorter than extreme tips of posterior pelvic fin lobes. Gonads (*testes*) small, whitish, sperm *ducts* straight and thread-like.
2 - IMMATURE (DEVELOPING) *Claspers*: flexible, partially calcified, as long as/longer than pelvic fins. *Testes*: developing, may start to segment in sharks, rays - lobules visible but don't occupy whole surface. *Ducts*: developing, beginning to coil.	**DEVELOPING (TESTES BEGINNING TO GROW AND DEVELOP)** Small testes but easily identified. Spermatocysts evident along lobules. Sg2, sc1, sc2, st, and sz can be present in spermatocysts. Sz not present in lumen of lobules or in sperm ducts. Ge continuous throughout. Early developing subphase: sg1, sg2, and sc1 only.	**2 – MATURING** *Claspers* longer than pelvic fins, soft and un-calcified, unable or difficult to splay open or rotate forwards.	**B OR 2 – MATURING, ADOLESCENT, SUBADULT** *Claspers* becoming extended, longer than tips of posterior pelvic fin lobes, their tips (glans) becoming structured, but their skeleton still soft and flexible. Gonads enlarged, sperm *ducts* beginning to meander.

ICES (2012) Key	Brown-Peterson et al (2011) Key	NIWA key	Stehmann (2002) Key
3A - MATURE (CAPABLE TO REPRODUCE) *Claspers*: rigid, fully calcified, longer than pelvic fin. *Testes*: fully developed (some sharks - fully segmented). *Ducts*: tightly coiled, filled with sperm.	**SPAWNING CAPABLE (FISH ARE DEVELOPMENTALLY AND PHYSIOLOGICALLY ABLE TO SPAWN IN THIS CYCLE)** Large and firm testes. Sz in lumen of lobules and/or sperm ducts. All stages of spermatogenesis (sg2, sc, st, sz) can be present. Spermatocysts throughout testis, active spermatogenesis. Ge can be continuous or discontinuous. Actively spawning subphase (macroscopic): milt released with gentle pressure on abdomen. Histological subphases based on structure of ge. Early ge: continuous ge in all lobules throughout testes. Mid-ge: continuous ge in spermatocysts at testis periphery, discontinuous ge in lobules near ducts. Late-ge: discontinuous ge in all lobules throughout testes.	**3 – MATURE** *Claspers* longer then pelvic fins, hard and calcified, able to splay open and rotate forwards to expose clasper spine.	**C OR 3 – MATURE, ADULT** *Claspers* fully formed and stiff, eventually present cartilaginous hooks, claws or spines of glans free and sharp. Gonads enlarged, well rounded, filled with flowing sperm and often reddish in colour. Sperm *ducts* tightly coiled and well filled with sperm.

ICES (2012) Key	Brown-Peterson et al (2011) Key	NIWA key	Stehmann (2002) Key
3B - MATURE (ACTIVE) *Claspers*: similar to 3a, clasper glands dilated, sometimes swollen, sperm may be present in clasper groove or glands. *Testes*: similar to 3a. *Ducts*: sperm observed inside, or flowing out of cloaca.	**N/A**	**N/A**	**D OR 4 – ACTIVE** Glans *clasper* often dilated and swollen, with free cartilaginous spines mostly erect; sperm flowing from cloaca under pressure on seminal vesicle and/or present in clasper groove.
4 - MATURE (REGRESSING) *Claspers*: fully formed (same as in 3). *Testes*: shrunken, flaccid, sperm doesn't flow on pressure. *Ducts*: empty, flaccid. Seminal vesicle developed but empty.	**REGRESSING (CESSATION OF SPAWNING)** Small and flaccid testes, no milt release with pressure. Residual sz present in lumen of lobules and in sperm ducts. Widely scattered spermatocysts near periphery containing sc2, st, sz. Little to no active spermatogenesis. Spermatogonial proliferation and regeneration of ge common in periphery of testes.	**N/A**	**N/A**

ICES (2012) Key	Brown-Peterson et al (2011) Key	NIWA key	Stehmann (2002) Key
N/A	**REGENERATING (SEXUALLY MATURE, REPRODUCTIVELY INACTIVE)** Small testes, often threadlike. No spermatocysts. Lumen of lobule often non-existent. Proliferation of spermatogonia throughout testes. Ge continuous throughout. Small amount of residual sz occasionally present in lumen of lobules and in sperm.	**N/A**	**N/A**

1.6 Research Objectives

The objective of this thesis was to gain a better understanding of the general biology, reproduction and maturity of a range of poorly studied deep-sea elasmobranch and batoid species. This study aims to test the hypothesis that sperm storage occurs in all deep-sea elasmobranch and batoid species. This study also aims to assess the validity and quality of the macroscopic maturity staging key used in New Zealand, towards determining the onset of maturity and accurately distinguishing between macroscopic stages. Additional measurements of uterus width and sperm storage are hypothesised to facilitate the differentiation of maturity stages 2 (maturing/resting) and stage 6 (post-partum). Multiple elasmobranch and batoid species were examined to look for common patterns, as well as mitigate generally small sample sizes available for deep-sea species. This study aims to provide baseline biological information for these poorly studied species.

In the following chapters, histological (Chapter 3) and biological data (Chapter 4) were analysed for viviparous elasmobranchs (*D. calcea, C. crepidater* and *C. squamosus*), oviparous elasmobranchs (*Apristurus ampliceps, Apristurus exsanguis* and *Apristurus melanoasper*) and a batoid (*Brochiraja asperula*). Data for an additional five species where sample sizes were smaller are detailed in Appendix A (*Brochiraja leviveneta, Brochiraja spinifera, Amblyraja hyperborea, Bathyraja shuntovi, Apristurus sinensis* and *Apristurus garricki*).

Chapter 2 Methods

2.1 Sampling at sea

Shovelnose dogfish (*Deania calcea*), longnose velvet dogfish (*Centroselachus crepidater*), leafscale gulper shark (*Centrophorus squamosus*), five deep-sea catshark species (*Apristurus* spp.) and five deep-sea batoid species (Rajiformes spp.) were collected from the 24th Chatham Rise hoki and middle depth fish trawl survey (TAN1601) conducted by the National Institute for Water and Atmospheric Research (NIWA) for MPI, carried out by the RV *Tangaroa,* from 3 January – 2 February 2016 (Figure 2.1- 1).

The survey was part of a time series of trawl surveys conducted on Chatham Rise, and is the most comprehensive time series of species abundance at depths 200 – 800 m in New Zealand's Exclusive Economic Zone (EEZ). The survey fulfils a crucial role in ecosystem monitoring as well as providing data for single-species stock assessments (O'Driscoll et al., 2011; Stevens et al., 2017; Tuck et al., 2009).

The 2016 survey followed a two-phase stratified random design with a core sampling area of 200 – 800 m depth divided into 23 strata, with 84 phase one and 9 phase two core tows (200 – 800 m) carried out during daylight hours (as defined by (Hurst et al., 1992)) (Stevens et al., 2017). The survey duration was of 31 days and also covered deep strata from 800 – 1300 m around the entire Chatham Rise. Deep-sea strata were also sampled at night, with 46 phase one tows; no phase two allocations were made for deep strata (Stevens et al., 2017). RV *Tangaroa* was used to conduct the survey, a purpose-built research stern trawler of 70 m total length (Stevens et al., 2017). The bottom trawl was the same as that used on all previous trawl surveys conducted in the time series (Stevens et al., 2017). The net was an eight-seam hoki bottom trawl with 100 m sweeps, 50 m bridles, 12 m backstrops, 58.8 m groundrope, 45 m headline and 60 mm codend mesh (Stevens et al., 2017). The net was towed for a total of 3 nautical miles (nmi) at 3.5 knots at each station (Stevens et al., 2017).

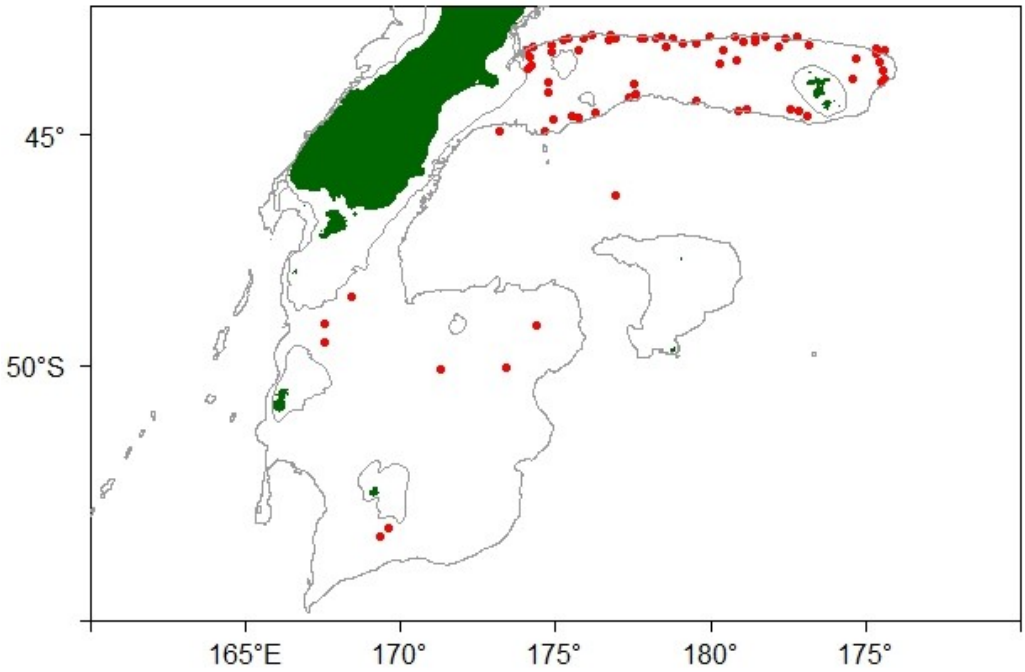

Figure 2.1- 1: Catch area where red dots highlight sampled stations, where specimens for this study were collected.

Additional *Brochiraja asperula* (n = 6), *Brochiraja spinifera* (n = 3) and *Apristurus exsanguis* (n = 1) samples were requested from the Sub-Antarctic trawl survey of hoki and middle depths species (TAN1614), analogous to the Chatham Rise survey, conducted by NIWA, carried out on the RV Tangaroa in November and December 2016 (Figure 2.1- 1) (Bagley et al., 2013; MacGibbon et al., 2017). In 2016, 57 phase one tows were completed in 20 strata divided by depth (300 – 600 m; 600 – 800 m; 800 – 1000 m) (MacGibbon et al., 2017).

At all stations, catch was identified and sorted by species (where possible) and species catches weighted on Marel motion-compensating electronic scales accurate to approximately 0.04 kg (Stevens et al., 2017). More detailed biological data were then collected from a randomly selected subset of each species (the sub-catch; Table 2.1- 1), which included measurement of fish weight, total length (TL – measured from the snout to the distal end of the straightened caudal fin; TL for batoids was measured as disk width), sex (by the presence (males) or absence (females) of claspers) and maturity assessment (by macroscopic inspection of the gonad (following the 6-stage classification for chondrichthyans, based on the NIWA protocols (Francis & Lyon, NIWA, pers. comm.; Table 2.1- 2)).

Table 2.1- 1: Numbers of sharks sampled in sub-catches for biological measurements each species, and sex.

SPECIES	FEMALE	MALE	TOTAL
Apristurus spp.*	**17**	**47**	**64**
Centrophorus squamosus	**33**	**11**	**44**
Centroselachus crepidater	**356**	**448**	**804**
Deania calcea	**345**	**421**	**766**
Other species*	**16**	**12**	**28**
TOTAL	**767**	**939**	**1706**

* Catches were not identified to species level at sea, but samples returned and in the laboratory species identification and biological data were recorded.

Table 2.1- 2: Macroscopic maturity staging key used to assess maturity in chondrichthyan species (Francis and Lyon, NIWA, pers. comm.).

STAGE	NAME	MALE	FEMALE
1	**IMMATURE**	Claspers shorter than pelvic fins, soft and un-calcified, unable or difficult to splay open.	Ovaries small and undeveloped. Oocytes not visible, or small (pin-head sized) and translucent whitish.
2	**MATURING/RESTING**	Claspers longer than pelvic fins, soft and un-calcified, unable or difficult to splay open or rotate forwards.	Some oocytes enlarged, up to about pea-sized or larger, and white to cream.
3	**MATURE**	Claspers longer then pelvic fins, hard and calcified, able to splay open and rotate forwards to expose clasper spine.	Some oocytes large (greater than pea-sized) and yolky (bright yellow).
4	**GRAVID I**	N/A	Uteri contain eggs or egg cases but no embryos are visible.
5	**GRAVID II**	N/A	Uteri contain visible embryos. N/A for egg layers.
6	**GRAVID III**	N/A	Uteri flaccid and vascularised indicating recent birth.

From the TAN1601 survey (once sub-catch sampling was complete), as many shovelnose dogfish (*D. calcea*), longnose velvet dogfish (*C. crepidater*) and leafscale gulper shark (*C. squamosus*) females as practicable were taken at random for further measurement (Table 2.1- 3), including:

- Uterus width – at the widest part of the uterus
- Size of the largest ovarian follicle diameter
- Number of vitellogenic follicles (> 1 cm in diameter) present
- Length and width of each oviducal gland – where distinguishable from the isthmus and oviduct
- Gonad weight

Table 2.1- 3: Returned samples by species and sex (where D. calcea, C. crepidater and C. squamosus samples were selected from the sub-catches). Further biological data were collected and female reproductive tracts dissected for histological assessment.

SPECIES	FEMALE	MALE	TOTAL
Apristurus ampliceps	**10**	**9**	**19**
Brochiraja asperula	**8**	**7**	**15**
Brochiraja leviveneta	**0**	**1**	**1**
Brochiraja spinifera	**2**	**2**	**4**
Centrophorus squamosus	**8**	**0**	**8**
Centroselachus crepidater	**49**	**3**	**52**
Apristurus exsanguis	**7**	**8**	**15**
Apristurus garricki	**5**	**2**	**7**
Amblyraja hyperborea	**1**	**1**	**2**
Apristurus melanoasper	**10**	**46**	**56**
Bathyraja shuntovi	**5**	**1**	**6**
Apristurus sinensis	**1**	**4**	**5**
Deania calcea	**57**	**0**	**57**
TOTAL	**163**	**84**	**247**

All female reproductive tracts (n = 114) from the aforementioned *D. calcea, C. crepidater* and *C. squamosus* samples (n = 57; 49; and 8, respectively) were dissected and frozen at sea, then returned to the laboratory for later histological assessment.

All deep-sea catsharks and batoids (n = 92) (from both the Chatham Rise and Sub-Antarctic surveys – TAN1601 and TAN1614) were returned whole, for later species identification and laboratory analyses. All were thawed and identified to species level using the keys outlined in Roberts et al. (2015) (Table 2.1- 3). Biological data were then collected similarly to that collected for *D. calcea, C. crepidater* and *C. squamosus*.

2.2 Sampling of reproductive tracts in the laboratory

Reproductive tracts from all females were dissected, retained for histological assessment and preserved in 10 % neutrally buffered formalin (Table 2.1- 3).

Thawing led to some disintegration and damage to ovaries. The ovaries of some macroscopic stages 3 – 6 females were considered to be too badly damaged and were discarded after measurements were taken.

Comments on each sample were also recorded, such as the presence of large external parasites, undetectable oviducal glands, gut contents, and the presence of egg cases (Appendix C). Where oviducal glands were not distinguishable from the isthmus, a default value of 0.01 cm was given for both oviducal gland width and length measurements.

2.3 Histological analyses for sperm storage

The presence of sperm storage in females was evaluated following (Finucci et al., 2017, 2016; Pratt Jr, 1979; Storrie et al., 2008). From each preserved reproductive tract, and where oviducal gland was distinguishable from the isthmus and oviduct, one of the two preserved oviducal glands was removed and cut on the coronal plane then placed in biopsy cassettes for histological processing. Depending on size, either both ovidcual gland halves were able to fit inside a single cassette, or

each half required its own cassette. Some of the oviducal glands in the sample were not preserved well enough for histological processing. No histology was done for *A. hyperborea* or *B. shuntovi* due to the very large oviducal glands, which were unable to be processed using conventional cassettes for tissue embedding, and for which no other histological processing methodology was available. The final histological subsample is summarised in Table 2.3- 1.

Table 2.3- 1: Number of fish sampled for histological assessment, where the oviducal gland was distinguishable from the isthmus and oviduct.

SPECIES	HISTOLOGY
Apristurus ampliceps	**1**
Brochiraja asperula	**8**
Brochiraja leviveneta	**0**
Brochiraja spinifera	**2**
Centrophorus squamosus	**4**
Centroselachus crepidater	**14**
Apristurus exsanguis	**6**
Apristurus garricki	**3**
Amblyraja hyperborea	**0**
Apristurus melanoasper	**0**
Bathyraja shuntovi	**0**
Apristurus sinensis	**1**
Deania calcea	**3**
TOTAL	**42**

Tissue dehydration (through a series of ascending alcohol concentrations: 70 – 100 %) and wax impregnation were performed using an automated Sakura Tissue Tek VIP 5 Processor, before tissues were embedded into paraffin wax blocks. Sections of embedded tissues were taken 4 µm thick, using a Leica microtome and mounted on glass slides. Slides were left to dry overnight and were then stained with Harris's haematoxylin and eosin (H&E). The H&E staining schedule is shown in Table 2.3- 2 (Anderson, Otago, pers. comm.). Finally, coverslips were attached

with DPX mounting medium. Each oviducal gland was sectioned once (if both halves were in the same cassette, thus providing two sections on the same slide) or twice (if oviducal glands required two cassettes due to large size). Eight *C. crepidater* oviducal glands were re-sectioned, with 4 additional sections per tissue block, with 0.5 mm between each of the sections. Between 1 and 5 sections were taken from each oviducal gland.

Table 2.3- 2: Haematoxylin and Eosin (H&E) staining schedule for ovidcual gland sections. Two identical reagents in series means slides were placed in a fresh batch of reagent following the first exposure. Samples were agitated at each stage, unless comments note otherwise.

REAGENT	EXPOSURE TIME	COMMENTS
Xylol	**5**	
Xylol	**5**	
100 % ethanol	**3**	
100 % ethanol	**3**	
70 % ethanol	**3**	
Tap water	**3**	
Harris's haematoxylin	**10 – 12**	
Tap water	**–**	**RINSE**
1 % acid alcohol	**–**	**5 – 10 DIPS**
Tap water	**10**	
1 % eosin	**3 – 4**	
Tap water	**–**	**RINSE**
70 % ethanol	**3**	
100 % ethanol	**3**	
100 % ethanol	**3**	
Xylol	**5**	
Xylol	**5**	

Histological slides were examined at 0.5 – 400 × magnification through transmitted light. All histological slides were examined for the presence of sperm-storage tubules and spermatozoa. Spermatozoa were stained dark blue using H & E. The dark blue colour occurs when H & E stains the nucleus, and the presence of the distinctive tail, highlights spermatozoa.

Samples were photographed on a Proscitech OXJS500T light microscope with a Toupcam U3CMOS camera attachment.

2.4 Statistical analyses

All plotting, analyses and mapping was done in R, including the use of CRAN libraries: car, mapplots, nzplots and plyr (R Core Team, 2013).

The relationship between fish length and weight was modelled using the formula:

$$W = aL^b,$$

where W = weight (g)

L = length (cm)

a and b are constants

The model parameters a and b were estimated using least-squares estimation and the optim function (L-BFGS-B optimisation).

Length-weight relationships were modelled for the: a) whole sample (males and females), b) female-only sample and c) for divisions between macroscopic stages, in order to assess the growth forms of fish in different 'maturity' groupings. In the NIWA macroscopic maturity staging scale (Table 2.1- 2), maturity was considered to start at stage 3 (Finucci et al., 2017). However, stage 2 can represent either 'maturing' or 'resting' fish. Therefore, in the macroscopic analyses, 'mature' animals were classified into two groups: 1) stages two and above or 2) stages 3 and above. Length-weight curves were fitted to assess the difference in growth forms when maturity was considered to start at either stage 2 or stage 3.

Proportion mature at length was modelled for females using logistic ogives. A two-parameter logistic ogive is a sigmoidal model and was fitted to the proportion of

mature fish at length using least-squares estimation (again using optim in R). Maturity ogives were fitted to macroscopic data using the female-only data for each species, as outlined in Table 2.1- 3. The distinction between whether stage 2 or stage 3 should represent the onset of 'maturity' was also considered when modelling the proportion mature at length ogives.

The two-parameter logistic ogives had the form:

$$p_i <- 1/(1+19^{(L50 - L_i)/Lto95},$$

where p_i = the proportion of mature fish in size class i

L_i = the length of size class i

L50 = the length at which 50% of the fish are mature (either stage 2 +, or stage 3 +)

Lto95 = the difference between the length at which 95% of the fish are mature and the *L50*.

Confidence intervals for the parameters of the length-weight relationship and length-at-maturity ogive were initially estimated from the Hessian matrix (the matrix of the second partial derivative of the model parameters) (Haddon, 2010). However, the intervals were not credible (implausibly precise or imprecise). The most likely case of this result was that the model and data choices led to the Hessian matrix being non-invertible. When the Hessian matrix is non-invertible for data reasons (i.e. too few data – as in this study), the model being estimated is too demanding given the data (Haddon, 2010). As a result, an alternative method of non-parametric bootstrapping was used to derive more plausible confidence intervals (Haddon, 2010). Each bootstrapped sample was taken randomly with replacement. An ogive was then fitted to each new sample and parameter estimates obtained. The process of data resampling, model fitting and parameter estimation was repeated 1000 times. Parameter estimates were ordered from lowest to highest, and the 25th and 975th values were taken as credible intervals (Haddon, 2010).

In this study Analysis of Variance (ANOVA) (Fisher, 1918) models were used to determine whether there were significant differences in fish size across macroscopic stages. Normal-based ANOVA analyses assume observations are independent, residuals are normally distributed and there is homogeneity of variances.

These assumptions were tested using:

- The Shaprio-Wilk normality test (Shapiro and Wilk, 1965) which tests the null hypothesis that the sample was selected from a normally distributed population: if the p-value is less than the chosen alpha level ($\alpha = 0.05$), there is evidence the data are not from a normally distributed population.

- The Levene's test for Homogeneity of Variance (Levene, 1960), where if the p-value is greater than the chosen alpha level ($\alpha = 0.05$), homogeneity is assumed since there is no evidence to suggest variance across the groups to be statistically significantly different.

Where the above assumptions were met, ANOVA and Tukey HSD (Tukey, 1949) tests were used to estimate the differences in mean length and weight for each macroscopic stage. Tukey HSD performs pair-wise comparisons between the means of groups, and can be used in conjunction with ANOVA to find those means that are significantly different from one another.

Where the assumptions of normality and/or homogeneity were violated, the non-parametric alternative to ANOVA, the Kruskal-Wallis rank sum test was used to determine whether statistically significant differences in mean length and weight were present across macroscopic stages. Pairwise comparisons were then performed using the Wilcoxon rank sum test.

Standardisation of biological data was conducted in order to control for effects of fish size, by dividing the measurement in question (e.g. uterus width) by TL, body weight or somatic weight, depending on the fish size effect being explored. Both standardised and unstandardized data were analysed. Where results for

standardised biological data were not materially different to unstandardised results, and for brevity of the results, only unstandardised results were reported. Only standardised results for the largest dataset are reported in Appendix B.

Both right and left sides of the reproductive system were functional in females, therefore left and right measurements were plotted against each other to test for differences.

Chapter 4 includes the histological results from Chapter 3. Analyses were conducted for each species separately, and the format of Chapters 4.1 to 4.7 has been kept similar to assist reporting. Short conclusions are reported at the end of each section. A summary of results and conclusions across species is presented in the Discussion (Chapter 5). Where data were considered insufficient to conduct analyses, raw data are reported in Appendix A.

Chapter 3 Histological Analyses for Sperm Storage

Histology was conducted on the oviducal glands of 42 fish (Table 2.3- 1). There were obvious macroscopic differences in oviducal gland shape and histological differences between maturity stages. However, the observed histological differences in oviducal gland structure, between stages, were subtle, therefore the only structural differences reported were those most notable.

The oviducal gland of live-bearing species was elliptical, whereas in deep-sea batoids it was bean-shaped. *Apristurus* spp. had the most variability in shape, with some being longer and more elliptical, as in *A. garricki,* and others bean-shaped.

Across all species, the oviducal gland was composed of four zones: the club, papillary, baffle and terminal zones; with a central lumen connecting the oviduct to the isthmus (Hamlett et al 1999; Moura et al 2011; Sierra-Pereira et al 2011) (Figure 3-1). As in other studies, the club zone consisted of distinctive club shaped lamellae. The papillary zone was distinct from the club zone through the presence of digit-shaped lamellae, although gland tubules in both zones were similar. The baffle zone was composed of serous secretory cells and mucous secretory cells (vacuolated cells), with both cell types having an epithelium lining in the lumen composed of ciliated and secretory cells. In histologically mature samples, secretory material was visible in the secretory cells and secretory ducts (Figure 3-2). The secretory material was visible in the oviducal gland lumen, as egg jelly (viviparous) or egg capsule material (oviparous) (Figure 3-2). The terminal zone consisted of similar secretory cells, as those found in the baffle zone, although gland tubules were elongated and secretory ducts had no lamellae.

The transition between zones was not always clear due to the plane of the section (i.e. tissue embedding led to some distortion of the oviducal gland). The oviducal glands of macroscopically mature fish had highly distinct zonation patterns. Zonation of the oviducal gland was particularly distinctive in *Apristurus* spp. and deep-sea batoids.

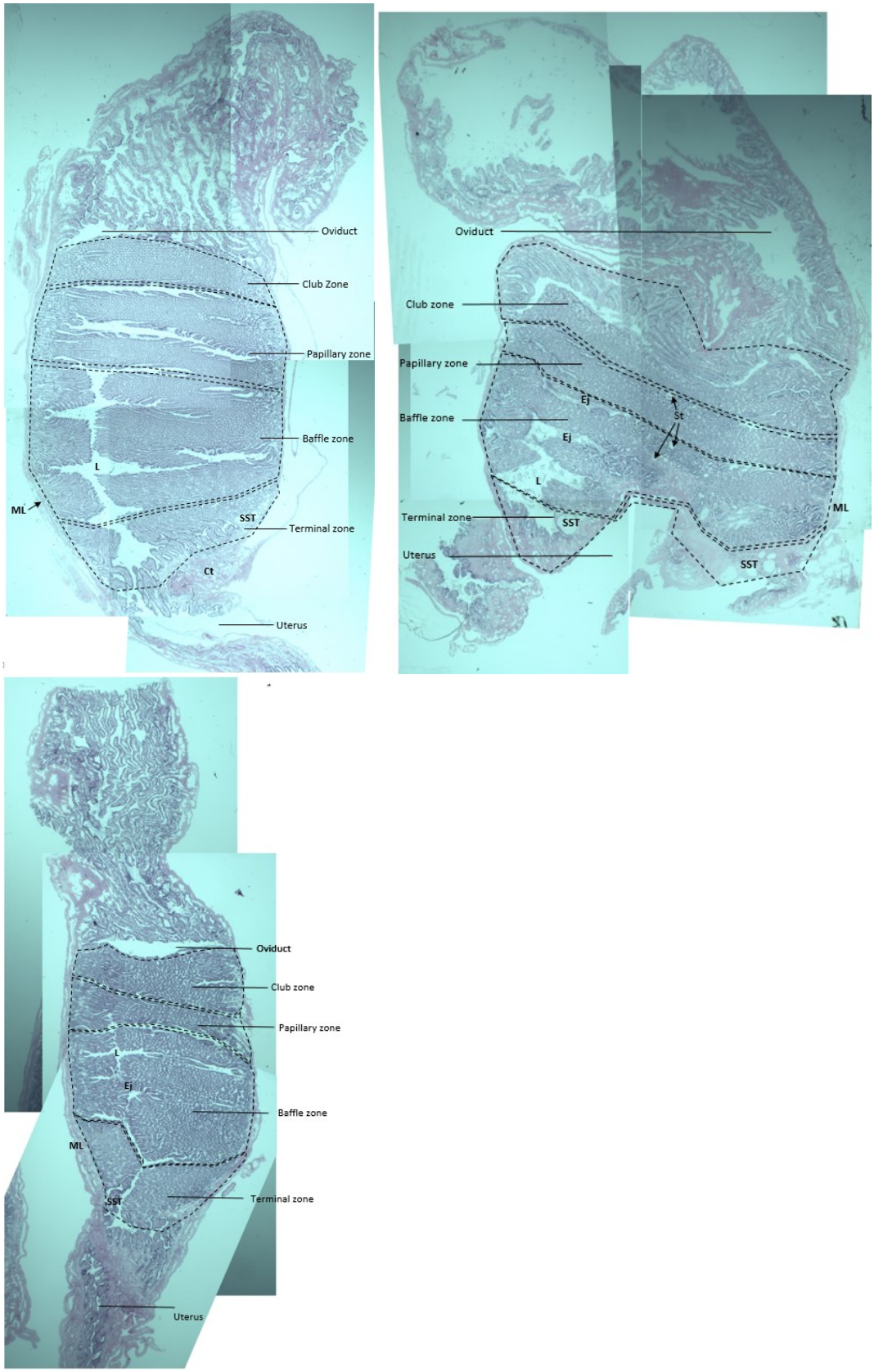

Figure 3-1: Sagittal sections of oviducal glands showing the internal structural organisation and histological staining of different zones of the oviducal gland of: top) macroscopic stage 3 C. crepidater; and bottom) macroscopic stage 2 C. crepidater. H & E. SST – Sperm-storage tubules; Ct – connective tissue; Ej – Egg jelly; L – Lumen; ML – Muscle layer; St – Secretory tubules.

Egg jelly was obvious in all oviducal glands classified as macroscopically mature, which included some fish classified at sea as macroscopically stage 2 (Figure 3-2). The egg secretions were primarily observed in the lumen of the baffle zone and within secretory cells throughout the oviducal gland (Figure 3-2). In all deep-sea batoids sampled histologically, the presence of an unidentified brown matter was observed (Figure 3-3).

Sperm storage was detected in three species: two viviparous species (*C. crepidater, C. squamosus)* and one deep-sea batoid (*B. asperula).* In all cases, sperm-storage tubules were located in the terminal zone, with tubules also bordering the baffle zone in macroscopic stage 3 fish. Sperm-storage tubules had a relatively thin epithelium, composed of secretory cells and lined with ciliary cells.

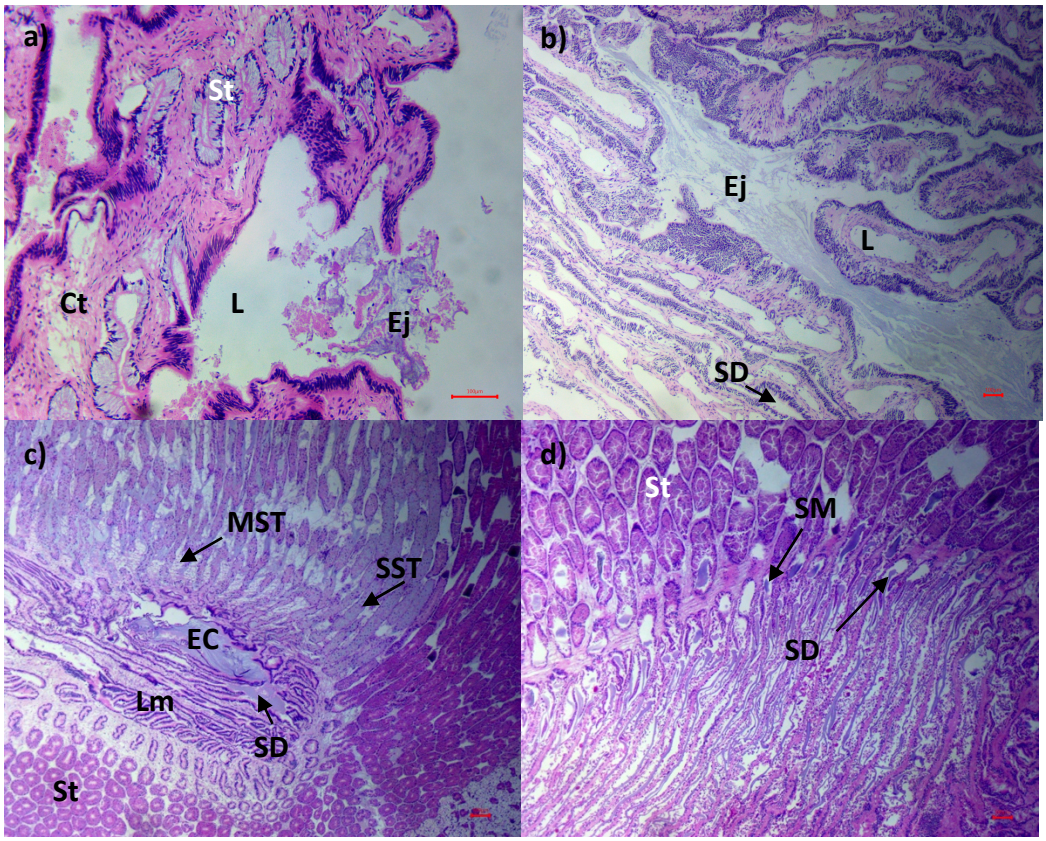

Figure 3-2: Secretory material produced by secretory tubules, and accumulations in the tubules lumen of histologically mature females. H & E. a) egg jelly secretions in the lumen of the terminal zone of a stage 4 female C. squamosus; b) baffle zone secretory ducts secreting egg jelly material into the gland lumen of a stage 2 female C. crepidater; c) structural organisation of the baffle zone, and secretory ducts secreting egg capsule material into the gland lumen of a stage 6 female B. asperula; d) baffle zone secretory ducts secreting egg capsule material into the gland lumen of a stage 6 female A. exsanguis. Ej – Egg jelly; EC – Egg capsule material; Ct – Connective tissue; L – Lumen; Lm – Lamellae; MST – Mucous secretory tubules; SST – Serous secretory tubules; SD – Secretory ducts; SM – Secretory material; St – Secretory tubule. All scale bars 100 µm.

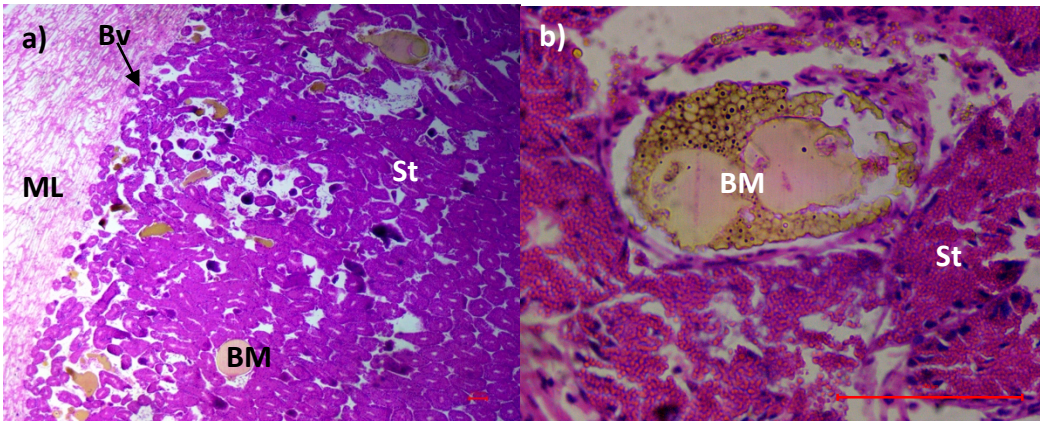

Figure 3-3: Brown material accumulations. H & E. a) in the baffle zone of a stage 2 female B. spinifora; b) in the central baffle zone of a stage 2 female B. asperula. BM – Brown matter; Bv – Blood vessel; ML – Muscle layer; St – Secretory tubule. All scale bars 100 µm.

3.1 Centroselachus crepidater

A total of 14 oviducal glands from eleven macroscopic stage 2 and three macroscopic stage 3 were selected for histological examination. Sperm storage was identified in six stage 2 and three stage 3 fish. Sperm-storage tubules varied in size, with the smallest observed being approximately 50 µm in diameter and the largest around 500 µm in diameter. Spermatozoa were evenly distributed throughout the sperm-storage tubules in both stage 2 and stage 3 fish (Figure 3.1-1; Figure 3.1- 2). However, one stage 2 fish had spermatozoa arranged in bundles (Figure 3.1- 1 d).

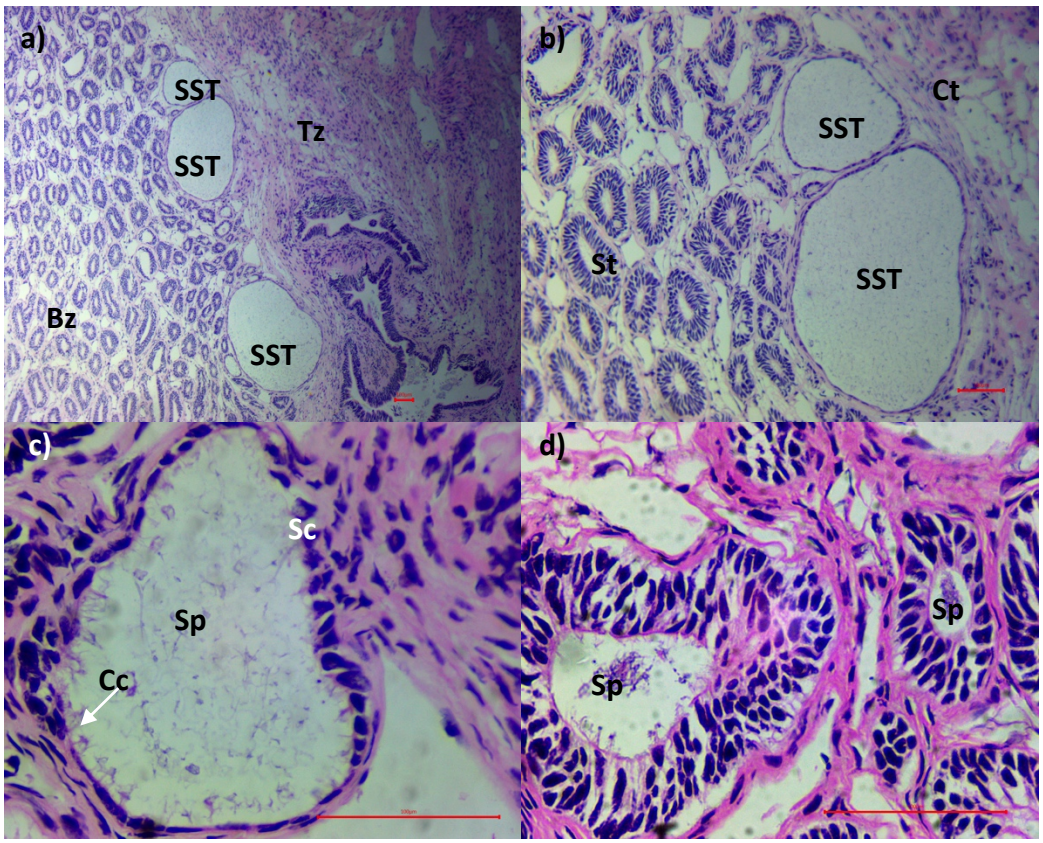

Figure 3.1- 1 C. crepidater: Spermatozoa observed in the oviducal gland of macroscopic stage 2 fish. H & E. a) sperm-storage tubules at the edge of the baffle and terminal zones; b) sperm-storage tubules located amongst secretory tubules of the baffle zone and connective tissue in the terminal zone; c) sparsely aggregated spermatozoa inside the sperm storage tubule; d) the epithelium of sperm-storage tubules consist of both secretory cells and ciliary cells. Tubule has a sperm bundle. SST – Sperm-storage tubules; Tz – Terminal zone; Bz – Baffle zone; St – Secretory tubule; Ct – Connective tissue; Sp – Spermatozoa; Sc – Secretory cell; Cc – Ciliary cell. All scale bars 100 µm.

The zones were at similar levels of differentiation in all fish. Stage 2, 3 and 4 fish all exhibited signs of maturity beyond zone differentiation, with egg jelly present in the oviducal gland lumen and building up in secretory cells (Figure 3.1- 2). Histologically, particularly with regards to those fish exhibiting sperm storage, there were few histological differences between stages 2 and 3 that were observable with H & E staining.

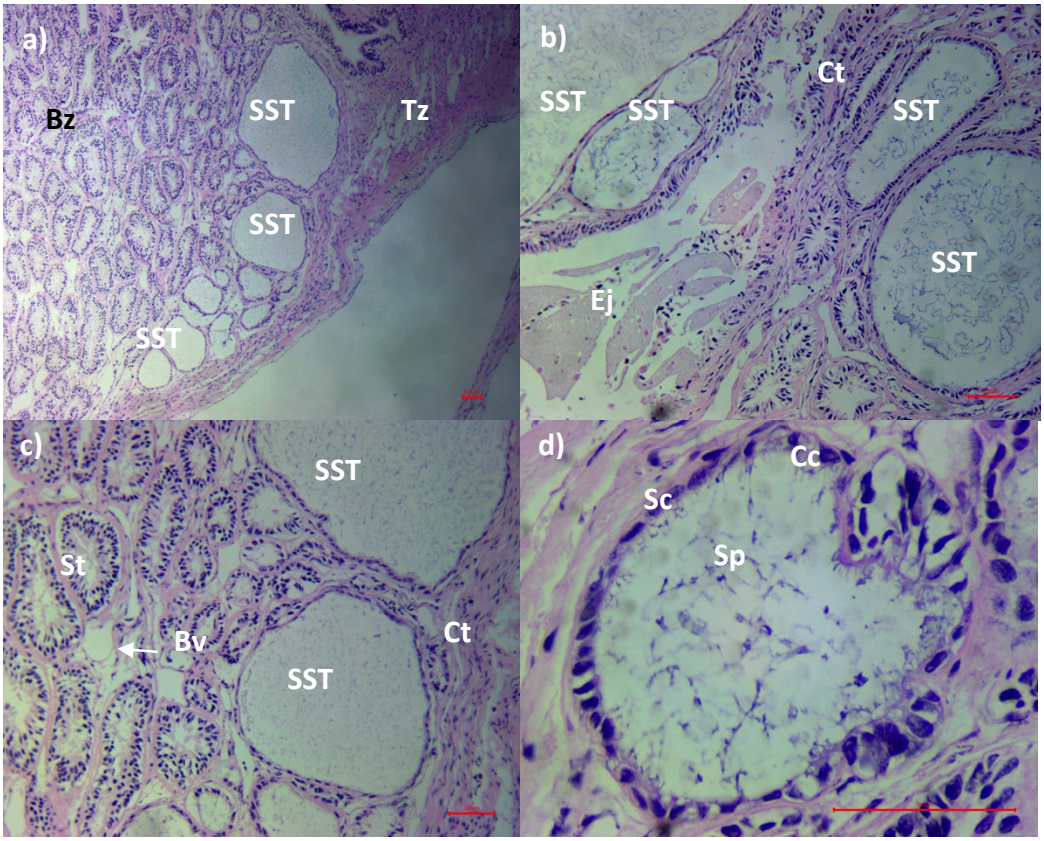

Figure 3.1- 2 C. crepidater: Spermatozoa observed in the oviducal gland of macroscopic stage 3 fish. H & E. a) sperm-storage tubules at the edge of the baffle and terminal zones; b) sperm-storage tubules located amongst secretory tubules of the baffle zone and connective tissue in the terminal zone; c) sperm-storage tubules located amongst secretory tubules and blood vessels of the baffle zone and connective tissue in the terminal zone; d) the epithelium of sperm-storage tubules consist of both secretory cells and ciliary cells. Tubule has aggregated spermatozoa. SST – Sperm-storage tubules; Tz – Terminal zone; Bz – Baffle zone; St – Secretory tubule; Ct – Connective tissue; Bv – Blood vessel; Sp – Spermatozoa; Sc – Secretory cell; Cc – Ciliary cell. All scale bars 100 µm.

3.2 Centrophorus squamosus

A total of 4 oviducal glands from three macroscopic stage 3 and one macroscopic stage 4 were selected for histological examination. Sperm storage was identified in two stage 3 and the stage 4 fish. Sperm-storage tubules observed were approximately 100 µm by 150 µm. Spermatozoa were evenly distributed throughout the sperm-storage tubules, without forming obvious bundles (Figure 3.2- 1).

Zonation of the oviducal gland was similar in all fish. All had egg jelly present in the oviducal gland lumen and in secretory cells. Histologically, particularly with regards to those fish exhibiting sperm storage, there were few differences between stages 3 and 4 that were observable with H & E staining.

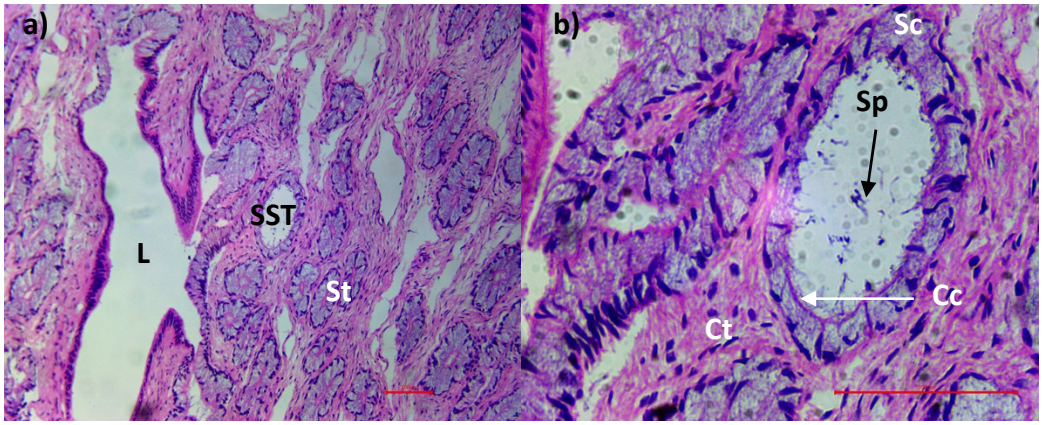

Figure 3.2- 1 C. squamosus: Spermatozoa observed in the oviducal gland of macroscopic stage 4 fish. H & E. a) sperm-storage tubules near the lumen of the terminal zone; b) the epithelium of sperm-storage tubules consist of both secretory cells and ciliary cells. Tubule lumen has dispersed spermatozoa. SST – Sperm-storage tubules; St – Secretory tubule; Ct – Connective tissue; L – Lumen; Sp – Spermatozoa; Sc – Secretory cell; Cc – Ciliary cell. All scale bars 100 µm.

3.3 Brochiraja asperula

A total of 8 oviducal glands from one macroscopic stage 2, four macroscopic stage 3 and three macroscopic stage 6 fish were selected for histological examination. Sperm storage was identified in one stage 6 fish. Sperm-storage tubules varied in size from between 50 µm and 200 µm. Spermatozoa were distributed throughout the sperm-storage tubules without forming obvious bundles (Figure 3.3- 1).

Zonation was similar in all fish. The presence of egg capsule material in the oviducal gland lumen and in secretory cells also histologically confirmed maturity. There were few histological differences between stages 2, 3 and 6 that were observable with H & E staining.

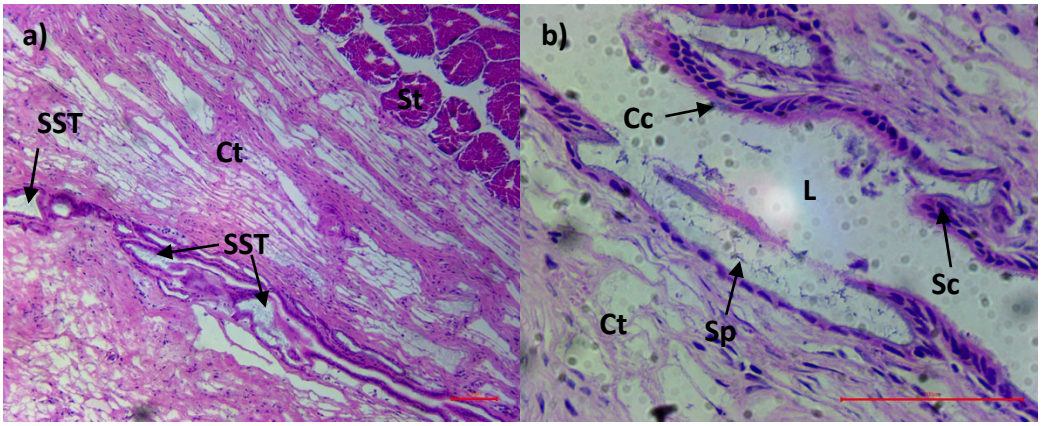

Figure 3.3- 1 B. asperula: Spermatozoa observed in the oviducal gland of macroscopic stage 6 fish. H & E. a) sperm-storage tubules located amongst secretory tubules of the baffle zone and connective tissue in the terminal zone; b) spermatozoa aggregating in a sperm-storage tubule forming in the lumen of the terminal zone, surrounded by connective tissue. SST – Sperm-storage tubules; St – Secretory tubule; Ct – Connective tissue; Sp – Spermatozoa; Sc – Secretory cell; Cc – Ciliary cell. All scale bars 100 µm.

3.4 Histology conclusions

Zonation was similar in all fish irrespective of macroscopically assigned stage, suggesting development in the oviducal gland had attained maturity. Histology was conducted for 42 individuals across nine species and was used to successfully identify sperm storage in 14 fish across three species: *C. crepidater*, *C. squamosus* and *B. asperula*. These are the first records of sperm storage in these species and the first report of sperm storage in a deep-sea batoid of the order *Arhynchobatidae*. Sperm storage could not be histologically confirmed in 28 fish.

Chapter 4 Reproductive Biology

4.1 Centroselachus crepidater

A sub-catch from the TAN1601 survey of male (n = 448) and female (n = 356) *C. crepidater* were caught principally on the northern Chatham Rise (Figure 4.1- 1). Stage 1 females (n = 146) were relatively evenly distributed throughout the catch area and there was a higher proportion of stage 2 and stage 3 females in the eastern Chatham Rise (Figure 4.1- 1; Table 4.1- 1). Stage 3 males were predominantly caught from the central northern Chatham Rise, along with stage 4, 5 and 6 females (Figure 4.1- 1). Most males were stage 1 (n = 152) and 3 (n = 157) (Table 4.1- 1). Female fish from all macroscopic stages (1 – 6) were sampled, but stage 1 – 3 females were most abundant (n = 271) (Table 4.1- 1).

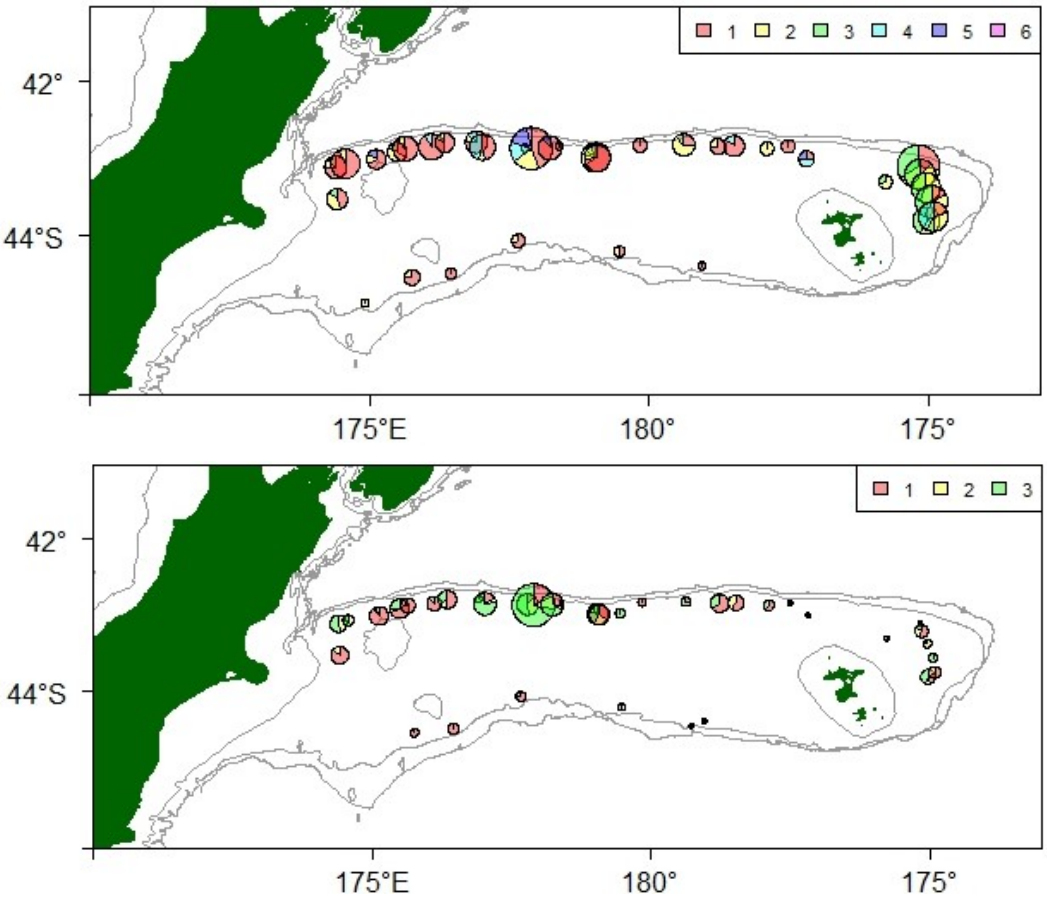

Figure 4.1- 1 C. crepidater: Sample distribution for C. crepidater top: females (n = 356); bottom: males (n = 448) on Chatham Rise with 200 m, 1000 m and 1200 m contours (grey). Pie charts denote the proportion of each macroscopic stage (colours for each macroscopic stage defined in legend) caught at each tow where samples were collected. Pie chart area – sample sizes collected relative to other tows.

There were 54 females and 88 males that were only sampled for length, weight and sex. 52 were sampled for full biological measurements. The remaining results use data from these 52 fish (n = 49 for females and n = 3 for males). Stage 1 – 4 females within this sample were predominantly caught in the northwestern Chatham Rise (Figure 4.1- 2). Stage 2 and 3 females were taken from northeastern and stage 3 females were taken from southern Chatham Rise (Figure 4.1- 2), consistent with the distribution of maturity stages seen in Figure 4.1- 1. No Stage 5 or 6 females were included in the random sample.

A very small random male sample was used as part of this study (n = 3), and only mature males (stage 3) were kept for measurements, since this study predominantly focused on female reproduction. All three males were taken from the northwestern Chatham Rise (Appendix B).

Table 4.1- 1 C. crepidater: The number of fish in the sub-catch at each macroscopic stage for both males and females.

MACROSCOPIC STAGE FEMALES	NUMBER OF FISH	MACROSCOPIC STAGE MALES	NUMBER OF FISH
1 (IMMATURE)	**146**	1 (IMMATURE)	**152**
2 (MATURING/RESTING)	**84**	2 (MATURING)	**51**
3 (MATURE)	**41**	3 (MATURE)	**157**
4 (GRAVID I)	**21**	–	**–**
5 (GRAVID II)	**9**	–	**–**
6 (POST-PARTUM)	**1**	–	**–**
NOT ASSIGNED	**54**	NOT ASSIGNED	**88**
TOTAL	**356**	**TOTAL**	**448**

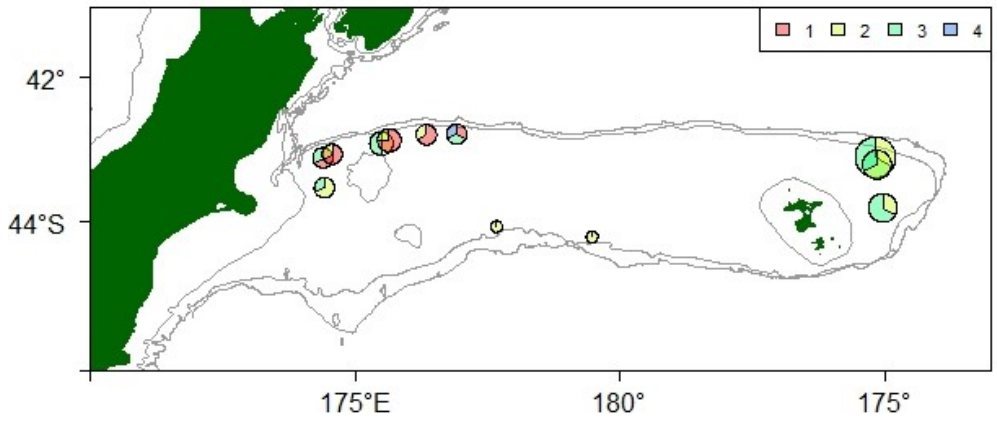

Figure 4.1- 2 C. crepidater: Chatham Rise area with 200 m, 1000 m and 1200 m contours (grey), showing sample distribution for C. crepidater females. Pie charts denote the proportion of each macroscopic stage (colours for each macroscopic stage defined in legend) caught at each tow where samples were collected. Pie chart area – sample sizes collected relative to other tows.

Females ranged in total length (TL) from 49.8 cm to 93.9 cm, with a mode at around 80 – 95 cm (Figure 4.1- 3). Median length was 84.8 cm for females with a median weight of 2822 g (Figure 4.1- 3).

C. crepidater failed to meet the ANOVA and Tukey HSD test assumptions, therefore Kruskal-Wallis rank sum (KW) and Wilcoxon pairwise comparison (Wilcoxon) tests were conducted. The KW test indicated significant differences in mean length and weight distributions for females across macroscopic stages ($p < 0.05$; Figure 4.1- 3). The Wilcoxon test indicated there were significant mean size differences between stage 1 and stages 2 and 3 (Wilcoxon $p < 0.05$ for both length and weight), and stage 2 was significantly different to stage 3 mean weight (Wilcoxon $p < 0.05$) and length (Wilcoxon $p = 0.031$). There was a large gap in size between stage 1 and stage 2, which was less pronounced in weight (Figure 4.1- 3).

The correlation between total weight and somatic weight was close to 1:1 for stage 1 and stage 2 fish, but stage 3 fish had a higher and more variable body weight (Figure 4.1- 4). All fish that showed sperm storage sat well on the 1:1 line, meaning that gonad weight in these fish was not a large component of total body weight (Figure 4.1- 4).

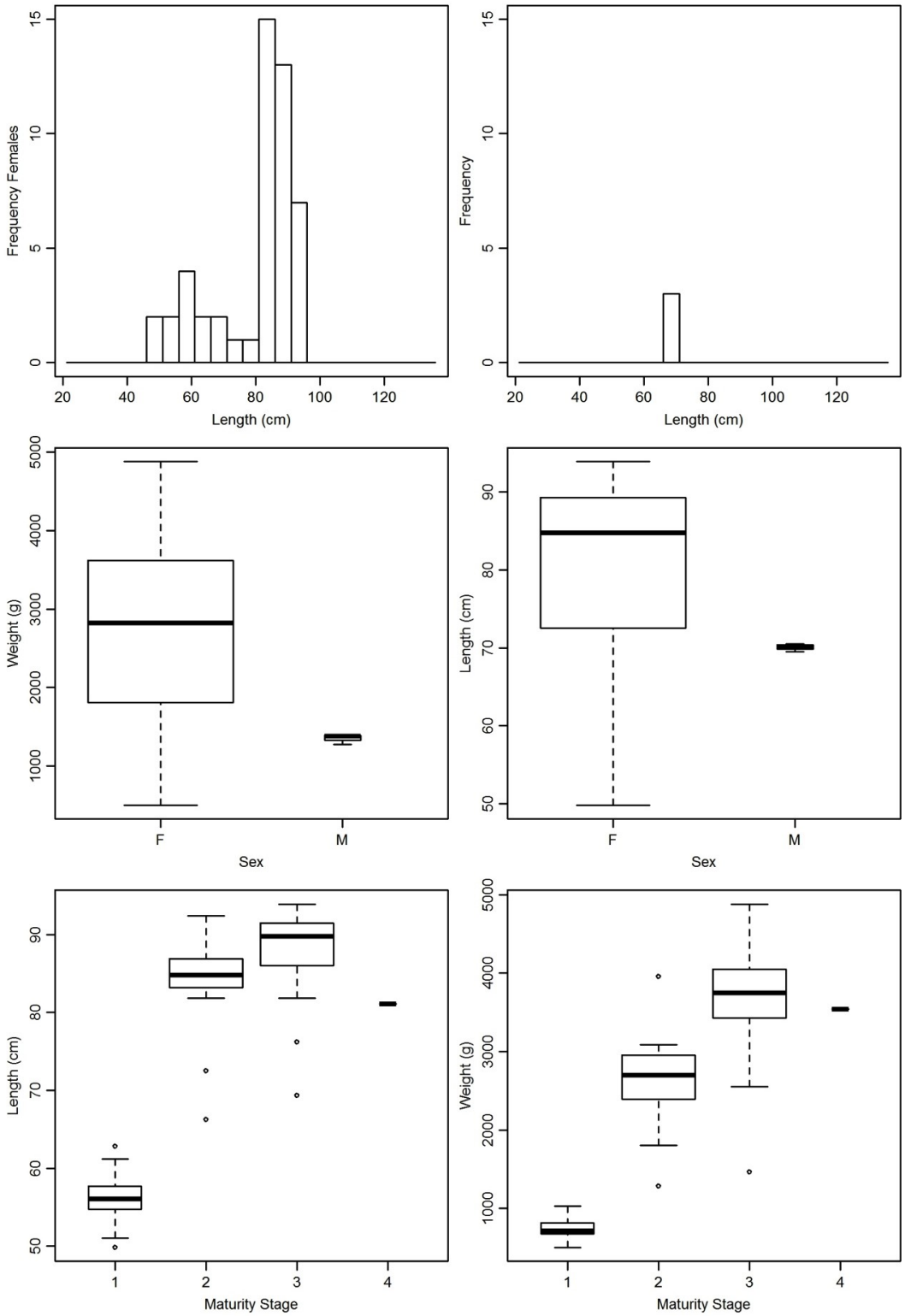

Figure 4.1- 3 C. crepidater: Total length (TL) frequency for C. crepidater (n=52) within the sample from the Chatham Rise, with females (n= 49; top left panel) and males (n=3; top right); Weight and length distributions for males and females (middle panels), where the darkest black line represents the median. Length and weight distributions across females in relation to macroscopic maturity staging (bottom panels).

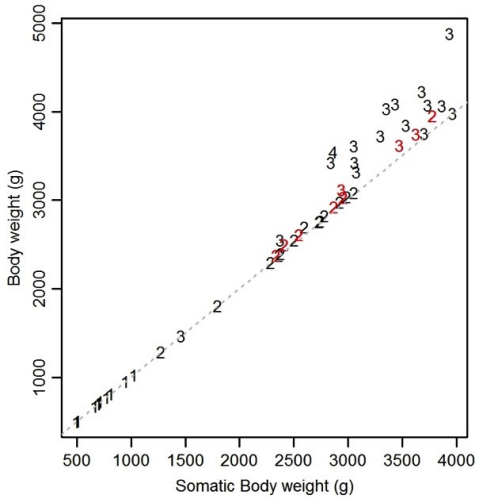

Figure 4.1- 4 C. crepidater: Relationship body weight and somatic body weight. The grey line indicates the 1:1 relationship. Numbers represent the macroscopic stage assigned to each fish in the sample. Red numbers indicate those fish where sperm storage was detected.

Length and weight were found to be well-correlated. Those fish sitting above the length-weight curves were "fatter" than average, and those below "skinnier" (Figure 4.1- 5). Weight in larger fish (i.e. greater than 75 cm TL) was more variable and larger fish were more numerous (Figure 4.1- 5 B). The greater variability in weight at length of stage 3 indicated length was not a good predictor of weight for those fish. The residuals were larger for longer fish and the fitted curve gave more weight to these data; the fit to smaller fish was compromised, where weight at length was slightly over-estimated by the model (Figure 4.1- 5 B). All fish exhibiting sperm storage were greater than 80 cm TL (Figure 4.1- 5).

The length-weight curve of mature fish (stage 3 +) was less steep (b = 2.35; CI = 2.33 – 2.86) (Figure 4.1- 5 C). Two stage 3 fish were outliers. However, when removed, the estimated length-weight relationship remained unchanged (b = 2.35 (CI = 2.33 – 2.86)) (Appendix B). Stage 1 and 2 fish were well-fitted by a length-weight model, indicating similar body forms and allometric growth (b = 3.16; CI = 2.94 – 3.35) (Figure 4.1- 5 D). All stage 2 fish, in which sperm storage was observed (with one exception), fitted on this curve. When maturity was considered to start at stage 2, the length-weight curve was a compromise between growth forms (b = 2.99; CI = 2.34 – 3.38) (Figure 4.1- 5 E). A number of stage 3 individuals (two exhibiting sperm storage) had a length-weight relationship similar to stage 1 and 2. A length-weight curve fitted to stage 1 females only, had similar parameters to stage 1 and 2 females, confirming their similar growth form (Figure 4.1- 5 F).

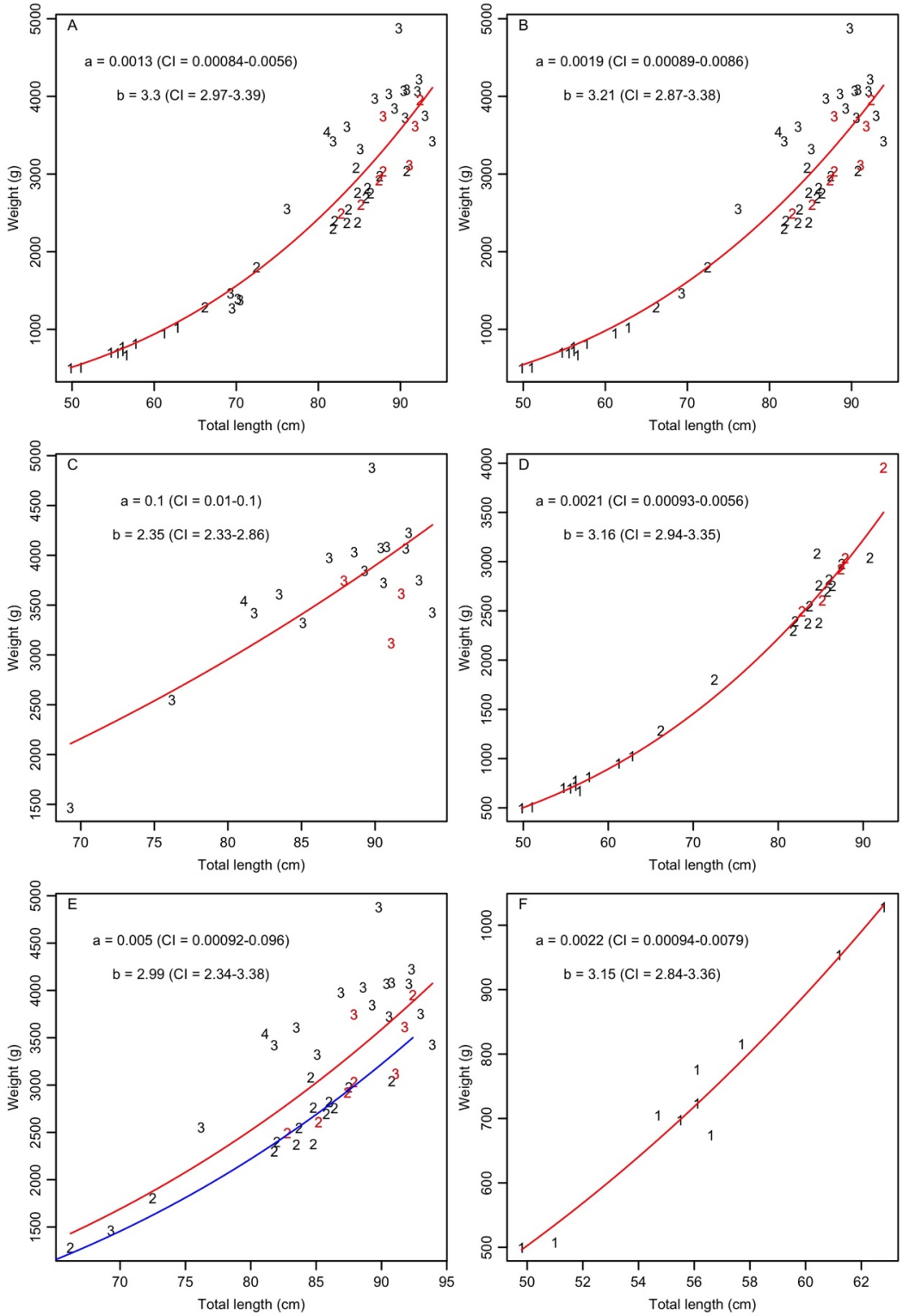

Figure 4.1- 5 C. crepidater: Length-weight relationships for C. crepidater. a) across the whole sample (males and females; n = 52); b) for all females (n = 49); c) for females stages 3 and above (n = 20); d) for females stages 1 and 2 (n = 29); e) for females stages 2 and above (n = 39); f) for stage 1 females (n = 10). The red line represents the bootstrapped sum of least-squares fit for each group. Note: e) the blue line represents the bootstrapped sum of least squares fit for plot d). Numbers represent the macroscopic staging assigned to each individual in the sample. Red numbers indicate those individuals where sperm storage was detected.

When maturity was considered to start at stage 2, 50 % of females were predicted to be mature at 65 cm (95 % CI = 61 – 70 cm) (Figure 4.1- 6). When maturity was considered to start at stage 3, maturity was estimated at 82.5 cm (95 % CI = 72.7 – 90.2 cm) (Figure 4.1- 6). Therefore, there was a 20 cm 'gap' in fish size (65 – 82.5 cm) between the onset of 'maturity' (stage 2) and when follicles exceeded 1 cm (stage 3).

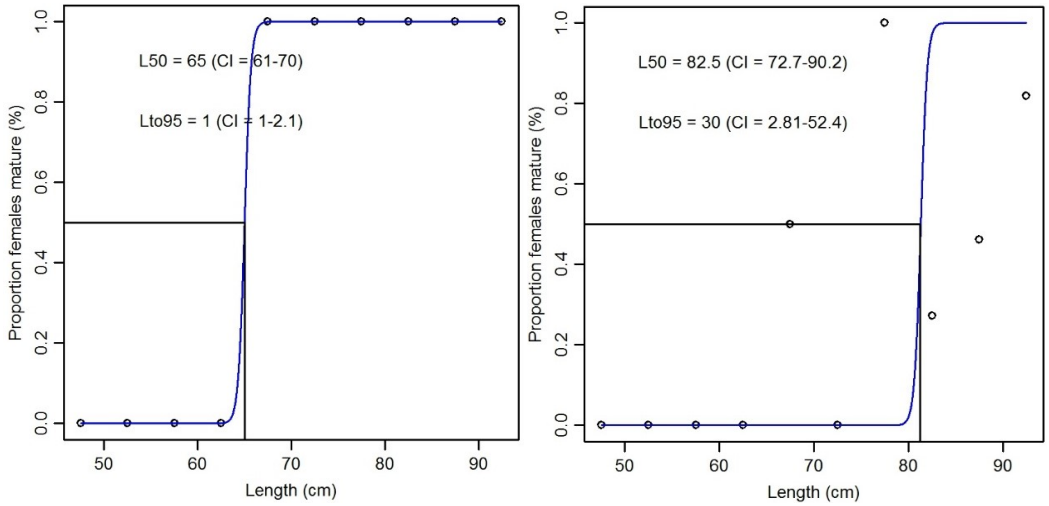

Figure 4.1- 6 C. crepidater: Estimated length-at-maturity for C. crepidater females on Chatham Rise (TAN1601 survey), where macroscopic maturity is considered to start at left: stage 2 (n = 39); right: stage 3 (n = 20). Points were proportion mature in 5 cm length bins. Line fitted is the maturity ogive. The black lines indicate the L50 value. The 95% confidence intervals were estimated by bootstrapping.

On average, the right oviducal gland was longer than the left (Figure 4.1- 7). However, the left oviducal gland appeared to be slightly wider on average (Figure 4.1- 7). When oviducal gland area was calculated, the left and right were similar in size and a couple of outliers were reduced (Figure 4.1- 7). This indicated the two oviducal glands were different in shape rather than overall size. As a result average oviducal gland area (left area + right area/2) was used for the subsequent analyses.

Oviducal gland area was larger in more 'mature' and larger females (> 75 cm TL) (Figure 4.1- 8). However, across all stages (excluding stage 4) and sizes, some oviducal glands were very small (allocated a nominal area of 0.001 cm^2) (Figure 4.1- 8); these appeared as a cluster of data points in the bottom left-hand corner of Figure 4.1- 7.

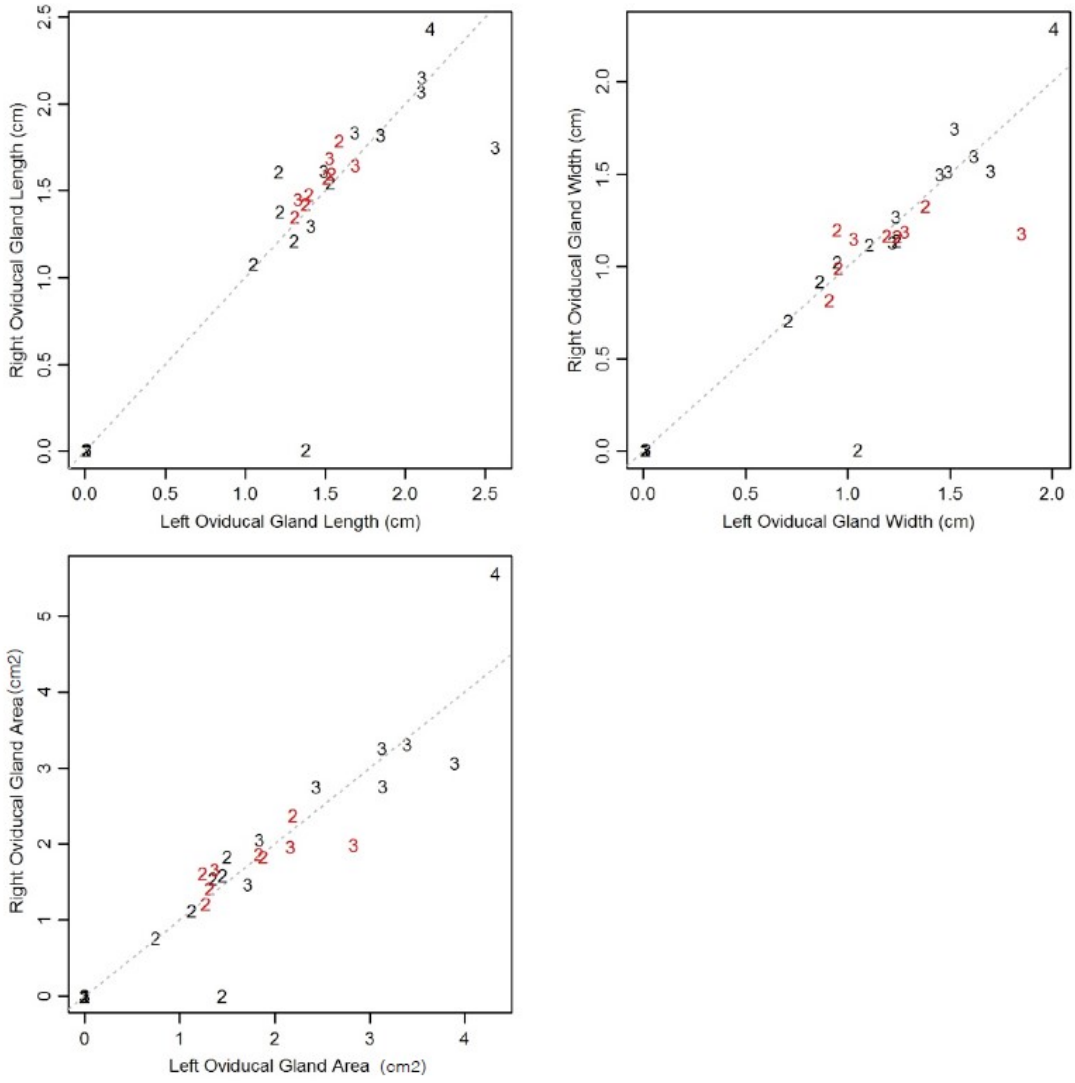

Figure 4.1- 7 C. crepidater: The relationships between left and right oviducal gland lengths (top left), widths (top right) and areas (bottom). The dashed grey lines represent the 1:1 relationship. Numbers represent the macroscopic staging assigned to each individual in the sample. Red numbers indicate those individuals where sperm storage was detected.

Irrespective of changes in fish size, stage 2 and 3 fish appeared to be split into two distinct groups, where: 1) oviducal size was small (close to zero) and 2) average oviducal gland area was relatively large (Stage 2: mean = 0.92 cm^2; 0.76 – 2.29 cm^2; Stage 3: mean = 2.51 cm^2; 1.52 – 3.5 cm^2) (Figure 4.1- 8). There was more overlap between stage 2 and 3 fish when plotted against fish length than weight (Figure 4.1- 8).

Gonad weight and average oviducal gland area were correlated: as oviducal gland area increased, gonad weight increased, although there were a number of stage 3 fish that had small, undetectable oviducal glands that did not follow the same patterns observed in other stage 3 fish (Figure 4.1- 9). A similar correlation was

observed with follicle size (Figure 4.1- 9). However, there appeared to be differences in the onset of follicle and oviducal gland development (Figure 4.1- 9).

Oviducal gland area increased with follicle size once follicles attained sizes greater than 1 cm in diameter, with some overlap between adjacent stages, and with the exception of stage 3 fish with undetectable oviducal glands (Figure 4.1- 9). Stage 2 fish tended to have oviducal glands less than 2 cm^2, whereas stage 3 with measurable oviducal glands were greater than 2 cm^2 on average (Figure 4.1- 9). Those fish showing sperm storage had oviducal glands ranging in size from 1.24 cm^2 to 2.41 cm^2 and follicles between 0.58 and 3.04 cm (Figure 4.1- 8). The stage 4 fish had the largest follicles (6.67 cm) and average oviducal gland area (4.95 cm^2) (Figure 4.1- 9).

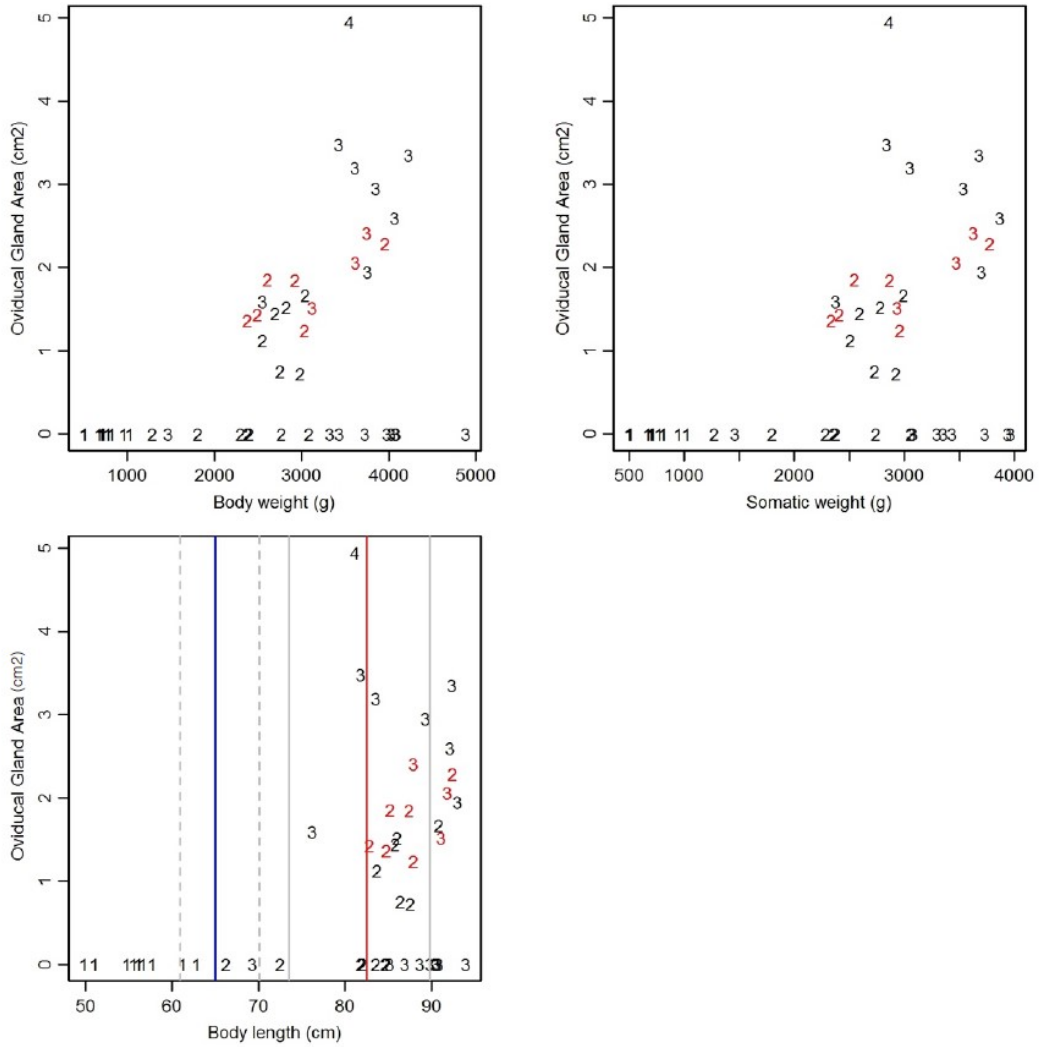

Figure 4.1- 8 C. crepidater: The relationships between average oviducal gland area (cm^2) and body weight, somatic weight and length (clockwise from top left). The numbers denote the macroscopic maturity stages assigned to each individual. Red numbers indicate those individuals where sperm storage was detected. Lines represent length-at-maturity estimates for maturity occurring at stage 2 (blue) and at stage 3 (red). Grey lines represent the 95 % confidence intervals (Figure 4.1- 6).

There were three groups that appeared in the relationship between uterus width and average oviducal gland area: 1) small uteri (< 0.5 cm) and oviducal glands (< 0.2 cm^2), 2) small oviducal glands (< 0.2 cm^2) and large uteri (> 0.5 cm) and 3) large oviducal glands (> 0.2 cm^2) and uteri (> 0.5 cm) (Figure 4.1- 9). However, there was some overlap between adjacent stages (i.e. stages 2 and 3 demonstrated similar variability in uterus width when average oviducal gland area was greater than 1 cm^2) (Figure 4.1- 9). When average oviducal gland size was small (< 0.2 cm^2), stage 2 fish had uterus widths less than 0.5 cm, whereas stage 3 had uterus widths comparable to all other stage 3 fish (> 0.5 cm) (Figure 4.1- 9). For fish with follicle counts less than approximately 10, average oviducal gland size was highly variable (< 0.01 – 4.95 cm^2; Figure 4.1- 9). At counts greater than 10, average oviducal gland area was approximately 2 cm^2 (Figure 4.1- 9). Those fish that showed sperm storage had oviducal glands around 2 cm^2 and follicle counts up to 22 (Figure 4.1- 9).

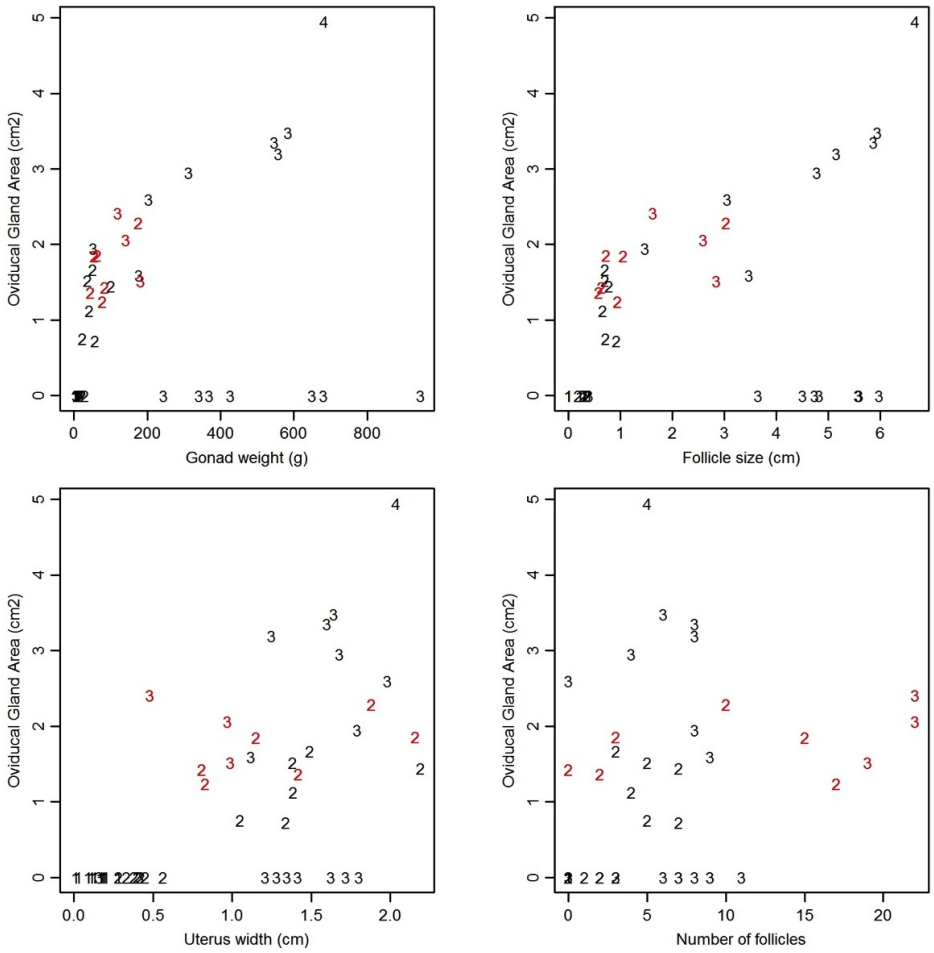

Figure 4.1- 9 C. crepidater: The relationships between average oviducal gland area and gonad weight, follicle size, number of follicles and uterus width (clockwise from top left). The numbers denote the macroscopic maturity stages assigned to each individual. Red numbers indicate those individuals where sperm storage was detected.

As follicle size increased, gonad weight increased (Figure 4.1- 10). Follicle growth appeared to asymptote when follicles were approximately 6 cm in diameter (Figure 4.1- 10). All stage 2 fish (bar one anomaly) had follicles less than 1 cm in diameter; this does not indicate a developmental threshold, but is the criterion for assigning stage 3 versus stage 2, and therefore demonstrates the accurate use of the macroscopic staging scale (Figure 4.1- 10). The estimate of size-at-maturity from the length-at-maturity ogive estimate (stage 3 +) was consistent with increases in gonad weight (Figure 4.1- 11).

Up to approximately 0.5 cm, uterus width slightly increased with length (Figure 4.1- 11). At lengths greater than approximately 75 cm, uterus width variability increased substantially, with some overlap between adjacent stages (Figure 4.1- 11). In stage 3 fish, follicle size increased with uterus width, with all fish exhibiting sperm storage having moderate uterus widths, irrespective of changes in fish size (0.48 – 0.99 cm; Figure 4.1- 10; Figure 4.1- 12). There was no consistent uterine width for stage 2 fish (0.28 cm – 2.19 cm), although follicle size remained relatively consistent at 1 cm (Figure 4.1- 10; Figure 4.1- 12).

The number of follicles varied greatly at lengths greater than 80 cm, with a maximum of 22 (Figure 4.1- 11). There was some overlap in between stages 2, 3 and 4 (Figure 4.1- 11). Five of the nine fish that demonstrated sperm storage had the greatest follicle counts in the sample, although follicles did not exceed 3 cm in diameter (Figure 4.1- 10; Figure 4.1- 11). As follicle number decreased to around 10 follicles, uterus width continued to increase despite the high variability in follicle number (Figure 4.1- 10). In stage 2 fish, those fish that showed sperm storage had uterus widths with a mean 1.38 cm (0.81 – 2.16 cm), slightly larger than that for all other stage 2 fish (1.05 cm; 0.28 – 2.19 cm) (Figure 4.1- 13).

All fish exhibiting sperm storage were larger than the estimated length-at-maturity (based on the stage 3 + maturity ogive) and had similar uterine widths, gonad weights and follicle sizes (Figure 4.1- 11).

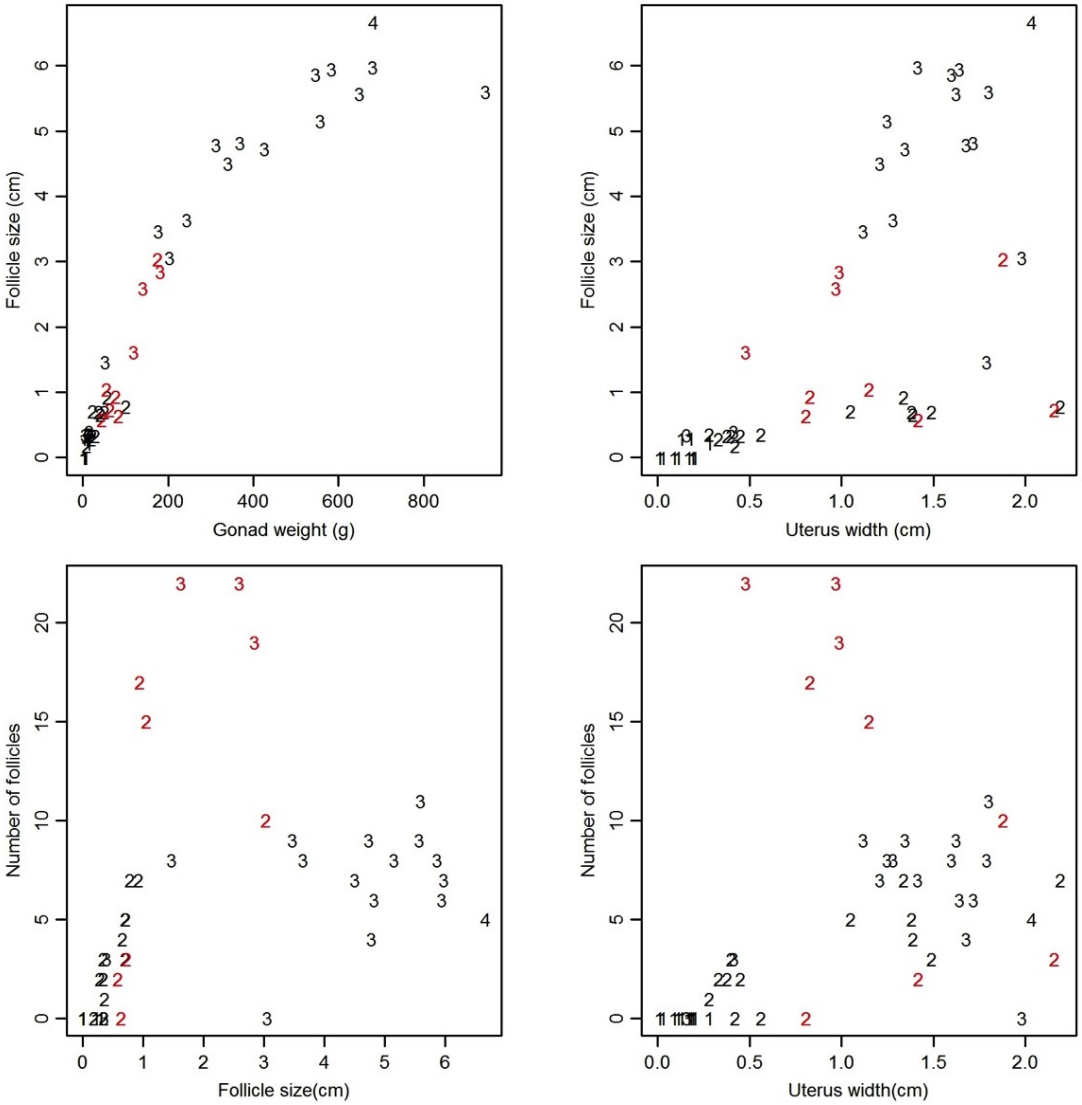

Figure 4.1- 10 C. crepidater: Relationships between follicle size, gonad weight, uterus width and number of follicles. The numbers denote the macroscopic maturity stages assigned to each individual. Red numbers indicate those individuals where sperm storage was detected.

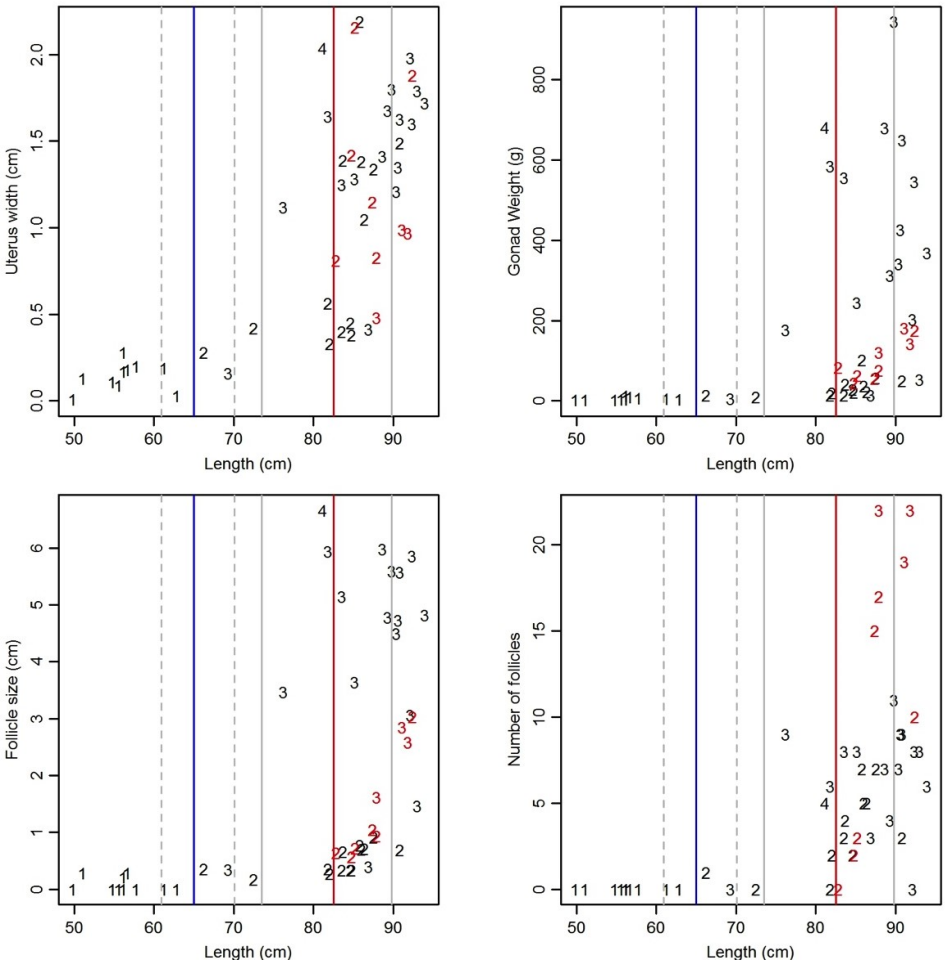

Figure 4.1- 11 C. crepidater: Relationships between length and uterus width, gonad weight, number of follicles and follicle size (clockwise from top left). The numbers denote the macroscopic maturity stages assigned to each individual. Red numbers indicate those individuals where sperm storage was detected. Lines represent length-at-maturity estimates for maturity occurring at stage 2 (blue) and at stage 3 (red). Grey dashed lines represent the 95 % confidence intervals Figure 4.1- 6).

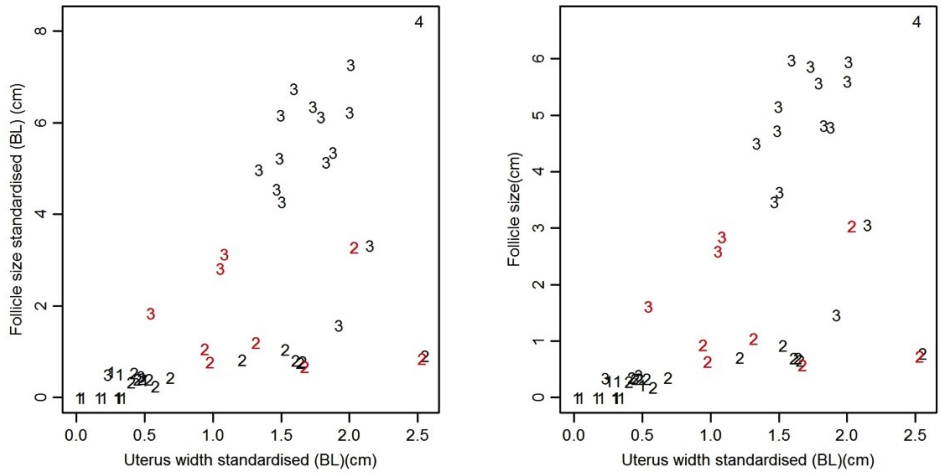

Figure 4.1- 12 C. crepidater: Relationship between length-standardised measures of follicle size and uterus width. The numbers denote the macroscopic maturity stages assigned to each individual. Red numbers indicate those individuals where sperm storage was detected.

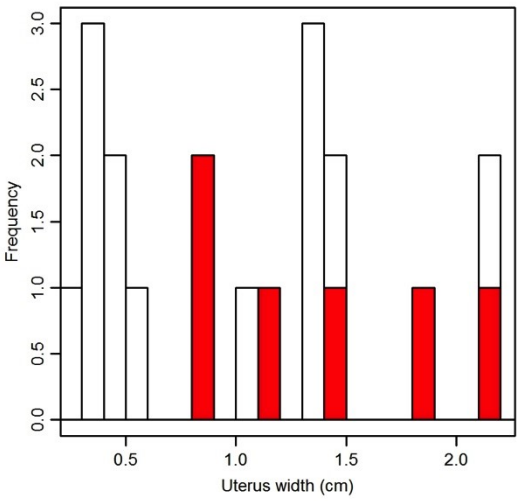

Figure 4.1- 13 C. crepidater: Uterus width frequency plot for stage 2 fish. The red bars show the uterus width frequencies of those stage 2 fish that showed sperm storage.

4.1.1 *Conclusions*

Following the onset of 'maturity' (stage 3), weight and weight variability increased, indicating gonad weight represented a greater and variable proportion of body weight. As a result, the length-weight curve/growth form changed between stages 2 and 3, supporting a change in biology between stages 2 and 3.

Gonad weight, uterus width, follicle size, follicle number, oviducal gland area and length-weight relationship, all showed step changes (or transitions) at around the fish length consistent with maturity (as measured at stage 3 of the macroscopic scale).

Length-at-maturity was estimated at between 65 cm (stage 2 onset) and 82.5 cm (stage 3 onset), suggesting a 20 cm 'gap' between the onset of 'maturity' (stage 2), and when follicles exceeded 1 cm (stage 3).

Sperm storage was detected in macroscopically assigned stage 2 and stage 3 fish, and if confirmed as an indicator of maturity, would suggest some fish identified as immature, by the macroscopic staging key, were in fact mature. Those stage 2 fish, likely to be mature (exhibiting sperm storage), had uterus widths consistent with mature fish as indicated by maturity at stages 3 +.

Five of the six fish exhibiting sperm storage demonstrated a similar length-weight relationship as those fish considered to be immature (stage 1).

Stage 2 fish had the greatest variability in uterus width, suggesting some are likely immature and some mature (post-partum, stage 6) (i.e. two groups). Therefore, uterus width may be a useful attribute in distinguishing between immature and mature/resting stage 2 fish.

Peak follicle number coincided with sperm storage, although follicles were relatively small and in the early stages of development.

Some stages 1 – 3 had undetectable oviducal glands, although follicle size and uterus width (particularly in stage 3 fish) were comparable with fish with developed oviducal glands. Oviducal gland area varied in fish of similar size and follicle development. The onset of oviducal gland development and follicle development may be different and oviducal development may continue after mating. Oviducal gland size is probably not a good indicator of maturity due to variability in size over all stages.

Follicle size appeared to asymptote at 6 cm in diameter. Follicle size alone may be insufficient in describing reproductive processes occurring particularly in stage 2 and 3 fish. The distinction between stage 1, 2 and 3 in the current macroscopic staging key is based solely on follicle size, which may cut the maturity cycle arbitrarily.

4.2 *Denia calcea*

A sub-catch from the TAN1601 survey of male (n = 421) and female (n = 345) *D. calcea* were caught principally on the northern Chatham Rise (Figure 4.2- 1). Stage 1 (n = 132) and 2 (n = 161) females were relatively evenly distributed throughout the catch area, as were stage 3 males (n = 346), which were more abundant than immature (stage 1 and 2) males (n = 73) (Figure 4.2- 1; Table 4.2- 1). Female fish from all macroscopic stages (1 – 6) were sampled, but stage 1 – 3 females were more abundant (n = 336) (Table 4.2- 1).

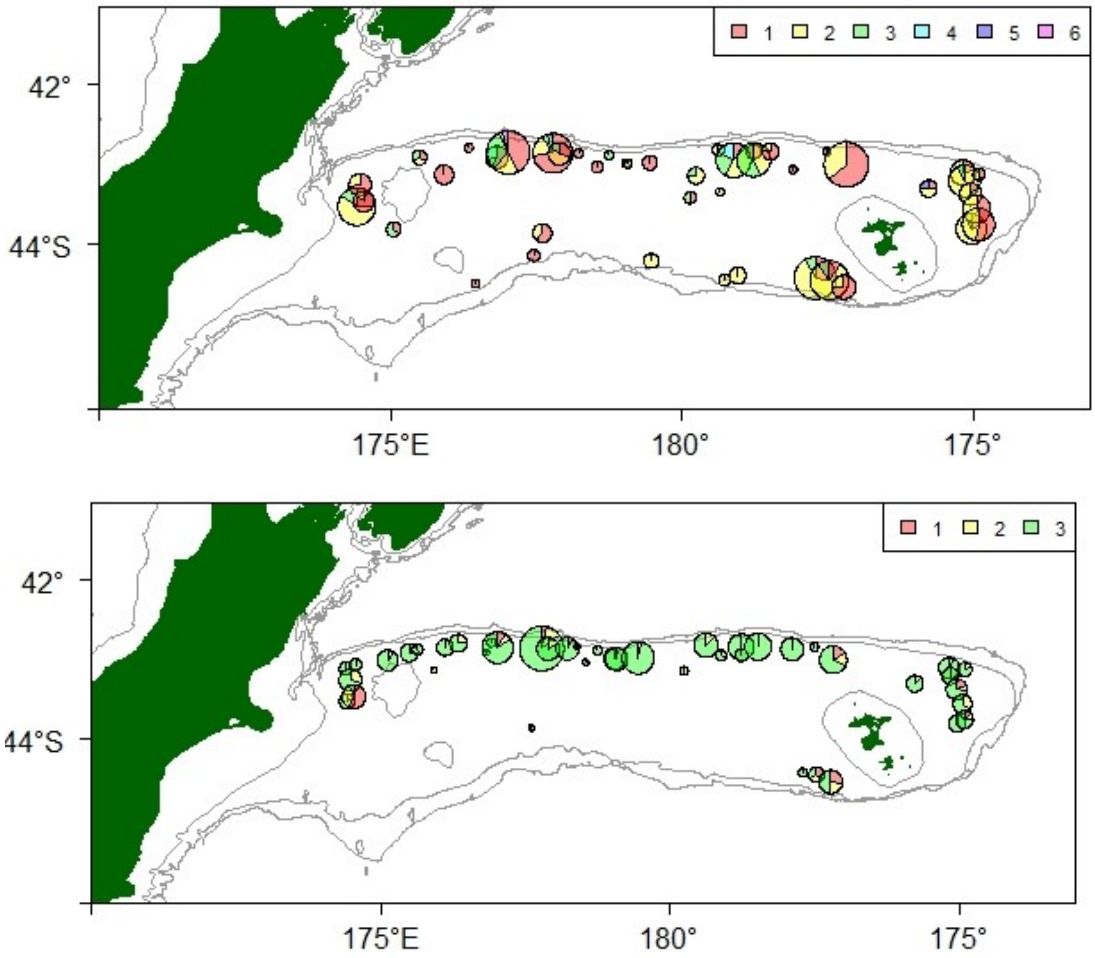

Figure 4.2- 1 D. calcea: Sample distribution for D. calcea top: females (n = 345); bottom: males (n = 421) on Chatham Rise with 200 m, 1000 m and 1200 m contours (grey). Pie charts denote the proportion of each macroscopic stage (colours for each macroscopic stage defined in legend) caught at each tow where samples were collected. Pie chart area – sample sizes collected relative to other tows.

Table 4.2- 1: D. calcea: The number of fish in the sub-catch at each macroscopic stage for both males and females.

MACROSCOPIC STAGE FEMALES	NUMBER OF FISH	MACROSCOPIC STAGE MALES	NUMBER OF FISH
1 (IMMATURE)	**132**	1 (IMMATURE)	**27**
2 (MATURING/RESTING)	**161**	2 (MATURING)	**46**
3 (MATURE)	**39**	3 (MATURE)	**346**
4 (GRAVID I)	**7**	–	**–**
5 (GRAVID II)	**1**	–	**–**
6 (POST-PARTUM)	**1**	–	**–**
NOT ASSIGNED	**4**	NOT ASSIGNED	**2**
TOTAL	**345**	**TOTAL**	**421**

There were 4 females and 2 males that were only sampled for length, weight and sex, and 57 were sampled for full biological measurements. The remaining results use data from these 57 females. Stage 1 – 3 females within this sample were predominantly caught in the northwestern, southern and eastern Chatham Rise (Figure 4.2- 2). Stage 3 females were taken from the eastern and western Chatham Rise at two stations, whereas stages 1 and 2 were taken throughout the area (Figure 4.2- 2). No stage 4, 5 or 6 females were included in the random sample. No males were a part of this sample, since this study focuses predominantly on female reproduction.

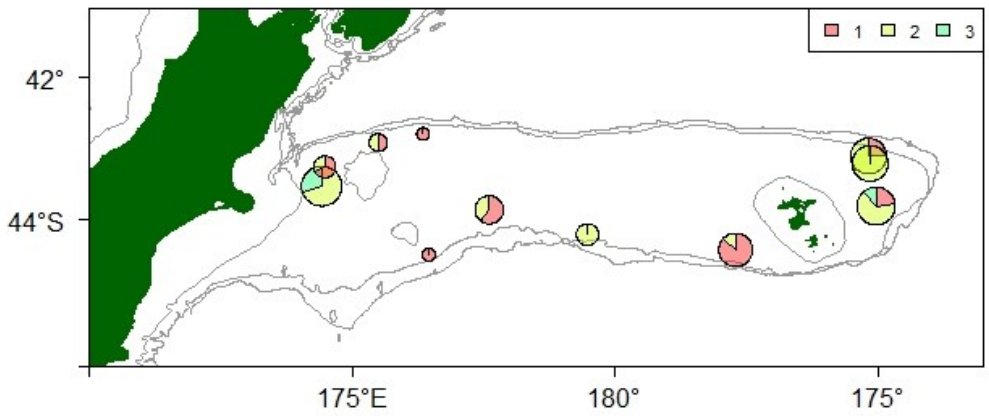

Figure 4.2- 2 D. calcea: Chatham Rise area with 200 m, 1000 m and 1200 m contours (grey), showing sample distribution for D. calcea females. Pie charts denote the proportion of each macroscopic stage (colours for each macroscopic stage defined in legend) caught at each tow where samples were collected. Pie chart area – sample sizes collected relative to other tows.

Females ranged in total length (TL) from 48 cm to 112.4 cm, with a mode at around 80 – 100 cm (Figure 4.2- 3). Median length was 87.9 cm with a median weight of 2680 g (Figure 4.2- 3).

D. calcea failed to meet the ANOVA and Tukey HSD test assumptions, therefore KW and Wilcoxon tests were conducted. The KW test indicated significant differences in mean length and weight distributions for females across macroscopic stages ($p < 0.05$; Figure 4.2- 3), specifically there were significant mean size differences between stage 1 and stages 2 and 3 (Wilcoxon $p < 0.05$ for both length and weight for all pairwise comparisons). Differences in mean size were also significant between stage 2 and stage 3 (Wilcoxon $p < 0.05$ for both length and weight).

The correlation between total weight and somatic weight was close to 1:1 for stage 1 and stage 2 fish, but stage 3 fish had a higher and more variable body weight (Figure 4.2- 4).

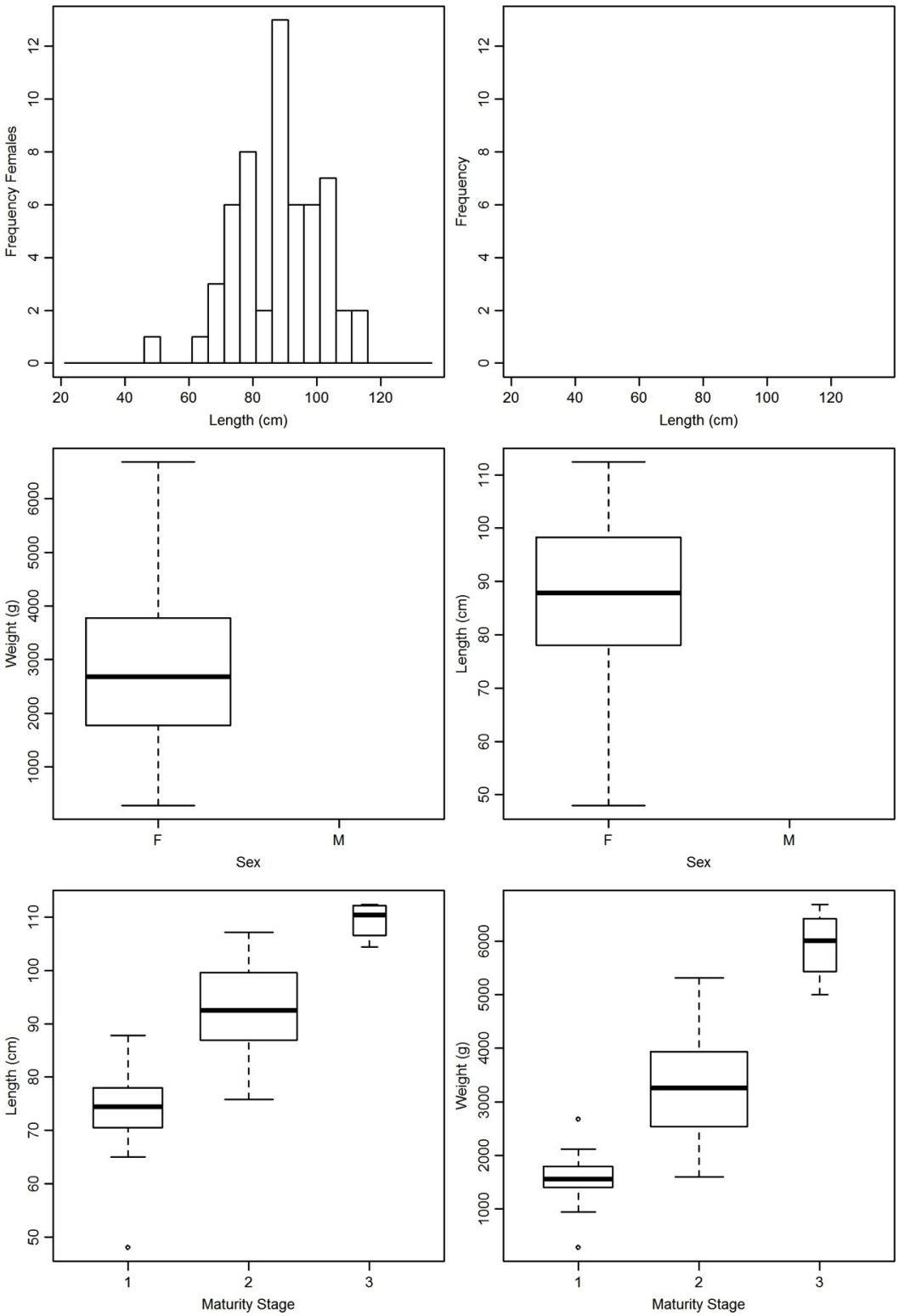

Figure 4.2- 3 D. calcea: Total length (TL) frequency for D. calcea (n=52) within the sample from the Chatham Rise, with females (n=57; top left panel) and males (n=0; top right); Weight and length distributions for females (middle panels), where the darkest black line represents the median; Length and weight distributions across females in relation to macroscopic maturity staging (bottom panels).

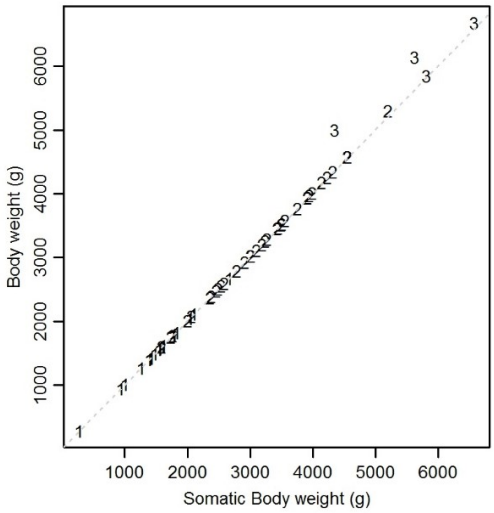

Figure 4.2- 4 D. calcea: Relationship body weight and somatic body weight. The grey line indicates the 1:1 relationship. Numbers represent the macroscopic staging assigned to each individual in the sample.

Length and weight were found to be well-correlated. Since there were no males in the sample, length-weight relationships could only be described for females. There was little variability in fish weight at length and all stages were well-fitted to by a length-weight model, indicating similar growth forms and allometric growth (Figure 4.2- 5 A – b = 3.31 (CI = 3.14 – 3.36); Figure 4.2- 5 B – b = 3.31 (CI = 3.14 – 3.36); Figure 4.2- 5 D – b = 3.19 (CI = 2.98 – 3.32)). Most macroscopic stage 2 + fish were greater than 80 cm (Figure 4.2- 5).

When maturity was considered to start at stage 2, the length-weight curve was slightly steeper (b = 3.31; CI = 3.07 – 3.36; Figure 4.2- 5 E), although there was insufficient stage 3 data to estimate weight at length for mature fish (stage 3 +) (Figure 4.2- 5 C). A length-weight curve fitted to stage 1 females only was less steep (b = 2.97; CI = 2.24 – 3.32; Figure 4.2- 5 F).

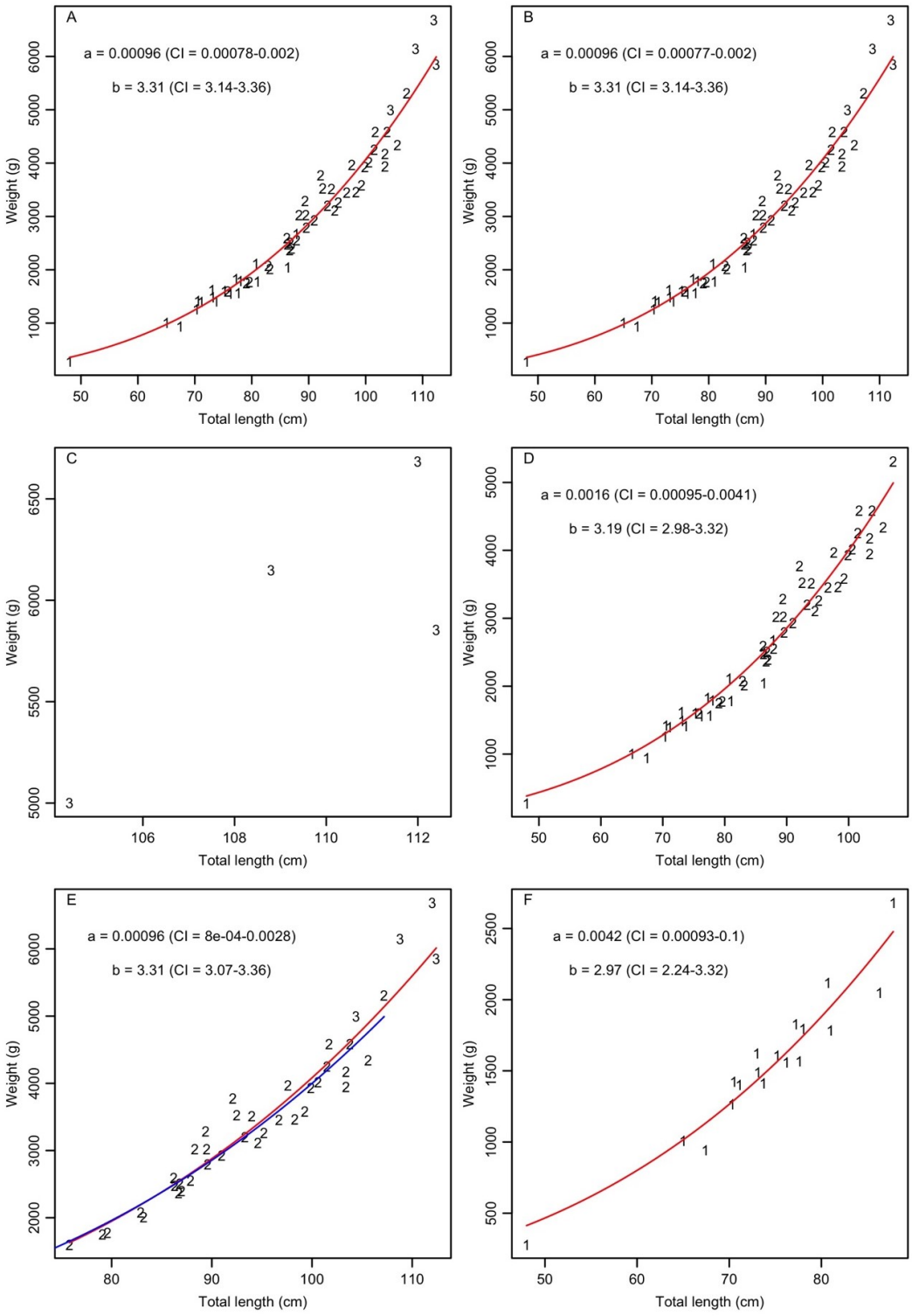

Figure 4.2- 5 D. calcea: Length-weight relationships for D. calcea. a) across the whole sample (n = 57); b) for all females (n = 57); c) for females stages 3 and above (n = 4); d) for females stages 1 and 2 (n = 53); e) for females stages 2 and above (n = 39); f) for stage 1 females (n = 18). The red line represents the bootstrapped sum of least-squares fit for each group. Note: e) the blue line represents the bootstrapped sum of least squares fit for plot d). Numbers represent the macroscopic staging assigned to each individual in the sample.

When maturity was considered to start at stage 2, 50 % of females were predicted to be mature at 81 cm (95 % CI = 77 – 87 cm) (Figure 4.2- 6). When maturity was considered to start at stage 3, maturity was estimated at 108 cm (95 % CI = 103 – 150 cm) (Figure 4.2- 6). Therefore, there was a 30 cm 'gap' in fish size (81 – 108 cm) between the onset of 'maturity' (stage 2) and when follicles exceeded 1 cm (stage 3).

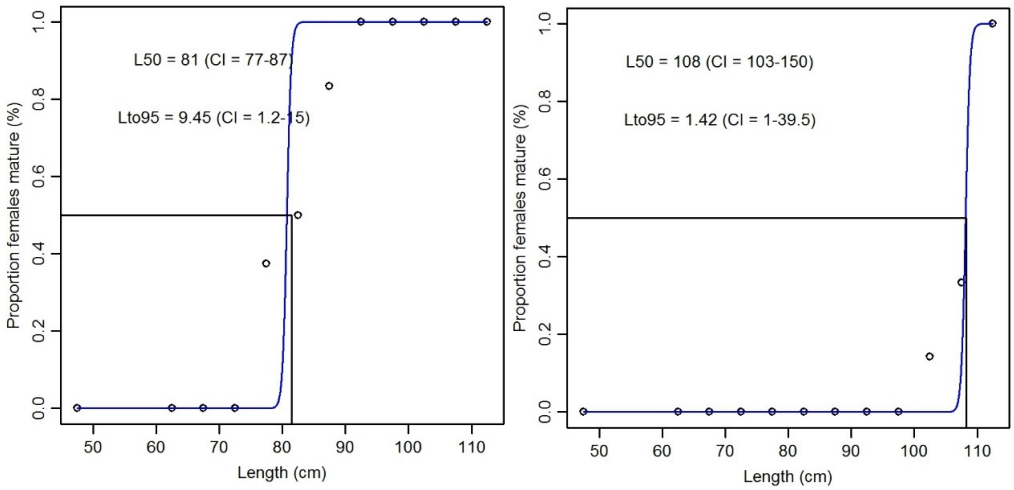

Figure 4.2- 6 D. calcea: Estimated length-at-maturity for D. calcea females on Chatham Rise (TAN1601 survey), where macroscopic maturity is considered to start at left: stage 2 (n = 39); right: stage 3 (n = 4). Points were proportion mature in 5 cm length bins. Line fitted is the maturity ogive. The black lines indicate the L50 value. The 95% confidence intervals were estimated by bootstrapping.

On average, the left oviducal gland was longer and wider (Figure 4.2- 7). When oviducal gland area was calculated, the left and right were similar in size, although the stage 3 outlier was not reduced (Figure 4.2- 7). This indicated the two oviducal glands were different in shape, rather than overall size, although there was minimal data for oviducal gland area, due to the small sample of fish that had ovidcual glands that were differentiated from the rest of the reproductive tract (Figure 4.2- 7). For consistency and comparability with other species in this study, the average oviducal gland (left area + right area/2) was used for subsequent analyses.

Oviducal gland area was larger in more 'mature' and larger females (> 100 cm TL) (Figure 4.2- 8). However, across all stages and sizes, some oviducal glands were very small (allocated a nominal area of 0.001 cm^2) (Figure 4.2- 8); these appeared as a cluster of data points in the bottom left-hand corner of Figure 4.2- 7.

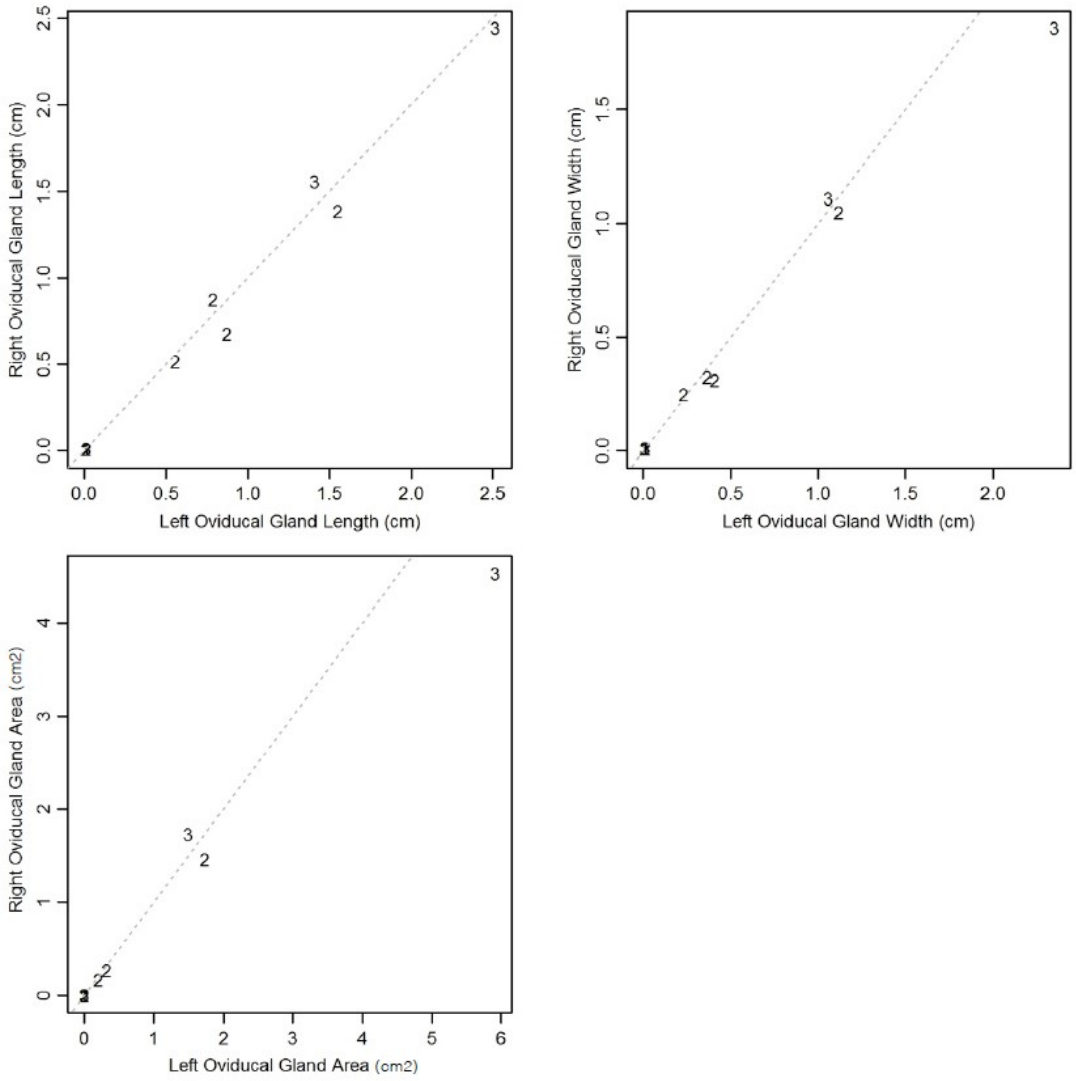

Figure 4.2- 7 D. calcea: Relationships between left and right oviducal gland lengths (top left), widths (top right) and areas (bottom left). The dashed grey lines represent the 1:1 relationship. Numbers represent the macroscopic staging assigned to each individual in the sample.

Irrespective of changes in fish size, stage 2 and 3 fish appeared to be split into two distinct groups, where: 1) oviducal size was small (close to zero) and 2) average oviducal gland area was relatively large (Stage 2: mean = 0.57 cm^2; 0.19 – 1.60 cm^2, Stage 3: mean = 3.42 cm^2; 1.61 – 5.22 cm^2) (Figure 4.2- 8). There was some overlap between adjacent stages particularly stages 1 and 2 (Figure 4.2- 8).

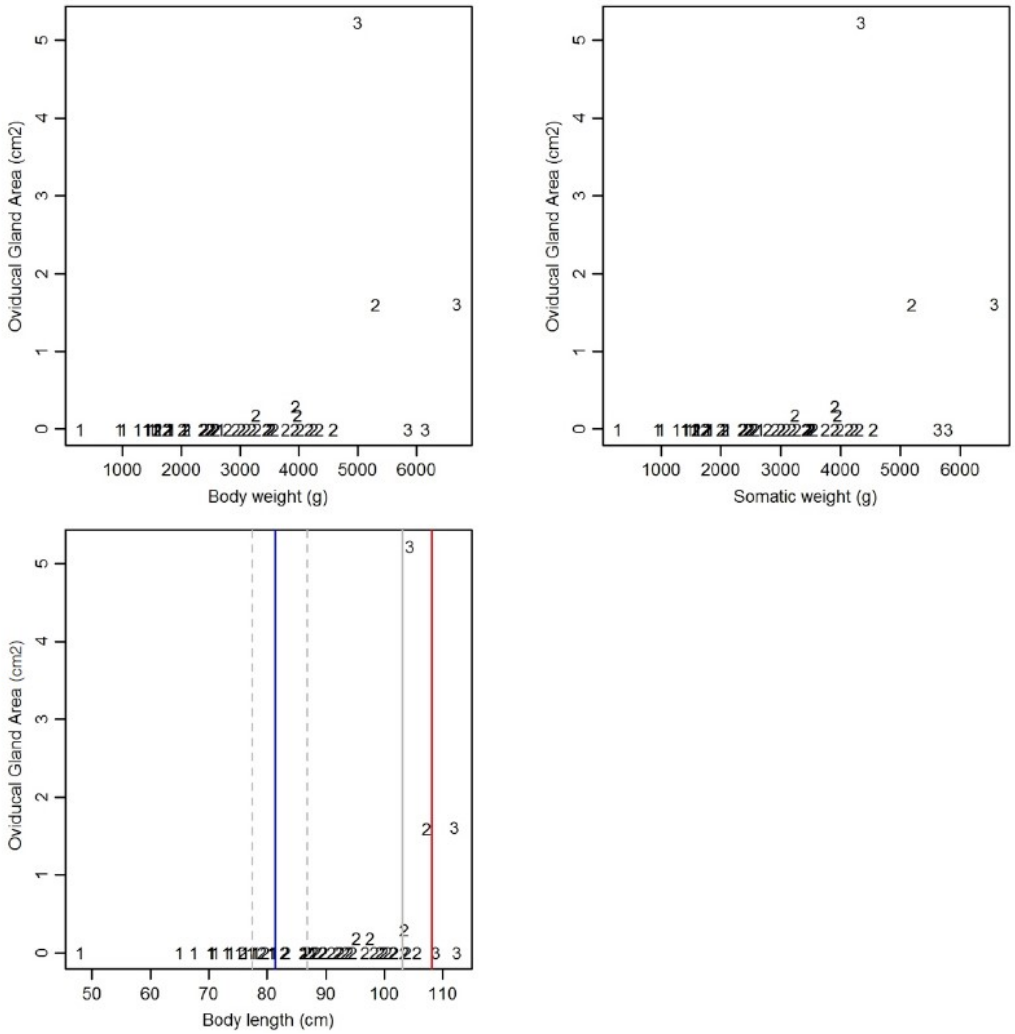

Figure 4.2- 8 D. calcea: The relationships between average oviducal gland area (cm^2) and body weight, somatic weight and length (clockwise from top left). The numbers denote the macroscopic maturity stages assigned to each individual. Lines represent length-at-maturity estimates for maturity occurring at stage 2 (blue) and at stage 3 (red). Grey lines represent the 95 % confidence intervals (Figure 4.2- 6).

The majority of the data had average oviducal gland area at 0.001 cm^2, with gonad weight less than 100 g and follicles under 1 cm in diameter (Figure 4.2- 9). There were few data for fish with an average oviducal gland area greater than 2 cm^2, therefore the relationships between gonad weight and follicle size and average oviducal gland area could not be assumed (Figure 4.2- 9).

There were three distinct groups that appeared in the relationship between uterus width and average oviducal gland area: 1) small uteri (< 0.5 cm) and oviducal glands (< 0.2 cm^2), 2) small oviducal glands (< 0.2 cm^2) and large uteri (> 0.5 cm) and 3) large oviducal glands (> 0.2 cm^2) and uteri (> 0.5 cm) (Figure 4.2- 9). However, there was some overlap between adjacent stages (i.e. stages 2 and 3 demonstrated similar variability in uterus width when average oviducal gland area

was greater than 1 cm^2) (Figure 4.2- 9). When average oviducal gland area was small (< 0.2 cm^2), stage 2 had uterus widths less than 0.5 cm, whereas stage 3 had uterus widths comparable to all other stage 3 fish (> 0.5 cm) (Figure 4.2- 9).

For fish with follicle counts less than approximately 10, average oviducal gland area was consistently less than 0.01 cm^2 (Figure 4.2- 9). At counts greater then 10, average oviducal gland area was highly variable (< 0.01 – 5.22 cm^2; Figure 4.2- 9).

As follicle size increased, gonad weight increased (Figure 4.2- 10). All stage 2 fish had follicles less than 1 cm in diameter; this does not indicate a developmental threshold, but is the criterion for assigning stage 3 versus stage 2, and therefore demonstrates the accurate use of the macroscopic staging scale (Figure 4.2- 10).

The estimate of size-at-maturity (from the length-at-maturity ogive estimate (stage 3 +)) was consistent with increases in gonad weight (Figure 4.2- 11).

Up to approximately 0.5 cm, uterus width slightly increased with length (Figure 4.2- 11). At lengths greater than approximately 100 cm, uterus width variability increased substantially, with some overlap between adjacent stages (Figure 4.2- 11).

In stage 3 fish, follicle size increased with uterus width, irrespective of changes in fish size (Figure 4.2- 10; Figure 4.2- 12). There was no consistent uterine width for stage 2 fish (0.15 cm – 1.65 cm), although follicle size remained relatively consistent at 1 cm (Figure 4.2- 10; Figure 4.2- 12).

The number of follicles varied greatly in fish greater than 80 cm TL, with a maximum of 20 (Figure 4.2- 11). There was some overlap between stages 2 and 3 (Figure 4.2- 11). Follicle number appeared to increase as uterus width increased, up to approximately 1 cm (Figure 4.2- 10). As follicle number decreased to around 10 follicles, uterus width continued to increase despite the high variability in follicle number (Figure 4.2- 10).

The relationships between length and uterus width, gonad weight, follicle size and number of follicles were not an artefact of changes in female size.

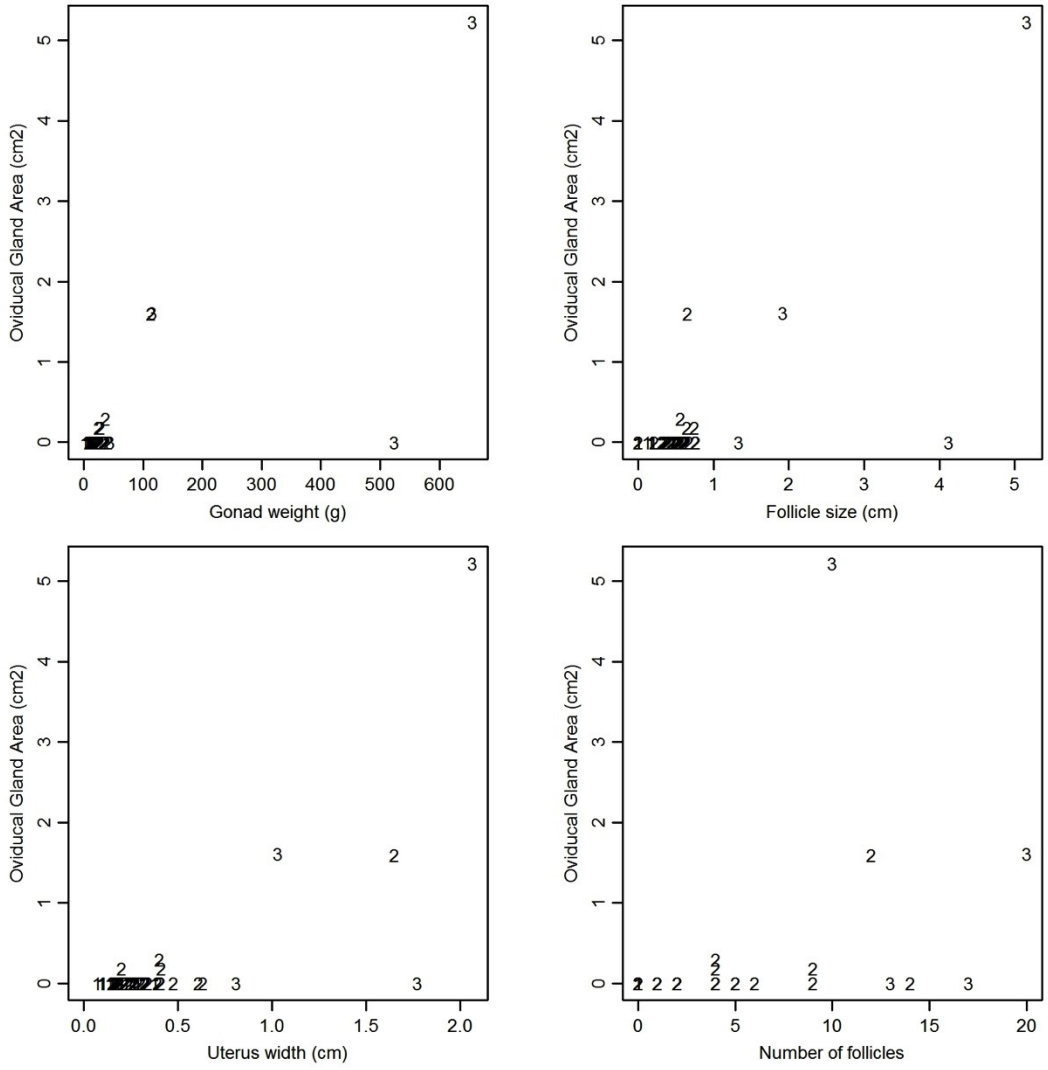

Figure 4.2- 9 D. calcea: The relationships between average oviducal gland area and gonad weight, follicle size, number of follicles and uterus width (clockwise from top left). The numbers denote the macroscopic maturity stages assigned to each individual.

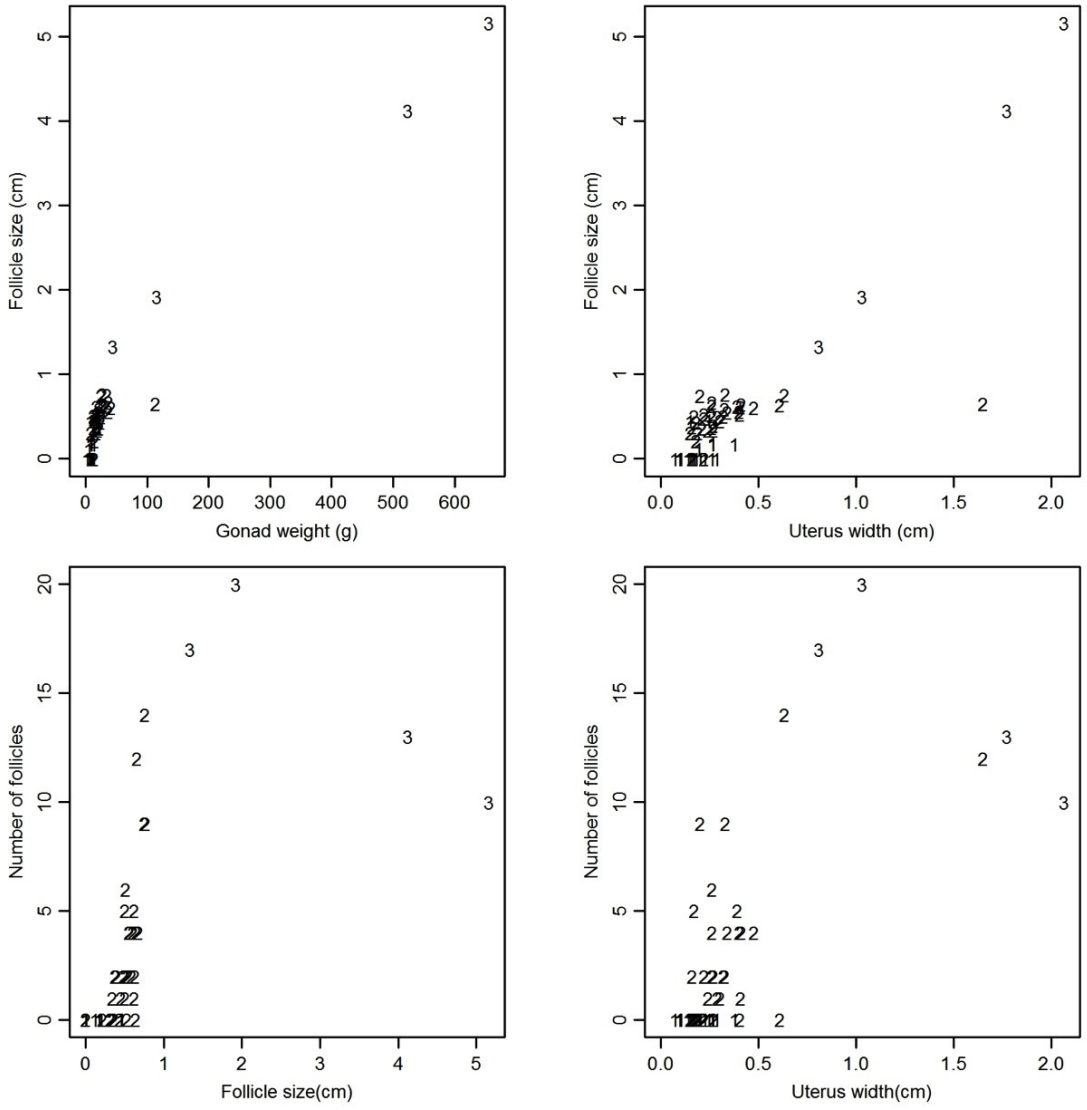

Figure 4.2- 10 D. calcea: Relationships between follicle size, gonad weight, uterus width and number of follicles. The numbers denote the macroscopic maturity stages assigned to each individual.

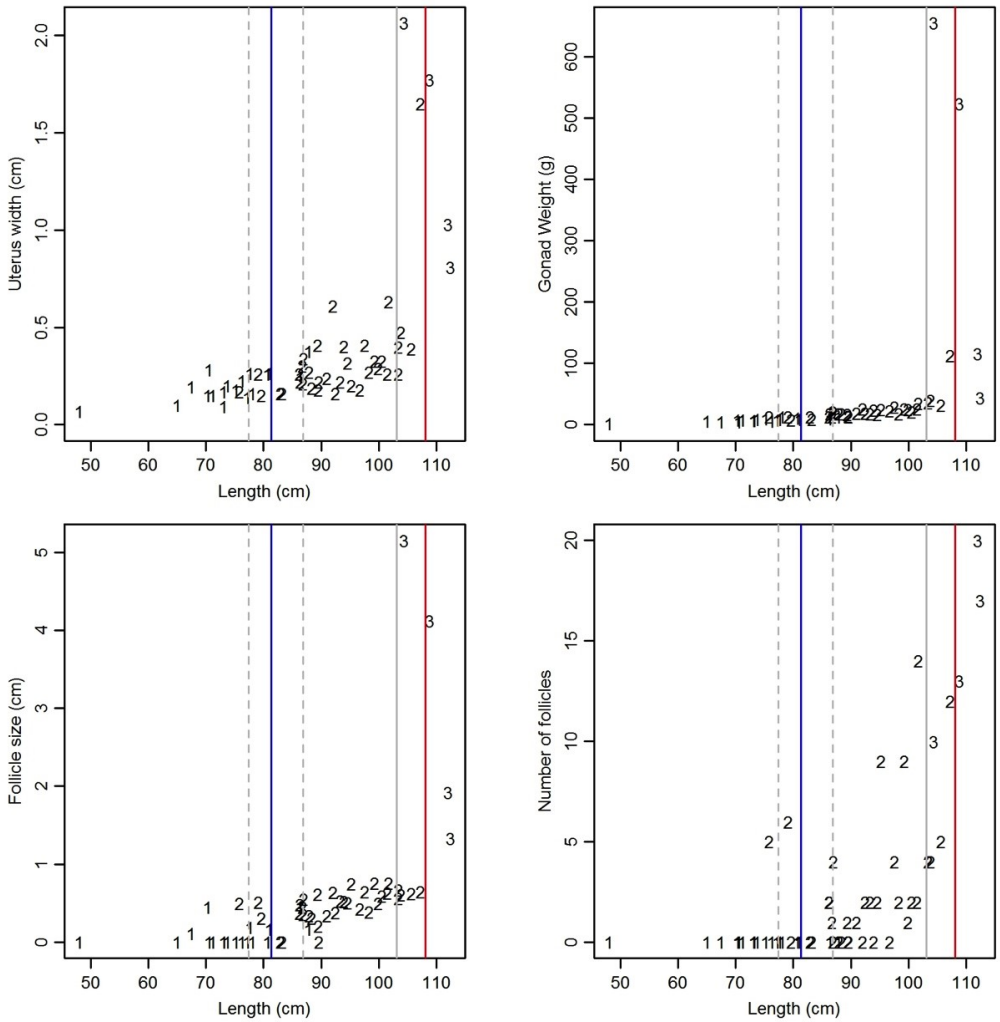

Figure 4.2- 11 D. calcea: Length relationships with uterus width, gonad weight, number of follicles and follicle size (clockwise from top left). The numbers denote the macroscopic maturity stages assigned to each individual. Lines represent length-at-maturity estimates for maturity occurring at stage 2 (blue) and at stage 3 (red). Grey lines represent the 95 % confidence intervals (Figure 4.2-6).

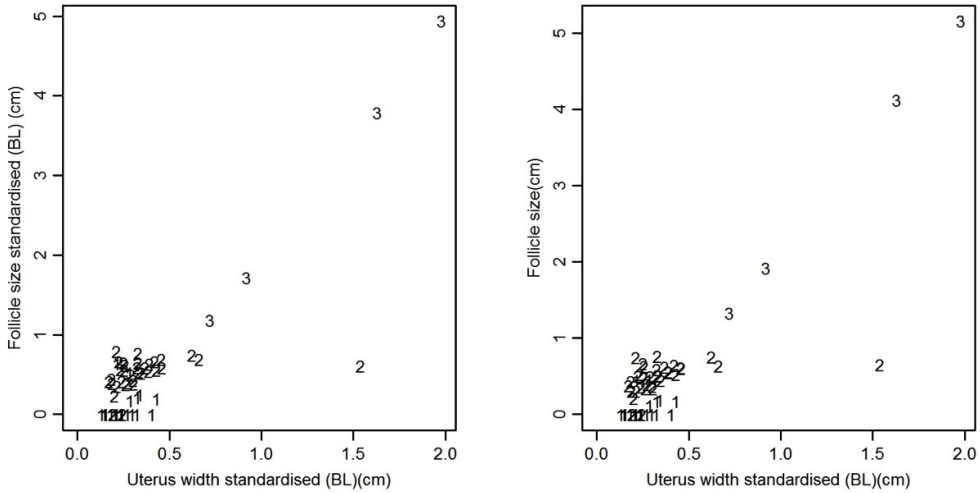

Figure 4.2- 12 D. calcea: Relationship between length-standardised measures of follicle size and uterus width. The numbers denote the macroscopic maturity stages assigned to each individual.

4.2.1 *Conclusions*

Gonad weight, uterus width, follicle size, follicle number, oviducal gland area and length-weight relationship, all showed step changes (or transitions) at around the fish length consistent with maturity (as measured at stage 3 of the macroscopic scale).

Length-at-maturity was estimated at between 81 cm (stage 2 onset) and 108 cm (stage 3 onset), suggesting a 30 cm 'gap' between the onset of 'maturity' (stage 2) and when follicles exceeded 1 cm (stage 3).

Stage 2 fish had the greatest variability in uterus width, suggesting some are likely immature and some mature (post-partum, stage 6) (i.e. two groups). Therefore, uterus width may be a useful attribute in distinguishing between immature and mature/resting stage 2 fish.

Some stages 1 – 3 had undetectable oviducal glands, although follicle size and uterus width (particularly in stage 3 fish) were comparable to fish with developed oviducal glands. Oviducal gland area varied in fish of similar size and follicle development. The onset of oviducal gland development and follicle development may be different and oviducal development may continue after mating. Oviducal gland size is probably not a good indicator of maturity due to variability in size over all stages.

Follicle size alone may be insufficient in describing reproductive processes occurring particularly in stage 2 and stage 3 fish. The distinction between stage 1, 2 and 3 in the current macroscopic staging key is based solely on follicle size, which may cut the maturity cycle arbitrarily.

4.3 Centrophorus squamosus

A sub-catch from the TAN1601 survey of male (n = 11) and female (n = 33) *C. squamosus* were caught principally on the northern Chatham Rise (Figure 4.3- 1). Stage 3 males were predominantly caught from the central northern Chatham Rise, along with stage 3 and 4 females (Figure 4.3- 1). A higher proportion of stage 3 females were taken from the northeastern Chatham Rise where relatively few males were taken, compared to other areas (Figure 4.3- 1). Few stage 1 immature fish were taken (n = 5 for females; n = 7 for males). No stage 2 males, stage 5 or stage 6 females were caught.

Two females were sampled only for length, weight and sex, and 8 were sampled for full biological measurements. The remaining results use data from these 8 females (stages 1 – 4).

Females ranged in total length (TL) from 73.5 cm to 135.4 cm (Figure 4.3- 2). Median length was 116.84 cm with a median weight of 11046.25 g (Figure 4.3- 2).

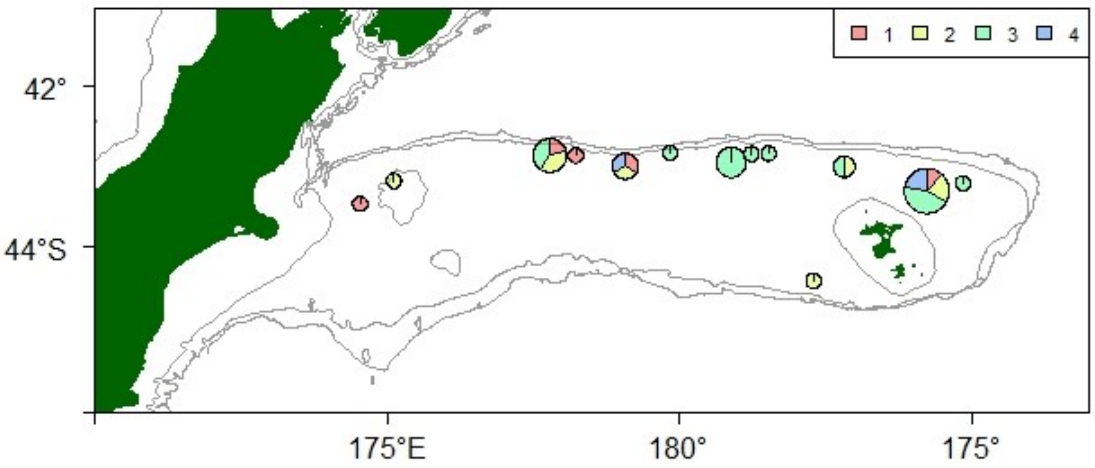

Figure 4.3- 1 C. squamosus: Chatham Rise area with 200 m, 1000 m and 1200 m contours (grey), showing sample distribution for C. squamosus females (n = 33). Pie charts denote the proportion of each macroscopic stage (colours for each macroscopic stage defined in legend) caught at each tow where samples were collected. Pie chart area – sample sizes collected relative to other tows.

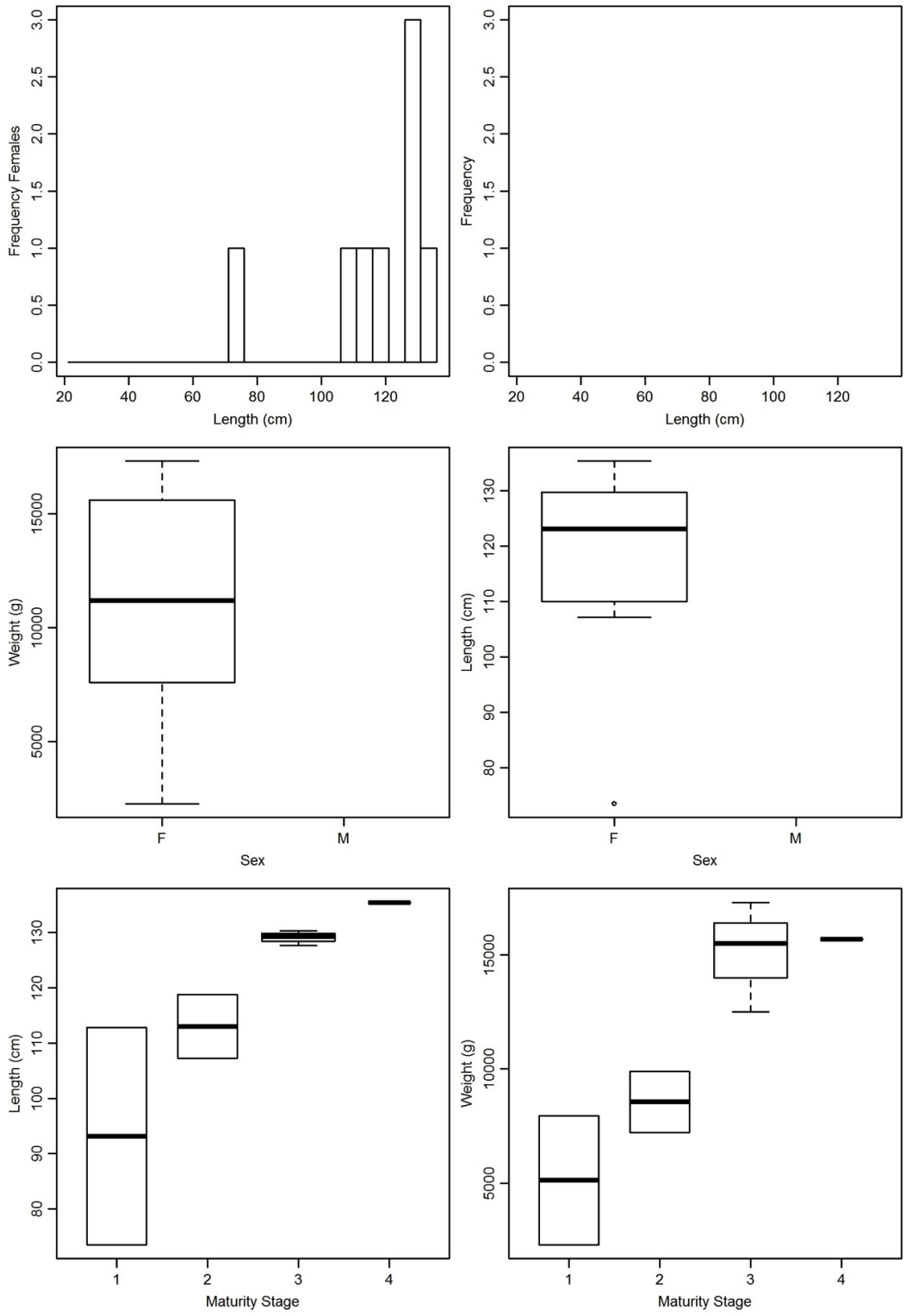

Figure 4.3- 2 C. squamosus: Total length (TL) frequency for C. squamosus (n=8) within the sample from the Chatham Rise, with females (n=8; top left panel) and males (n=0; top right); Weight and length distributions for females (middle panels), where the darkest black line represents the median. Length and weight distributions across females in relation to macroscopic maturity staging (bottom panels).

When maturity was considered to start at stage 2, the data estimates length-at-maturity at 100 – 110 cm and 120 – 130 cm when maturity is considered to start at stage 3 (Figure 4.3- 3). There was a 20 – 30 cm 'gap' between the onset of 'maturity' (stage 2) and when follicles exceeded 1 cm (stage 3).

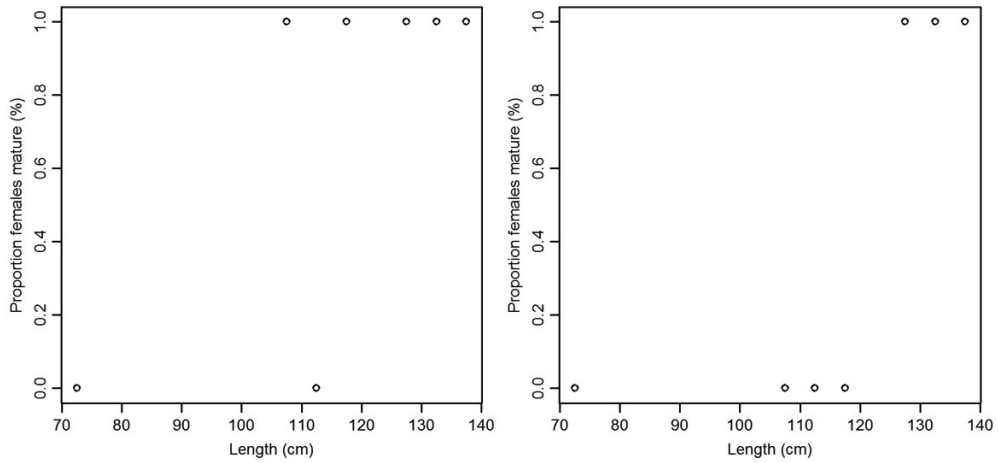

Figure 4.3- 3 C. squamosus: Estimated length-at-maturity for C. squamosus females on Chatham Rise (TAN1601 survey), where macroscopic maturity is considered to start at left: stage 2 (n = 6); right: stage 3 (n = 4). Points were proportion mature in 5 cm length bins. Line fitted is the maturity ogive. The black lines indicate the L50 value. The 95% confidence intervals were estimated by bootstrapping.

Results for analyses pertaining to the oviducal gland were not shown because of the small sample size (n = 8). Despite the small sample size, oviducal gland development was only observed in stage 3 and 4 fish, greater than approximately 130 cm TL, and where follicles were greater than 5 cm in diameter. Two of the three stage 3 and one stage 4 fish exhibited sperm storage.

As follicle size increased, gonad weight increased (Figure 4.3- 4). Follicle growth appeared to asymptote when follicles were approximately 6 cm in diameter (Figure 4.3- 4. The relationship between gonad weight and length suggests an increase in variability in gonad weight once length was greater than approximately 125 cm TL (Figure 4.3- 4; Figure 4.3- 5).

Irrespective of fish size, follicle size increased with uterus width (Figure 4.3- 5): stages 3 and 4 had larger uteri than stages 1 and 2, although uterus width was smaller in the single stage 4 fish than stage 3 fish (Figure 4.3- 4). At lengths greater than approximately 120 cm, uterus width increased (Figure 4.3- 4; Figure 4.3- 5).

Sperm storage was observed in fish that had follicles greater than 2 cm, and uterus widths greater than 2 cm (Figure 4.3- 4).

The number of follicles varied greatly at lengths greater than 120 cm, with a maximum of 12 (Figure 4.3- 4; Figure 4.3- 5). Follicle number was greatest in those fish exhibiting sperm storage and appeared to increase as uterus width increased, up to approximately 1 cm (Figure 4.3- 5). As follicle number decreased to around 8 follicles in the stage 4 fish, uterus width continued to increase despite the high variability in follicle number (Figure 4.3- 5).

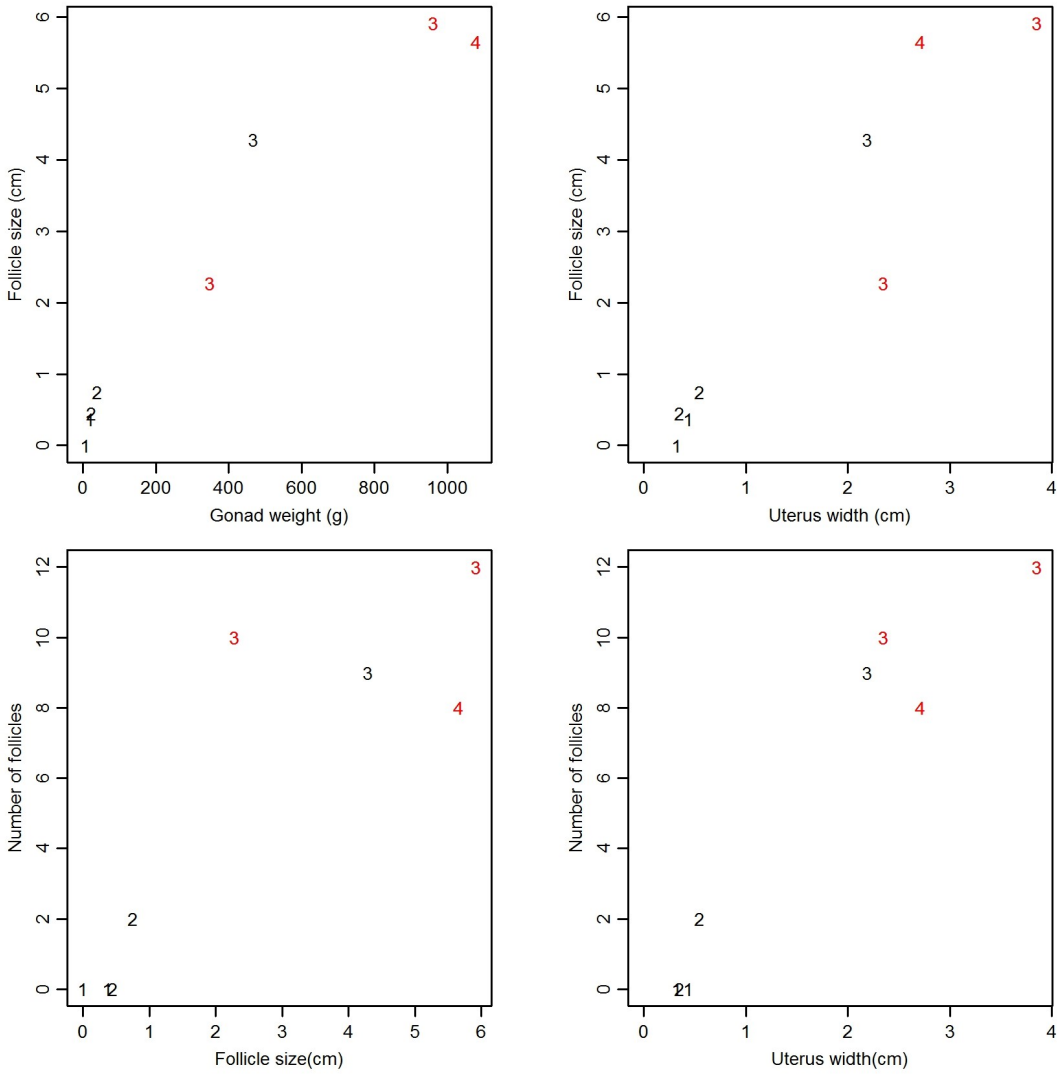

Figure 4.3- 4 C. squamosus: Relationships between follicle size, gonad weight, uterus width and number of follicles. The numbers denote the macroscopic maturity stages assigned to each individual. Red numbers indicate those individuals where sperm storage was detected.

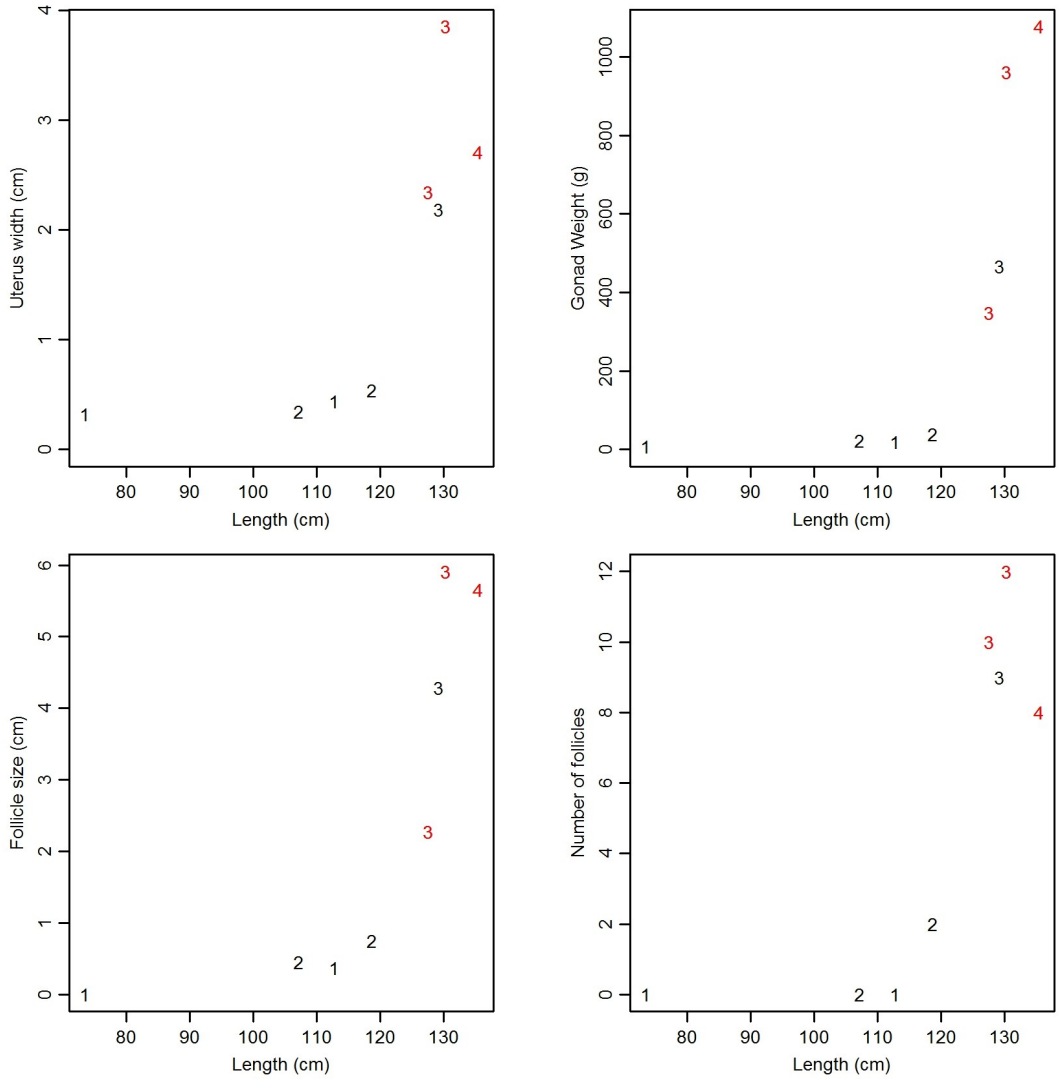

Figure 4.3- 5 C. squamosus: Length relationships with uterus width, gonad weight, number of follicles and follicle size (clockwise from top left). The numbers denote the macroscopic maturity stages assigned to each individual. Red numbers indicate those individuals where sperm storage was detected.

4.3.1 *Conclusions*

Gonad weight, uterus width, follicle size, follicle number and oviducal gland area all showed step changes (or transitions) at around the fish length consistent with maturity (as measured at stage 3 of the macroscopic scale).

Length-at-maturity was estimated at between 100 – 110 cm (stage 2 onset) and 120 – 130 cm (stage 3 onset), suggesting a 20 – 30 cm 'gap' between the onset of 'maturity' (stage 2) and when follicles exceeded 1 cm (stage 3).

Sperm storage was detected in macroscopically assigned stage 3 and stage 4 fish.

Uterus width increased at lengths greater than 120 cm, but was smaller in the single stage 4 fish than stage 3 fish, suggesting uterine development may occur before pup development.

Peak follicle number coincided with sperm storage, although follicles were relatively small and in the early stages of development.

Some stage 1, 2 and 4 fish had undetectable oviducal glands, although follicle size and uterus width (particularly in stage 4 fish) were comparable with fish with developed oviducal glands. The onset of oviducal gland development and follicle development may be different. Oviducal gland size is probably not a good indicator of maturity due to variability in size over all stages.

Follicle size appeared to asymptote at 6 cm in diameter. Follicle size alone may be insufficient in describing reproductive processes occurring particularly in stage 2 and stage 3 fish. The distinction between stage 1, 2 and 3 (in the current macroscopic staging key) is based solely on follicle size, which may cut the maturity cycle arbitrarily.

4.4 *Brochiraja asperula*

B. asperula were collected from both Chatham Rise (n = 9) and the Sub-Antarctic (n = 6) (Figure 4.4- 1). Stage 2, 3 and 6 females were principally caught throughout the Chatham Rise and from the Sub-Antarctic, as were stage 3 males (Figure 4.4- 1).

Full biological measurements were taken for all fish sampled (n = 15). The remaining results use data from these 15 fish (n = 8 for females; n = 7 for males). No stage 1 or 4 females were taken, nor were any stage 1 males. The male data was not analysed since this study focuses on female reproduction.

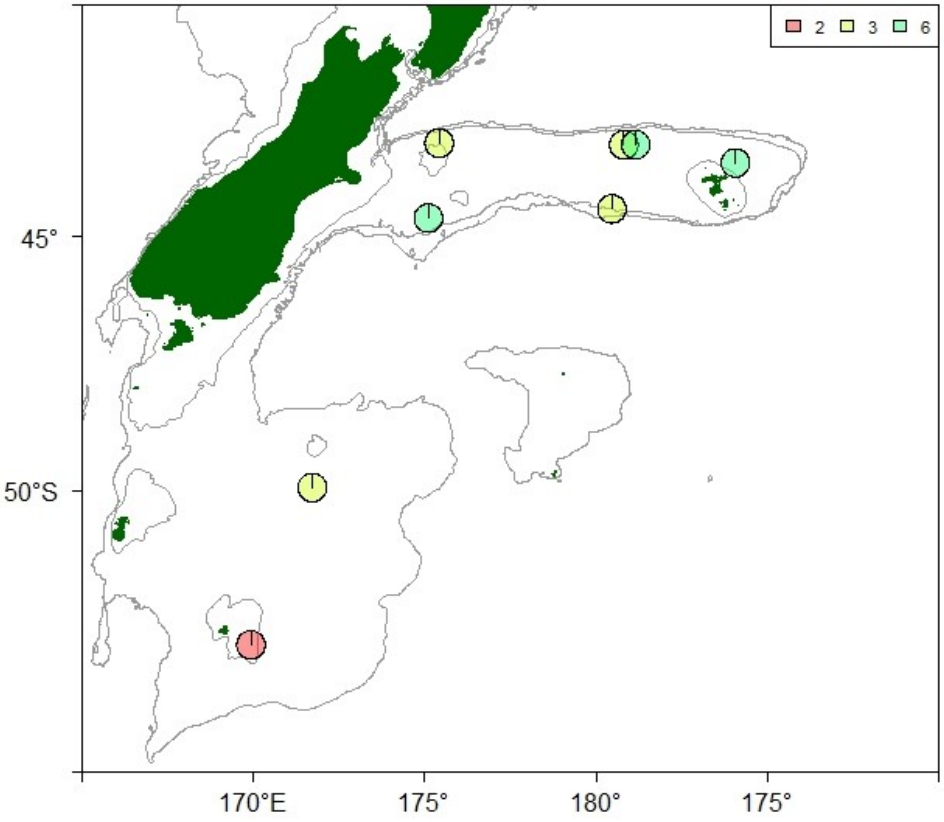

Figure 4.4- 1 B. asperula: Chatham Rise and Sub-Antarctic areas with 200 m, 1000 m and 1200 m contours (grey), showing sample distribution for B. asperula (n = 15). Pie charts denote the proportion of each macroscopic stage (colours for each macroscopic stage defined in legend) caught at each tow where samples were collected. Pie chart area – sample sizes collected relative to other tows.

Females ranged in total length (TL) from 27.5 cm to 32.5 cm, with a median length of 30 cm and a median weight of 577 g (Figure 4.4- 2). Males ranged in total length (TL) from 27 cm to 33.5 cm, with a median length of 33 cm and median weight of 675 g (Figure 4.4- 2).

B. asperula met the ANOVA and Tukey HSD test assumptions and the analyses indicated there were no statistically significant differences in mean fish size between macroscopic stages (ANOVA p = 0.0645 for length; 0.394 for weight) (Figure 4.4- 2).

Results for length-at-maturity and length-weight relationships were not estimated because of the small sample size (n = 8). Despite the small sample size, the relationships between oviducal gland area, follicle size, uterus width, gonad weight and follicle number, were analysed.

On average, the left oviducal gland was larger than the right (Figure 4.4- 3). Irrespective of fish size and changes in uterus width, follicle size, gonad weight and follicle number, average oviducal gland area appeared to be consistently between 1.62 cm^2 and 2.57 cm^2 (Figure 4.4- 4). Both stage 6 fish exhibiting sperm storage had developed oviducal glands (> 1.5 cm^2; Figure 4.4- 3).

Follicle size and gonad weight were not well-correlated (Figure 4.4- 5). When follicles were approximately 2 cm in diameter, gonad weight was highly variable, irrespective of fish size and macroscopic stage (Figure 4.4- 5). The single stage 2 fish had similar sized follicles as stage 3 and 6 fish, and similar gonad weight to stage 6 fish (Figure 4.4- 5). There was one stage 3 outlier fish that had follicles 13.69 cm in diameter (Figure 4.4- 5). When follicles were approximately 2 cm in diameter, follicle number (0 – 14) and uterus width (0.7 – 1.2 cm) were more variable (Figure 4.4- 5). Uterus width was similar in all stage 3 fish (approximately 1 cm) (Figure 4.4- 5). Stage 6 fish had the greatest variability in uterus width, with one having a similar uterus width as the stage 2 fish (Figure 4.4- 5). Those fish exhibiting sperm storage had the largest uteri (1.1 – 1.2 cm), although follicles were approximately 2 cm in diameter (Figure 4.4- 5; Figure 4.4- 6).

Follicle number varied greatly in fish greater than 29 cm TL (with a maximum of 14) and when uterus width was between 0.6 and 1.2 cm (Figure 4.4- 5; Figure 4.4- 6). Stage 2 had the highest follicle count, and stage 6 (including those exhibiting sperm storage) had the lowest (0 – 6) (Figure 4.4- 5).

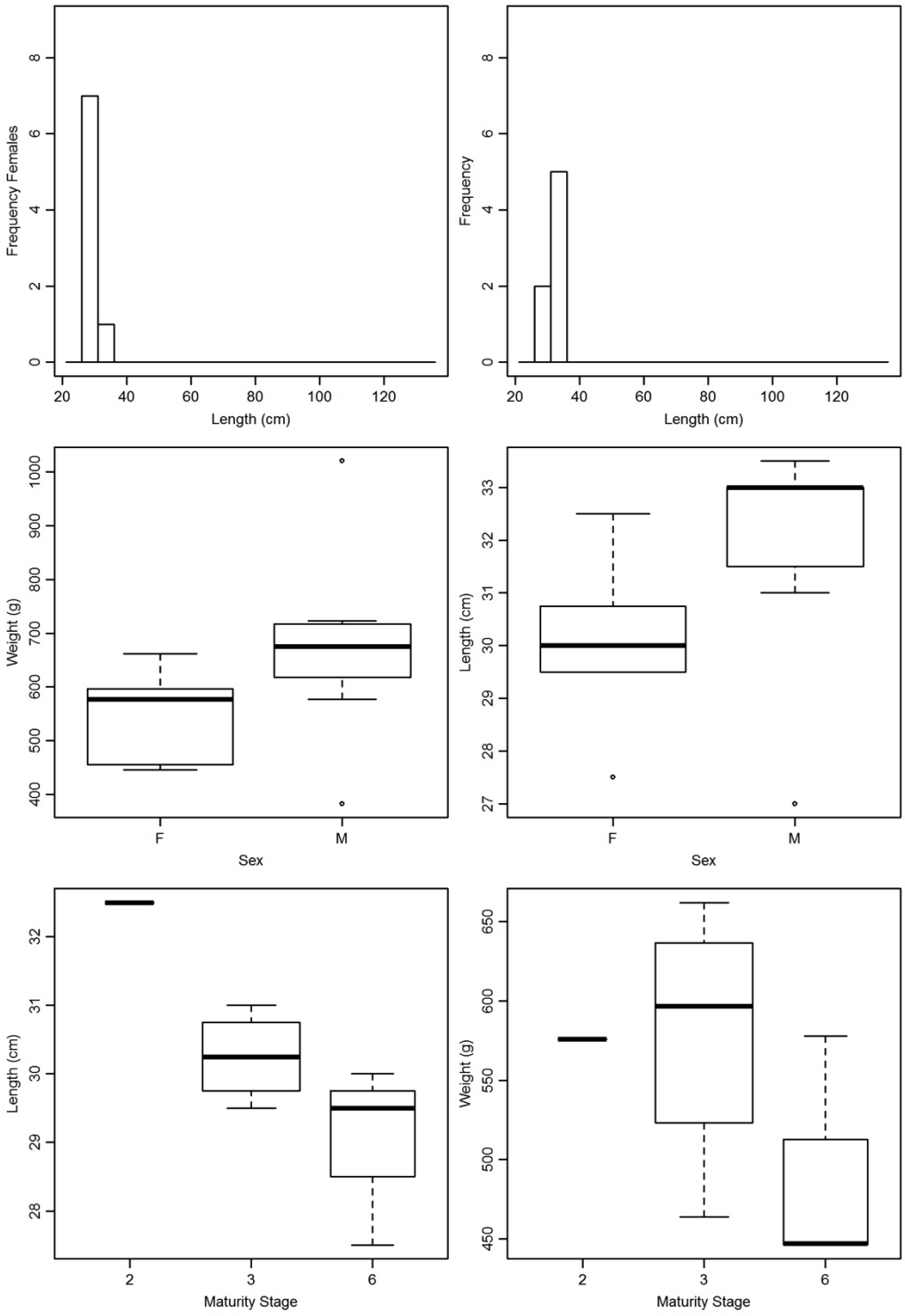

Figure 4.4- 2 B. asperula: Total length (TL) frequency for B. asperula (n=15) within the sample from the Chatham Rise, with females (n= 8; top left panel) and males (n=7; top right); Weight and length distributions for males and females (middle panels), where the darkest black line represents the median; Length and weight distributions across females in relation to macroscopic maturity staging (bottom panels).

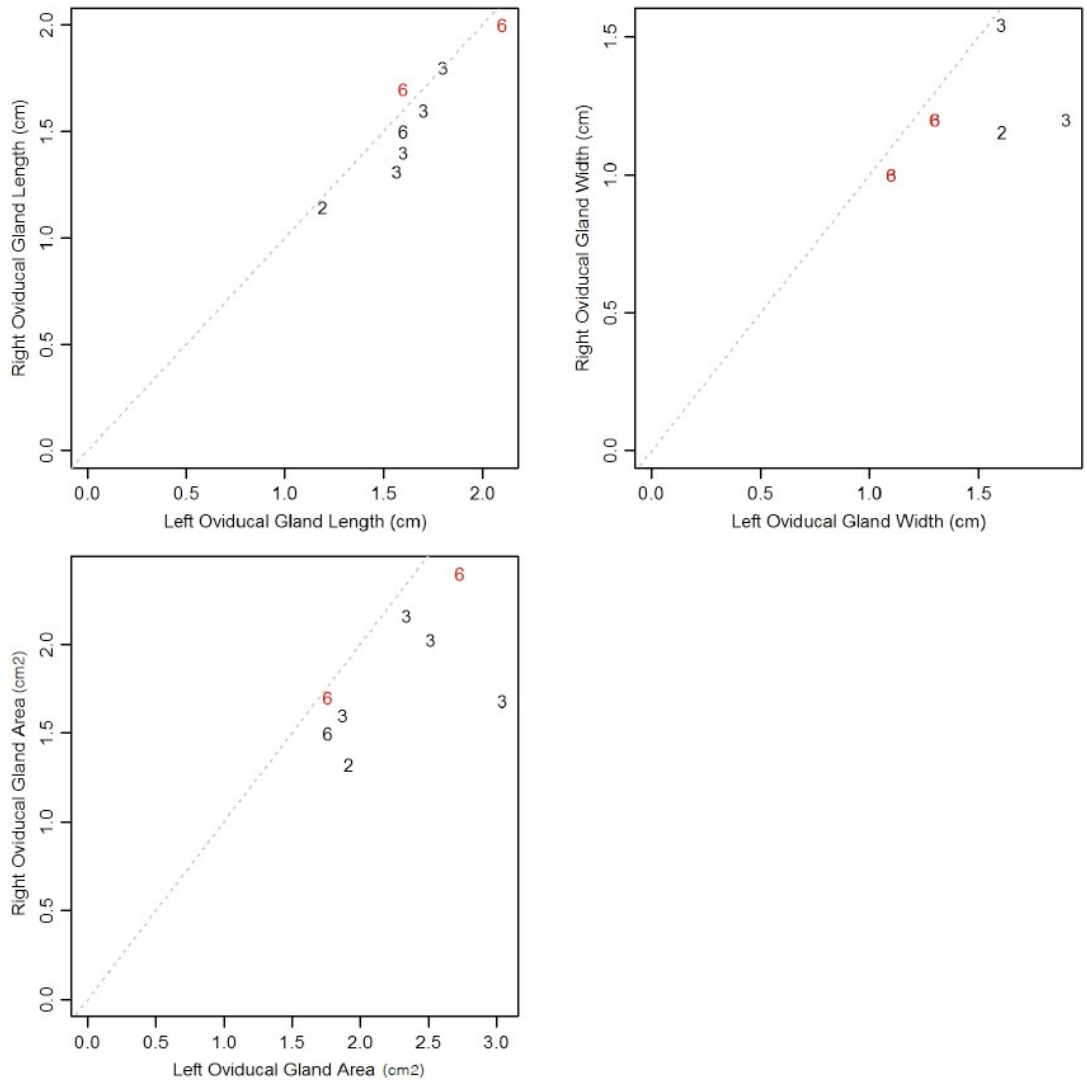

Figure 4.4- 3 B. asperula: Relationships between left and right oviducal gland lengths (top left), widths (top right) and areas (bottom left). The dashed grey lines represent the 1:1 relationship. Numbers represent the macroscopic staging assigned to each individual in the sample. Red numbers indicate those individuals where sperm storage was detected.

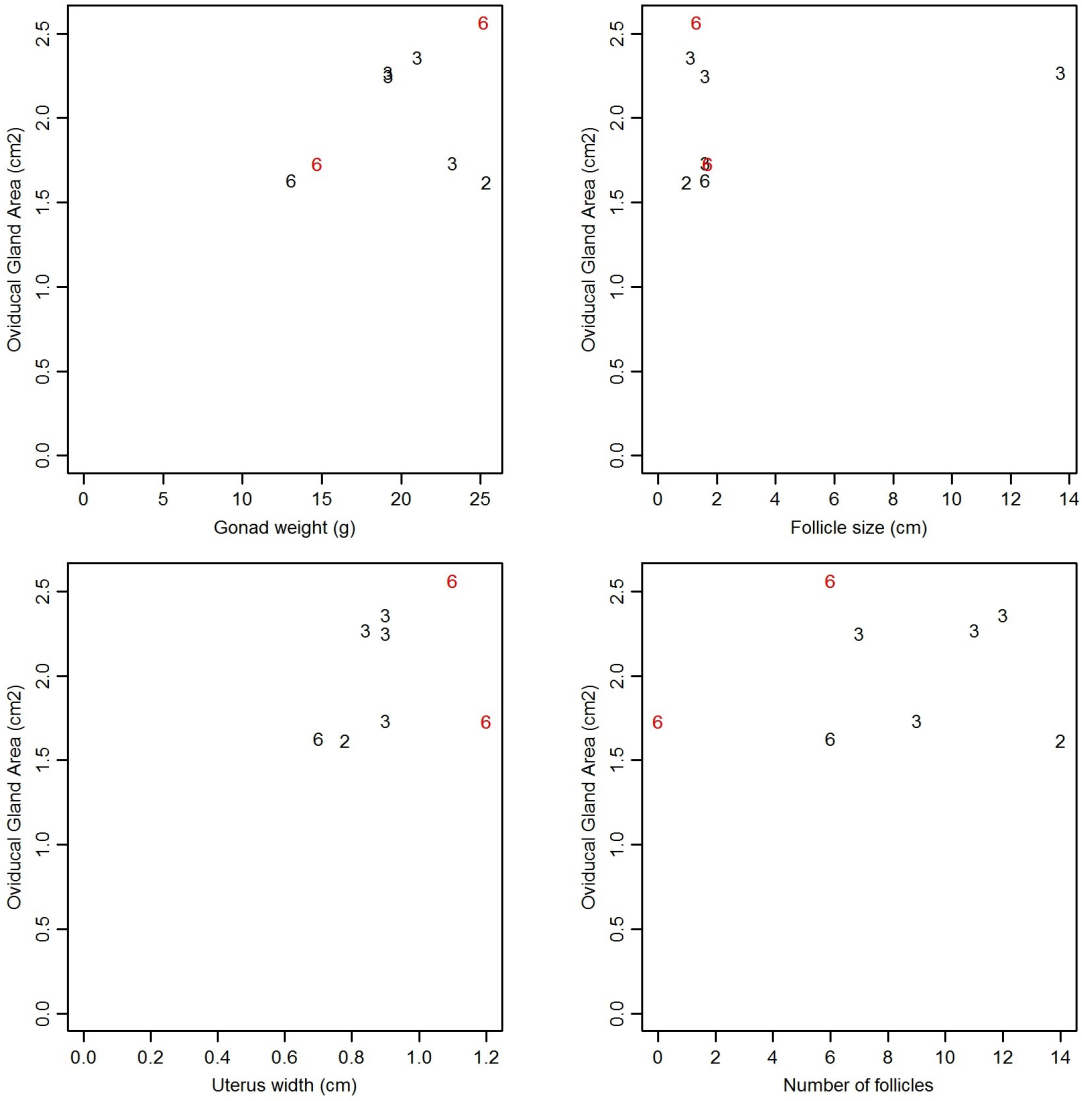

Figure 4.4- 4 B. asperula: The relationships between average oviducal gland area and gonad weight, follicle size, number of follicles and uterus width (clockwise from top left). The numbers denote the macroscopic maturity stages assigned to each individual. Red numbers indicate those individuals where sperm storage was detected.

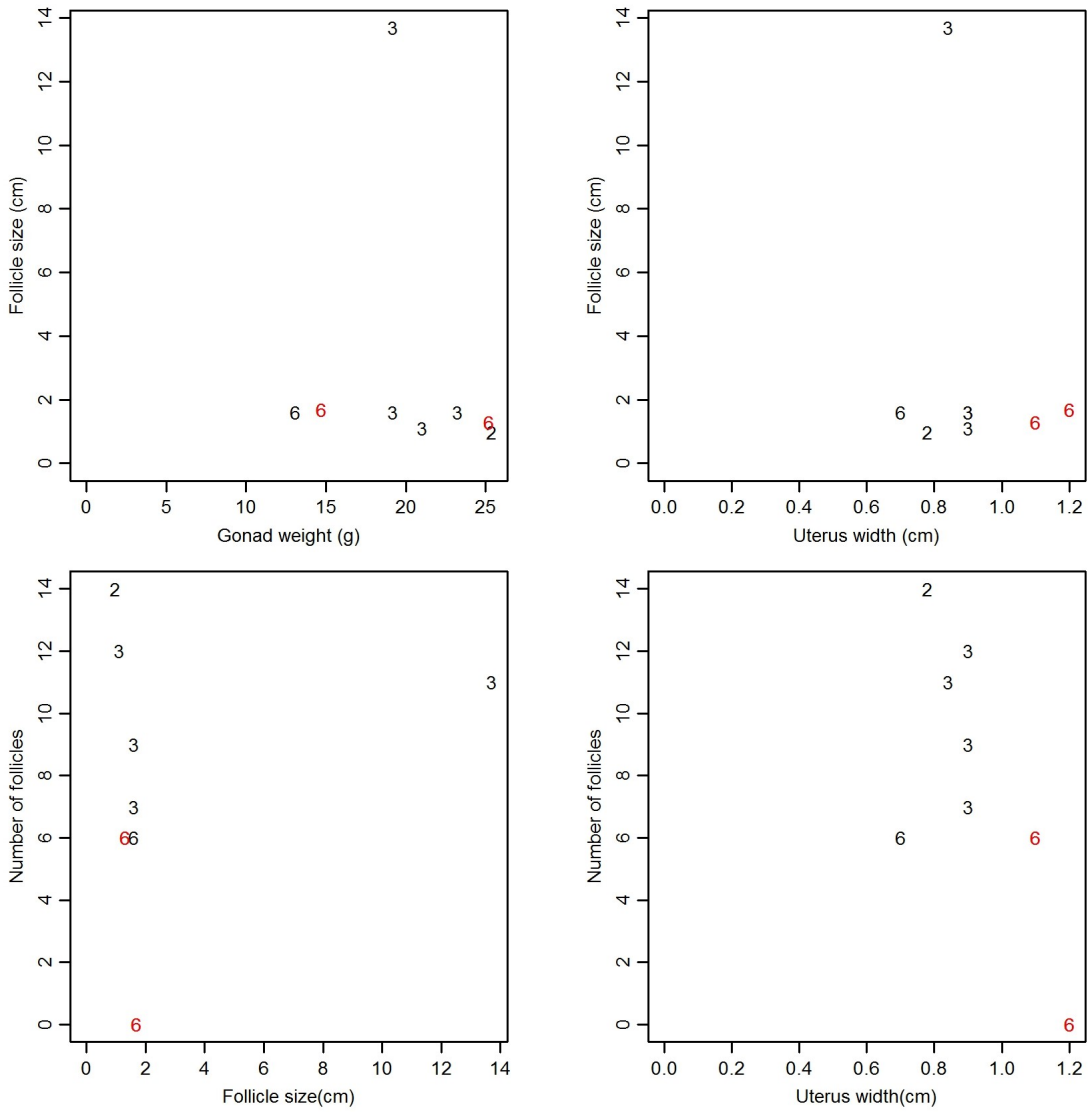

Figure 4.4- 5 B. asperula: Relationships between follicle size, gonad weight, uterus width and number of follicles. The numbers denote the macroscopic maturity stages assigned to each individual. Red numbers indicate those individuals where sperm storage was detected.

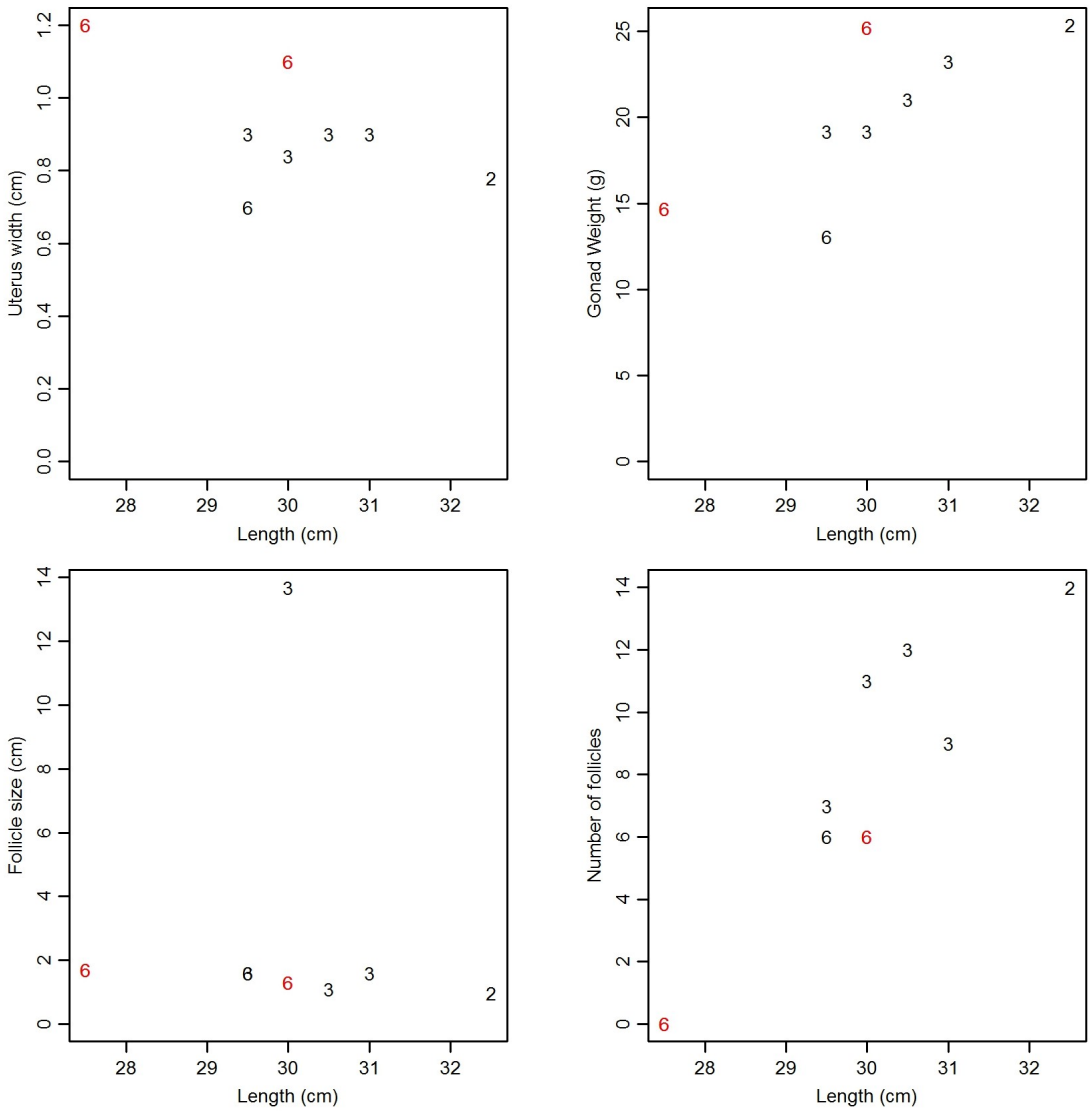

Figure 4.4- 6 B. asperula: Relationships between length and uterus width, gonad weight, number of follicles and follicle size (clockwise from top left). The numbers denote the macroscopic maturity stages assigned to each individual. Red numbers indicate those individuals where sperm storage was detected.

4.4.1 *Conclusions*

Length-at-maturity could not be estimated since no immature fish were available in the sample.

Sperm storage was detected in macroscopically assigned stage 6 fish.

Stage 6 fish had the greatest variability in uterus width, with some likely to be stage 2. Therefore, uterus width may be a useful attribute in distinguishing between stage 2 and stage 6 fish.

Sperm storage was detected in fish where follicles were relatively small and in the early stages of development.

The onset of oviducal gland development and follicle development may be different, and oviducal development may be independent to other developmental processes occurring in the reproductive tract. Oviducal gland size is probably not a good indicator of maturity due to variability in size over all stages.

Follicle size alone may be insufficient in describing reproductive processes occurring particularly in stage 2 and stage 6 fish.

4.5 Apristurus ampliceps

A. ampliceps (n = 10 for females) and (n = 9 for males) were caught principally on the southwestern Chatham Rise (Figure 4.5- 1). No stage 1 immature fish were taken, nor were any stage 2 females. The male data were not analysed since this study focuses on female reproduction.

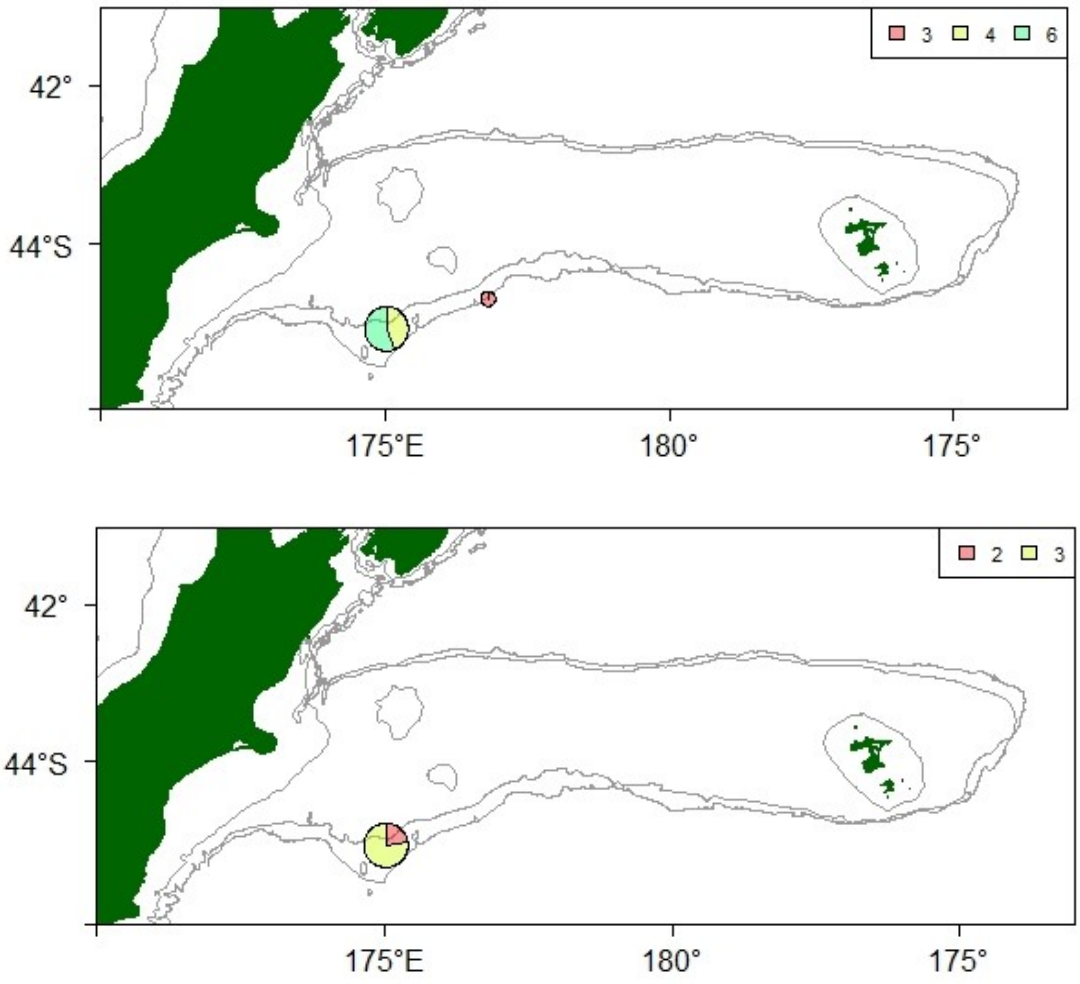

Figure 4.5- 1 A. ampliceps: Sample distribution for A. ampliceps top: females (n = 10); bottom: males (n = 9) on Chatham Rise with 200 m, 1000 m and 1200 m contours (grey). Pie charts denote the proportion of each macroscopic stage (colours for each macroscopic stage defined in legend) caught at each tow where samples were collected. Pie chart area – sample sizes collected relative to other tows.

Females ranged in total length (TL) from 75 cm to 88.5 cm, with a median length of 77.75 cm and a median weight of 1900 g (Figure 4.5- 2). Males ranged in total length (TL) from 74 cm to 84 cm, with a median length of 81 cm and a median weight of 2272 g (Figure 4.5- 2).

A. ampliceps met the ANOVA and Tukey HSD test assumptions, and the analyses indicated there were no statistically significant differences in mean fish size between macroscopic stages (ANOVA p = 0.793 for length; p = 0.532 for weight; Figure 4.5- 2).

Results for length-at-maturity and length-weight relationships were not estimated because of the small mature sample size (n = 10). Despite the small sample size, the data that suggested maturity-at-length was estimated at 69 cm regardless of whether maturity was considered to start at stage 2 or stage 3.

On average, the left oviducal gland was longer and wider than the right (Figure 4.5- 3). Stage 6 fish infested with *A. squalicola* (Appendix C.2) had among the shortest and widest oviducal glands, whereas stage 6 fish without *A. squalicola,* had the longest and thinnest (Figure 4.5- 3). Oviducal gland size appeared to remain consistent irrespective of fish size and changes in uterus width, follicle size, gonad weight, and follicle number (Figure 4.5- 4; Figure 4.5- 5).

As follicle size increased, gonad weight increased (Figure 4.5- 6). Three of the five stage 6 fish had follicles less than 1 cm in diameter, consistent with the criterion for stage 2 fish in the NIWA macroscopic staging key (Figure 4.5- 6). Follicle size was highly variable in stage 6 fish and largest follicles were found in stage 3 and 4 fish (Figure 4.5- 6). Follicle size and number varied at lengths greater than 76 cm, across all stages, with a maximum of 35 follicles (Figure 4.5- 6; Figure 4.5- 7). Follicle number increased with uterus width, up to approximately 1 cm (Figure 4.5- 7). As follicle number decreased to around 10 follicles, uterus width continued to increase despite the variability in follicle number (Figure 4.5- 6).

The relationship between gonad weight and length suggests an increase in gonad weight variability in fish greater than approximately 76 cm TL (Figure 4.5- 6).

Uterus width was similar in all stage 6 fish (0.6 – 1.1 cm), despite variability in follicle size (0 – 1.5 cm; Figure 4.5- 6). Irrespective of fish size, stage 3 and 4 fish had variable uterus widths (0.6 – 2.62 cm) although follicle size was similar (1.3 – 2.2 cm; Figure 4.5- 6). Stage 6 fish had smaller uteri than stage 4 (Figure 4.5- 6).

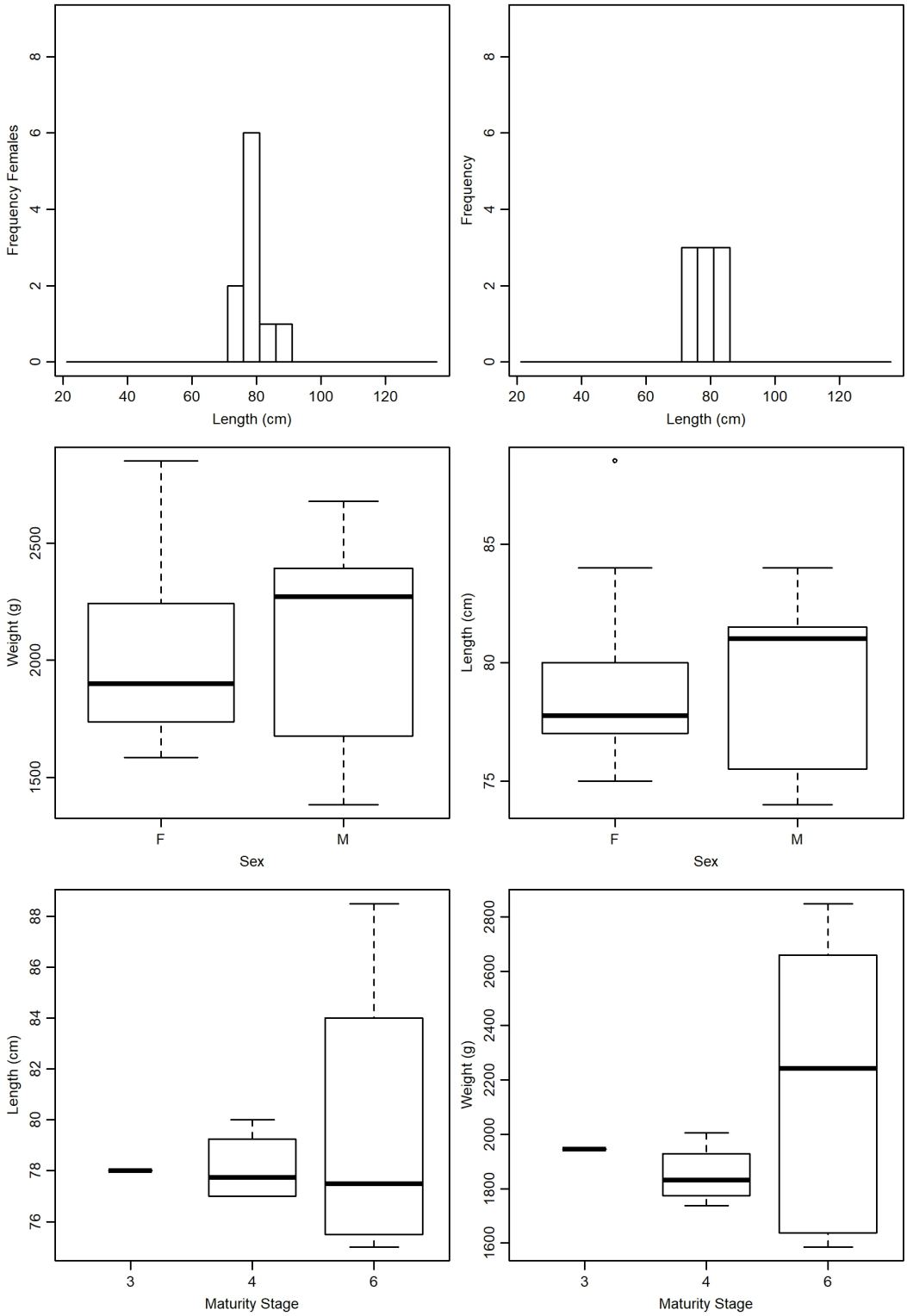

Figure 4.5- 2 A. ampliceps: Total length (TL) frequency for A. ampliceps (n=19) within the sample from the Chatham Rise, with females (n= 10; top left panel) and males (n=9; top right); Weight and length distributions for males and females (middle panels), where the darkest black line represents the median; Length and weight distributions across females in relation to macroscopic maturity staging (bottom panels).

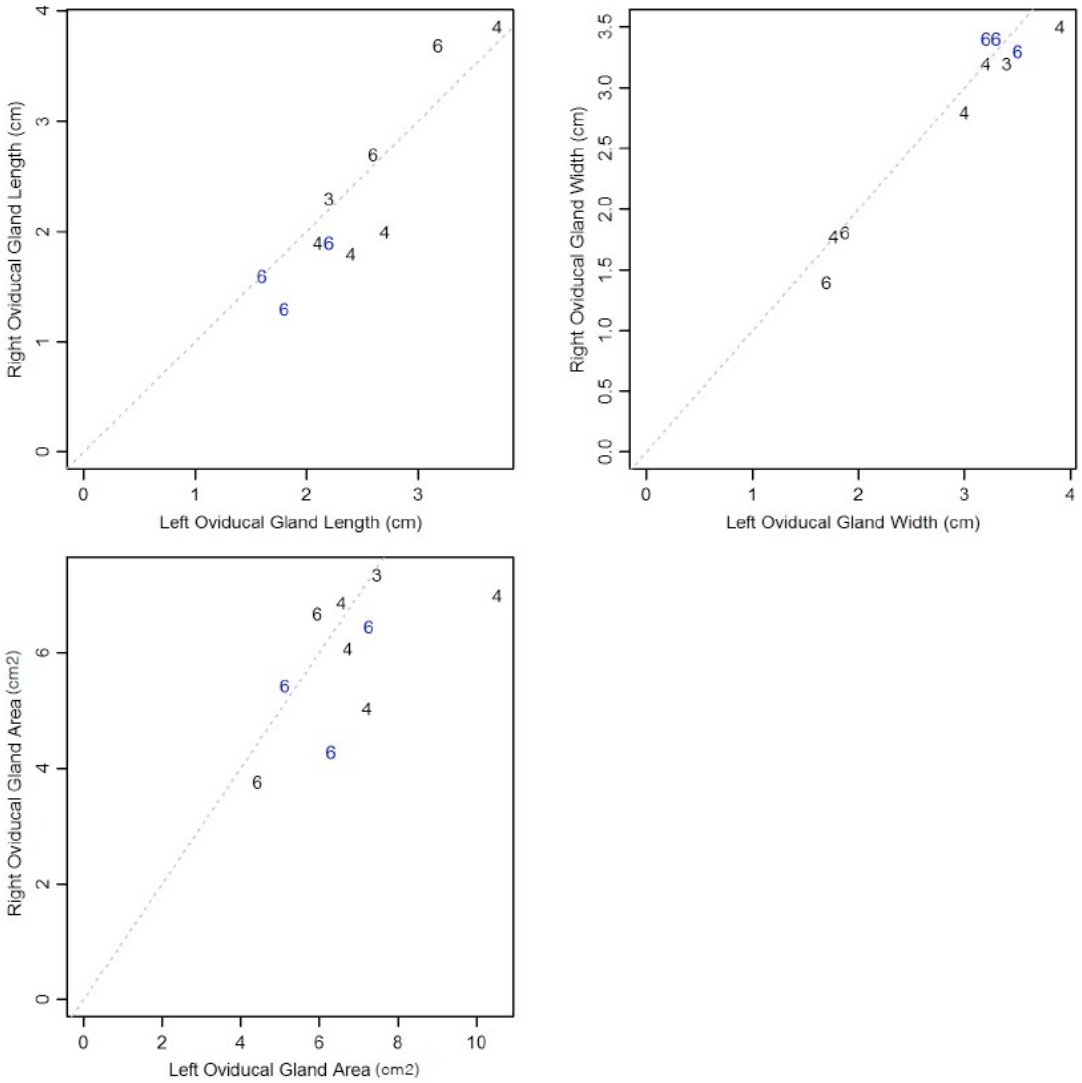

Figure 4.5- 3 A. ampliceps: Relationships between left and right oviducal gland lengths (top left), widths (top right) and areas (bottom left). The dashed grey lines represent the 1:1 relationship. Numbers represent the macroscopic staging assigned to each individual in the sample Blue numbers indicate those individuals infested with the parasite A. squalicola.

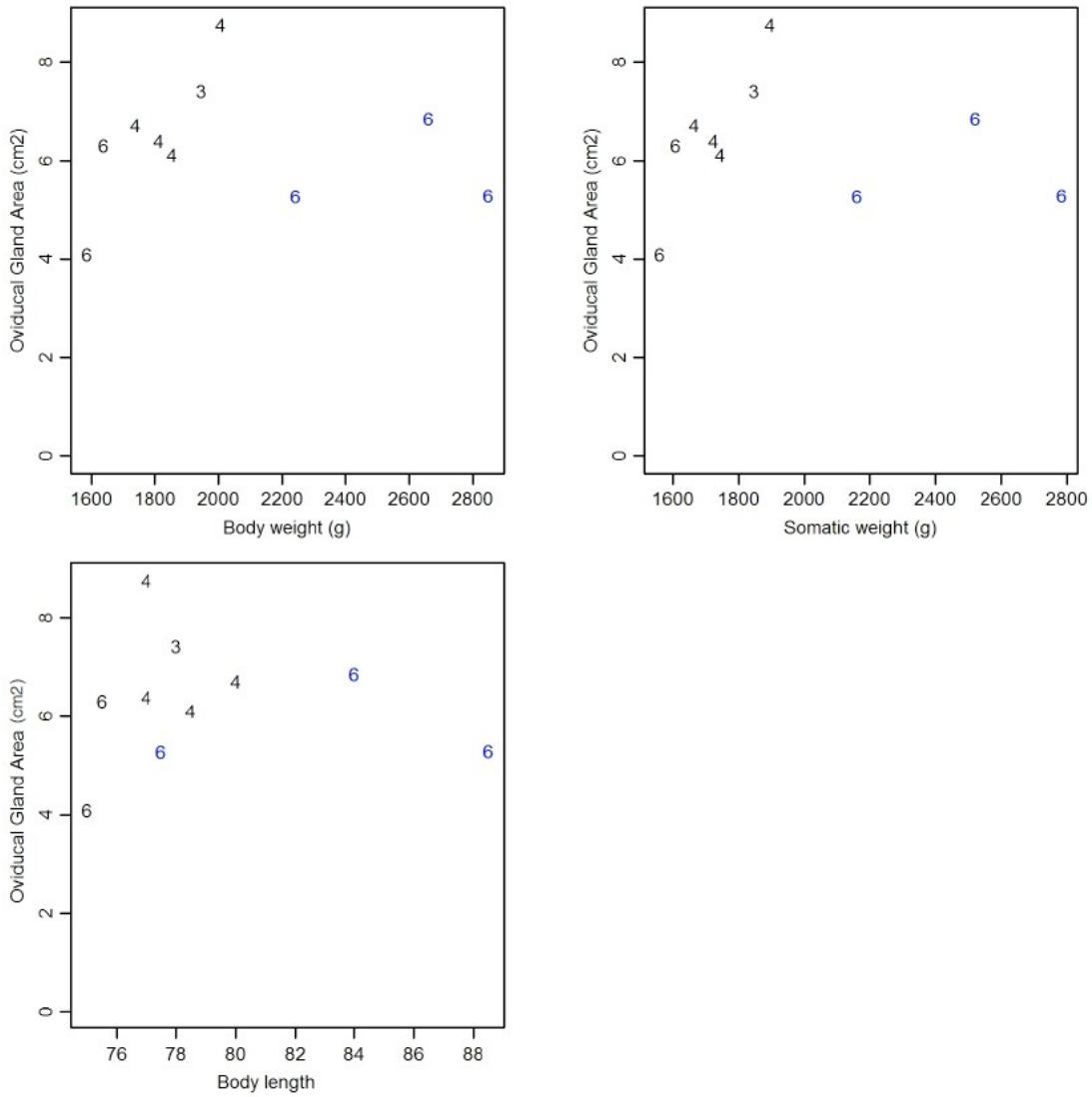

Figure 4.5- 4 A. ampliceps: The relationships between average oviducal gland area (cm^2) and body weight, somatic weight and length (clockwise from top left). The numbers denote the macroscopic maturity stages assigned to each individual. Blue numbers indicate those individuals infested with the parasite A. squalicola.

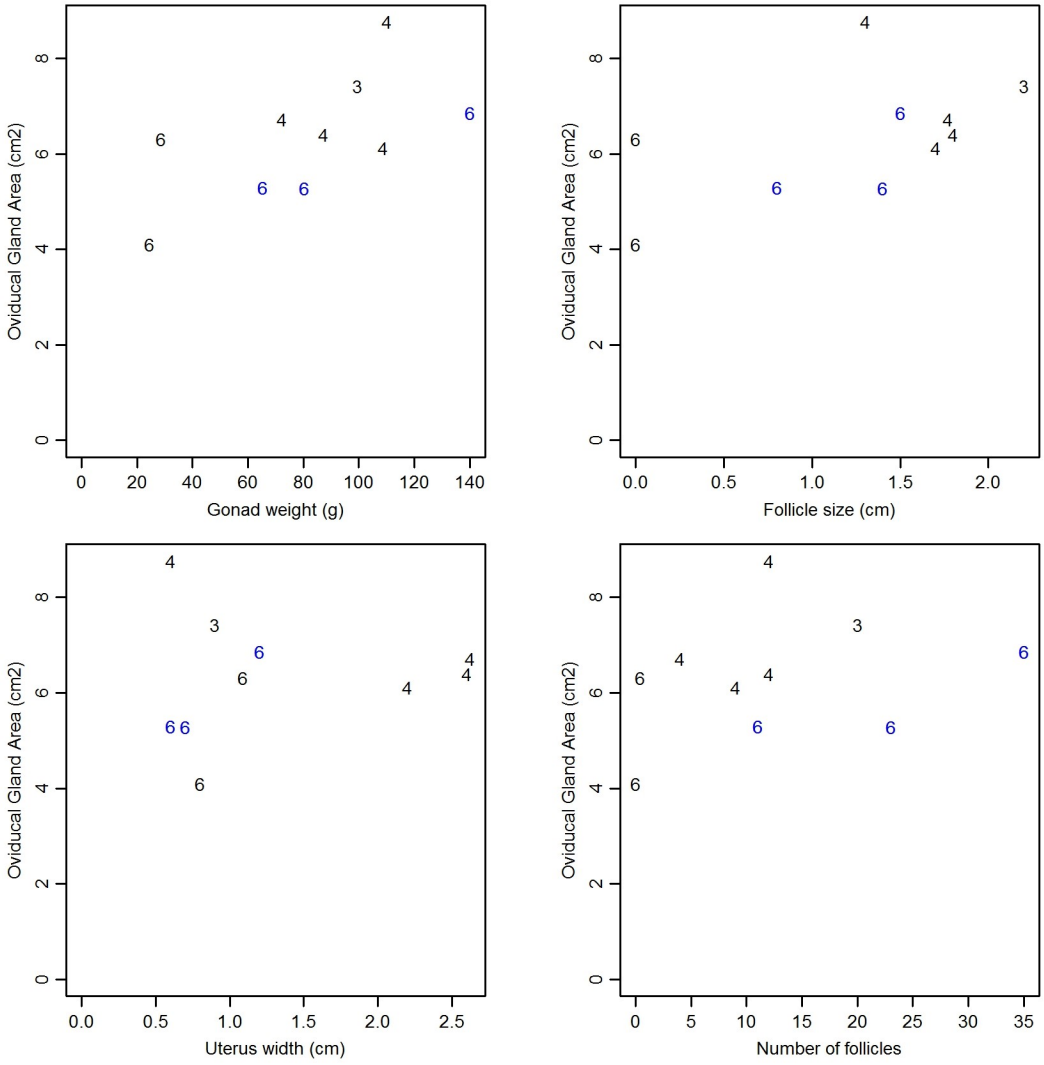

Figure 4.5- 5 A. ampliceps: The relationships between average oviducal gland area and gonad weight, follicle size, number of follicles and uterus width (clockwise from top left). The numbers denote the macroscopic maturity stages assigned to each individual Blue numbers indicate those individuals infested with the parasite A. squalicola.

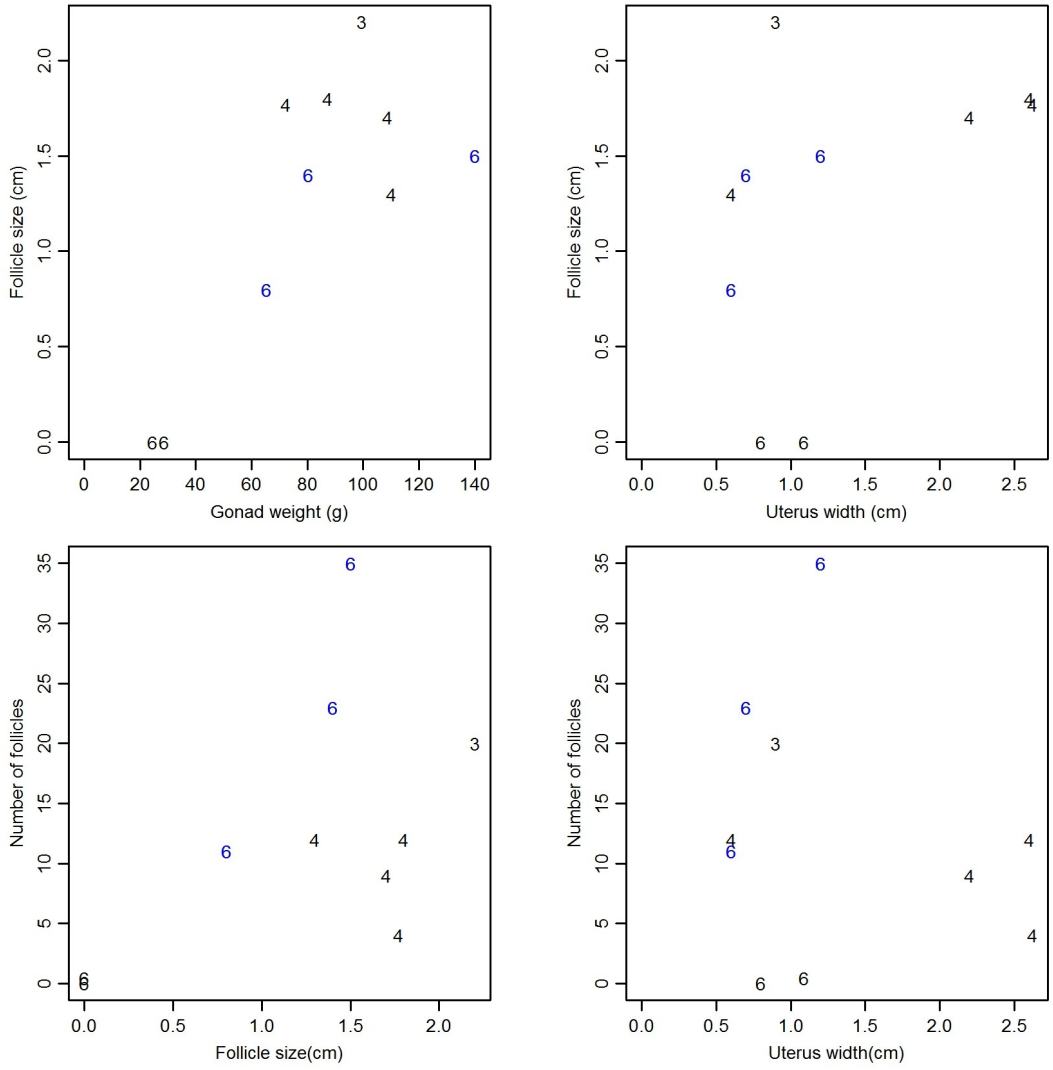

Figure 4.5- 6 A. ampliceps: Relationships between follicle size, gonad weight, uterus width and number of follicles. The numbers denote the macroscopic maturity stages assigned to each individual. Blue numbers indicate those individuals infested with the parasite A. squalicola.

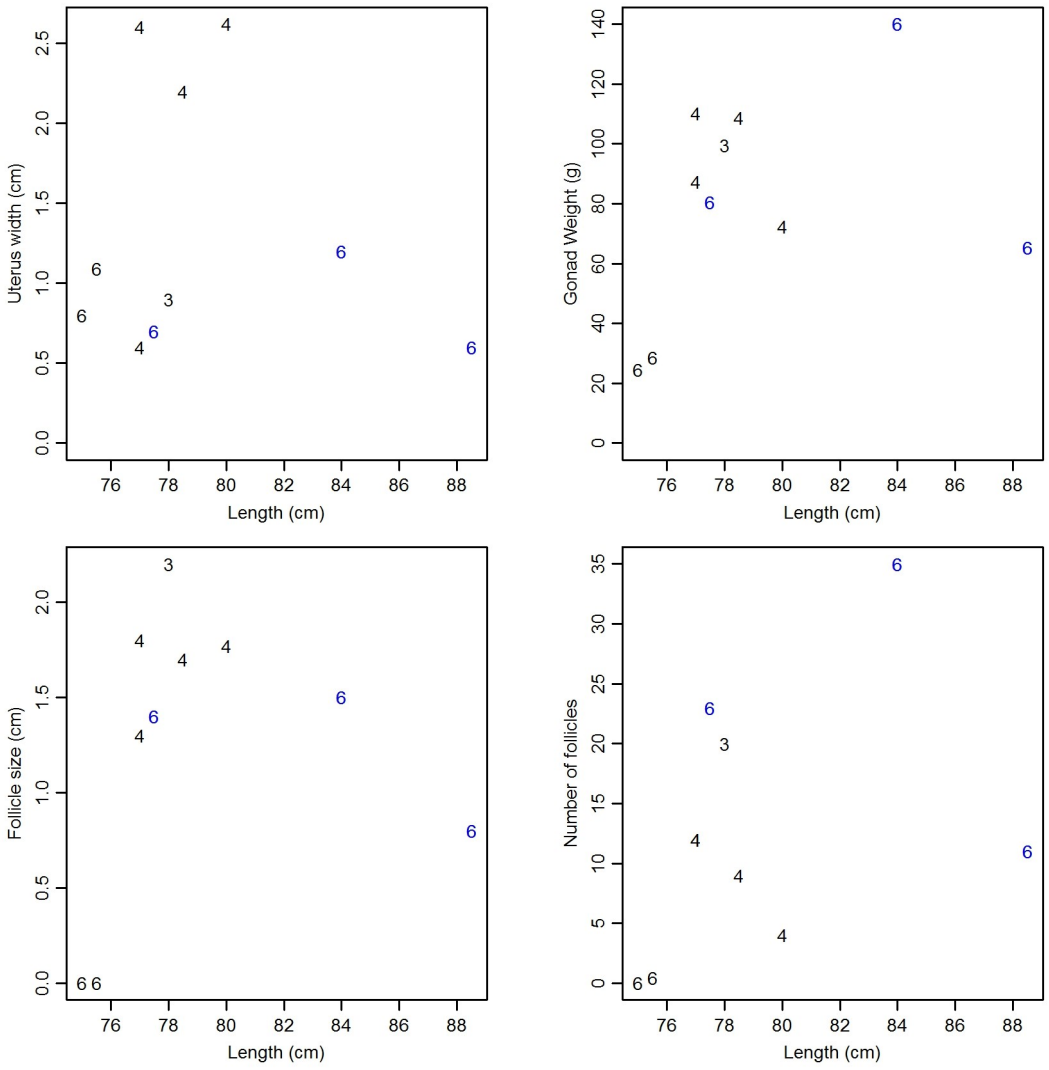

Figure 4.5- 7 A. ampliceps: Relationships between length and uterus width, gonad weight, number of follicles and follicle size (clockwise from top left). The numbers denote the macroscopic maturity stages assigned to each individual. Blue numbers indicate those individuals infested with the parasite A. squalicola.

4.5.1 *Conclusions*

Length-at-maturity was estimated to be at 69 cm, irrespective of whether or not the onset of maturity was considered to start at stage 2 or stage 3.

Uterus width was largest in stage 4 fish and decreased in stage 6, suggesting uterine development during egg case development and uterine regression post-partum. Stage 6 fish also had the greatest variability in uterus width, with some likely to be stage 2. Therefore, uterus width may be a useful attribute in distinguishing between stage 2 and stage 6 fish.

The onset of oviducal gland development and follicle development may be different. Oviducal gland size is probably not a good indicator of maturity due to variability in size over all stages.

Follicle size alone may be insufficient in describing reproductive processes occurring particularly in stage 6 fish.

Additionally, the presence of parasitic barnacles (*A. squalicola*; Appendix C.2) did not appear to have any effects on the reproductive biology of *A. ampliceps*.

4.6 Apristurus exsanguis

A. exsanguis were caught throughout the Chatham Rise, with Stage 2 – 6 females (n = 7) principally taken from the eastern Chatham Rise, whereas a predominantly stage 3 male sample (n = 8) was principally taken from the western Chatham Rise (Figure 4.6- 1). The male data were not analysed since this study focuses on female reproduction.

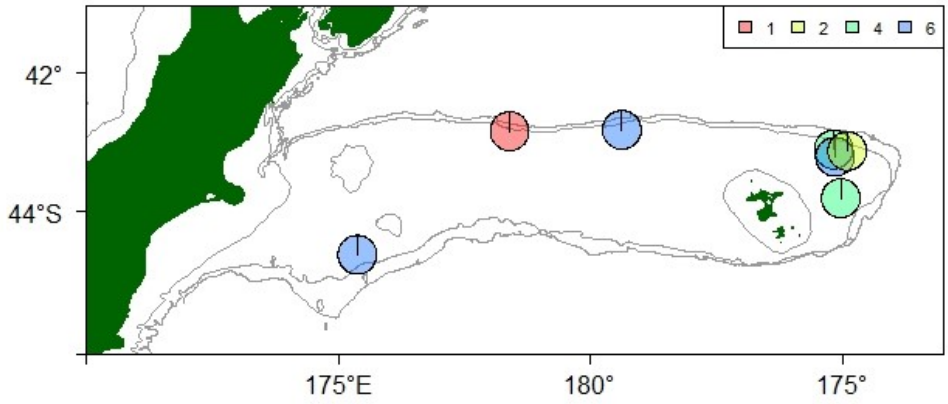

Figure 4.6- 1 A. exsanguis: Chatham Rise area with 200 m, 1000 m and 1200 m contours (grey), showing sample distribution for A. exsanguis females (n = 15). Pie charts denote the proportion of each macroscopic stage (colours for each macroscopic stage defined in legend) caught at each tow where samples were collected. Pie chart area – sample sizes collected relative to other tows.

Females ranged in total length (TL) from 57 cm to 76 cm, with a median length of 68 cm and a median weight of 1101 g (Figure 4.6- 2). Males ranged in total length (TL) from 52 cm to 87.5 cm, with a median length of 82 cm and a median weight of 1506 g (Figure 4.6- 2).

A. exsanguis met the ANOVA and Tukey HSD test assumptions, and the analyses indicated there were no statistically significant differences in mean fish size between macroscopic stages (ANOVA p = 0.207 for length; 0.406 for weight).

When maturity was considered to start at stage 2, 50 % of females were predicted to be mature at 60 cm (95 % CI = 10 – 64 cm) (Figure 4.6- 3). When maturity was considered to start at stage 3, maturity was estimated at 62.5 cm (95 % CI = 10 – 66.3 cm) (Figure 4.6- 3).

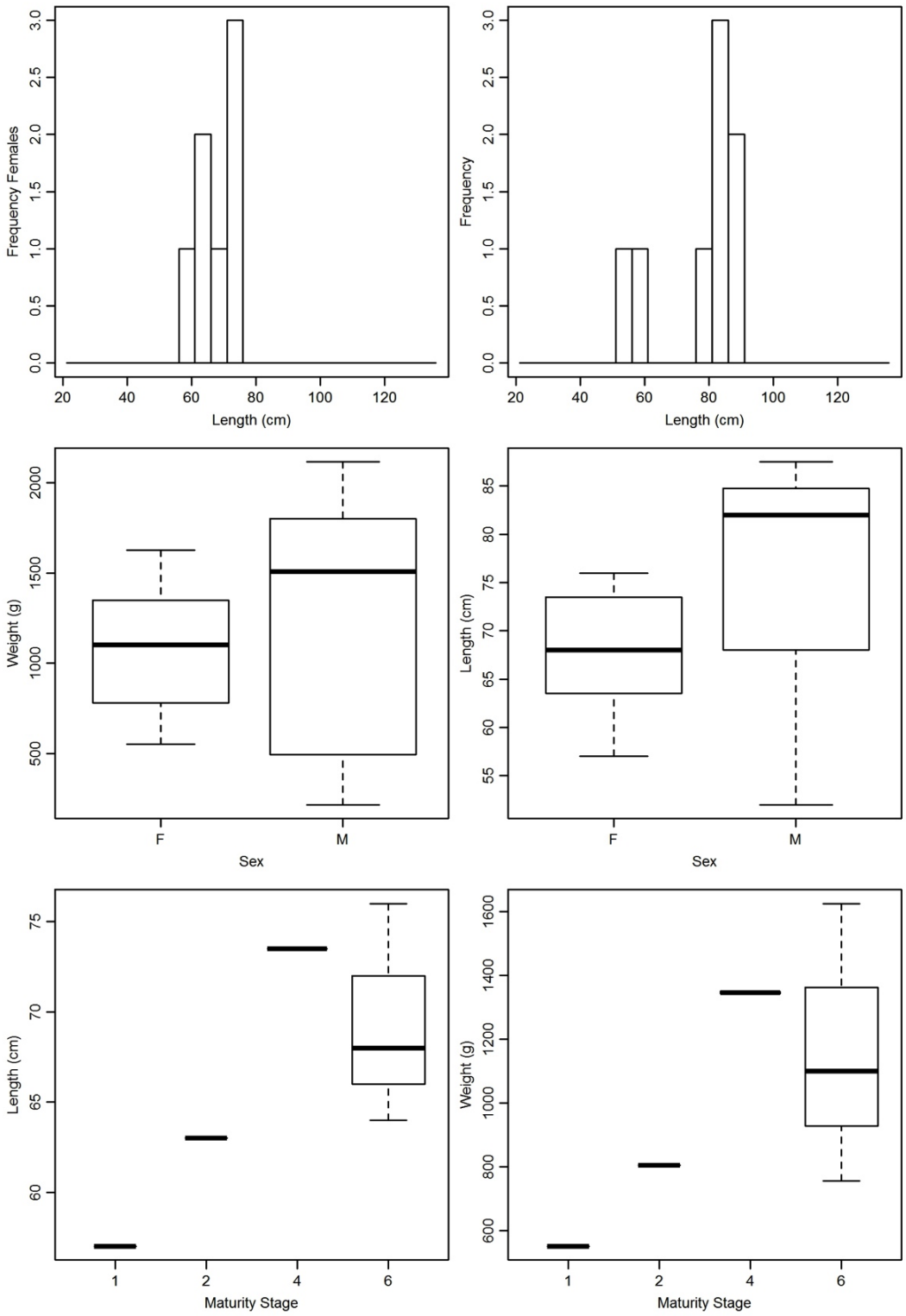

Figure 4.6- 2 A. exsanguis: Total length (TL) frequency for A. exsanguis (n=15) within the sample from the Chatham Rise, with females (n= 7; top left panel) and males (n=8; top right); Weight and length distributions for males and females (middle panels), where the darkest black line represents the median; Length and weight distributions across females in relation to macroscopic maturity staging (bottom panels).

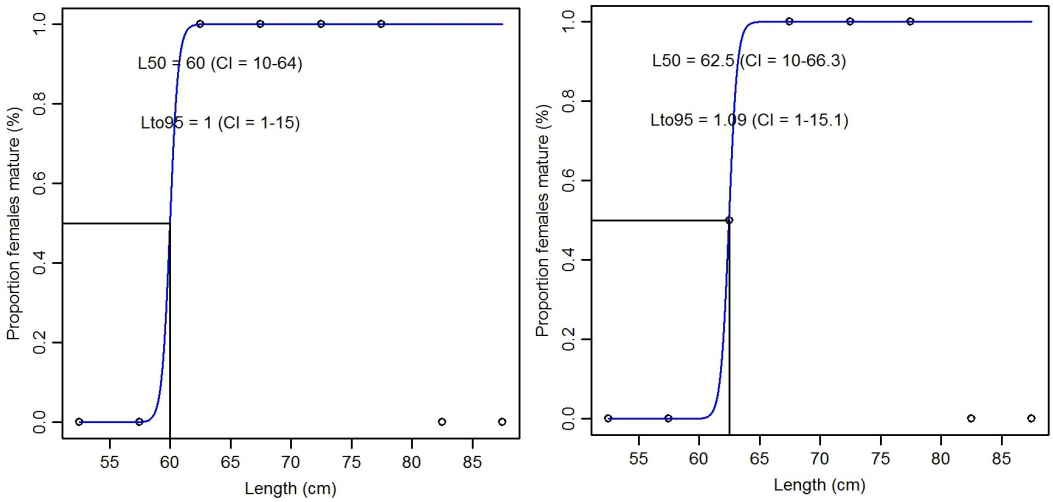

Figure 4.6- 3 A. exsanguis: Estimated length-at-maturity for A. exsanguis females on Chatham Rise (TAN1601 survey), where macroscopic maturity is considered to start at left: stage 2 (n = 6); right: stage 3 (n = 5). Points were proportion mature in 5 cm length bins. Line fitted is the maturity ogive. The black lines indicate the L50 value. The 95% confidence intervals were estimated by bootstrapping.

On average, the left oviducal gland was longer and the right wider, indicating some asymmetry in oviducal gland shape, despite average area being similar (Figure 4.6- 4). Oviducal gland area appeared to increase with fish size, gonad weight and follicle size (up to approximately 2 cm in diameter), and appeared to asymptote at approximately 8 cm^2 when follicle size was greater than 2 cm in diameter and uterus width was up to 1.5 cm (Figure 4.6- 5; Figure 4.6- 6). When average oviducal gland area was approximately 8 cm^2, uterus width variability increased (Figure 4.6- 6). Oviducal gland area also increased with follicle number (Figure 4.6- 6).

Stage 6 appeared to be split into two distinct groups, where 1) oviducal size was large (area ~ 8 cm^2) and 2) oviducal gland area was approximately 3 cm^2 (Figure 4.6- 6).

Follicle size increased when gonad weight was greater than 60 g (Figure 4.6- 7). Two of the three stage 6 fish had follicles less than 1 cm in diameter, consistent with the criterion for assigning macroscopic stage 2 in the NIWA macroscopic staging key (Figure 4.6- 7). The single stage 2 fish had follicles smaller than 1 cm in diameter, demonstrating accurate use of the macroscopic staging scale. The largest follicles were found in stage 4 fish (Figure 4.6- 7).

Follicle size variability was greatest when uteri were approximately 0.5 cm (Figure 4.6- 7). Stage 2 and 6 fish had uteri of comparable widths, although one stage 6 had follicles larger than 1 cm (Figure 4.6- 7). One stage 4 fish had a uterus width similar to the stage 2 fish, although follicle size was up to 4 times larger (Figure 4.6- 7).

Gonad weight, uterus width and follicle size variability increased in fish greater than approximately 70 cm TL (Figure 4.6- 8). Follicle number varied greatly at lengths greater than 65 cm, with a maximum of 24 (Figure 4.6- 7; Figure 4.6- 8). Follicle number increased with uterus width, up to approximately 1 cm (Figure 4.6- 7). As follicle number decreased to around 15 follicles, uterus width continued to increase (Figure 4.6- 7).

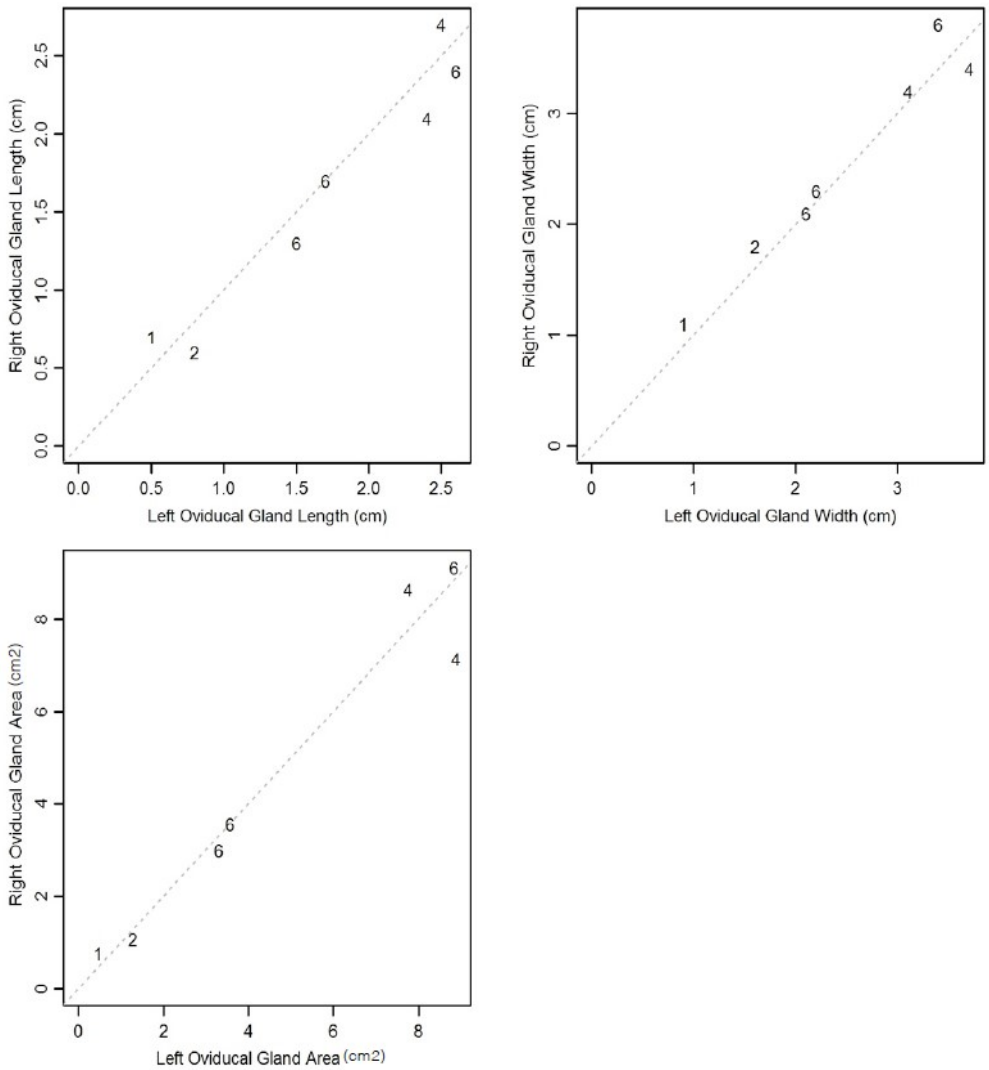

Figure 4.6- 4 A. exsanguis: Relationships between left and right oviducal gland lengths (top left), widths (top right) and areas (bottom left). The dashed grey lines represent the 1:1 relationship. Numbers represent the macroscopic staging assigned to each individual in the sample.

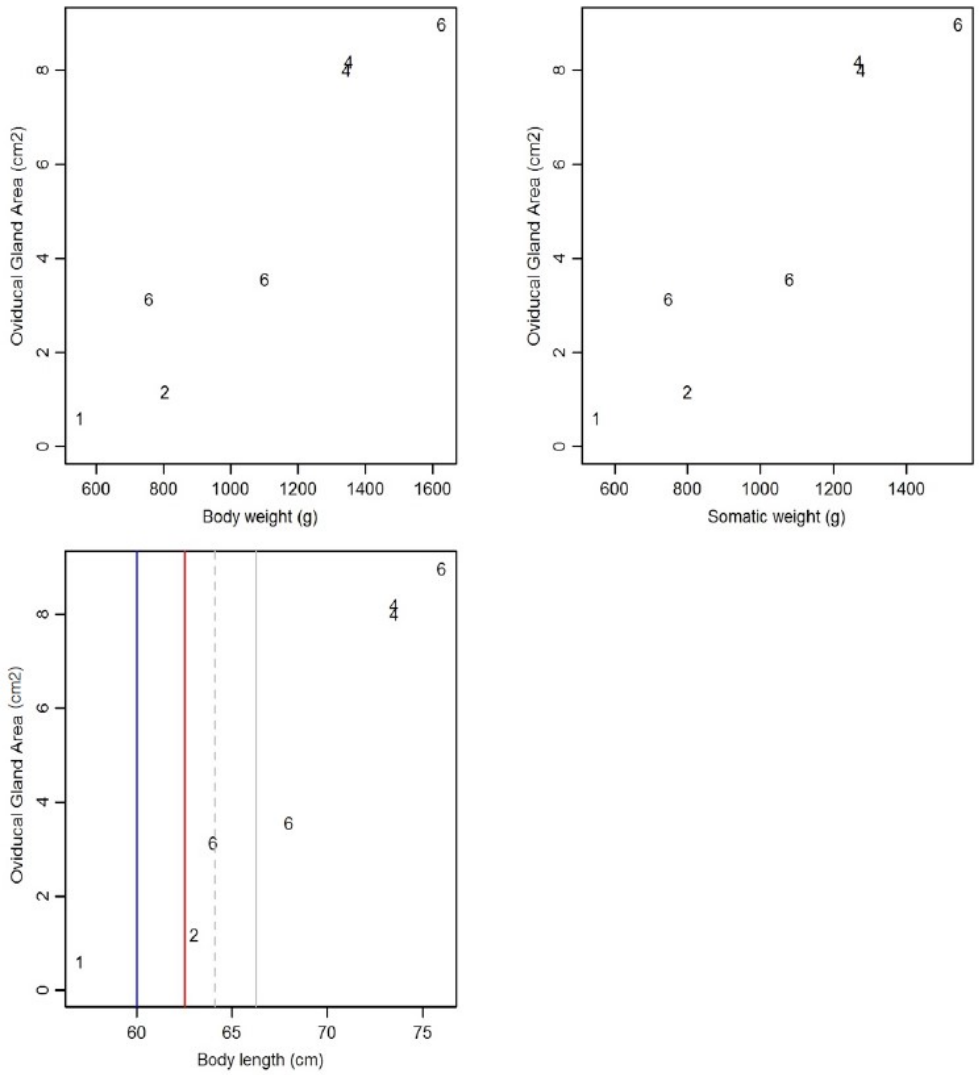

Figure 4.6- 5 A. exsanguis: The relationships between average oviducal gland area (cm^2) and body weight, somatic weight and length (clockwise from top left). The numbers denote the macroscopic maturity stages assigned to each individual. Lines represent length-at-maturity estimates for maturity occurring at stage 2 (blue) and at stage 3 (red). Grey lines represent the 95 % confidence intervals (Figure 4.6- 3).

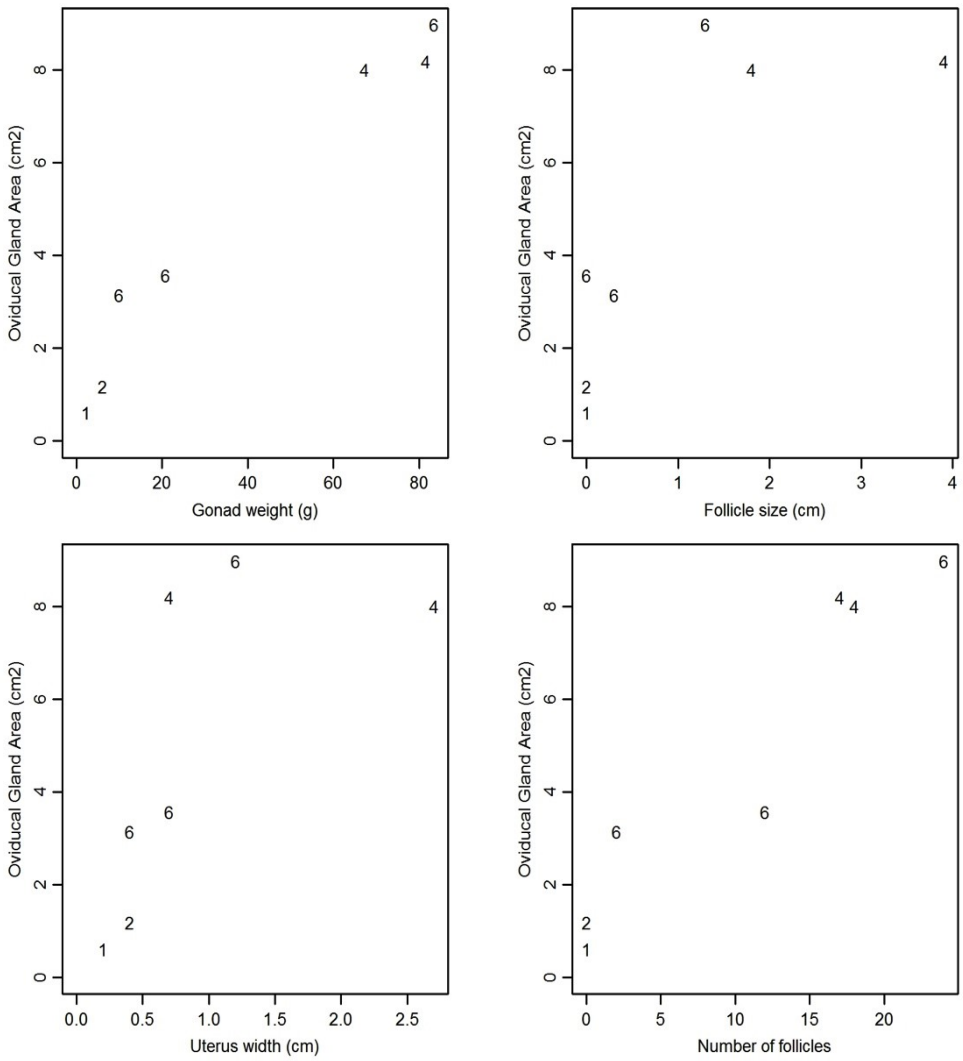

Figure 4.6- 6 A. exsanguis: The relationships between average oviducal gland area and gonad weight, follicle size, number of follicles and uterus width (clockwise from top left). The numbers denote the macroscopic maturity stages assigned to each individual.

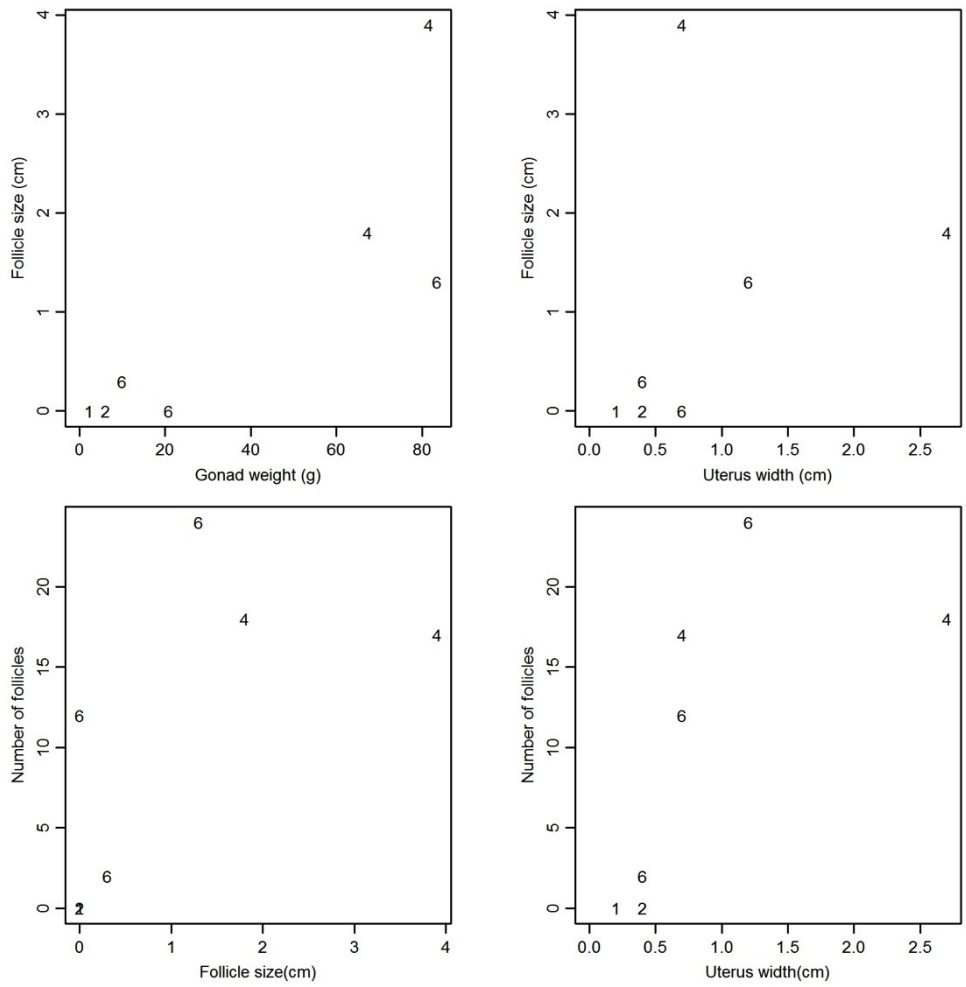

Figure 4.6- 7 A. exsanguis: Relationships between follicle size, gonad weight, uterus width and number of follicles. The numbers denote the macroscopic maturity stages assigned to each individual.

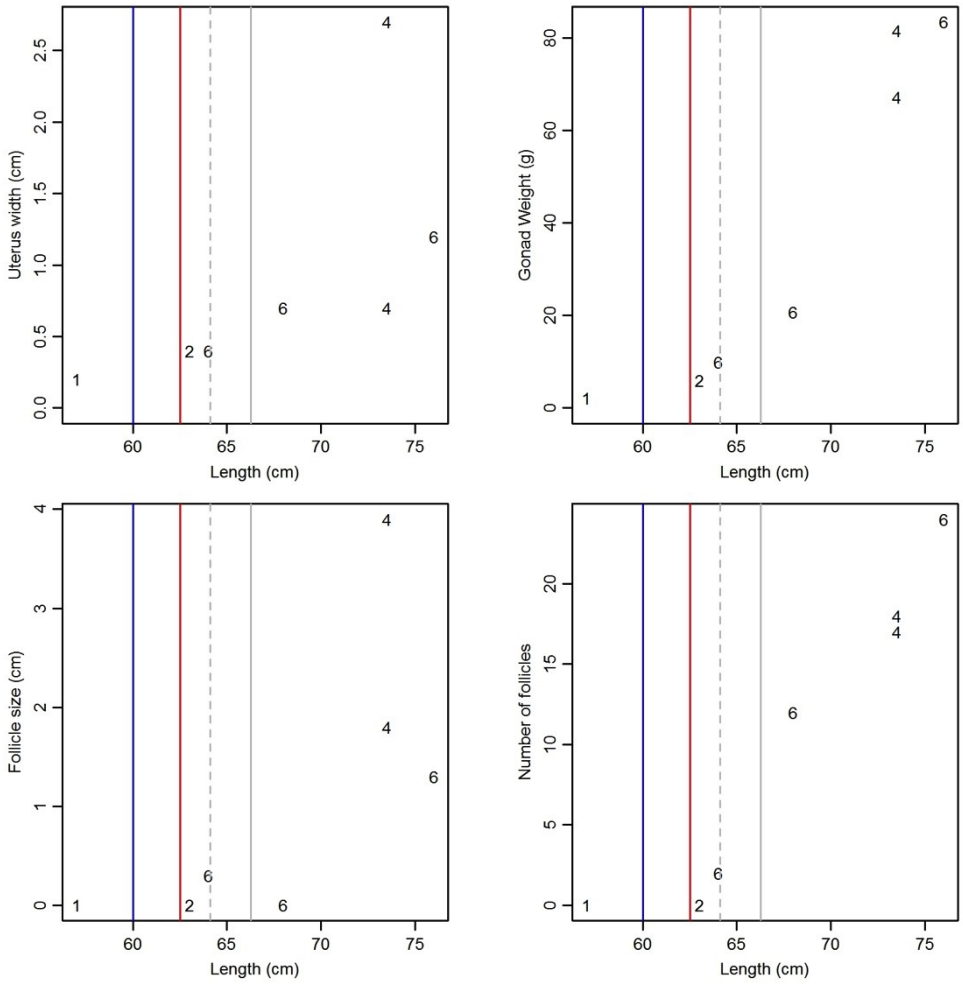

Figure 4.6- 8 A. exsanguis: Relationships between length and uterus width, gonad weight, number of follicles and follicle size (clockwise from top left). The numbers denote the macroscopic maturity stages assigned to each individual. Lines represent length-at-maturity estimates for maturity occurring at stage 2 (blue) and at stage 3 (red). Grey lines represent the 95 % confidence intervals (Figure 4.6- 3).

4.6.1 *Conclusions*

Gonad weight, uterus width, follicle size, follicle number and oviducal gland area, all showed step changes (or transitions) at around the fish length consistent with maturity (as measured at stage 3 of the macroscopic scale).

Length-at-maturity was estimated at between 60 cm (stage 2 onset) and 62.5 cm (stage 3 onset).

Uterus width was largest in stage 4 fish and decreased in stage 6, suggesting uterine development coincides with egg case development, and that uterine regression post-partum may confound stages 2 and 6. Therefore, uterus width may be a useful attribute in distinguishing between stage 2 and stage 6 fish.

Oviducal gland varied in size in fish of similar size and follicle development. Therefore, oviducal gland size is probably not a good indicator of maturity due to variability in size particularly in stage 6.

Follicle size alone may be insufficient in describing reproductive processes occurring particularly in stage 2 and stage 6 fish.

4.7 Apristurus melanoasper

A. melanoasper (n = 9 for females; n = 45 for males) were principally caught on the southwestern Chatham Rise (Figure 4.7- 1). The sample comprised of stage 3 – 6 females, stage 3 males and a single stage 1. The male data were not analysed since this study focuses on female reproduction.

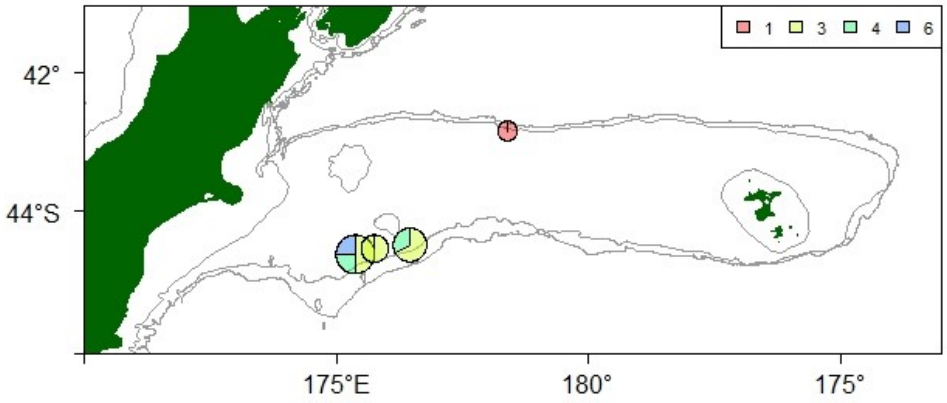

Figure 4.7- 1 A. melanoasper: Chatham Rise area with 200 m, 1000 m and 1200 m contours (grey), showing sample distribution for A. melanoasper females (n = 9). Pie charts denote the proportion of each macroscopic stage (colours for each macroscopic stage defined in legend) caught at each tow where samples were collected. Pie chart area – sample sizes collected relative to other tows.

Females ranged in total length (TL) from 37 cm to 72 cm, with a median length of 68.5 cm and a median weight of 1061 g (Figure 4.7- 2). Males ranged in total length (TL) from 43 cm to 81.5 cm, with a median length of 76.75 cm and a median weight of 1367 g (Figure 4.7- 2).

A. melanoasper met ANOVA and Tukey HSD test assumptions and the analyses indicated statistically significant differences in mean size across macroscopic stages (ANOVA $p < 0.05$ for both length and weight) (Figure 4.7- 2). Specifically there were significant mean size differences between stage 1 and stages 3, 4 and 6 (Tukey HSD $p < 0.05$ for both length and weight for all pairwise comparisons). Differences in mean length were not statistically significant between stages 3, 4 and 6 (Tukey HSD $p = 0.973$ (Stage 3 and 4); $p = 0.942$ (Stage 3 and 6); $p = 0.996$ (stage 4 and 6)), nor were they for mean weight differences (Tukey HSD $p = 0.877$ (Stage 3 and 4); $p = 0.924$ (Stage 3 and 6); $p = 0.999$ (stage 4 and 6)).

Results for length-at-maturity and length-weight relationships were not estimated because of the small mature sample size (n = 10). Despite the small sample size,

the data suggested that maturity-at-length was estimated between 40 and 70 cm regardless of whether maturity was considered to start at stage 2 or stage 3.

On average, the left oviducal gland was larger than the right (Figure 4.7- 3). Oviducal gland area variability increased at lengths greater than 65 cm (Figure 4.7- 4). Average oviducal gland area appeared to increase with gonad weight, follicle size, follicle number and uterus widths greater than 0.5 cm (Figure 4.7- 5). At sizes greater than 4 cm^2, uterus width variability increased and oviducal gland area was largest when follicles were greater than 1.5 cm in diameter (Figure 4.7- 5).

Follicle size increased when gonad weight was greater than 40 g (Figure 4.7- 6). There was some overlap between adjacent stages, although all fish had follicles greater than 1 cm, bar the stage 1 fish (Figure 4.7- 6). The largest follicles were found in stage 3 and 4 fish (Figure 4.7- 6).

Gonad weight, follicle size, follicle number and uterus width variability increased in fish greater than approximately 65 cm TL (Figure 4.7- 7).

Follicle size variability increased when uterus width was approximately 0.5 cm, although once uterus width was greater than 1 cm, variability decreased and follicles remained at approximately 2 cm in diameter (Figure 4.7- 6). The largest uterus width was in a stage 4 fish, approximately 1.5 cm larger than the stage 6 fish (Figure 4.7- 6).

At follicle sizes greater than 1 cm in diameter, follicle number increased to a maximum of 20 (Figure 4.7- 6). The majority of fish had follicle counts of between 7 and 13 (Figure 4.7- 6).

Follicle number appeared to increase with uterus width, up to approximately 1.5 cm (Figure 4.7- 6). As follicle number decreased to around 13 follicles, uterus width continued to increase (Figure 4.7- 6).

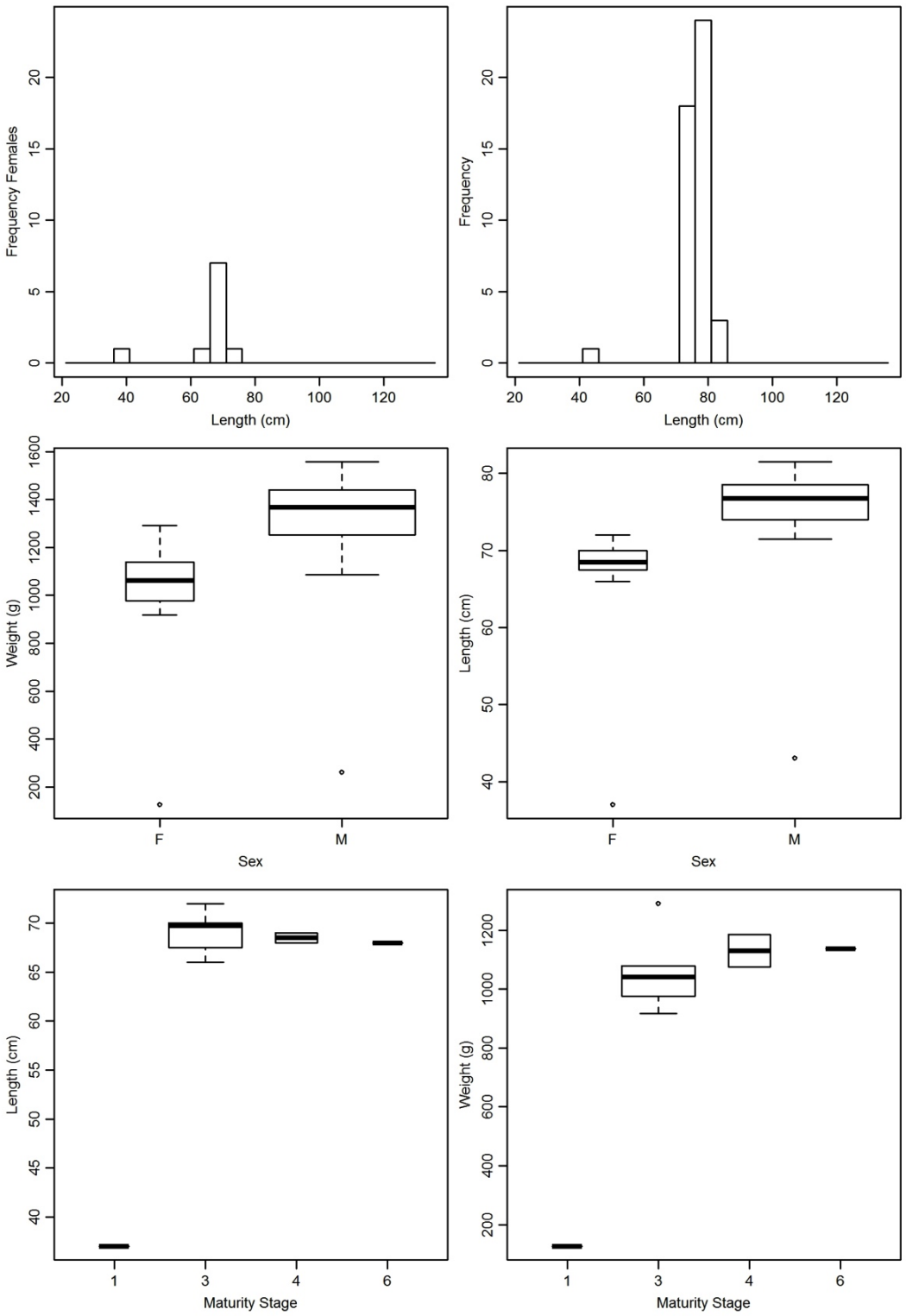

Figure 4.7- 2 A. melanoasper: Total length (TL) frequency for A. melanoasper (n=56) within the sample from the Chatham Rise, with females (n= 10; top left panel) and males (n=46; top right); Weight and length distributions for males and females (middle panels), where the darkest black line represents the median; Length and weight distributions across females in relation to macroscopic maturity staging (bottom panels).

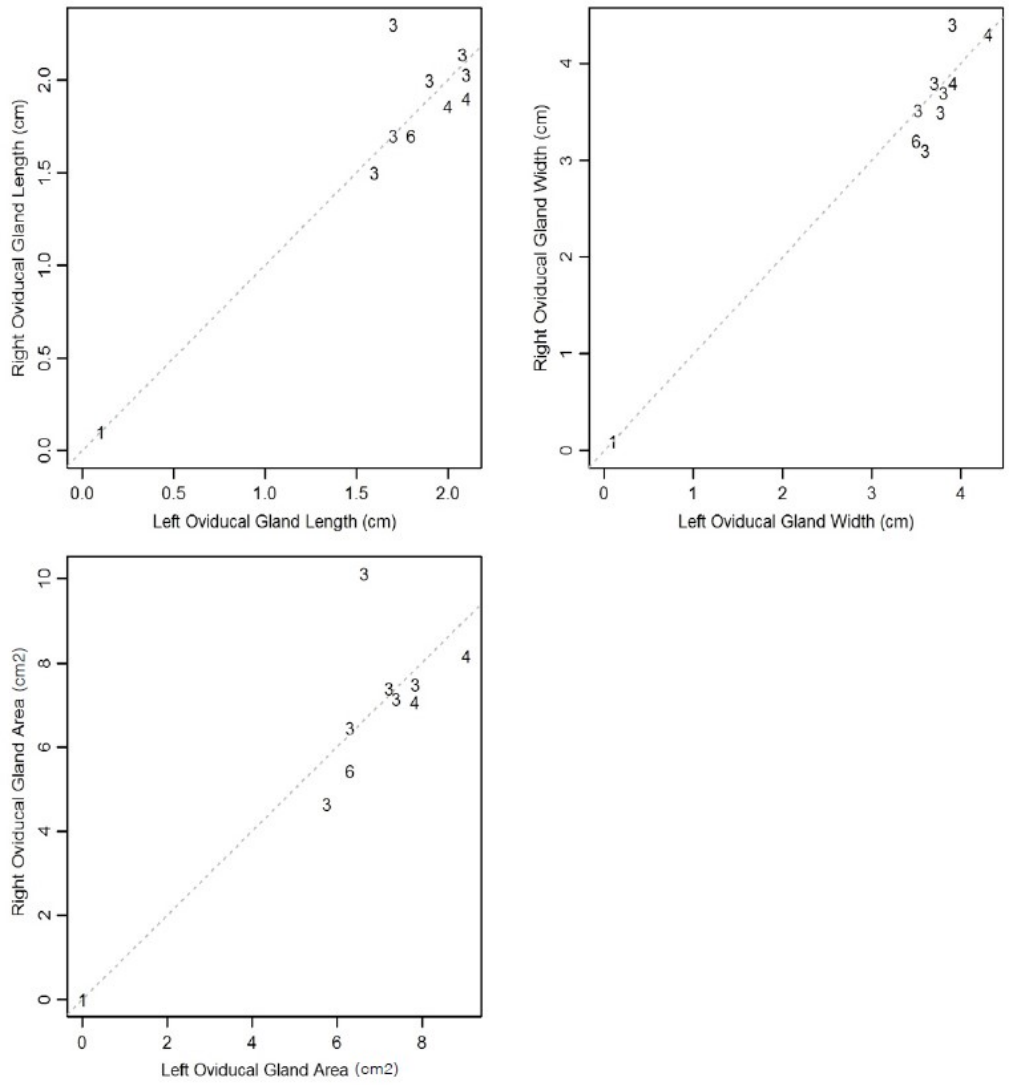

Figure 4.7- 3 A. melanoasper: Relationships between left and right oviducal gland lengths (top left), widths (top right) and areas (bottom left). The dashed grey lines represent the 1:1 relationship. Numbers represent the macroscopic staging assigned to each individual in the sample.

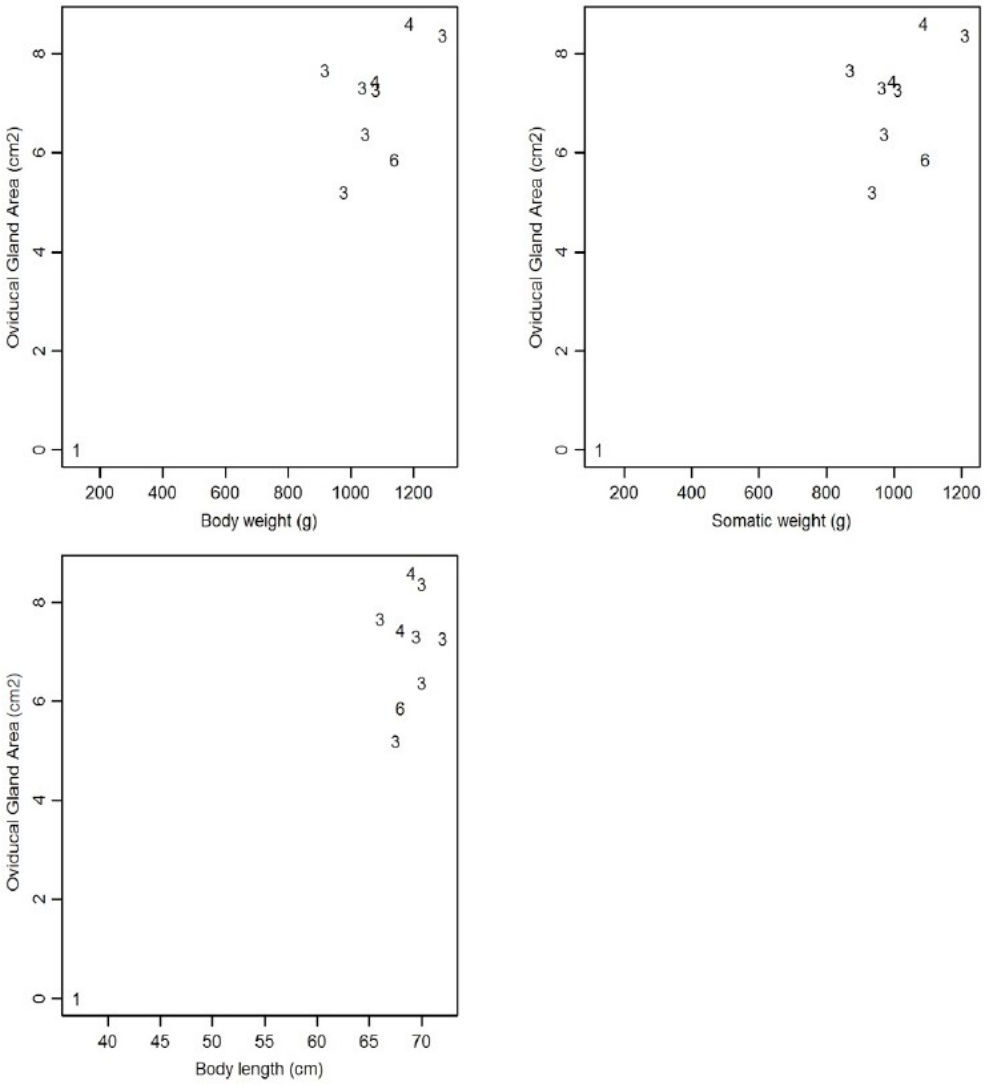

Figure 4.7- 4 A. melanoasper: The relationships between average oviducal gland area (cm^2) and body weight, somatic weight and length (clockwise from top left). The numbers denote the macroscopic maturity stages assigned to each individual.

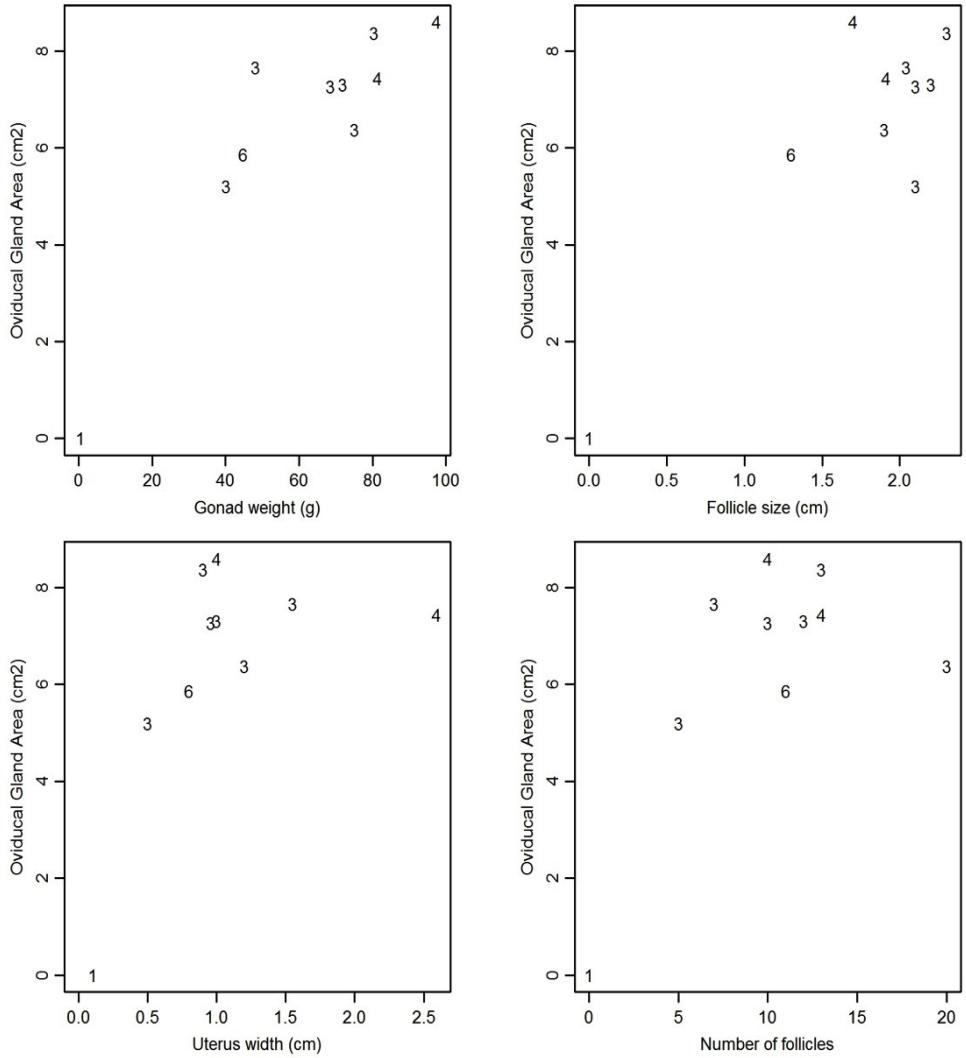

Figure 4.7- 5 A. melanoasper: The relationships between average oviducal gland area and gonad weight, follicle size, number of follicles and uterus width (clockwise from top left). The numbers denote the macroscopic maturity stages assigned to each individual.

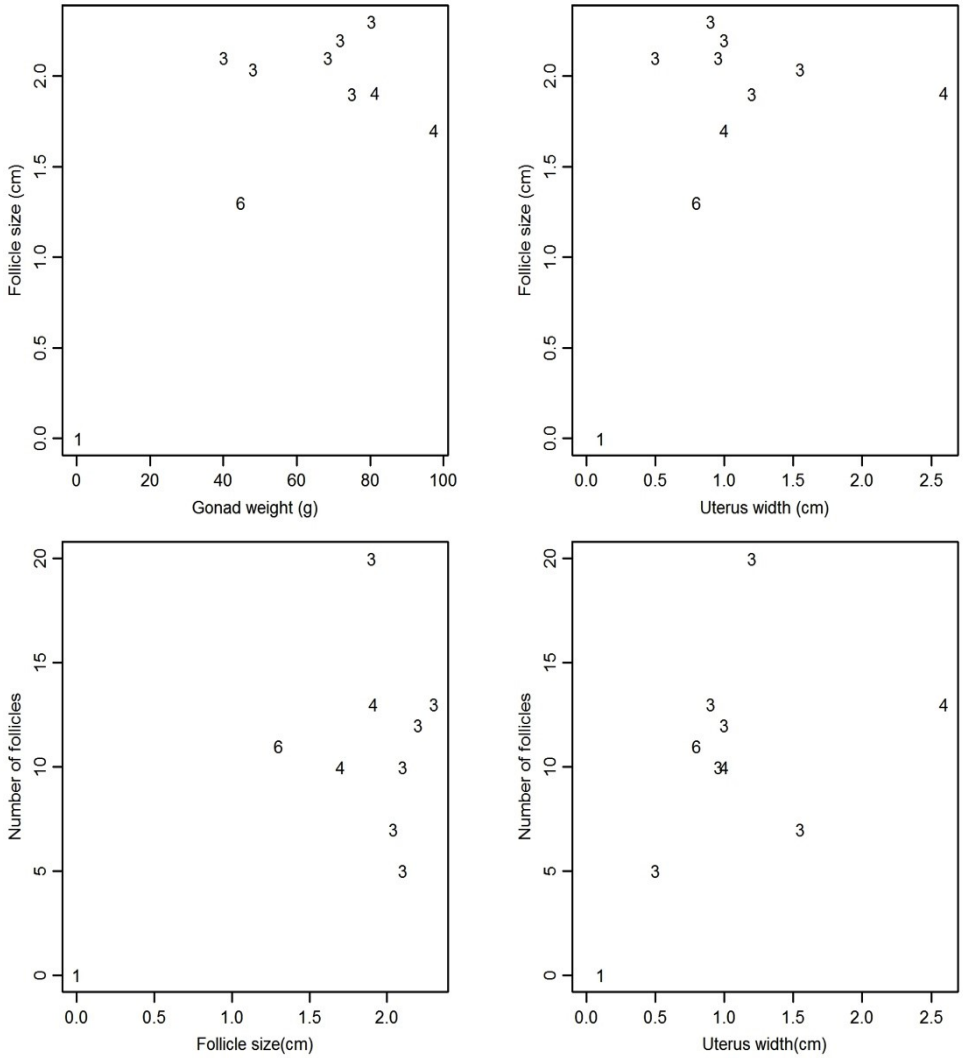

Figure 4.7- 6 A. melanoasper: Relationships between follicle size, gonad weight, uterus width and number of follicles. The numbers denote the macroscopic maturity stages assigned to each individual.

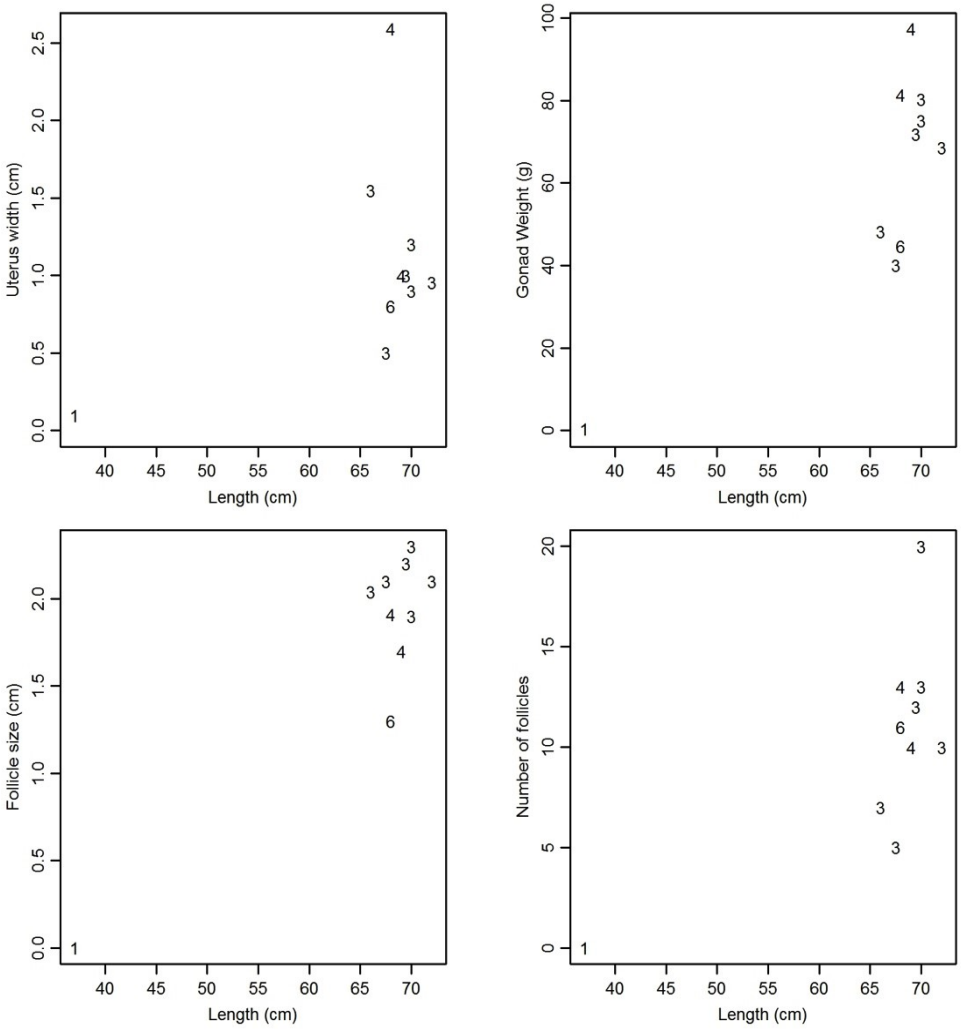

Figure 4.7- 7 A. melanoasper: Relationships between length and uterus width, gonad weight, number of follicles and follicle size (clockwise from top left). The numbers denote the macroscopic maturity stages assigned to each individual.

4.7.1 *Conclusions*

There was only one juvenile in the sample, and the patterns observed were similar for all mature fish.

Gonad weight, uterus width, follicle size, follicle number and oviducal gland area, all showed step changes (or transitions) at around the fish length consistent with maturity (as measured at stage 3 of the macroscopic scale).

Length-at-maturity was estimated to be between 40 and 70 cm, irrespective of whether maturity onset was considered at stages 2 or 3.

Uterus width was largest in stage 4 fish and decreased in stage 6, suggesting uterine development coincides with egg case development and that uterine regression post-partum may confound stages 2 and 6. Therefore, uterus width may be a useful attribute in distinguishing between stage 2 and stage 6 fish.

Oviducal gland varied in size in fish of similar size and follicle development. The onset of oviducal gland development and follicle development may be different. Oviducal gland size is probably not a good indicator of maturity due to variability in size over all stages.

Follicle size alone may be insufficient in describing reproductive processes occurring particularly in stage 3 and stage 6 fish.

Chapter 5 Discussion

Reproductive biology was assessed for thirteen deep-sea elasmobranch species: three viviparous species (*C. crepidater, C. squamosus* and *D. calcea)*, five deep-sea catsharks from the *Apristurus* genus, and five deep-sea skate species. Additionally, this study is the first species specific data for *Apristurus* spp. on Chatham Rise (McMillan et al., 2011; Roberts et al., 2015). A total of 102 deep-sea catsharks from *Apristurus* spp. were identified as five different species: *A. ampliceps, A. exsanguis, A. garricki, A. melanoasper, and A. sinensis*. A total of 28 deep-sea batoids were identified as: *B. asperula, B. leviveneta, B. spinifera, A. hyperborea, and B. shuntovi.*

Sperm storage was confirmed in three of the nine species examined histologically: *C. crepidater, C. squamosus* and *B. asperula.* The onset of maturity was determined to occur in stage 3 fish, but was also observed in some stage 2 fish. Differences between mature and immature fish were identified through histological confirmation of sperm storage and uterus and follicle measurements. The results demonstrated that although some characteristics gave some indication of the onset of maturity (i.e. follicle size), no attributes should be considered alone, supporting the recommendation in Finucci et al. (2017) that the use of multiple measurements should result in better maturity assessment.

Overall, length was a better indicator of fish size than weight, since weight measurements in this study were whole weight (i.e. not gutted weight) and may not be truly representative of fish size. Based on observations made in the field and in the laboratory, gut contents varied greatly. Since gut content influences body weight and somatic weight (body weight minus gonad weight) and since gutted weight was not recorded, length should be a more accurate and representative measure of fish size – particularly for stage 3 fish, where gonad weight formed a greater proportion of body weight than at other stages. In oviparous species where male data was available, males were on average larger than females, consistent with such sexual dimorphism already demonstrated in

deep-sea catsharks, notably *Apristurus* spp. (Flammang et al., 2008; Kyne and Simpfendorfer, 2007).

There were a number of stage 3 fish that fit well with the underlying length-weight curves seen for stage 2 fish, suggesting these fish may have a growth form more similar to that seen in stage 2 fish (including those exhibiting sperm storage) than more mature (4 +) fish. This may indicate these fish may have either: 1) been assigned incorrect macroscopic maturity stages, 2) development of the reproductive tract in fish that demonstrate sperm storage may be more consistent with assumed juvenile growth, 3) be reproducing for the first time, or 4) be about to ovulate. The last hypothesis (4) suggests the underlying length-weight curve of all fish remains constant regardless of macroscopic stage, but the variation observed, particularly in stage 3 fish, may simply be due to increases in gonad weight.

This research has not only allowed for a better understanding of the biology of a diverse range of deep-sea species throughout Chatham Rise and the Sub-Antarctic region, it is also the first to describe the distribution of members of *Apristurus* spp. in these areas (McMillan et al., 2011; Roberts et al., 2015). There was some distributional overlap between *A. ampliceps* and *A. melanoasper*, which were both predominantly distributed in the southwestern Chatham Rise. *A. exsanguis* has the broadest distribution, being taken throughout Chatham Rise. Overall, the majority of samples were collected east of the Chatham Islands. *A. garricki* were predominantly located in the northeastern Chatham Rise, with some distributional overlap with *A. melanoasper*. Depth distribution was not analysed here, therefore it remains plausible that although some species are similarly distributed geographically, there may be some level of depth distribution. The diverse morphology of egg cases was also described in Appendix C.1. Additionally, the first observation of infestation of the parasitic *A. squalicola* barnacle on *A. ampliceps* was recorded in Appendix C.2.

5.1 Evaluating maturity

Maturity is currently assumed to be represented as abrupt changes in length across a range of biological parameters (Finucci et al., 2017). Abrupt changes in length across different parameters measured here (oviducal gland size, follicle size, uterus width, gonad weight, follicle number) were recorded for all species and compared with length-at-maturity estimates (Table 5.1- 1). Across all species (and all biological parameters), abrupt changes in length were found to differ from estimated length-at-maturity, which suggests the development of some reproductive structures is uncoupled and may not correspond to those structures used in the rubric for macroscopic maturity assignment (Table 5.1- 1).

Table 5.1- 1: Abrupt changes in fish length using different biological parameters.

	LENGTH OF ABRUPT CHANGE					
SPECIES	**LENGTH-AT-MATURITY**	**GONAD WEIGHT**	**UTERUS WIDTH**	**FOLLICLE SIZE**	**FOLLICLE NUMBER**	**OVIDUCAL GLAND AREA**
C. crepidater	65 CM (STAGE 2) 82.5 CM (STAGE 3)	**~ 80 CM**	**~ 75 CM**	**~ 75 CM**	**~ 75 CM**	**~ 75 CM**
D. calcea	81 CM (STAGE 2) 108 CM (STAGE 3)	**~ 105 CM**	**~ 105 CM**	**~ 105 CM**	**~ 95 CM**	**~ 105 CM**
C. squamosus	~ 100 CM (STAGE 2) ~ 120 CM (STAGE 3)	**~ 130 CM**	**~ 130 CM**	**~ 130 CM**	**~ 130 CM**	**~ 130 CM**
A. ampliceps	~ 69 CM (STAGE 2) ~ 69 CM (STAGE 3)	**~ 75 CM**	**N/A**	**~ 75 CM**	**N/A**	**N/A**
A. exsanguis	60 CM (STAGE 2) 62.5 CM (STAGE 3)	**~ 74 CM**	**~ 74 CM**	**~ 74 CM**	**~ 69 CM**	**~ 74 CM**
A. garricki	49 CM (STAGE 2) 63.2 CM (STAGE 3)	**~ 65 CM**	**~ 55 CM**	**~ 65 CM**	**~ 65 CM**	**~ 65 CM**
A. melanoasper	~ 40 – 70 CM	**~ 70 CM**	**~ 70 CM**	**~ 70 CM**	**~ 70 CM**	**~ 70 CM**

5.2 Structures used to evaluate maturity

This research used objective biological measures (oviducal gland size, follicle size, follicle number, uterus width and gonad weight) to assess the quality of the NIWA macroscopic staging key in determining the onset of maturity and in distinguishing between macroscopic stages. Histological observations, notably the presence of spermatozoa in sperm-storage tubules in the terminal zone of the oviducal gland, were also used to inform the quality of the NIWA staging key in assessing maturity.

5.2.1 *Oviducal gland*

Oviducal gland development was not coupled with follicle development, especially in *C. crepidater* and *D. calcea*. Oviducal gland development has been found to be coincidental with follicle development in a variety of oviparous species including *H. ocellatum*, *R. erinacea*, *L. ocellata*, *A. radiata* and *C. laticeps* (Awruch, 2013). It has been suggested that if both are developing at similar rates, they should give a good indication of maturity (Awruch, 2013). Although the sample sizes here were small, this study found no clear correlations between oviducal gland size and follicle size, predominantly due to the high variability in size across stages, regardless of follicle development and in agreement with (Flammang et al., 2008); oviducal gland was not considered a good indicator of maturity.

Across all species, the data showed asymmetry between the left and right oviducal glands, in terms of shape and size. Therefore, oviducal gland area may provide a more accurate representation of oviducal gland size than average oviducal gland width or length. However, most studies only measure oviducal gland width. No differences in width were observed between the right and left oviducal glands of *Chimaera carophila* and *Hydrolagus homonycteris*, and only width was utilised in analyses (Finucci et al., 2017). There was no clear justification why width was chosen over length. McCully et al. (2012) and ICES (2017a, 2013, 2010) highlighted the value of oviducal gland width in validating macroscopic maturity assessment. Barnett et al. (2009), Finucci et al. (2017) and

King and McPhie (2015) also concluded oviducal gland width was a promising indicator of maturity, in conjunction with gonad mass. Serra-Pereira et al. (2011b) found oviducal gland size (width, height and wall thickness) was statistically different between maturity stages: the smallest in developing females and the largest in the spawning stage – where macroscopic oviducal gland development was completed. Moura et al. (2011) found oviducal glands in *Centroscymnus coelolepis* became distinguishable when females were developing. Once maturity was attained, oviducal glands were found to greatly increase in volume and attain maximum size before ovulation began; post-ovulation, oviducal glands regressed to the diameter they were in maturing fish (Moura et al., 2011). Again, only oviducal gland width was used in these studies.

The oviducal glands of batoids and *Apristurus* spp. tended to be larger in macroscopically mature fish, and the majority of oviducal glands measured in oviparous species were large and considered developed (Serra-Pereira et al., 2011a). In viviparous species, few oviducal glands were developed; a majority of which were undetectable. Oviducal gland regression post-ovulation may explain why some stage 3 and stage 4 fish, especially *C. crepidater,* had undetectable oviducal glands. The oviducal glands of these fish may have completely regressed back to 'maturing' sizes and are ready to re-expand once ovulation recommences. It is possible that the oviducal gland enlargement which occurs when viviparous females move from developing or regenerating (post-partum) stages to mature stages, is due to an increase in the production of secretory products in oviducal gland tubules, as observed in Moura et al. (2011).

Oviducal gland size varied greatly across stages with drastically different follicle development, and may change throughout the reproductive cycle; therefore the applicability of oviducal gland size, as a key attribute in determining maturity and its use as a validation tool, is questionable. Macroscopic definitions for oviducal gland development use vague terminology (i.e. 'oviducal glands were enlarged or developing'), making the interpretation of oviducal gland development both difficult and subjective (Bromley, 2003; Brown-Peterson et al., 2011; Gracan et al., 2013; Hilge, 1977; ICES, 2017a, 2017b; Kjesbu, 2009;

Lowerre-Barbieri et al., 2011; Sieiro et al., 2014; Storrie et al., 2008). Oviducal gland development can only be confirmed through histology, a relatively expensive method rarely used for maturity assessment in chondrichthyans, particularly on surveys and commercial sampling (Brown-Peterson et al., 2011; Finucci et al., 2017, 2016; ICES, 2017b; Kyne and Simpfendorfer, 2007). Here, the presence of egg jelly, tertiary egg casing and other secretory materials accumulated in oviducal gland tubules and in the lumen, was prevalent in mature (stage 3 +) fish, as well as in some stage 2 fish – suggesting maturity in those stage 2 fish.

5.2.2 *Sperm storage*

Sperm storage was observed for the first time in the terminal zone of the oviducal glands of *C. crepidater, C. squamosus* and *B. asperula*. Sperm storage in chondrichthyans typically occurs in the terminal zone of the oviducal gland and has been confirmed in other Squaliformes, such as *C. coelolpis,* the first reported incident of sperm storage in this family (Finucci et al., 2017; Hamlett et al., 1998; Holt and Lloyd, 2010; Moura et al., 2011; Pratt Jr, 1979; Serra-Pereira et al., 2011a; Storrie et al., 2008). This research is, to the best of our knowledge, the first to describe sperm storage in the order *Arhynchobatidae.*

Although sperm storage was only confirmed in three of the nine species examined histologically in this study, sperm storage has been confirmed and is considered ubiquitous throughout chondrichthyans, with widespread observations reported in females in all orders of holocephalans and elasmobranchs (Carrier et al., 2012). Long-term sperm storage is considered an advantageous and opportunistic strategy used (particularly in deep-sea species), allowing for fertilization to occur even when females are not ovulating at the time of insemination (Bernal et al., 2015; Storrie et al., 2008). Chondrichthyans are thought to have particularly long sperm storage durations, with several chondrichthyan species known to store spermatozoa for months or even years (Bernal et al., 2015; Holt and Lloyd, 2010; Moura et al., 2011; Storrie et al., 2008). The only substantive estimates for the duration of sperm storage have been made for *M. antarcticus* at 13 months and at greater than 2 years for

Scyliorhinus rotifer (Bernal et al., 2015; Holt and Lloyd, 2010; Moura et al., 2011; Storrie et al., 2008). Sperm storage is thought to be a particularly important strategy for oviparous species where the timing between mating and ovulation is often decoupled (Awruch, 2013).

There are many hypotheses as to why females store spermatozoa in their reproductive tracts, including (but not limited to): 1) allowing for spermatozoa to be used for fertilisation after mating due to an asynchrony between mating events and fertilisation, varying anywhere from hours in most mammals, up to 60 days in some marine birds and up to several years in some reptiles and insects (Holt and Lloyd, 2010; Storrie et al., 2008), 2) in social systems where males and females are largely solitary, sperm storage is obviously advantageous, since insemination could occur at any point (regardless of ovulation) and still result in fertilisation (Holt and Lloyd, 2010; Storrie et al., 2008), 3) genetic and reproductive advantages including females having access to spermatozoa from multiple males (e.g. the coupling of sperm storage with female cryptic choice - maximising genetic quality of offspring whilst also ensuing maximum number of offspring. Some turtles are able to select which cohort of spermatozoa is to be used for subsequent fertilisation) (Holt and Lloyd, 2010; Storrie et al., 2008) and 4) guaranteed reproductive success, ensuring optimisation times for copulation, fertilization and parturition (Figueiredo et al., 2008; Moura et al., 2011; Storrie et al., 2008). However, the mechanisms allowing for such a strategy to evolve and be so successful, as well as the benefits of such a strategy, remain unknown despite the wide research on this topic (Holt and Lloyd, 2010). Caution is needed when comparing physiologies of different taxonomic groups, despite results being similar, since the mechanisms through which sperm storage has arisen and those involved may be quite different (Holt and Lloyd, 2010).

Several *C. crepidater* stage 2 ('maturing', i.e. not mature) females exhibited sperm storage, suggesting at least behavioural maturity since copulation had occurred. Storrie et al. (2008) reported the presence of spermatozoa in maturing and immature *M. antarcticus*, although not in the sperm-storage tubules of the oviducal glands, but rather, in the vaginal sphincter and body of

the uterus. Females with observable spermatozoa in the reproductive tract could be considered behaviourally mature, but not yet functionally mature (where follicles develop and ovulate). However, it was not determined whether the presence of spermatozoa throughout the reproductive tract was associated with recent mating events, or whether they may eventually be stored in the sperm-storage tubules of the oviducal glands (Storrie et al., 2008). The sections examined in this thesis suggest the sperm-storage tubules in *B. asperula* might form through the budding off of sections of lamellae lining the lumen of the terminal zone, once spermatozoa aggregated inside.

Histology indicated that spermatozoa were not always found in clear bundles, nor in any other distinctive forms of organisation, rather spermatozoa appeared to be evenly dispersed throughout sperm-storage tubules. Bundled spermatozoa were only observed in a single *C. crepidater* female. Differences in spermatozoon arrangement may be an indicator for storage duration, since it has been hypothesised that the matrix produced in the formation of sperm bundles (or spermatozeugmata) may increase the lifespan of spermatozoa by acting as a protective layer (Holt and Lloyd, 2010; Moura et al., 2011). Hypothetically, spermatozoa may enter sperm-storage tubules and disperse evenly throughout the tubule, eventually forming a bundle; or vice versa (Holt and Lloyd, 2010; Moura et al., 2011). Commonly in chondrichthyans, spermatozoa are organised into bundles, where spermatozoa adhere to one another within a sticky matrix and only the tails of peripheral spermatozoa protrude (Holt and Lloyd, 2010). Other forms of organisation include single layers of radially aligned spermatozoon clumps (i.e. *Carcharhinus plumbeus*) and several layers of spermatozoon clumps (i.e. *Sphyrna lewini*) (Holt and Lloyd, 2010; Moura et al., 2011).

5.2.3 *Gonad weight*

Gonad weight had a direct correlation with follicle size and maximum follicle size was variable across species; therefore, absolute gonad weight was highly variable both within and across macroscopic maturity stages and species. The variability across species was inferred to be a consequence of relative fish size,

as well as reproductive strategy. Gonad weight was comparatively larger in viviparous fish, since they require more reproductive investment to produce offspring (Irvine et al., 2012; Kyne and Simpfendorfer, 2007). Since *C. squamosus* are considerably larger animals than other viviparous deep-sea dogfish and oviparous *Apristurus* spp. studied here, it was determined that relative gonad size, (Gonadosomatic Index (GSI) values) may be a more compelling indicator of fish maturity than either gonad weight or follicle size. GSI is a relative measure, allowing for high variability in follicle sizes across species, where gonad mass is represented as a proportion of total body weight ((gonad weight / body weight) x 100).

GSI is a common metric of reproductive allocation and condition in fisheries biology, although it assumes a close relationship between gonad mass and gonad developmental stages, as well as a isometry between somatic weight and gonad weight (Flores et al., 2014; Zeyl et al., 2013). Although GSI provides a more accurate representation of gonad size across species of different body sizes, GSI differs with reproductive strategy (Flores et al., 2014; Zeyl et al., 2013). Maximum GSI for oviparous species in this study was approximately 6 %, and approximately 13 % for viviparous species, consistent with GSI values of 12.6 % for *Oxynotus brunensis* (Finucci et al., 2016).

GSI can help distinguish between gross reproductive processes (i.e. vitellogenesis) when there is considerable gonad growth at a particular developmental stage (Flores et al., 2014; Zeyl et al., 2013). GSI may also be useful in distinguishing between immature and mature fish, rather than differences between specific stages (Zeyl et al., 2013). Furthermore, GSI has been found to change seasonally, since GSI increases when fish ovulation commences and subsequently decreases post-partum (Zeyl et al., 2013).

5.2.4 *Follicle size*

Since follicle size progressively increased with macroscopic maturity, it was considered a good indicator of maturity. However, the relationship between follicle size and uterus width indicated that follicle size alone was insufficient in

determining processes occurring in the reproductive tract, but rather one component of many which indicated maturity (e.g. uterus expansion, production of tertiary egg casing or jelly in the oviducal gland and the presence of sperm storage).

The criteria for macroscopic stages in the NIWA key suggests the criteria for stages 2 and 3 are solely based on follicle size, but give no definitive measurements, which, in this instance, opens the rubric of maturity to subjective interpretation. The criteria for assessing stage 2 describe follicles as being *"up to about pea-sized or larger…"* and *"greater than pea-sized…"* for stage 3 (Francis & Lyon, NIWA, pers. comm.). Here, 'pea-sized' was assumed to mean approximately 1 cm in diameter. The results demonstrated the quality of this rubric in practice, with the majority of stage 2 individuals sitting below 1 cm follicle size. However, the grouping of stage 2 fish with follicles less than 1 cm in diameter may simply be an artefact of the macroscopic staging criteria, where follicle size thresholds may be arbitrary. Various studies utilise different follicle size thresholds to distinguish between immature (i.e. stage 2) and mature (i.e. stage 3) fish, as well as between oviparous and viviparous species (McCully et al., 2012; Moura et al., 2011).

The results also indicated follicles may asymptote at their maximum size, which was estimated for *C. crepidater* and *C. squamosus* at approximately 6 cm in diameter. Although estimates for maximum follicle size could not be estimated for other species (due to small sample sizes), the data suggested that maximum follicle size in oviparous species was, on average, smaller than that of viviparous species (Irvine et al., 2012; Kyne and Simpfendorfer, 2007).

The ovaries of gravid (stage 4), post-partum (stage 6) and some stage 2 females lacked follicles or had atresic follicles, demonstrating that the reproductive cycle may be non-continuous, since it assumes a period of inactivity in follicle development between pregnancies (Finucci et al., 2016; Irvine et al., 2012; Kyne and Simpfendorfer, 2010; Rigby and Simpfendorfer, 2015). The presence of small follicles and enlarged uteri in post-partum and resting females also provides some evidence for non-continuous reproductive cycles, which are

considered typical of deep-sea chondrichthyans (Finucci et al., 2016; Irvine et al., 2012; Kyne and Simpfendorfer, 2010; Rigby and Simpfendorfer, 2015). For two species with the same fecundity and lifetime reproductive output, non-continuous reproducers will presumably have shorter gestation periods than those with continuous cycles of follicle development.

Currently, follicle size measures maturity based on the most developed, largest ova in the ovary, rather than the proportion of developed follicles relative to those showing no signs of development (i.e. follicles lacking yolk) (Brown-Peterson et al., 2011; Finucci et al., 2017, 2016; ICES, 2017a). As a consequence of this, the number of follicles present and the size of the largest follicle may not accurately represent actual ovarian processes; therefore, relative measures of egg size to egg number and body size, may be required to assess and compare different species – in which maximum egg sizes are inconsistent. Berrigan (1991) observed different relationships between egg volume and egg number in three taxa of insect, concluding that although larger insects absolutely lay larger eggs, they are proportionally smaller than for smaller insects. Egg volume and body size were strongly correlated for *Hymenoptera* and *Coleoptera*, in which individuals consistently had large eggs, whereas in *Diptera*, the relationship demonstrated negative allometry – indicating that *Diptera* could either have many small eggs, or few large ones, at the same body sizes (Berrigan, 1991). Consideration of all follicles present and their relative size may be required to compare reproductive output in other taxonomic groups, including chondrichthyans.

5.2.5 *Uterus width*

Presently, uterine development is not considered as part of the macroscopic NIWA staging key for stages 1 through 3. Additionally, the uterine processes considered noteworthy for stages 4 and 5 simply highlight the presence of ova in the uterus and the development of embryos or egg cases – processes that occur in the uterus and do not necessarily reflect uterine development, but rather relate to follicle development in the uterus. Stage 6 is the sole stage where the uterus is considered a key attribute in identifying post-partum fish.

The results concluded that uterus width might be a useful attribute in identifying 'immature' and 'mature' fish, particularly in differentiating between stage 2 'maturing' and stage 2 'resting' fish. Uterus width was highly variable in stage 2, indicating the presence of two homogeneous groups: some fish with small uteri (similar to stage 1 fish) and others with similar (or larger) uteri to stage 3 and stage 4 fish. All fish exhibiting sperm storage (including stage 2 fish considered to be at least biologically, if not functionally mature) had uteri wider than 0.48 cm. Those stage 2 fish exhibiting sperm storage should not be grouped with their immature (maturing) counterparts, as they are likely a part of the reproductive population.

A cyclical relationship between follicle size and uterus width was suggested. Follicle and uterine sizes were negligible when fish were immature (stage 1) and developing (stage 2, maturing). Follicle size then increased with uterus width as fish matured (stage 3), up to the point where follicles entered the uterus (stage 4) and embryos (stage 5) or egg cases developed (stage 4 for oviparous species). Once parturition was complete, follicle size decreased rapidly, whereas the uteri remained expanded, indicating post-partum (stage 6) fish. It is possible that the uteri then regress throughout the resting period, until ovulation recommences and the uteri re-expand to accommodate fertilised ova.

The cyclical nature of the uterus width-follicle size relationship also suggested that when development was synchronous, uterus development occurred with pup development. Conversely, when asynchronous, the onset of uterus development occurred before the onset of follicle development; thus, the uteri prepare for the movement of ova into the uteri after fertilisation. It is hypothesised that expansion of the uterus prior to ova entry into the uterus may allow the uterus to accommodate large ova, which – if entering the uterus without prior expansion – could cause rupturing of the uterine wall (Hamlett and Hysell, 1998). In some post-partum fish, uteri remain distended until the fish enters the 'resting' phase (Serra-Pereira et al., 2011b). Oviparous species demonstrated more synchronous uterine and follicle development, since stage 4 fish with partially developed egg cases were observed with wider uteri

anteriorly (where the uterus connects to the oviducal gland) than posteriorly, suggesting uterine expansion occurs as the egg case develops and moves further into the uterus. Viviparous species demonstrated more asynchronous development of the uteri and follicles, where uterus width was on average smaller in stage 4 fish than stage 3 fish and uterus width was found to be larger in some stage 2 individuals, specifically in *C. crepidater*. Stage 4 is the 'candle' phase (where there is a clear expectation of development within the uterus) and stage 3 is the 'ovulation' phase (where uterine development is not expected); therefore, a priori, stage 4 uteri are expected to be larger than that of stage 3 fish (Serra-Pereira et al., 2011b).

The uterine development and size differences between stage 2 'maturing' and stage 2 'mature, resting', indicated uterine regression post-partum. Uteri of all elasmobranchs display similar characteristics, regardless of reproductive strategy (Hamlett and Hysell, 1998). When gravid, uterine surface area increases (due to either increased vascularity, or to the development of uterine folds or villous extensions), in order to enhance gas exchange and provide embryos with higher oxygen levels, which generally leads to the thinning of the uterine epithelium (Hamlett and Hysell, 1998). Uterine regression occurs when the post-partum uterus returns to its pre-pregnant state and is well documented in vertebrates, particularly in mammals (Al-Bassam, 2009; Hsu et al., 2014). In humans, regression occurs rapidly during the first week post-partum and returns to its pre-pregnant state within six weeks (Al-Bassam, 2009). During pregnancy, the human uterus muscle fibres become ten times as long, and five times as broad than in resting or pre-pregnant uteri (Al-Bassam, 2009). However, despite returning to pre-pregnant sizes, there is evidence of increases in overall uterine weight post-partum (Hsu et al., 2014). In early pregnancy, more uterine muscle tissue is formed to accommodate for embryonic development (no myocyte apoptosis has been shown to occur post-partum), therefore uterine size or weight cannot regress completely to pre-pregnant values (Hsu et al., 2014). Uterine sizes in multiparous females are generally larger than in females reproducing for the first time (Hsu et al., 2014). Should no muscular apoptosis

occur post-partum in chondrichthyans, expansion of the uteri should lead to larger uterine sizes in multiparous chondrichthyans.

Although uterine regression has been proposed in chondrichthyans, there is contradiction in the literature (ICES, 2013). The results demonstrate that understanding the processes pertaining to uterine regression may provide critical information to fully understand chondrichthyan reproductive cycles, particularly during the 'resting' phase. However, the most recent ICES (2013) keys removed all mention of regression, as the working group found the term to be confusing. The report also noted that the 'resting' and 'regressing' phases are the least-adequately developed in macroscopic staging keys, due to the lack of information available (ICES, 2013).

Here, the results demonstrated the importance of understanding the level of uterine regression to help describe the key characteristics of fish that are capable to reproduce. Moreover, a better understanding of processes such as uterine regression may also enable differentiation of 'maturing' and 'mature, resting' fish, which are currently amalgamated into the same classification (stage 2) in the NIWA key. Separating 'mature, resting' fish from 'maturing' fish may also allow for more accurate biological parameters to be estimated, since the entire mature sample can be used in models. Currently, due to the grouping of 'maturing' and 'resting' fish, the latter are excluded from such models and assumed to be immature.

5.2.6 *Follicle number*

Follicle number was not well-correlated with other reproductive measures or across macroscopic stages; rather, patterns in follicle number appeared to be cyclical. Follicle number was found to: 1) increase with the onset of ovulation, 2) decrease post-mating and post-fertilisation and 3) return to zero developing follicles (some atresic follicles were present) as fish were post-partum and resting. Therefore, follicle number may not be an important attribute in determining maturity.

Follicle number has been used to provide estimates of fecundity (Kyne and Simpfendorfer, 2007). Where litter sizes are unavailable (as for the majority of species in this study, particularly *Apristurus* spp.), estimates of ovarian fecundity (the number of developed follicles in the ovaries) have been used to provide proxies of uterine fecundity (the number of fertilised eggs or developing embryos in the uterus) and reproductive output (Kyne and Simpfendorfer, 2007). Here, the results showed follicle number increased in stage 2 fish and decreased in stages 3 and 4. Therefore, follicle number may not provide accurate estimates of fecundity (particularly in stage 2 fish), since follicle number dropped substantially during later stages.

5.2.7 *Length-at-maturity*

The application of the NIWA staging key in estimating length-at-maturity determines that stage 3 is presumed the onset of maturity, and all stage 2 fish, including 'mature/resting' fish, are assumed to be immature (Finucci et al., 2017, 2016). Although the onset of maturity was found to occur in stage 3 fish, assuming all stage 2 fish are immature removes the 'mature/resting' females from the reproductive population, when estimating biological parameters such as length-at-maturity.

When estimating length-at-maturity (using the NIWA key) a 'gap' between the proposed onset of maturity (stage 2) and the development of follicles (stage 3) was observed. Such a 'gap' suggests there is a period of growth in stage 2 fish, where no reproductive development occurs. However, since the NIWA key only uses follicle size as the key attribute for stages 2 and 3, some reproductive development could be occurring in structures other than follicles, or in follicles smaller than 1 cm in diameter. In particular, it was found in *C. crepidater* that abrupt changes in length, across all biological parameters (bar gonad weight), did not correspond well with length-at-maturity estimates, since abrupt changes were observed to be approximately 10 cm larger (when 'mature' at stage 2) and 10 cm smaller (when 'mature' at stage 3) than the modelled estimates, suggesting over-or-underestimations of length-at-maturity (Table 5.1- 1).

Across all species, with the exception of *C. squamosus* and *A. melanoasper*, abrupt changes in length with uterus width occurred at lengths shorter than the estimated length-at-maturity (stage 3 onset) (Table 5.1- 1). Since uterus width is not a key attribute for determining macroscopic maturity in the NIWA key, length-at-maturity may be overestimated using current criteria for stage 3 maturity onset (as defined in the NIWA key); therefore, the key may not be accurately determining maturity. Conversely, Finucci et al. (2017), who also used the NIWA key to assess maturity in *Harriotta raleighana* and *Rhinochimaera pacifica*, found good correspondence between length-at-maturity estimates (stage 3 onset), oviducal gland and uterus width, suggesting accuracy in using a macroscopic key to determine length-at-maturity.

The inclusion of 'mature resting' fish in stage 2 (maturing/resting), may lead to an overestimation of length-at-maturity. When stage 3 is considered the onset of maturity, length-at-maturity estimates do not include all fully developed fish, since stage 2 resting fish are excluded and classified as stage 2 'maturing'. Consequentially, mature fish are grouped with maturing fish. Recently, several studies have combined maturing fish with later maturity stages when estimating length-at-maturity (McCully et al., 2012). Since these estimates include fish that are not fully developed in stages determined to be indicative of the onset of maturity, reported length-at-maturity estimates are much lower than earlier works. Staging keys like the NIWA scale impose the opposite problem and potentially overestimate length-at-maturity.

Length-at-maturity estimates are known to differ across studies (ICES, 2017a, 2017b, 2013; McCully et al., 2012). Temporal, spatial and other differences in methodology (i.e. fishing effort, gear type, maturity staging etc.) may explain comparative differences (ICES, 2017a, 2017b, 2013; McCully et al., 2012). Given the array of populations of the same species studied worldwide (e.g. *S. acanthias*), the presumptions that geography may present populations with different environmental stimuli (e.g. temperature, depth, currents, fishing effort etc.), and given the pertinent inconsistencies in maturity assessment keys, comparisons across populations should be avoided in order to reduce confusion

that arises when comparing studies with inconsistent methodologies (Kyne and Simpfendorfer, 2007). Despite this, ICES (2017a) compared length-at-maturity estimates for *S. acanthias* from two studies that used different keys and were conducted at different sites, concluding the true length-at-maturity estimate was an average of the two.

Some uncertainty remains as to whether differences in length-at-maturity for various species are attributed to real spatial or temporal differences; whether they simply reflect subtle differences in sampling (i.e. staging assessment, sample sizes), or whether overexploitation through fishing may be responsible (ICES, 2017a, 2017b, 2013; McCully et al., 2012).

5.3 Improving the macroscopic maturity key

The results of this study have successfully highlighted potential problems with the macroscopic staging criteria used in New Zealand. Given the observed cyclical relationship between follicle size and uterus width and the presence of sperm storage in some stage 2 fish, the results suggest interactions between different reproductive processes – the development of reproductive structures, when considered alone, may not indicate functional maturity. Since the NIWA macroscopic staging criteria does not include consideration for such interactions, it may not be accurately describing the processes occurring in the reproductive tract, particularly in stage 2 fish. Finucci et al. (2017) concluded that – given the overlap in maturity stages in measurements of oviducal gland, uterus width and GSI – no single measurement gives a clear-cut indicator of maturity.

As a consequence of these findings, an improved maturity key (Figure 5.2- 1) was developed in an attempt to define which characteristics best define maturity stages for each species group. The approach was consistent with ICES (2013) recommendations that two macroscopic staging keys (one for oviparous, one for viviparous species) should be developed to allow for differences in reproductive strategies and investment.

An improved macroscopic key was developed using key attributes identified in this study as being good indicators of maturity, with consideration of reproductive differences between viviparous and oviparous species (Figure 5.2-1). Stage 2 demonstrated two homogeneous groups, which implied the presence of both 'immature' and 'mature' stage 2 – consistent with the definition of stage 2 as either maturing (immature), or resting (mature). This study showed some fish, previously assigned stage 2, were very likely 'mature'. Some stage 2 fish had similar characteristics indicative of maturity, such as large uterus widths and the presence of sperm storage. For this reason, and since sperm storage is considered ubiquitous among deep-sea chondrichthyans, sperm storage was used to inform maturity.

The improved key (Figure 5.2- 1) aims to add objectivity to current macroscopic maturity criteria and to particularly assist in distinguishing between stage 2 maturing, stage 2 resting and stage 6 (post-partum) females, by examining the same key reproductive structures across all macroscopic stages.

Gonad weight was considered an important characteristic in distinguishing between immature and mature fish. GSI was found to provide a more objective and relative measure of gonad weight, removing some of the variability in gonad weight across species. GSI appeared to be the single distinguishing attribute that differed considerably between reproductive strategies (Irvine et al., 2012; Kyne and Simpfendorfer, 2007). However, GSI is best utilised when distinguishing between immature and mature fish, rather than between specific stages (Zeyl et al., 2013). No existing GSI thresholds were available for chondrichthyans. Since the maximum GSI score for oviparous species was approximately 6 %, the GSI threshold for mature fish was set at half of this (3 %). The GSI threshold for mature viviparous fish was set at 5 %, since it is expected that reproductive investment in viviparous species is higher than oviparous species. The GSI threshold for both immature oviparous and viviparous fish was set at 2 %. These thresholds are largely arbitrary and need further research and validation.

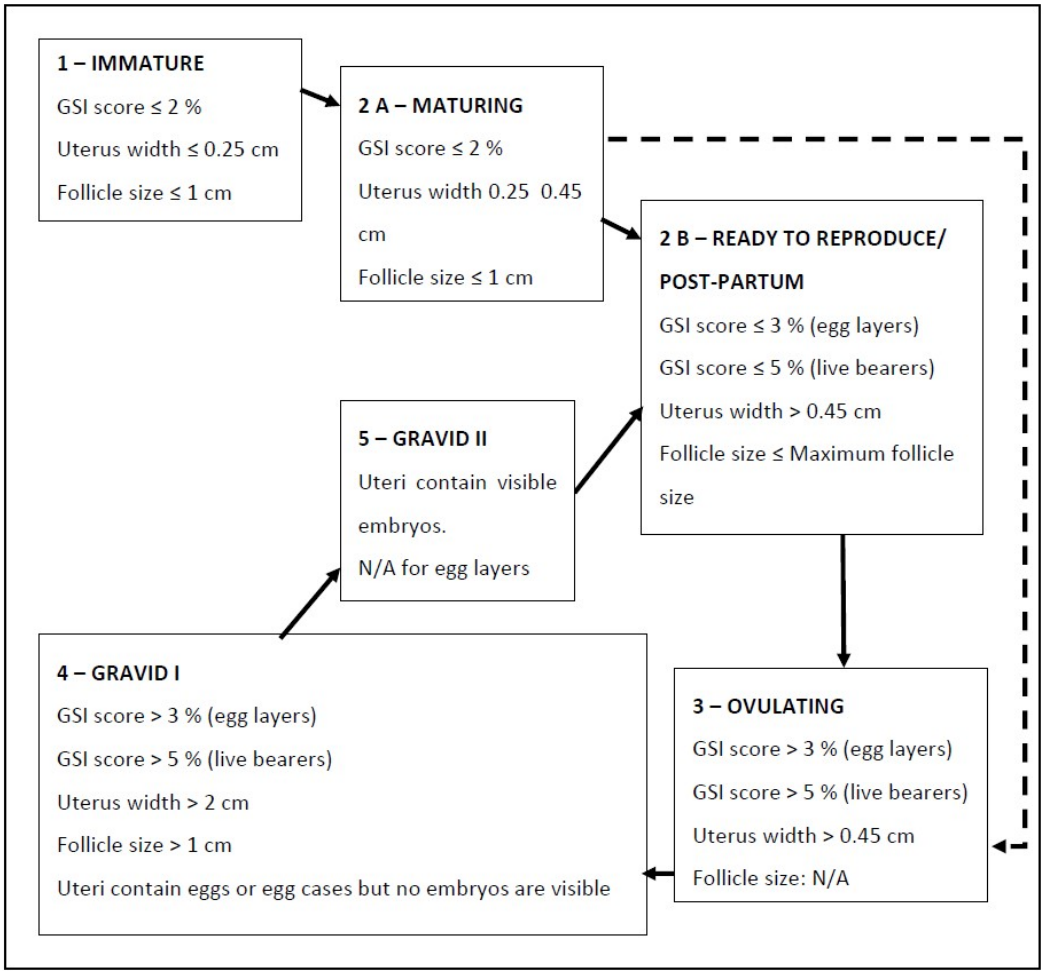

Figure 5.2- 1: The cyclical nature of the reproductive cycle and the relationship between each stage as defined in the improved key. The dotted line indicates the possibility that stage 2A progresses directly to stage 3 when first maturity is reached.

GSI was not as well-correlated with follicle size as gonad weight; therefore, follicle size was retained as a key attribute. Since follicle size was found to increase with uterus width in ovulating fish (NIWA stage 3), it was not included in the new key for stage 3 in order to remove an arbitrary threshold. The removal of the follicle size threshold for stage 3 also allows for gonad weight and uterus width to determine maturity. This is important across different reproductive strategies, where maximum follicle size can differ.

The relationship between follicle size and uterus width was apparent across all species; therefore, uterus was considered a key attribute, particularly in distinguishing between immature and mature stage 2 fish. The proposed key imposes a fairly arbitrary threshold to uterine development, set at 0.45 cm – the smallest uterus width for a fish exhibiting sperm storage (across all species in this study). Since there were differences in the onset of uterus enlargement

relating to reproductive strategy, the new key includes an additional characteristic for stage 4: the presence of eggs or egg casings in the uterus.

Some NIWA stage 3 fish had undetectable oviducal glands. Excluding oviducal gland size from the maturity rubric allowed maturity to be assigned based on the level of development of other reproductive structures. Should oviducal gland size be included in the key, such fish may be classified as being less mature, although other reproductive structures may indicate maturity. The removal of oviducal gland as a key attribute, as is seen in the ICES (2013) key, may, to some extent, remove the subjectivity involved in its inclusion – allowing for fish with small, undetectable oviducal glands and large well developed follicles (as in the NIWA and Stehmann (2002) keys (Table 5.2- 1)).

The improved key (Figure 5.2- 1) split NIWA stage 2 into two stages as indicative in the results: stage 2A for all stage 2 fish considered 'immature', and stage 2B, for all stage 2 fish considered 'mature'. Despite the small sample sizes, stage 6 fish shared similar characteristics with stage 2 fish considered 'mature': similar uterine widths and follicle sizes. Changes in the reproductive tract post-partum (stage 6), particularly in follicle size, may occur more rapidly than at other stages (i.e. large follicles are "used" in the production of offspring and therefore there are no developed follicles left post-partum). Uterus width may take longer to regress, since changes in uterus width are muscular (or structural); therefore, uterus width and follicle size appear to be similar in stage 2 'resting' and stage 6 fish. The Stehmann (2002) key highlights similarities between post-partum fish (stage G/7 (post-natal, spent)) and immature (stage A/1 (immature, juvenile)) or maturing (B/2 (maturing**,** adolescent)) fish (Table 5.2- 1). However, there are no criteria for resting fish in the Stehmann (2002) and ICES (2013) keys, suggesting either the inclusion of resting fish in another stage or their total exclusion (Table 5.2- 1). The key diagnostics for stage 2B are similar to those defined in stage G/7 of the Stehmann (2002) key (Table 5.2- 1). The thresholds set for key attributes as defined in the improved key were found to be similar in stage 6 and 'mature' stage 2 fish; therefore, these stages were combined into a single stage, stage 2B.

Since stage 2B fish were considered to be either functionally mature or behaviourally mature, this study considers these fish capable of reproduction, although some may have not reproduced for the first time, distinguishing these fish from 'maturing' fish (stage 2A – not yet biologically or functionally mature). To clarify the inclusion of those fish entering the reproductive cycle for the first time, as well as those that have already reproduced (i.e. post-partum/resting), stage 2B was labelled 'capable of reproduction'. However, it is possible the cycle goes from stage 2A directly through to stage 3, when reaching first maturity (Figure 5.2- 1). Stage 3 was changed from 'mature' to 'ovulating' since the results indicated all fish in this category were currently ovulating (i.e. follicles were developed), although fertilisation may have not yet occurred.

The key proposed here uses objective measures of maturity, rather than more subjective diagnostics used in existing macroscopic keys (Table 5.2- 1). The collection of additional biological data (uterus width, follicle size, follicle number, oviducal gland size (length and width) and gonad weight) could help inform and improve existing macroscopic maturity keys and establish more accurate thresholds for distinguishing between stages across a wide range of species. The use of objective macroscopic measures of maturity could also be used in conjunction with existing macroscopic maturity keys and may remove some researcher bias that is often associated with the use of more subjective keys, including: 1) the assignment of more 'mature' stages to larger fish, even though reproductive structures may not indicate maturity, or 2) the assignment of fish to grouped stages (e.g. NIWA stage 2) when maturity is difficult to assign, since these stages encompass both immature (maturing) and mature (resting) fish. Here, for example, fish with small follicles and larger uteri were more readily assigned to stage 2 in viviparous fish and stage 6 in oviparous fish.

The improved key was developed using limited data and small sample sizes. Further research and validation are required. If the improved key is to be considered adequate in determining maturity, the following hypotheses should be met: 1) fish size should increase with maturity stage (i.e. stage 2B fish should be on average larger than 2A), since larger fish are generally more 'mature', as is

expected in species with indeterminate growth (Charnov and Berrigan, 1991), 2) uterus width should be greater in more 'mature' fish (i.e. stages 2B, 3 and 4 compared to 1 and 2A), although some fish capable of reproduction (stage 2 B) may have uteri of a similar size to 'immature' fish (stage 2A), due to uterine regression between pregnancies, 3) follicles should be larger in more 'mature' fish (i.e. stages 2B, 3 and 4) – indicative of maturity, ovulation, or pregnancy, 4) follicle number variability should increase with 'maturity', since developing follicles undergo vitellogenesis asynchronously, with only some progressing through to fertilisation, 5) fish exhibiting sperm storage should be classified as 'mature', as copulation has already occurred, even though ovulation may have not yet occurred, 6) GSI values should be higher in ovulating fish, since GSI accounts for changes in gonad size relative to body size, which tend to be greater when follicles are developed during ovulation and 7) the improved key should provide more realistic length-at-maturity estimates, since all 'mature' fish would be included in the model (the onset of maturity considered to be at stage 2B).

Table 5.2- 1: Table of the key diagnostics for macroscopic maturity assessment using the proposed key, with comparison with three key maturity staging keys in literature: the NIWA key (Francis & Lyon, NIWA, pers. comm.), the ICES (2013) key and the Stehmann (2002) key.

Proposed Key	NIWA Key	ICES (2013) Key	Stehmann (2002) Key
1 – IMMATURE *GSI score* ≤ 2 % *Uterus width* ≤ 0.25 cm *Follicle size* ≤ 1 CM	**1 – IMMATURE** *Ovaries* small and undeveloped. Oocytes not visible, or small (pin-head sized) and translucent whitish	**1 - IMMATURE** *Ovaries:* small whitish, undistinguishable follicles *Oviducal gland:* not visible - some see thickening of uteri where glad will develop *Uteri:* thread-like, narrow	**A OR 1 - IMMATURE, JUVENILE** *Ovaries*: small, their internal structure gelatinous or granulated. No oocytes differentiated or all uniformly small, granular *Uteri*: narrow, thread-like
2 A – MATURING *GSI score* ≤ 2 % *Uterus width* 0.25 0.45 cm *Follicle size* ≤ 1 CM	**2 – MATURING/RESTING** Some oocytes enlarged, up to about pea-sized or larger, and white to cream	**2 - IMMATURE (DEVELOPING)** *Ovaries:* follicles different stages of development (small, med yolky) *Oviducal gland:* distinguishable and developing *Uteri:* enlarging	**B OR 2 - MATURING**, **ADOLESCENT** *Ovaries*: somewhat enlarged, walls more transparent. Oocytes becoming differentiated to various small sizes *Uteri:* largely as stage A/1 but may become widened posteriorly
2 B – READY TO REPRODUCE *GSI score* ≤ 3 % (oviparous) *GSI score* ≤ 5 % (viviparous) *Uterus width* > 0.45 cm *Follicle size* ≤ maximum follicle size	**N/A**	**N/A**	**N/A**

Proposed Key	NIWA Key	ICES (2013) Key	Stehmann (2002) Key
3 – OVULATING *GSI score* > 3 % (oviparous) *GSI score* > 5 % (viviparous) *Uterus width* > 0.45 cm *Follicle size* N/A	**3 – MATURE** Some oocytes large (greater than pea-sized) and yolky (bright yellow)	**3 - MATURE (CAPABLE TO REPRODUCE)** *Ovaries:* presence large yolked follicles ready to be ovulated *Oviducal gland:* fully developed *Uteri:* fully developed	**C OR 3 - MATURE ADULT** *Ovaries* large, well rounded. Oocytes obviously enlarged, all to about the same size, can easily be counted and measured Oviparous - *Uteri* enlarged and widening over nearly their entire length
4 – GRAVID I *GSI score* > 3 % (oviparous) *GSI score* > 5 % (viviparous) *Uterus width* > 2 cm *Follicle size* > 1 cm *Uteri* contain eggs or egg cases but no embryos are visible	**4 – GRAVID I** *Uteri* contain eggs or egg cases but no embryos are visible	**4A - MATERNAL (EARLY PREGNANCY)** *Ovaries:* not considered *Oviducal gland:* not considered *Uteri:* well filled and rounded with yolk content (candle shaped), embryos not observed	**D OR 4 – DEVELOPING OR ACTIVE** Viviparous – *uteri* well filled and rounded with seemingly unsegmented yolk content ("candle"). Oviparous – A distinctly large yolk-egg present in one or both Fallopian tubes. No egg capsule yet visible in shell gland, or beginning formation of egg capsule at most

Proposed Key	NIWA Key	ICES (2013) Key	Stehmann (2002) Key
N/A	**N/A**	**4B - MATERNAL (MID PREGNANCY)** *Ovaries:* not considered *Oviducal gland:* not considered *Uteri:* well filled and rounded, embryos always visible, small with large yolk sac	**E OR 5 – DIFFERENTIATING OR ADVANCED** Viviparous – *uteri* well filled and rounded with segmented content of large yolk balls, can easily be counted and measured. Embryos variously small, atop their huge yolk balls, larger ones with external gills filaments and unpigmented (still "candle") Oviparous - Large yolk-eggs in Fallopian tubes, or already passing through into egg capsules. Egg capsules about fully formed in one or both oviducts but still soft at upper end and located very close to Fallopian tubes

Proposed Key	NIWA Key	ICES (2013) Key	Stehmann (2002) Key
5 – GRAVID II *Uteri* contain visible embryos. N/A for oviparous	**5 – GRAVID II** *Uteri* contain visible embryos. N/A for egg layers	**4C - MATERNAL (LATE PREGNANCY)** *Ovaries*: not considered *Oviducal gland*: not considered *Uteri*: embryos fully formed, yolk sacs reduced/absent	**F OR 6 – EXPECTING OR EXTRUDING** Viviparous – embryos more or less fully formed, pigmented, external gill filaments lost, yolk sacs obviously reduced. Can be counted, measured and sexed easily Oviparous – Completed, hardened egg capsules in one or both oviducts, more or less separated from Fallopian tubes. Skate capsule surface mostly covered with dense silky fibres. Either no enlarged oocytes in Fallopian tubes, or one or two in position. If oviducts empty but still much enlarged and wide, capsules have probably just been extruded
STAGE REMOVED	**6 – POST-PARTUM** *Uteri* flaccid and vascularised indicating recent birth	**5 - MATERNAL (POST-PARTUM)** *Ovaries*: shrunken without follicle development and with atresic follicles *Oviducal gland*: not considered *Uteri*: enlarged and flaccid	**G OR 7 - POST-NATAL, SPENT** *Ovaries:* at resting stage, similar to stages A/1 or B/2 *Uteri:* empty but still widened considerably over their full length in contrast to stages A/1 or B/2

5.4 Ecological implications

Life history traits, including internal fertilisation, long gestation and late sexual maturity, make chondrichthyans more vulnerable to exploitation (i.e. overfishing) than teleosts (Moya et al., 2017). Sperm storage is known to directly influence a species' reproductive potential and constitutes an important input in the assessment of stocks, particularly for those species considered to be vulnerable to exploitation (Moura et al., 2011). Further data collection and understanding of different aspects of reproduction are critical in bettering the management of sustainable fisheries and conservation tools (Moya et al., 2017).

Overexploitation can result in density-dependent changes to some life-history attributes, in which regulation can be achieved through compensatory increases in fecundity and growth rates, leading to a reduced length-at-maturity, as seen in some elasmobranch populations (ICES, 2017a, 2017b; McCully et al., 2012; Osaer et al., 2015; Stephan et al., 2014). The reduction in length-at-maturity (in response to fishing pressure) coupled with decreasing population sizes, have been well discussed for a number of elasmobranch species including *S. acanthias* and *Dipturus chilensis* (ICES, 2017a, 2017b; McCully et al., 2012; Osaer et al., 2015; Stephan et al., 2014). However, it is recognised that although overexploitation may be a reasonable explanation for reductions in length-at-maturity over time, little evidence for this is available, since studies that serve as 'before' snapshots (e.g. Steven (1934)) were poorly designed, compared to the quality of maturity studies seen today (McCully et al., 2012). Comparative studies relating to life-history parameters, and how exploitation may affect populations, remain problematic, due to difficulties posed in biological data collection and inconsistencies in methodology (ICES, 2017a, 2017b; McCully et al., 2012; Osaer et al., 2015; Stephan et al., 2014).

Population viability relies on continued breeding success (ICES, 2017a, 2017b; Irvine et al., 2012; Moura et al., 2014). Understanding demographic distribution of species can assist in identifying breeding and nursery grounds, which may be particularly important in the implementation of spatial management initiatives

(ICES, 2017a, 2017b; Irvine et al., 2012; Moura et al., 2014). It is therefore essential to have methods that correctly identify demographic components (i.e. maturity assignment) before being able to understand the demographic distribution. Should maturity be incorrectly assigned, as is proposed here, demographic distribution could be misinterpreted and thus management initiatives utilising such parameters would be misinformed. This study highlighted problems in assigning maturity, using the rubric in the NIWA key. The assignment of stages 2 and 6 was found to be problematic, since characteristics were similar, yet the key attributes currently used to differentiate between these stages focus on different reproductive structures (i.e. follicles in stage 2 and uteri in stage 6). The current criteria may not be accurately determining the onset of maturity, since both immature and mature fish were grouped in the stage 2 classification. The potential implications of having both 'maturing' and 'resting' fish in the same category may lead to misinterpretations of the reproductive population and a species' reproductive biology, since models may exclude a reproductive portion of the population (stage 2 resting). The proposed improvement on the NIWA key aims to resolve some of these problems in utilising objective measures for determining macroscopic maturity.

5.5 Final Conclusions

This study has successfully described new aspects of maturity in deep-sea elasmobranchs and batoids, allowing for the development of a revised macroscopic maturity key. There remains much to be known about the deep-sea elasmobranch and batoid species presented here, however, this research has allowed for a better understanding of maturity assignment and the reproductive biology of species that are poorly studied. Additionally, this research is the first to describe the distribution of members of *Apristurus* spp. from the Chatham Rise and the Sub-Antarctic region of New Zealand.

This study confirmed sperm storage in *C. crepidater, C. squamosus,* and *B. asperula.* To the best of our knowledge, this is the first to describe sperm storage in the order *Arhynchobatidae.* Sperm storage was predominantly observed in

stage 3 and 4 fish, although some stage 2 fish were also found to exhibit sperm storage.

It is apparent that some reproductive organs develop asynchronously, and development may be cyclical. The results demonstrated that some stage 2 fish are very likely 'mature', since those fish had characteristics (i.e. large uterus widths) and exhibited sperm storage – indicative of maturity. Therefore, it is critical to assess development throughout the reproductive tract and the interactions between different structures. Even though singular characteristics (i.e. follicle size) gave some indication of the onset of maturity, no attributes should be considered alone. The macroscopic staging key for New Zealand uses a single characteristic approach (i.e. only changes in follicle development are used to define stages 1 – 3), which may lead to incorrect assessment of maturity and therefore life history parameters (i.e. length-at-maturity), resulting in management initiatives being misinformed. The life history traits of chondrichthyans make them more vulnerable to overexploitation than teleosts and the majority of species (particularly deep-sea species) that are considered to be data deficient. It is crucial that the data collected and modelled life history parameters are as accurate as possible. Since the species studied here are all considered to be at high risk to the impacts of fisheries in New Zealand and are all poorly studied, the ecological implications of incorrect maturity assessment may alter our understanding of the true risk fisheries pose to these populations.

Maturity assessment methods may not be accurately determining the onset of maturity, as demonstrated here and may result in misinformed understanding of a species' reproductive biology. Additionally, comparability of international studies has shown to be hugely difficult, because apparent spatial and temporal differences in life history characteristics between populations of the same species may be due to sampling methodology as well as regional differences. This study has demonstrated the importance of objective measures (particularly, uterus width, follicle size, GSI and sperm storage) in the determination of the onset of maturity in deep-sea elasmobranch and batoid species. An improvement on the existing macroscopic staging key used in New Zealand has been proposed here

and aims to add objectivity to macroscopic maturity criteria. The proposed improvement also aims to assist in distinguishing between stage 2 (maturing), stage 2 (resting) and stage 6 (post-partum) females, by examining the same key reproductive structures across all macroscopic stages. Although further data and validation is required to assess the quality of the proposed key, the implementation of an objective key, as proposed here, may help better inform management decisions, as well as better our understanding of the reproductive biology of deep-sea elasmobranchs and batoids.

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Appendix A Supplementary Data

A total of 28 deep-sea batoids were identified as the following species: *B. asperula, B. leviveneta, B. spinifera, A. hyperborea* and *B. shuntovi.* Only data for *B. asperula* were sufficient to conduct analyses. Five *Apristurus* species were identified as: *A. ampliceps, A. exsanguis, A. garricki, A. melanoasper and A. sinensis. A. sinensis* and *A. garricki* had insufficient data to conduct analyses. The raw data for species, where data were considered insufficient and male data are outlined in Table A-1. Male data was collected, although not used in the current research, since the focus was predominantly on female reproduction.

Table A-1 Other species: Table of all biological data collected for data deficient species in this study, and male specimens for all species, including whether histology was conducted and/or sperm storage was observed. LOWG – Left oviducal gland width; ROGW – right oviducal gland width; LOGL – left oviducal gland length; ROGL – right oviducal gland length; OG – oviducal gland.

SURVEY	STATION NUMBER	SPECIES	SEX	LENGTH (CM)	WEIGHT (G)	MACRO MATURITY STAGE	GONAD WEIGHT (G)	UTERUS WIDTH (CM)	FOLLICLE NUMBER	FOLLICLE SIZE (CM)	LOGW (CM)	ROGW (CM)	LOGL (CM)	ROGL (CM)	AVERAGE OG AREA (CM^2)	CLASPER LENGTH (CM)	CLASPER WIDTH (CM)	SPERM STORAGE	HISTOLOGY
1601	85	*A. ampliceps*	M	81	2360	3	–	–	–	–	–	–	–	–	–	6.8	0.9	–	–
1601	85	*A. ampliceps*	M	75	1665	3	–	–	–	–	–	–	–	–	–	6.43	1.17	–	–
1601	85	*A. ampliceps*	M	74	1385	2	–	–	–	–	–	–	–	–	–	3.36	0.78	–	–
1601	85	*A. ampliceps*	M	82	2272	3	–	–	–	–	–	–	–	–	–	6.29	1.28	–	–
1601	85	*A. ampliceps*	M	79.5	1922	3	–	–	–	–	–	–	–	–	–	6.91	1.05	–	–

SURVEY	STATION NUMBER	SPECIES	SEX	LENGTH (CM)	WEIGHT (G)	MACRO MATURITY STAGE	GONAD WEIGHT (G)	UTERUS WIDTH (CM)	FOLLICLE NUMBER	FOLLICLE SIZE (CM)	LOGW (CM)	ROGW (CM)	LOGL (CM)	ROGL (CM)	AVERAGE OG AREA (CM^2)	CLASPER LENGTH (CM)	CLASPER WIDTH (CM)	SPERM STORAGE	HISTOLOGY
1601	85	*A. ampliceps*	M	84	2677	3	–	–	–	–	–	–	–	–	–	6.6	0.9	–	–
1601	85	*A. ampliceps*	M	81	2391	3	–	–	–	–	–	–	–	–	–	6.53	1.11	–	–
1601	85	*A. ampliceps*	M	81.5	2655	3	–	–	–	–	–	–	–	–	–	6.79	1.41	–	–
1601	85	*A. ampliceps*	M	75.5	1677	2	–	–	–	–	–	–	–	–	–	5.6	0.8	–	–
1601	18	*B. asperula*	M	33.5	658	3	–	–	–	–	–	–	–	–	–	7.6	0.7	–	–
1614	32	*B. asperula*	M	33	675	3	–	–	–	–	–	–	–	–	–	7.206	0.608	–	–
1601	39	*B. asperula*	M	27	383	1	–	–	–	–	–	–	–	–	–	2.1	2.5	–	–
1614	48	*B. asperula*	M	32	577	3	–	–	–	–	–	–	–	–	–	7.578	0.624	–	–
1614	53	*B. asperula*	M	31	712	3	–	–	–	–	–	–	–	–	–	7.542	0.834	–	–
1601	99	*B. asperula*	M	33	723	3	–	–	–	–	–	–	–	–	–	8.2	0.7	–	–
1614	53	*B. asperula*	M	33	1020	3	–	–	–	–	–	–	–	–	–	7.882	0.686	–	–
1601	89	*B. leviveneta*	M	79.5	1922	3	–	–	–	–	–	–	–	–	–	8	0.6	–	–
1601	3	*B. spinifera*	M	84	2677	3	–	–	–	–	–	–	–	–	–	1.8	0.1	–	–
1614	22	*B. spinifera*	M	81	2391	3	–	–	–	–	–	–	–	–	–	12.993	0.911	–	–
1614	7	*B. spinifera*	F	38	1137	3	39.01	0.798	13	20.21	2.169	2.182	2.083	1.888	4.319	–	–	N	Y

SURVEY	STATION NUMBER	SPECIES	SEX	LENGTH (CM)	WEIGHT (G)	MACRO MATURITY STAGE	GONAD WEIGHT (G)	UTERUS WIDTH (CM)	FOLLICLE NUMBER	FOLLICLE SIZE (CM)	LOGW (CM)	ROGW (CM)	LOGL (CM)	ROGL (CM)	AVERAGE OG AREA (CM^2)	CLASPER LENGTH (CM)	CLASPER WIDTH (CM)	SPERM STORAGE	HISTOLOGY
1614	12	*B. spinifera*	F	38	979	2	34.36	0.671	11	1.319	2.078	2.01	1.499	1.382	2.946	–	–	N	Y
1601	95	*C. crepidater*	M	81.5	2655	3	–	–	–	–	–	–	–	–	–	5.77	1.18	–	–
1601	107	*C. crepidater*	M	75.5	1677	2	–	–	–	–	–	–	–	–	–	4.96	1.01	–	–
1601	122	*C. crepidater*	M	33.5	658	3	–	–	–	–	–	–	–	–	–	5.34	1.02	–	–
1614	1	*A. exsanguis*	M	33	675	3	–	–	–	–	–	–	–	–	–	6.145	0.956	–	–
1601	6	*A. exsanguis*	M	27	383	1	–	–	–	–	–	–	–	–	–	1.1	0.3	–	–
1601	7	*A. exsanguis*	M	32	577	3	–	–	–	–	–	–	–	–	–	0.6	0.3	–	–
1601	60	*A. exsanguis*	M	31	712	3	–	–	–	–	–	–	–	–	–	5.8	0.9	–	–
1601	84	*A. exsanguis*	M	33	723	3	–	–	–	–	–	–	–	–	–	5.82	1.33	–	–
1601	94	*A. exsanguis*	M	33	1020	3	–	–	–	–	–	–	–	–	–	6.7	0.9	–	–
1601	101	*A. exsanguis*	M	22	202	3	–	–	–	–	–	–	–	–	–	5.77	1.09	–	–
1601	88	*A. exsanguis*	M	37	806	1	–	–	–	–	–	–	–	–	–	6.2	1	–	–
1601	33	*A. garricki*	M	43	1617	3	–	–	–	–	–	–	–	–	–	5	2	–	–
1601	27	*A. garricki*	M	70.2	1392	3	–	–	–	–	–	–	–	–	–	3.5	1.1	–	–
1601	20	*A. garricki*	F	53.5	275	2	3.14	3	0	0	1.3	1.6	0.8	0.8	1.68	–	–	N	Y

SURVEY	STATION NUMBER	SPECIES	SEX	LENGTH (CM)	WEIGHT (G)	MACRO MATURITY STAGE	GONAD WEIGHT (G)	UTERUS WIDTH (CM)	FOLLICLE NUMBER	FOLLICLE SIZE (CM)	LOGW (CM)	ROGW (CM)	LOGL (CM)	ROGL (CM)	AVERAGE OG AREA (CM^2)	CLASPER LENGTH (CM)	CLASPER WIDTH (CM)	SPERM STORAGE	HISTOLOGY
1601	27	*A. garricki*	F	37.5	70	1	0	0.5	0	0	0.01	0.01	2	1.5	0.0275	–	–	N	N
1601	27	*A. garricki*	F	37.5	74	1	0	0.5	0	0	0.01	0.01	2	2	0.03	–	–	N	N
1601	27	*A. garricki*	F	53.5	241	2	1.69	0.1	0	0	1.4	0.9	1.3	1.1	2.315	–	–	N	Y
1601	46	*A. garricki*	F	68	569	4	40.28	1.6	11	1.2	2.2	1.9	1.7	1.3	4.975	–	–	N	Y
1601	78	*A. hyperborea*	M	69.5	1276	3	–	–	–	–	–	–	–	–	–	3.7	0.6	–	–
1601	92	*A. hyperborea*	F	70	9668	4	–	7.6	13	3.9	3.9	4.4	5.8	6.4	25.39			N	N
1601	77	*A. melanoasper*	M	70.5	1378	3	–	–	–	–	–	–	–	–	–	0.6	0.1	–	–
1601	79	*A. melanoasper*	M	82	1521	3	–	–	–	–	–	–	–	–	–	4.8	0.6	–	–
1601	79	*A. melanoasper*	M	58.5	571	1	–	–	–	–	–	–	–	–	–	5.2	0.9	–	–
1601	79	*A. melanoasper*	M	52	416	1	–	–	–	–	–	–	–	–	–	5.2	0.9	–	–
1601	87	*A. melanoasper*	M	77.5	1491	3	–	–	–	–	–	–	–	–	–	4.91	0.88	–	–
1601	79	*A. melanoasper*	M	87.5	2115	3	–	–	–	–	–	–	–	–	–	5.3	0.9	–	–
1601	79	*A. melanoasper*	M	82.5	1771	3	–	–	–	–	–	–	–	–	–	5.4	0.8	–	–
1601	79	*A. melanoasper*	M	82	1828	3	–	–	–	–	–	–	–	–	–	5.4	0.8	–	–
1601	87	*A. melanoasper*	M	87	216	3	–	–	–	–	–	–	–	–	–	5.57	0.9	–	–

SURVEY	STATION NUMBER	SPECIES	SEX	LENGTH (CM)	WEIGHT (G)	MACRO MATURITY STAGE	GONAD WEIGHT (G)	UTERUS WIDTH (CM)	FOLLICLE NUMBER	FOLLICLE SIZE (CM)	LOGW (CM)	ROGW (CM)	LOGL (CM)	ROGL (CM)	AVERAGE OG AREA (CM^2)	CLASPER LENGTH (CM)	CLASPER WIDTH (CM)	SPERM STORAGE	HISTOLOGY
1601	88	*A. melanoasper*	M	41	114	1	–	–	–	–	–	–	–	–	–	–	–	–	–
1601	79	*A. melanoasper*	M	53.5	235	1	–	–	–	–	–	–	–	–	–	5.2	0.8	–	–
1601	79	*A. melanoasper*	M	44.5	1717	2	–	–	–	–	–	–	–	–	–	5.1	0.9	–	–
1601	79	*A. melanoasper*	M	43	261	1	–	–	–	–	–	–	–	–	–	5.1	0.9	–	–
1601	80	*A. melanoasper*	M	78	1517	3	–	–	–	–	–	–	–	–	–	5.5	0.8	–	–
1601	87	*A. melanoasper*	M	76	1309	3	–	–	–	–	–	–	–	–	–	5.48	0.7	–	–
1601	79	*A. melanoasper*	M	76	1309	3	–	–	–	–	–	–	–	–	–	4.7	0.9	–	–
1601	79	*A. melanoasper*	M	78	1424	3	–	–	–	–	–	–	–	–	–	5.7	0.9	–	–
1601	79	*A. melanoasper*	M	75	1450	3	–	–	–	–	–	–	–	–	–	5.7	0.9	–	–
1601	80	*A. melanoasper*	M	78	1263	3	–	–	–	–	–	–	–	–	–	4.7	0.8	–	–
1601	87	*A. melanoasper*	M	78	1263	3	–	–	–	–	–	–	–	–	–	5.4	0.95	–	–
1601	88	*A. melanoasper*	M	81.5	1557	3	–	–	–	–	–	–	–	–	–	5	0.8	–	–
1601	79	*A. melanoasper*	M	76	1440	3	–	–	–	–	–	–	–	–	–	5.9	0.9	–	–
1601	79	*A. melanoasper*	M	75	1338	3	–	–	–	–	–	–	–	–	–	5.9	0.9	–	–
1601	80	*A. melanoasper*	M	76.5	1288	3	–	–	–	–	–	–	–	–	–	5.1	0.8	–	–

SURVEY	STATION NUMBER	SPECIES	SEX	LENGTH (CM)	WEIGHT (G)	MACRO MATURITY STAGE	GONAD WEIGHT (G)	UTERUS WIDTH (CM)	FOLLICLE NUMBER	FOLLICLE SIZE (CM)	LOGW (CM)	ROGW (CM)	LOGL (CM)	ROGL (CM)	AVERAGE OG AREA (CM^2)	CLASPER LENGTH (CM)	CLASPER WIDTH (CM)	SPERM STORAGE	HISTOLOGY
1601	87	*A. melanoasper*	M	76.5	1288	3	–	–	–	–	–	–	–	–	–	5.42	0.84	–	–
1601	88	*A. melanoasper*	M	80	1481	3	–	–	–	–	–	–	–	–	–	5	1.6	–	–
1601	79	*A. melanoasper*	M	71.5	1209	3	–	–	–	–	–	–	–	–	–	6.1	0.9	–	–
1601	79	*A. melanoasper*	M	75	1232	3	–	–	–	–	–	–	–	–	–	6.1	0.9	–	–
1601	80	*A. melanoasper*	M	78.5	1440	3	–	–	–	–	–	–	–	–	–	4.3	0.9	–	–
1601	79	*A. melanoasper*	M	78.5	1440	3	–	–	–	–	–	–	–	–	–	5.2	1.1	–	–
1601	79	*A. melanoasper*	M	74	1282	3	–	–	–	–	–	–	–	–	–	5.2	1.1	–	–
1601	80	*A. melanoasper*	M	79	1534	3	–	–	–	–	–	–	–	–	–	5.1	0.9	–	–
1601	79	*A. melanoasper*	M	78.5	1406	3	–	–	–	–	–	–	–	–	–	5.7	0.8	–	–
1601	79	*A. melanoasper*	M	74	1238	3	–	–	–	–	–	–	–	–	–	5.7	0.8	–	–
1601	79	*A. melanoasper*	M	74	1238	3	–	–	–	–	–	–	–	–	–	5.8	0.9	–	–
1601	79	*A. melanoasper*	M	81	1477	3	–	–	–	–	–	–	–	–	–	5.8	0.9	–	–
1601	79	*A. melanoasper*	M	77	1430	3	–	–	–	–	–	–	–	–	–	5.7	1	–	–
1601	79	*A. melanoasper*	M	77	1444	3	–	–	–	–	–	–	–	–	–	5.7	1	–	–
1601	79	*A. melanoasper*	M	78	1433	3	–	–	–	–	–	–	–	–	–	5.5	0.9	–	–

SURVEY	STATION NUMBER	SPECIES	SEX	LENGTH (CM)	WEIGHT (G)	MACRO MATURITY STAGE	GONAD WEIGHT (G)	UTERUS WIDTH (CM)	FOLLICLE NUMBER	FOLLICLE SIZE (CM)	LOGW (CM)	ROGW (CM)	LOGL (CM)	ROGL (CM)	AVERAGE OG AREA (CM^2)	CLASPER LENGTH (CM)	CLASPER WIDTH (CM)	SPERM STORAGE	HISTOLOGY
1601	79	*A. melanoasper*	M	78	1433	3	–	–	–	–	–	–	–	–	–	5.5	0.9	–	–
1601	79	*A. melanoasper*	M	76	1239	3	–	–	–	–	–	–	–	–	–	5.3	0.9	–	–
1601	79	*A. melanoasper*	M	81.5	1429	3	–	–	–	–	–	–	–	–	–	5.3	0.9	–	–
1601	79	*A. melanoasper*	M	81.5	1429	3	–	–	–	–	–	–	–	–	–	5.5	0.7	–	–
1601	79	*A. melanoasper*	M	74	1248	3	–	–	–	–	–	–	–	–	–	5.5	0.7	–	–
1601	79	*A. melanoasper*	M	79	1396	3	–	–	–	–	–	–	–	–	–	5.6	0.8	–	–
1601	79	*A. melanoasper*	M	79	1396	3	–	–	–	–	–	–	–	–	–	5.6	0.8	–	–
1601	77	*B. shuntovi*	M	53	1750	2	–	–	–	–	–	–	–	–	–	4	0.4	–	–
1601	4	*B. shuntovi*	F	66.5	3756	3	207.91	1.7	22	2.8	2.7	2.8	4.4	3.9	17.34	–	–	N	N
1601	71	*B. shuntovi*	F	83	6765	3	300.61	1.8	14	2.9	5	5	3.9	3.9	29.25	–	–	N	N
1601	85	*B. shuntovi*	F	74	5273	4	275.1	2.4	13	3.09	3.21	3.03	4.49	3.55	19.79115	–	–	N	N
1601	92	*B. shuntovi*	F	76.5	6183	6	114.54	1.1	0	0	2.9	3.2	4	4	18	–	–	N	N
1601	96	*B. shuntovi*	F	59.5	2891	3	92.12	0.9	13	1.7	2.6	2.2	3.9	4	14.54	–	–	N	N
1601	60	*A. sinensis*	M	79	1483	3	–	–	–	–	–	–	–	–	–	5.7	0.9	–	–
1601	80	*A. sinensis*	M	73	1169	3	–	–	–	–	–	–	–	–	–	6	1.2	–	–

SURVEY	STATION NUMBER	SPECIES	SEX	LENGTH (CM)	WEIGHT (G)	MACRO MATURITY STAGE	GONAD WEIGHT (G)	UTERUS WIDTH (CM)	FOLLICLE NUMBER	FOLLICLE SIZE (CM)	LOGW (CM)	ROGW (CM)	LOGL (CM)	ROGL (CM)	AVERAGE OG AREA (CM^2)	CLASPER LENGTH (CM)	CLASPER WIDTH (CM)	SPERM STORAGE	HISTOLOGY
1601	84	*A. sinensis*	M	73	1169	3	–	–	–	–	–	–	–	–	–	6.1	1.2	–	–
1601	101	*A. sinensis*	M	78.5	1518	3	–	–	–	–	–	–	–	–	–	6.1	1.2	–	–
1601	69	*A. sinensis*	F	75	1435	3	62.48	1	13	2	1.6	1.7	3.1	3.5	5.455	–	–	N	Y

Appendix B Supplementary Material

Supplementary material used in the statistical analyses for the largest dataset only (*C. crepidater*), are supplied here.

B.1 Centroselachus crepidater

Males (n = 3) were distributed along the northwestern Chatham Rise Figure B-1).

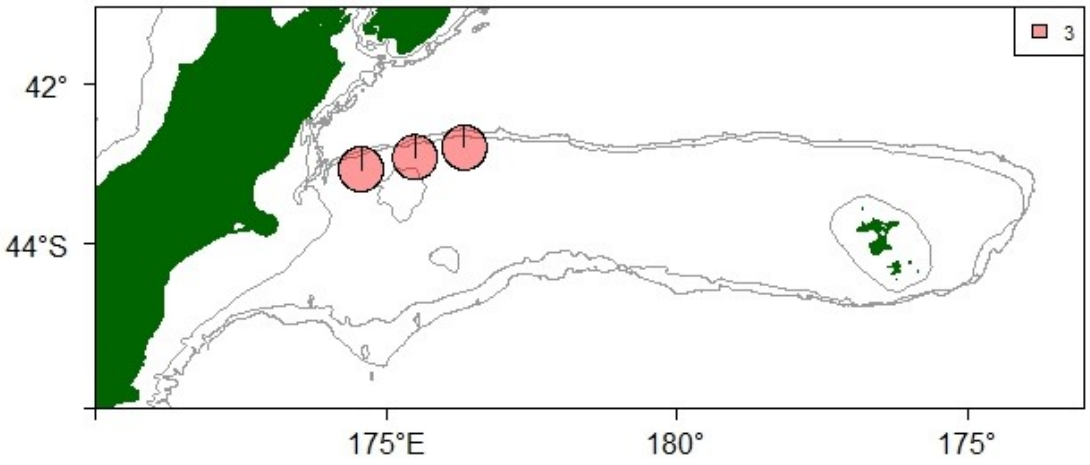

Figure B-1 C. crepidater: Chatham Rise area with 200 m, 1000 m and 1200 m contours (grey), showing sample distribution for C. crepidater males (n = 3). Pie charts denote the proportion of each macroscopic stage (colours for each macroscopic stage defined in legend) caught at each tow where samples were collected. The size of the pie chart is related to the number of samples collected relative to other tows.

When stage 3 outliers were removed, no change in to the estimated length-weight curve was observed (Figure B-2).

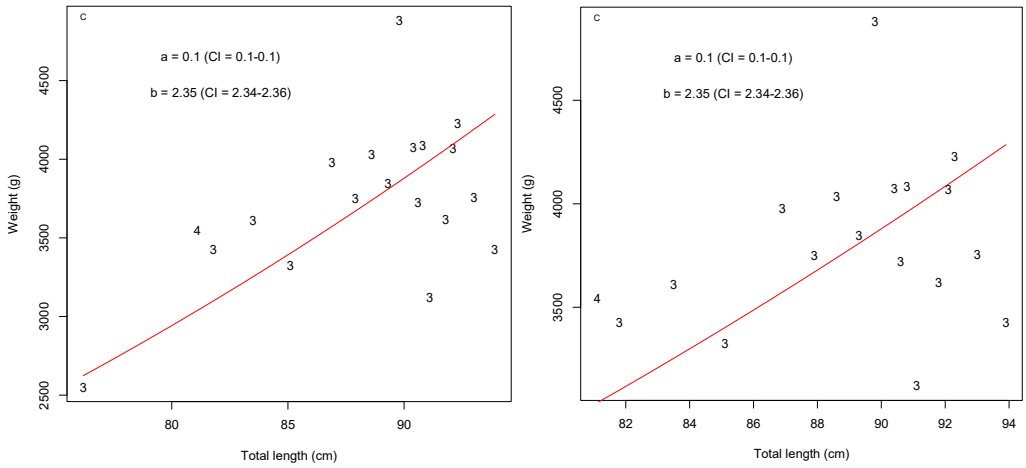

Figure B-2 C. crepidater: Length-weight relationships for C. crepidater for females stages 3 and above (n = 20), where stage 3 "outliers" have been removed in order to compare the growth curves with that seen in Figure C). The red line represents the bootstrapped sum of least-squares fit for each group. Numbers represent the macroscopic staging assigned to each individual in the sample.

All biological measurements were standardised by fish length, body weight and somatic weight (Figure B-3; Figure B-4; Figure B-5; Figure B-6; Figure B-7). All results demonstrate patterns consistent with those observed using unstandardised measures. Therefore, only unstandardised results were reported in Chapter 4.

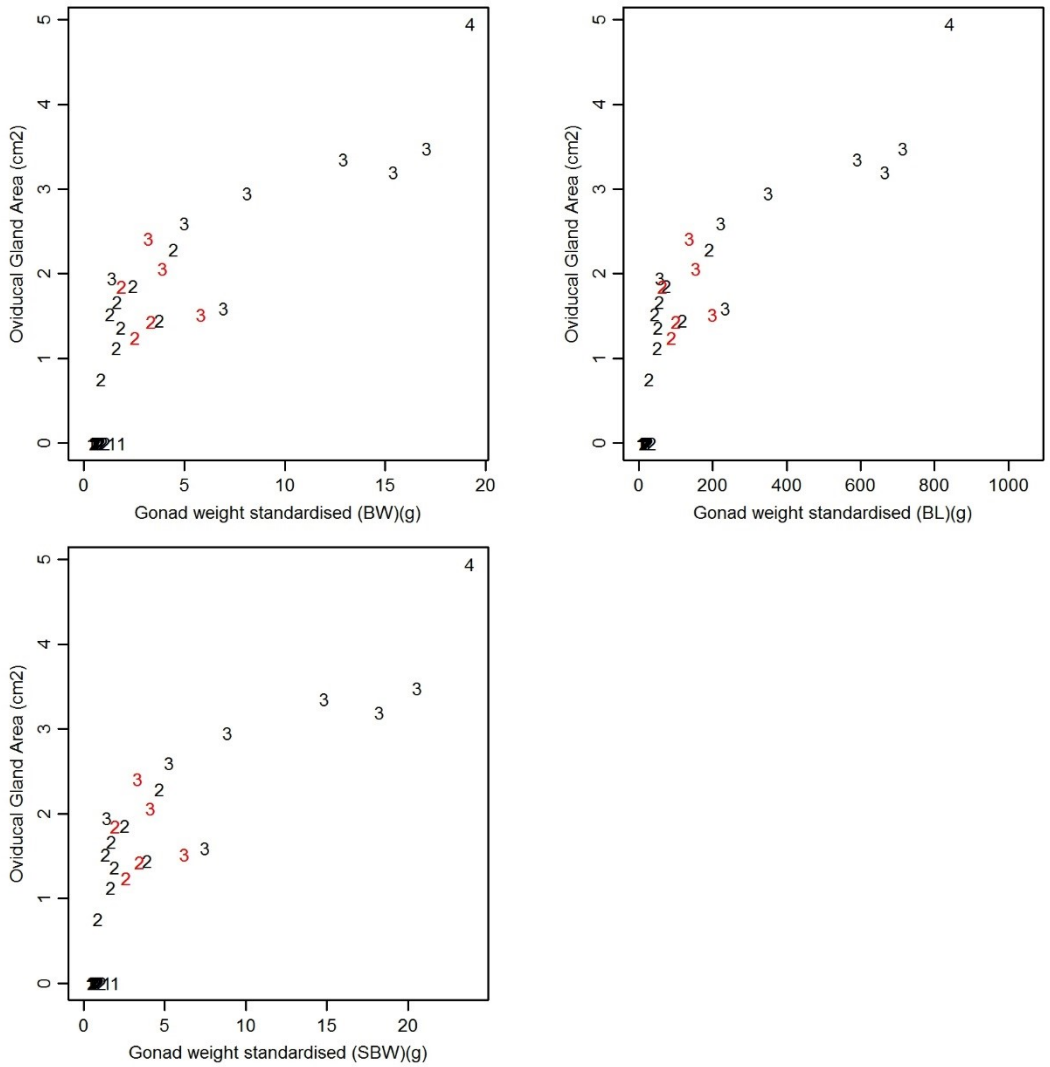

Figure B-3 C. crepidater: Relationship between average oviducal gland area and weight-, somatic weight- and length-standardised measures of gonad weight. The numbers denote the macroscopic maturity stages assigned to each individual. Red numbers indicate those individuals where sperm storage was detected.

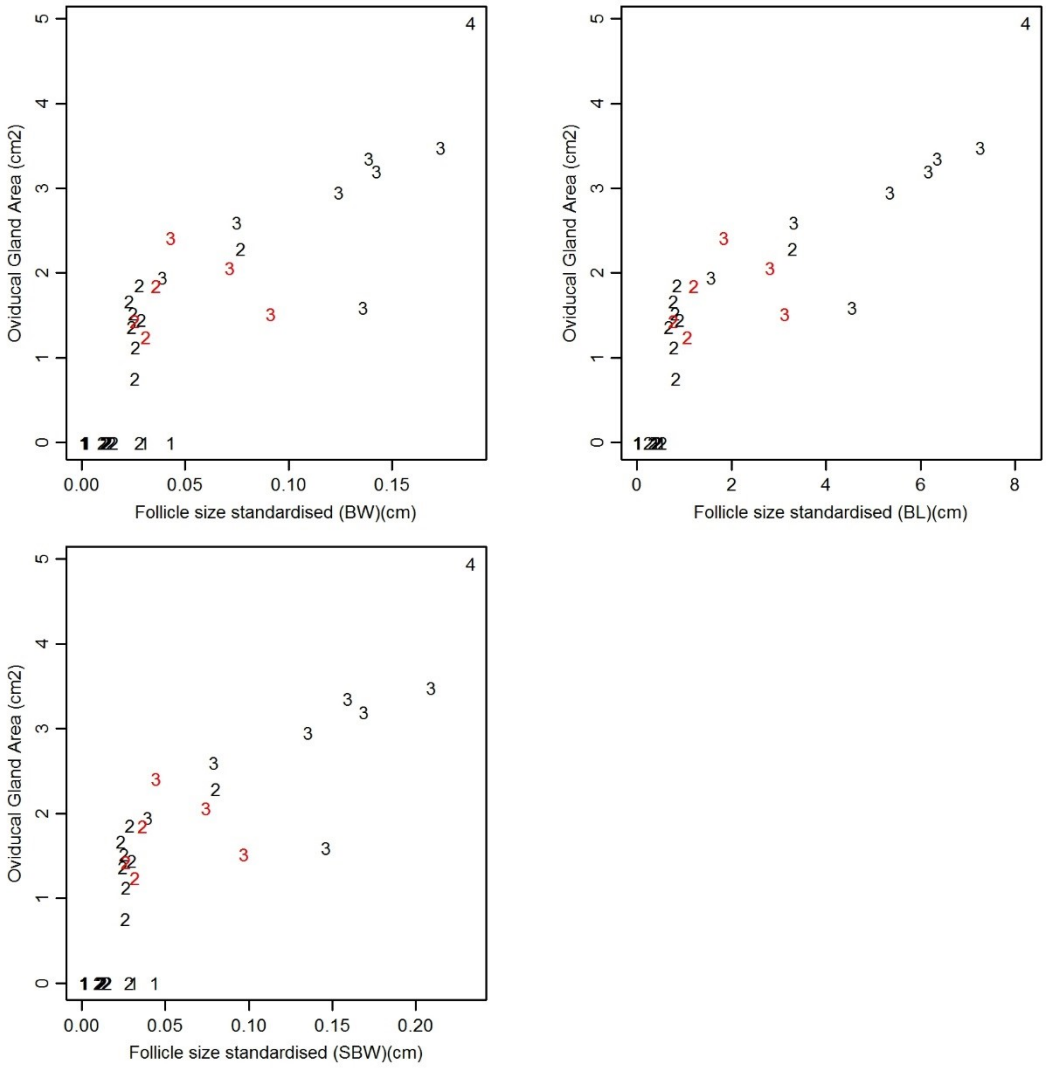

Figure B-4 C. crepidater: Relationship between average oviducal gland area and weight-, somatic weight- and length-standardised measures of follicle size. The numbers denote the macroscopic maturity stages assigned to each individual. Red numbers indicate those individuals where sperm storage was detected.

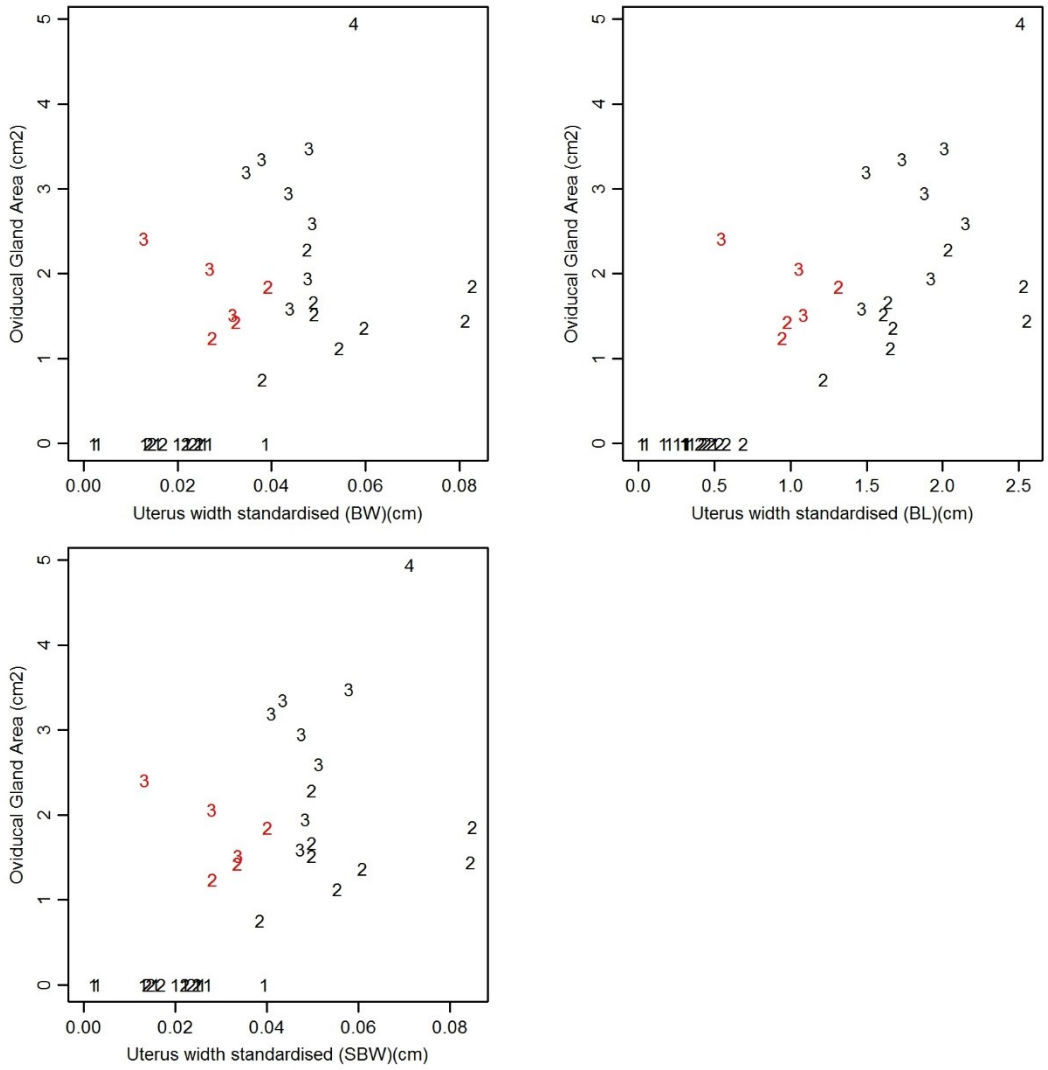

Figure B-5 C. crepidater: Relationship between average oviducal gland area and weight-, somatic weight- and length-standardised measures of uterus width. The numbers denote the macroscopic maturity stages assigned to each individual. Red numbers indicate those individuals where sperm storage was detected.

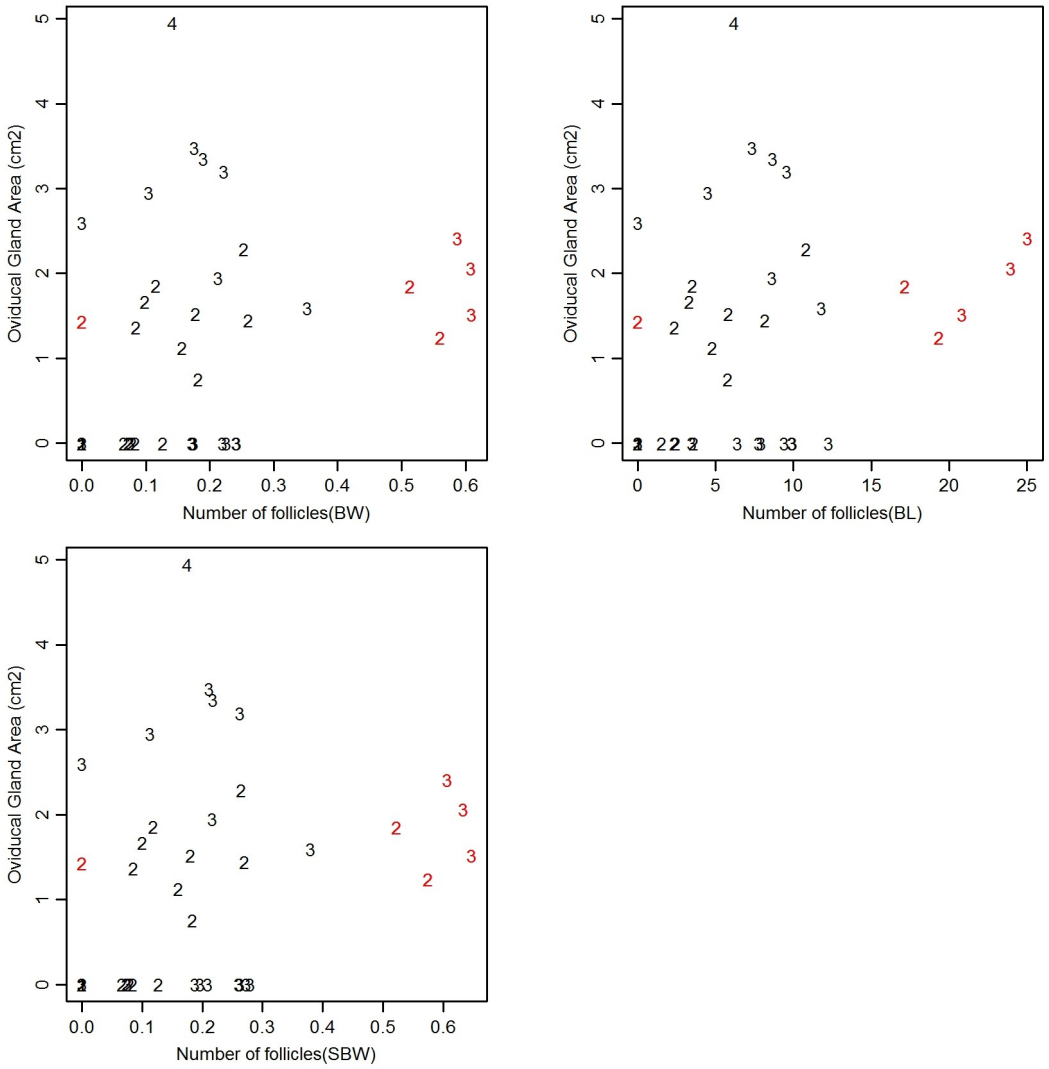

Figure B-6 C. crepidater: Relationship between average oviducal gland area and weight-, somatic weight- and length-standardised measures of follicle number. The numbers denote the macroscopic maturity stages assigned to each individual. Red numbers indicate those individuals where sperm storage was detected.

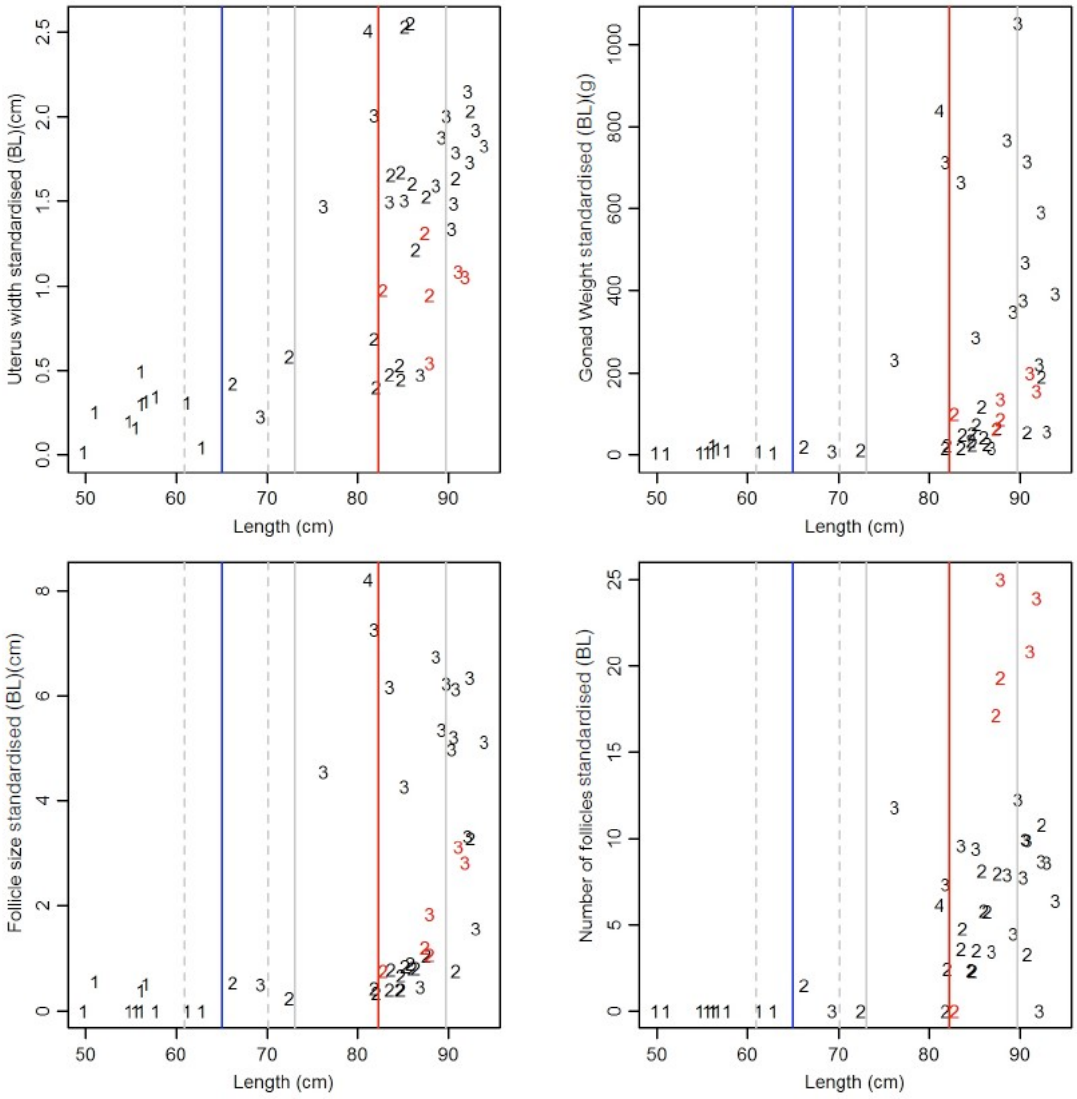

Figure B-7 C. crepidater: Length relationships with length-standardised measures of uterus width, gonad weight, number of follicle size and follicle size (clockwise from top left). The numbers denote the macroscopic maturity stages assigned to each individual. Red numbers indicate those individuals where sperm storage was detected. Lines represent length-at-maturity estimates for maturity occurring at stage 2 (blue) and at stage 3 (red). Grey lines represent the 95 % confidence intervals (Figure 4.1- 6).

Appendix C Additional Observations

C.1 Egg case morphology

C.1.1 *Deep-sea batoids*

Egg cases were found in two batoid species: *A. hyperborea* (n = 1) and *B. shuntovi* (n = 1). No photographs were taken of the egg cases in *B. shuntovi* because the egg case was only beginning to develop and the tertiary egg casing was still being produced by the oviducal gland, therefore no defining morphometric characteristics could be examined for this species. *A. hyperborea* egg cases were approximately 14 cm in length (excluding the horns), with horns the anterior and short tendrils at the posterior end (Figure C.1.1- 1). The cases were a dark green-brown in colour with distinctive striations on the exterior surface and had aprons at the posterior end of the body (Figure C.1.1- 1). *A. hyperborea* egg cases have been previously by Ishihara et al. (2012).

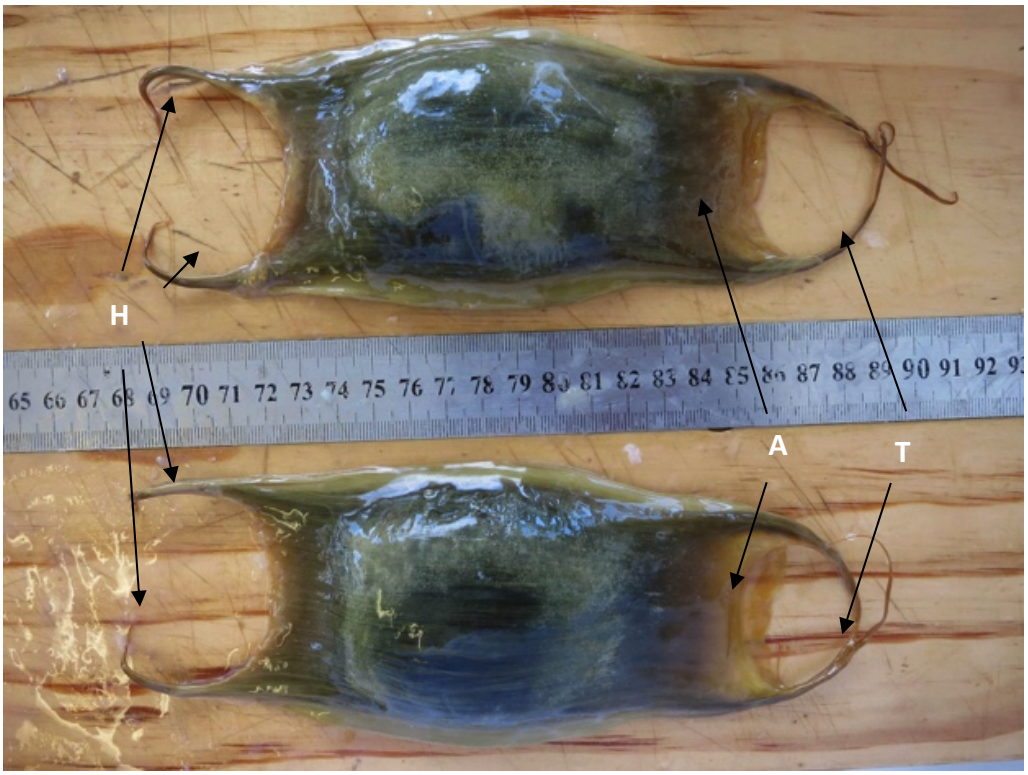

Figure C.1.1- 1 A. hyporborea: Egg cases. T – Tendrils; H – Hook; A - Apron.

C.1.2 *Apristurus spp.*

Egg cases were found in four of the five *Apristurus* species in this study, in a total of nine fish: *A. ampliceps* (n = 4)*; A. exsanguis* (n = 2)*; A. garricki* (n = 1)*;* and *A. melanoasper* (n = 2). No egg cases were identified in *A. sinensis*. Egg cases had species-specific morphometric characteristics. *A. ampliceps* eggs had a smooth green-brown casing with no tendrils or hooks, and were approximately 7 cm in length (Figure C.1.2- 1 a). There was one egg case per oviduct suggesting the species is single oviparous, which is presumed throughout the genus (Figure C.1.2- 1 b). *A. exsanguis* egg cases were approximately 5 cm long (excluding the tendrils), had distinctive striations on their outer surface and were ochre when developed (Figure C.1.2- 1 c & d). Colouration of the egg case appeared to occur once the egg was deposited in the oviduct and was independent from the oviducal gland (which produces the tertiary egg case) (Figure C.1.2- 1 c & d). *A. exsanguis* egg cases had long coiled tendrils at the posterior end and small hooks at the anterior end, suggesting that eggs from this species attach to 3-dimensional structures on the ocean floor (i.e. coral branches) (Flammang et al., 2007) (Figure C.1.2- 1 c & d). *A. garricki* had long and narrow cylindrical egg cases approximately 7.5 cm long, with visible striations, although much less pronounced than those seen in *A. exsanguis* (Figure C.1.2- 1 c, d & e). *A. garricki* egg cases were a brown-orange in colour and had no tendrils or hooks (as seen in *A. ampliceps*), which suggests that these species are likely to bury their eggs, or lodge them in rock crevices (Flammang et al., 2007) (Figure C.1.2- 1 e). *A. melanoasper* had egg cases that were approximately 6 cm in length and were a similar ochre colour to those of *A. exsanguis* (Figure C.1.2- 1 c, d & f). Striations on the exterior surface were distinctive, but less pronounced as those seen in *A. exsanguis* (Figure C.1.2- 1 c, d & f). *A. melanoasper* egg cases had tightly coiled tendrils at the posterior end and hooks at the anterior end that had tendril extensions (Figure C.1.2- 1 f). The egg cases observed here have all been previously described (Flammang et al., 2007; Iglesias et al., 2004; Sato et al., 2013, 1999).

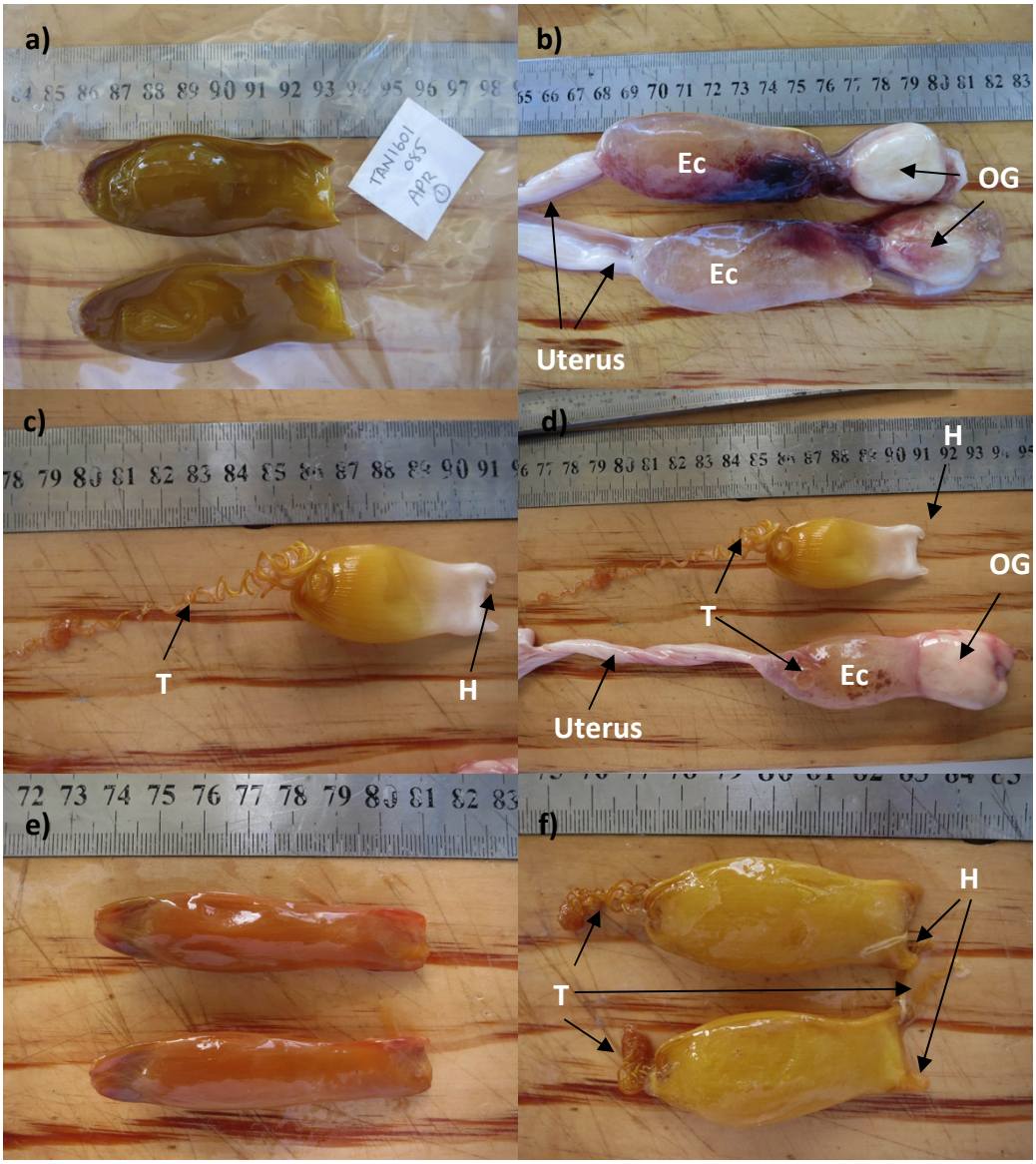

Figure C.1.2- 1 Apristurus spp.: Comparison of egg cases from a) A. ampliceps – egg cases without tendrils or hooks; b) A. ampliceps – egg cases in utero; c) A. exsanguis – single egg case with distinctive striations, tendrils and developing hooks; d) A. exsanguis – developing egg case (above) and egg case in utero (below); e) A. garricki – egg cases without tendrils or hooks; f) A. melanoasper – egg cases with tendrils and hooks. Ec – Egg case; OG – Oviducal gland; T – Tendrils; H – Hook.

C.2 Parasitic barnacle (Anelasma squalicola) infestation

The infestation of the parasitic barnacle, *Anelasma squalicola* (confirmed with DNA sequencing; Schnabel, NIWA, pers. comm.) on *A. ampliceps* was recorded for the first time in four fish: 3 females and 1 male (Figure C.2- 1) and is the first reported case of infestation in this order. The parasites were varied in size, with one per host and various attachment sites: two on the right flank anterior to the dorsal fin; one on the head, in line with the gills; and one on the left flank anterior to the pelvic fin (Figure C.2- 1). Previous reports have documented *A. squalicola* in the two genera of the same family, Etmopteridae: *Etmopterus* and *Centroscyllium,* and *A. squalicola* has been found to have a detrimental effect on reproductive organs (Yano and Musick, 2000). However, there appeared to be no effect of the parasite on the reproductive organs of *A. ampliceps* (Chapter 4.5).

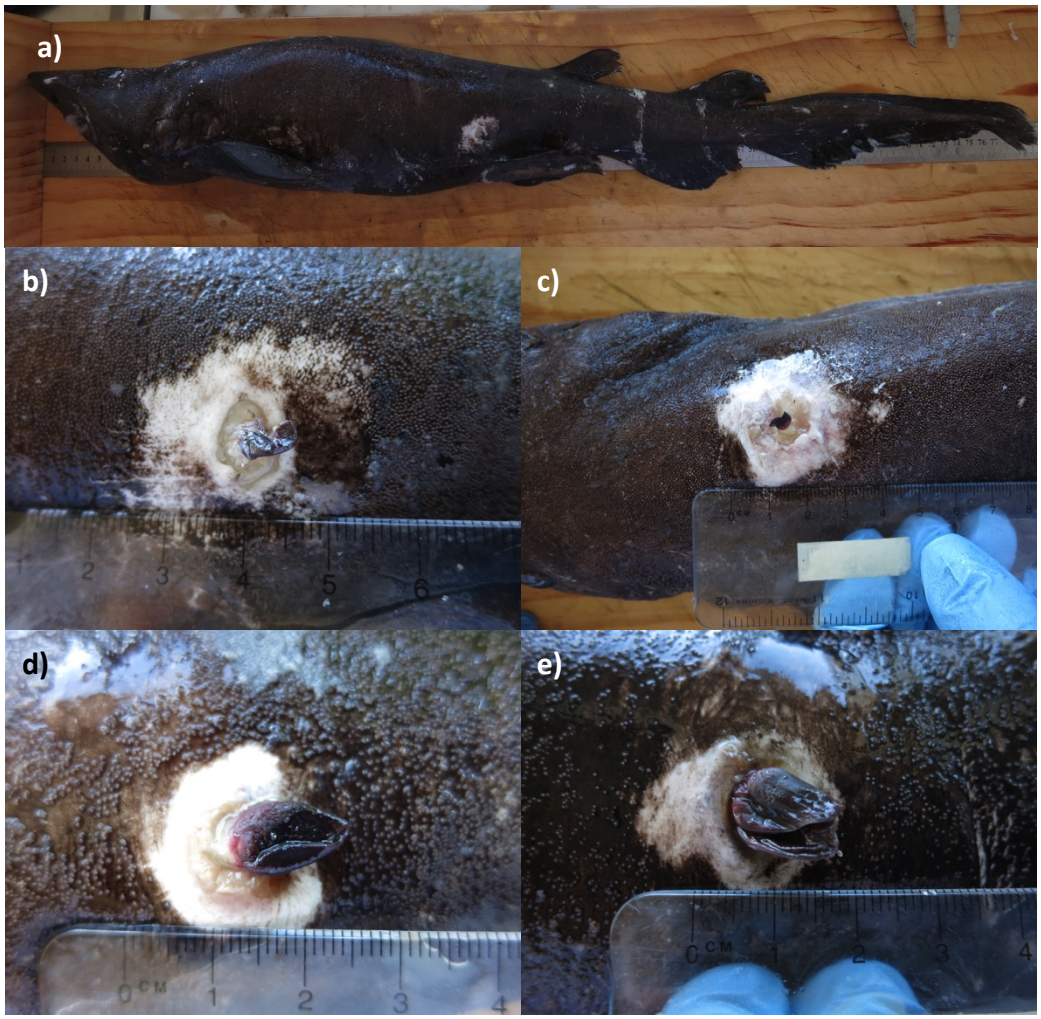

Figure C.2- 1 A. ampliceps: Observations of the parasitic barnacle, Anelasma squalicola, on four A. ampliceps hosts. a) whole male A. ampliceps specimen with a parasitic barnacle on the left flank anterior to the pelvic fin; b) detail of the parasite from a); c) parasite on the head of female host, in line with the gills; d) parasite on the right flank anterior to the dorsal fin of female host; e) parasite on the right flank anterior to the dorsal fin of a female host.