UNIVERSITY OF SOUTHAMPTON

FACULTY OF SCIENCE

School of Ocean and Earth Science

Reproductive Success in Antarctic Marine Invertebrates

By

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Dedicated to my Mum, Dad, Sam
and my one and only Mike.

UNIVERSITY OF SOUTHAMPTON
ABSTRACT
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REPRODUCTIVE SUCCESS IN ANTARCTIC MARINE INVERTEBRATES

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The nearshore Antarctic marine environment is unique, characterised by low but constant temperatures that contrast with an intense peak in productivity. As a result of this stenothermal environment, energy input has a profound ecological effect. These conditions have developed over several millions of years and have resulted in an animal physiology that is highly stenothermal and sometimes closely coupled with the seasonal food supply, e.g. reproductive periodicity and food storage. Therefore, Antarctic marine animals are likely to be amongst the most vulnerable species worldwide to environmental modifications and can be regarded as highly sensitive barometers for change. Reproductive success is a vital characteristic in species survival and evaluation of change in reproductive condition with time key to identifying vulnerable taxa. Characterising reproductive success with time is a major requirement in predicting species response to change and the early stages of species loss.

Some invertebrates are highly abundant in shallow water sites around the Antarctic and form conspicuous members of the Antarctic benthos. Three common echinoderms and one nemertean were sampled from sites adjacent to the British Antarctic Survey’s Rothera Research Station, Adelaide Island, on the West Antarctic Peninsula between 1997-2001. Reproductive patterns were determined by histological analyses of gonad tissue.

This study provided further evidence for inter-annual variation in Antarctic gametogenic development, which appeared to be driven to some extent by trophic position and reliance on the seasonal phytoplankton bloom. The largest variation in reproductive condition was demonstrated for the detritivorous brittle star, *Ophionotus victoriae*. The seasonal tempos of this echinoderm have been attributed in part, to the seasonal sedimentation events common in the high Antarctic. The reproductive patterns in the scavenging starfish, *Odontaster validus* and the predatory nemertean, *Parborlasia corrugatus* showed less inter-annual variation. The de-coupling of these invertebrates from the intensely seasonal phytoplankton bloom appeared to partially account for the reproductive trends observed. The lack of inter-annual variation in the reproduction of the filter-feeding sea-cucumber, *Heterocucumis steineni*, was somewhat counterintuitive, although problems with sample processing probably accounted for the majority of this anomaly.

Echinoderms were also collected during the Antarctic summer field seasons in 2003 and 2004. A series of fertilisation success studies were undertaken comparing the adaptations in an Antarctic and an equivalent temperate starfish to achieve optimal numbers of fertilised eggs, and elemental analyses were used to estimate the nutritional and energetic condition of the bodily and reproductive tissues in two Antarctic echinoderms.

Fertilisation studies indicated that Antarctic invertebrates require 1-2 orders of magnitude more sperm to ensure optimal fertilisation success. These sperm tended to be long-lived and capable of fertilising eggs 24+ hours after release. The study suggested that synchronous spawning, aggregations and specific pre-spawning behaviour are employed to help counter the deleterious effects of sperm limitation. The Antarctic eggs and sperm were also highly sensitive to even small modifications in temperature and salinity, affecting the

number of eggs fertilised. Such stenothermy is of particular relevance if the 1-2ºC rise in global temperature, predicted over the next century, is realised.

Biochemical composition of body components of two species of Antarctic echinoderm indicated a significant difference in the composition between the male and female gonad, particularly in the Antarctic brittle star *Ophionotus victoriae*. The ovaries contained a much larger proportion of lipid compared to the testes, and demonstrated a distinct seasonality in composition. Higher levels of lipid were observed in the ovary during the austral winter coincident with a period of reproductive investment and maturing oocytes in the gonad. *O. victoriae* exhibited lower amounts of lipid in the late austral spring suggesting the removal of mature oocytes from the ovary through spawning. The seasonality in composition and the high levels of lipid and protein measured in the ophiuroid gut tissue, suggested the gut might play a role in providing material and energy for metabolic function and possibly gametogenesis; higher lipid levels were apparent during the period of seasonal phytodetrital flux. The role of the pyloric ceaca in asteroids as a nutrient storage organ was also evident in the high levels of both protein and lipid observed in this bodily component in the star fish, *Odontaster validus*.

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Data are presented as overall means ± SD of three replicate egg counts. Data are based on normal, abnormal and unfertilised egg counts. Only the normal and abnormal fertilisation data are plotted. Temperature was maintained at 12˚C ± 0.5˚C.

Fig. 3.3.2 *Odontaster validus*. The effect of sperm dilution on fertilisation success. This experiment was repeated on three separate occasions using one male and one female starfish. A different male-female paring was used during each trial. Data are presented as overall means ± SD of three replicate egg counts. Data are based on normal, abnormal and unfertilised egg counts. Only the normal and abnormal fertilisation data are plotted. Temperature was maintained at 0.5˚C ± 0.5˚C.

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Chapter 4. Tissue Composition and Condition

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Tables

Chapter 2. Long-term Reproductive Cycles

Table. 2.1.1 Modified from Galley (2004). Reproductive periodicity and egg size in Antarctic shallow-water invertebrates. Length of oogenesis is referred to as Cycle; modes of development are listed as either AL = abbreviated lecithotrophy; B = brooding; L = lecithotrophic; P = planktotrophic. Periodicities are listed as either: A = aseasonal; S = seasonal.

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Table. 2.3.4 Correlations between reproductive (average gonad index and average oocyte size) and environmental variables (chlorophyll, phaeophytin and temperature) between 1997 and the end of 2000. All data were ranked prior to analysis and tested using Pearson's Product Moment Correlation. A $P < 0.05$ indicated that variables were significantly correlated.

Table. 2.3.5 *Odontaster validus*. Sex ratio of males and females collected between 1997 and the beginning of 2001 using a Chi Square statistical test

Table. 2.3.6 *Odontaster validus*. Comparisons in wet weight (±0.01g), radial length (R ±0.01mm) and body radius (r ±0.01mm) between individual years (1997-2001). The variation in wet weight, radial length and body radius of individual starfish was tested between years. A 2-sample T-test or a non-parametric Mann Whitney test was used depending on if the data were normally distributed (Appendix Table. 2). A significant difference was indicated by a $P < 0.05$. A test for equal variance was also undertaken taken when a 2-sample T-test was employed and the results included in the assumptions of the test (Appendix Table. 4).

Table. 2.3.7 *Odontaster validus*. Correlations between animal wet weight (±0.01g), radial length (R ±0.01mm) and body radius (r ±0.01mm) collected between 1997 and early 2001. All data were ranked and tested using Pearson's Product Moment Correlation. A $P < 0.05$ indicated that variables were significantly correlated.

Table 2.3.8 *Odontaster validus*. Comparisons in wet weight (±0.01g), radial length (R ±0.01mm) and body radius (r ±0.01mm) over the entire study period (1997-2001). The wet weight, radial length and body radius of individual starfish were compared. A One-way ANOVA or Kruskal-Wallis non-parametric analysis was used depending on if the data were normally distributed and a significant difference was indicated by a $P < 0.05$.

Table. 2.3.9 *Odontaster validus*. Reproductive condition is presented as the percentage decrease in the male and female gonad index between years (1997-2001), calculated from the average gonad index before (fecund) and after spawning (spent).

Table. 2.3.10 *Odontaster validus*. Comparisons in overall gonad index between individual years. The variation in gonad index of individual starfish was tested between years (1997-2001). A Mann Whitney non-parametric analysis was employed and a P < 0.05 indicated a significant difference in overall gonad index between years.

Table. 2.3.11 *Odontaster validus*. Comparisons in overall pyloric caeca index between individual years. The variation in pyloric caeca index of individual starfish was tested between years (1997-2001). A Mann Whitney non-parametric analysis was employed and a P < 0.05 indicated a significant difference in overall pyloric caeca index between years.

Table. 2.3.12 *Parborlasia corrugatus*. Sex ratio of males and females collected between 1997 and the end of 2000 using a Chi Square statistical test.

Table. 2.3.13 *Parborlasia corrugatus*. Comparisons in retracted length (±0.01mm) between individual years. The variation in retracted length of individual nemerteans was tested between years (1997-2000). A Mann Whitney non-parametric analysis was employed and a P < 0.05 indicated a significant difference in retracted length between years.

Table. 2.3.14 *Heterocucumis steineni*. Sex ratio of males and females collected between 1997 and early 2001 using a Chi Square statistical test.

Table. 2.3.15 *Heterocucumis steineni*. Comparisons in wet weight (±0.01g) and length (±0.01mm) between individual years. The variation in wet weight and length of individual holothurians was tested between years (1997-2001). A Mann Whitney non-parametric analysis was employed and a P < 0.05 indicated a significant difference in length between years.

Table. 2.3.16 *Heterocucumis steineni*. Correlations between animal wet weight (±0.01g), length (±0.01mm) and gonad index collected between 1997 and early 2001. All data were ranked and tested using Pearson's Product Moment Correlation. A P < 0.05 indicated that variables were significantly correlated.

Table 2.3.17 *Heterocucumis steineni*. Comparisons in wet weight (±0.01g) and length (R ±0.01mm) over the entire study period (1997-2001). The wet weight and length of individual holothurians were compared. A Kruskal-Wallis non-parametric analysis was used and a significant difference was indicated by a $P < 0.05$.

Table. 2.3.18 *Heterocucumis steineni*. Reproductive condition is presented as the percentage decrease in the male and female gonad index between years (1997-2001), calculated from the average gonad index before (fecund) and after spawning (spent).

Table. 2.3.19 *Heterocucumis steineni*. Comparisons in overall gonad index between individual years. The variation in gonad index of individual holothurians was tested between years (1997-2001). A 2-sample T-test was employed and a $P < 0.05$ indicated a significant difference in overall gonad index between years. A test for equal variance was also undertaken and the results included in the assumptions of the test (Appendix Table. 5). The 2-sample T-test was repeated (1997-1999, 1997-2000 and 1998-2000) assuming equal and unequal variance (*) when the test for equal variance returned p values both greater than and less than $P = 0.05$.

Chapter 3. Fertilisation Kinetics

Table. 3.1.1 Factors influencing fertilisation success (adapted from Levitan, 1995 and Powell, 2001).

Table. 3.1.2 Fertilisation studies using a number of temperate and tropical marine invertebrates.

Table. 3.1.3 Echinoid fertilisation studies.

Table. 3.1.4 Gamete-specific adaptations to maximise fertilisation success.

Table. 3.2.1 Separate experimental trials were designed using increasing concentrations of three chemical agents used to artificially alter seawater viscosity. Viscosity was calculated using a Falling Ball Viscometer and quantified by the passage of a weighted ball along the viscometer's length. Viscosity A = seawater viscosity maintained at 12ºC ± 0.5ºC, B = seawater viscosity maintained at 0.5ºC ± 0.5ºC.

Table. 3.2.2 Dry sperm concentrations (ml^{-1}) from individual males in 2003, using the haemocytometer counting cell, and in 2004 using the Coulter Multisizer II. Data are presented as overall means and ±SD from 3 replicate counts.

Table. 3.3.1 *Odontaster validus*. Test for equal variance in fertilisation success between normal seawater and PVP-seawater conditions

Table. 3.3.2 *Laternula elliptica*. Test for equal variance in fertilisation success between normal seawater conditions and enhanced seawater viscosities using PVP, glycerol and methylcellulose.

Table. 3.3.3 *Odontaster validus.* Test for equal variance in fertilisation success between normal egg conditions and the removal of the jelly coat.

Table. 3.3.4 *Odontaster validus*. Correlations between female fecundity and female wet weight (±0.01g) and size (R and r ±0.01mm) using Pearsons Product Moment Correlation. All variables were normally distributed.

Table. 3.3.5 *Marthasterias glacialis*. Male (mean = 1.80E+10 sperm, SD = 1.86E+10, n = 18) and female (mean = 1.59E+06 eggs, SD = 1.40E+06, n = 15) gamete release (into 1L seawater) over an hour. Data are presented as overall means. Male and female wet weight (+0.01g) and size (R and r ±0.01mm) are also presented.

Table. 3.3.6 *Marthasterias glacialis*. Correlations between the number of sperm and male wet weight (±0.01g) and size (R and r ±0.01mm) using Pearsons Product Moment Correlation. Male wet weight and egg number were the only variables to be normally distributed (Appendix Table. 8). Therefore all data were ranked prior to analysis and tested non-parametrically. Parametric testing on the non-ranked data produced the same statistical conclusions.

Table. 3.3.7 *Odontaster validus.* Male (mean = 1.41E+10, SD = 1.61E+10, n = 8) and female (mean = 8.40E+4 eggs, SD = 8.78E+4, n = 9) gamete release over an hour. Data are presented as overall means. Male and female wet weight (+0.01g) and size (R and r ±0.01mm) are also presented.

Table. 3.3.8 *Odontaster validus.* The correlation between number of sperm and male wet weight (±0.01g) and size (R and r ±0.01mm) using Pearsons Product Moment Correlation. All male variables were normally distributed. All female variables were normally distributed except egg number. Therefore, the female wet weight, size and egg number were ranked prior to analysis and tested non-parametrically. Parametric testing on the non-ranked data produced the same statistical conclusions.

Table. 3.3.9 *Odontaster validus*. A Mann Whitney statistical test was used to compare the size (R and r ±0.01mm) and wet weight (±0.01g) of competent and non-responsive starfish. The majority of the size variables were not normally distributed, except radial length in the competent and non-responsive individuals (Appendix Table. 9). Therefore, the data were tested non-parametrically.

Table. 3.3.10 *Odontaster validus*. A Mann Whitney statistical test was used to compare the size (R and r ±0.01mm) and wet weight (±0.01g) of competent males and females with the total number of starfish injected. The size and weight variables of the competent males and females and the total number of starfish injected were normally and not normally distributed (Appendix Table. 10).

Table. 3.3.11 *Odontaster validus*. Test for equal variance in radial length (R ±0.01mm) between competent males and injected starfish and competent females and injected starfish.

Table. 3.3.12 *Laternula elliptica* and *Parborlasia corrugatus*. Wet weight (±0.01g) and size (±0.01mm) of bivalves (n = 9) and nemerteans (n = 2) strip spawned to investigate sperm swimming speed.

Chapter 4. Tissue Composition and Condition

Table. 4.2.1 Individuals were collected over monthly intervals from shallow water sites adjacent to Rothera Research Station. 10-15 starfish and brittlestars were sampled over a 4 and 10 month period respectively.

Table. 4.2.2 The biochemical composition of the standard Acetanilide and the error boundaries accepted for each constituent after calibration.

Table. 4.3.1 *Odontaster validus*. Table of values for residual water and non-protein nitrogen (NPN) used to estimate proximate composition from elemental analysis based on stoichiometry (Gnaiger and Bitterlich, 1984). The values of residual water and NPN show the values that optimised the results and returned data within the boundary limits of the macro (original default = residual water 6, NPN 5). All tissues had a default value of 0.15 for the mass fraction of N in the NPN. The mean %ash and mean %C in ash are also included. The last column details the number of individuals that were removed from further analysis after returning consistently negative lipid or carbohydrate values.

Table. 4.3.2 *Ophionotus victoriae*. Table of values for residual water and non-protein nitrogen (NPN) used to estimate proximate composition from elemental analysis based on stoichiometry (Gnaiger and Bitterlich, 1984). The values of residual water and NPN show the values that optimised the results and returned data within the boundary limits of the macro (original default = residual water 6, NPN 5). All tissues had a default value of 0.15 for the mass fraction of N in the NPN. The mean %ash and mean %C in ash are also included. The last column details the number of individuals that were removed from further analysis after returning consistently negative lipid or carbohydrate values.

Table. 4.3.3 *Odontaster validus*. Descriptive statistics (mean ± SD, range, N) describing the composition of the gonad and pyloric caeca tissues in males and females.

Table. 4.3.4 *Odontaser validus* and *Ophionotus victoriae*. One Way ANOVA and Kruskal-Wallis test statistics testing for differences between sexes and sampling month in the composition of bodily, digestive and reproductive tissues (normal and non-normal data). A significant difference was recorded at a significance level P < 0.05.

Table. 4.3.5 *Odontaster validus* and *Ophionotus victoriae*. The results of one-way ANOVA and the Tukey Test for pairwise multiple comparisons testing for differences between month sampled in bodily, digestive and reproductive tissues. A significant difference was recorded at a significance level P < 0.05. Differences identified by the Tukey Test are described numerically, (1-10 for February 2003-March 2004 in *O. victoriae* and 1-5 for December 16th 2003-March 2004 for *Odontaster validus*) displaying the month tested against the most dissimilar months in brackets. If a general overall difference was recorded the Tukey comparison was recorded as 'none'.

Table. 4.3.6 *Odontaster validus*. Test for equal variance in energy content of the pyloric caeca between males and females. P < 0.05 indicates unequal variance.

Table. 4.3.7 *Ophionotus victoriae*. Descriptive statistics (mean ± SD, range, N) describing the composition of the bodily, gut and gonad tissues in males and females.

Table. 4.3.8 *Odontaster validus*. Test for equal variance in %nitrogen content between female gonad and pyloric caeca tissue. P < 0.05 indicates unequal variance.

Table 4.3.9 *Ophionotus victoriae*. Comparisons between the %carbon content in the gut and gonad tissues in males and females. A Mann-Whitney non parametric analysis was employed and P < 0.05 indicated a significant difference in tissue biochemical composition.

Table. 4.3.10 *Ophionotus victoriae*. Comparisons between the %carbohydrate content in the gut and gonad tissues. Comparisons in these tissues in males and females are also recorded. A Mann-Whitney non parametric analysis was employed and P < 0.05 indicated a significant difference in tissue biochemical composition

Table. 4.3.11 Test for equal variance in %protein content between the gut of *Ophionotus victoriae* and the pyloric caeca of *Odontaster validus*. P < 0.05 indicates unequal variance.

Table. 4.3.12 Test for equal variance in %lipid content between the gut of *Ophionotus victoriae* and the pyloric caeca of *Odontaster validus*. P < 0.05 indicates unequal variance

Table. 4.3.13 Biochemical and proximate composition of the gonad and pyloric caeca tissue of *Odontaster validus*. Mean and standard deviation are given for sample sizes > 1. The raw carbon and nitrogen data were used to determine the amounts of protein, lipid, carbohydrate and energy using the Gnaiger and Bitterlich (1984) algorithm. An average %ash content was calculated from 15 random pyloric caeca and gonad samples. Values of C in ash are not included as the default value 0.01 was used for all samples.

Table. 4.3.14 Biochemical and proximate composition of the bodily tissues of *Ophionotus victoriae*. Mean and standard deviation are given for sample sizes > 1. The raw carbon and nitrogen data were used to determine the amounts of protein, lipid, carbohydrate and energy using the Gnaiger and Bitterlich (1984) algorithm. The ash content was calculated for each individual sample and a mean and the standard deviation reported. Values of C in ash were calculated by running each ash sample through the autoanalyser. Any zero or negative values were recorded as the default value 0.01.

Table. 4.3.15 Biochemical and proximate composition of the gut of *Ophionotus victoriae*. Mean and standard deviation are given for sample sizes > 1. The raw carbon and nitrogen data were used to determine the amounts of protein, lipid, carbohydrate and energy using the Gnaiger and Bitterlich (1984) algorithm. An average %ash content was calculated from 30 random gut samples. Values of C in ash are not included as the default value 0.01 was used for all samples.

Table. 4.3.16 Biochemical and proximate composition of the gonad of *Ophionotus victoriae*. Mean and standard deviation are given for sample sizes > 1. The raw carbon and nitrogen data were used to determine the amounts of protein, lipid, carbohydrate and energy using the Gnaiger and Bitterlich (1984) algorithm. An average %ash content was calculated from 30 random gonad samples. Values of C in ash are not included as the default value 0.01 was used for all samples.

Appendix

Table. 1 The division of histological samples between different workers. Laura Grange (LG) completed histology for all four species and repeated image analysis for all of the samples described. This required new photographs to be prepared and calibrated for each species and 100 oocytes per female to be re-measured. New photographs could not be prepared and analysed for *Heterocucumis steineni* collected between April 1998-December 1999 owing to the original slides being missing. Therefore, the original blocks were re-cut and new slides prepared for analysis by LG. The original histology completed by LG was not repeated and all repeated image analysis was carried out by the original methods employed by LG. Several months contained additional individuals not processed by the original workers and this was especially common in *H. steineni* collected between 1998-1999. LG processed these individuals through histology and image analysis.

Table. 2 *Ophionotus victoriae, Odontaster validus, Parborlasia corrugatus* and *Heterocucumis steineni*. Anderson-Darling Normality test on reproductive data. Normally distributed data are given by P-values >0.05.

Table. 3 *Ophionotus victoriae*. Test for equal variance in actual fecundity between individual years (1997 to 2001). Years with equal variance were indicated by an F-test and a Levene's test when P > 0.05.

Table. 4 *Odontaster validus*. Test for equal variance in radial length (R ±0.01mm) between individual years (1997 to 2001). Years with equal variance were indicated by an F-test and a Levene's test when P > 0.05.

Table. 5 *Heterocucumis steineni*. Test for equal variance in overall gonad index between individual years (1997-2001). Years with equal variance were indicated by an F-test and a Levene's test when P > 0.05.

Table. 6 *Odontaster validus*, *Marthasterias glacialis* and *Laternula elliptica*. Anderson-Darling Normality test on fertilisation success variables.

Table. 7 *Odontaster validus*. Anderson-Darling Normality test on female wet weight (±0.01g), size (R and r ±0.01mm) and fecundity (egg number). Normally distributed data were indicated by P > 0.05.

Table. 8 *Marthasterias glacialis*. Anderson-Darling Normality test on male and female wet weight (±0.01g), size (R and r ±0.01mm) and on sperm and egg numbers released during gamete release experiments.

Table. 9 *Odontaster validus*. Anderson-Darling Normality test on the wet weight (±0.01mm) and size (R and r ±0.01mm) of both competent and non-responsive starfish.

Table. 10 *Odontaster validus*. Anderson-Darling Normality test on the wet weight (±0.01mm) and size (R and r ±0.01mm) of competent males and females, and the total number of starfish injected.

Table. 11 *Odontaster validus* and *Ophionotus victoriae*. The Anderson-Darling Normality test statistics and recorded p-values for the biochemical and proximate composition of bodily, digestive and gonad tissue in two shallow-water Antarctic echinoderms. Normally distributed data are indicated by P > 0.05.

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Antarctica is regarded as the largest wilderness on Earth and its discovery has been described many times (Jones, 1982; Fogg & Smith 1990; Fogg, 1992; Yelverton 2000). Very little was known about Antarctica until approximately 1780, when it was referred to as ‘Terra Australis Nondum Cognita’ (The Unknown Southern Land) and was imagined to be a large landmass that covered the southern hemisphere (Headland, 1999). This period of the unknown was brought to a conclusion by the voyages of James Cook and Yves-Joseph de Kerguelen-Trémarec. Cook’s circumpolar navigation of the continent in 1772-1775 proved that this was not a rich, temperate habitable land, as was widely thought of by Man. On the contrary he experienced extreme cold, strong winds and huge expanses of ice and fog that hindered his progress further south.

These are characteristics we recognise in the Antarctic today and Antarctica is still the focus of much debate. We now have a bright outlook on Antarctic science and acknowledge its exceptional value to many other areas of research. Data from the pole is crucial in our deliberations about the global earth system. The continent is proving key to our understanding and monitoring of global warming and predicted climate change, and therefore inevitably the Antarctic story still has much to tell.

1.1 The Antarctic Ecosystem

Southern Ocean: The ocean around Antarctica has played a pivotal role in the development of knowledge concerning the continent's climate system. The Southern Ocean has the highest southerly marine latitude and is extreme, comprising the water surrounding the Antarctic continent, including the southernmost parts of the Pacific, the Atlantic and the Indian Oceans (Zwally *et al*., 1983). The Weddell, Bellingshausen, Admundsen, and Ross Seas lie adjacent to the continent. The Southern Ocean is unique among the world's oceans because the configuration of land and water in the Southern Hemisphere permits a circumpolar oceanic flow (Deacon, 1982). At the northern limit of circumpolar waters the Antarctic surface water sinks beneath less dense southward flowing sub-Antarctic water forming the phenomenon of the circumpolar front (El-Sayed, 1985). The polar-frontal zone is characterised by large gradients in temperature, salinity and density associated with the Antarctic Circumpolar Current and several mid-ocean ridge systems. Its position is not fixed; it fluctuates with changing temperature, pack-ice and currents, moving between 54ºS and 62ºS (El-Sayed, 1985). The Southern Ocean overlies areas of deep ocean, where the sea-bed forming the continental shelf is limited meeting the shelf break at ~1000m (Peck, in press). The latter is mainly a result of ice activity and its gouging of the sea floor, and the depression of the continent under the huge Antarctic ice sheet. The biological processes commonly associated with the continental shelf (patterns of resource supply, disturbance e.t.c) occur to a depth of 400-500m in the Antarctic, and this depth is also comparable to the deepest continental shelves elsewhere. Despite vast expanses of the shelf being covered in ice, the average width of the shelf is 125km, more than double the width of other continental shelves, and on this premise 5% of the world's continental shelf is in Antarctica (Peck, in press). This area of the global ocean is also instrumental in the conveyor belt of deep ocean water that regulates many aspects of global climate. In Antarctica cold, saline water is produced in the high Antarctic in the vicinity of ice shelves as ice consolidates and the residual salt is leached into the surrounding seawater. This high-density water sinks near the coast and after mixing with meltwater from the base of the ice shelves forms Antarctic Bottom Water. The largest volume is produced at the margins of the Filchner-Ronne ice shelf in the Weddell Sea. Once formed Antarctic Bottom Water migrates equatorwards along the continental shelf into the Southern Ocean, now having access to the seaways of the north (Deacon, 1982; Orsi *et al*., 1999). This system also serves the deep-ocean currents of the global Thermohaline Circulation (Brix and Gerdes, 2003; Jacobs, 2004).

The Marine Ecosystem: The physical conditions in Antarctica show extreme stability (Tressler and Ommundsen, 1962; Littlepage, 1965). Surface water temperatures range from 3.5ºC at the Antarctic Polar Front, to –1.8ºC near the continents edge. At the highest latitude Antarctic sites, such as McMurdo Sound (77º 51’S, 166º 40’E), sea temperatures may only vary by 0.1ºC around a mean of –1.8ºC (Littlepage, 1965, Clarke *et al*., 1988; Clarke and Leakey, 1996), although recent studies suggest even here temperatures may vary as much a 1ºC annually (Hunt *et al*., 2003). The Southern Ocean typically contains significant concentrations of seasonally available dissolved nutrients (Priddle *et al*., 1986; 1994). The marine environment is characterised by a background of cold but constant temperatures contrasted against an intense seasonal productivity. The study of seasonal biological responses in polar-regions therefore affords a natural opportunity to distinguish between the different effects of food availability and temperature (Clarke, 1988). This seasonality has a large influence controlling the tempos and timing of many biological processes.

Despite the harsh environment, marine productivity is high, especially within coastal regions, e.g. the Antarctic Peninsula (El-Sayed, 1984, 1985; Clarke *et al*., 1988; Holm-Hansen *et al*., 1989; Tréguer and Jacques, 1992; Clarke and Leakey, 1996), exhibiting comparable values with the upwelling systems of Peru, southeast Arabia, Somalia and southwest Africa (El-Sayed, 1988), the main difference being the shortness of the growth period (~8-10 weeks) (Whitaker, 1982; Clarke and Leakey, 1996). However, the spatial distribution of productivity in Antarctic waters is patchy and can vary by 1-2 orders of magnitude, where more conservative levels are associated with the sea floor and benthic microalgae (Dayton *et al*., 1986; Gilbert, 1991a; Brockington, 2001), the open ocean water column and phytoplankton (Priddle *et al*., 1994) and with melting sea ice and ice algae (Bunt, 1964b; Sullivan *et al*., 1988). The productivity levels below and within the sea ice zone have also been studied in some detail. These blooms tend to be composed of microalgae that are shade adapted owing to the shadowing effect of ice (Bunt, 1964a). Fritsen *et al*., (1994) and Kattner *et al*., (2004) also commented on the high productivity found in surface ice and the gap layers in sea ice, which are ubiquitous in the Antarctic pack-ice zone. The productive communities of the ice are also important as they provide a vital 'seed-stock' to developing planktonic communities and as ice is ephemeral, it releases ice-associated material into the water column, which is seasonally influential (Garrison, 1991; Savidge *et al*., 1996; Lizotte, 2001; Garrison *et al*., 2003). Productivity levels can also vary over long temporal scales. Current evidence suggests that unique circumpolar oceanographic and atmospheric processes operating on a sub-decadal time scale may be important in forcing this variability (Murphy *et al*., 1995).

Food is only available during a limited period of the austral summer, with blooms being initiated as early as November, peaking in January and collapsing no later than March, leaving the remainder of the year essentially devoid of autotrophic production (Whitaker, 1982; Clarke, 1988). This poses heavy constraints on consumers and their ability to feed, reproduce and survive extended episodes of starvation. Light levels increase during the austral summer encouraging the onset of phytoplankton blooms. The levels of light received at the ocean surface are regulated over spatial, including depth, and temporal scales (El-Sayed, 1984). Irradiance levels are important as they govern the maximum amount of energy available for photosynthesis (Whitaker, 1982) and secondly regulate ambient temperature. Broad scale disparity is caused by latitudinal differences in the amount of daily solar radiation reaching the sea surface, as well as variability in the sea ice extent and its effect on the levels of sunlight reflected. Wind-induced mixing events may also carry pockets of water, laden with photosynthetic cells, to depths in the water column with sub-optimal light levels. However in contrast, water column stability can maintain phytoplankton at the sea surface where maximum irradiance is focussed (Pingree, 1978; Whitaker, 1982, Priddle *et al*., 1986). The vertical stability generated by meltwater during the retreat of the ice zone has been suggested to cause ice-edge blooms (Smith and Nelson, 1986; Smith *et al*., 1987; Brierely and Thomas, 2002). Turbidity levels and cloud cover can also obscure photosynthetic cells by absorbing and/or reflecting light (Clarke and Leakey, 1996). Finally, sea ice plays a very important role in determining the dynamics of the marine ecosystem and, owing to the complex nature of this system, most environmental parameters exhibit large intra-annual as well as large inter-annual variation and are closely inter-linked (Clarke *et al*., 1988; Clarke and Leakey, 1996).

Other subsidiary processes regulate phytoplankton growth in the Southern Ocean, which include low temperature effects arresting cell growth rates (Eppley, 1972), nutrient levels and the availability of trace elements such as iron (Smith and Dierssen, 1996), although most data suggest that production is not nutrient limited in the Southern Ocean (Knox 1970; Dell, 1972; El-Sayed, 1984, 1985; Clarke *et al*., 1988). Even when productivity levels are at their maximum, the availability of nutrients remains well above limiting values (Hayes *et al*., 1984). Recent findings demonstrate that iron supply controls phytoplankton growth and community composition during summer in polar Southern Ocean waters (Boyd *et al*., 2000; Coale *et al*., 2004). Grazing is probably the most equivocal control ranging between rates that vary from a few percent of daily phytoplankton production to more than a significant proportion of the available biomass. Grazing may also influence the size structure of phytoplankton in the Southern Ocean (Smith and Dierssen, 1996).

The Antarctic Benthos: The huge expanses of ice common across Antarctica's perimeter appear impressive and inhospitable, however in truth there is a wealth of life that exists beneath on the sea floor (Dearborn, 1965; Clarke and Johnston, 2003). Studies have also yielded evidence for the existence of benthic assemblages beneath the Antarctic ice shelves (reviewed by Dayton, 1990) and the fast ice (Littlepage and Pearse, 1962; Dearborn, 1963, 1967b). Benthic communities contain the true indigenous fauna of the Antarctic (Picken, 1985), and show a high incidence of endemism having evolved over millions of years. De Broyer and Jazdzewski (1996) reported an 80% level of endemism at the species level and 17% at the genus level for Antarctic amphipods. Even higher levels have been observed in the pycnogonids ~90% (Arntz *et al*., 1997).

A consensus of modern opinion suggests the limit of the Antarctic benthos to extend to South Georgia, and the sub-Antarctic islands of Heard, Macquarie and Kerguelen (Dell, 1972). White (1984) delimited the Antarctic benthos to the area south of the Antarctic Convergence/Polar Front. However, although a clear physical boundary to the pelagic, it is less absolute when it comes to the benthos, which connects to the northern seaways of the Pacific, Atlantic and Indian Oceans in the vicinity of the front. Therefore, areas of the Pacific, Indian and Atlantic Ocean floors are inevitably under Antarctic influence at the outer edge of the Antarctic continental shelf (Knox, 1970). Seawater temperatures below 30m in the Antarctic are comparable in temperature to the deep ocean sector of the world's oceans, and therefore temperature is an unlikely limiting factor in determining the northern extent of the benthos. Picken (1985) described the potential for sediment characteristics to determine benthic faunal composition and distribution. Much of these sediments are terrigenous in origin and are deposited as ice moves northwards and melts. Therefore, the northern limit of the pack ice may be a more realistic limit of the benthos. The basic biogeographic subdivisions of the Southern Ocean benthic fauna are South Georgia, the Antarctic Peninsula (including the South Orkney Islands) and high Antarctic, comprising the fauna on the continental shelf at high latitudes. However, noteworthy differences between East and West Antarctica are apparent (Clarke and Johnston, 2003). The intermediate latitudes of the Antarctic Peninsula and the South Orkney Islands are sometimes referred to as the maritime Antarctic (Fig. 1.1.1).

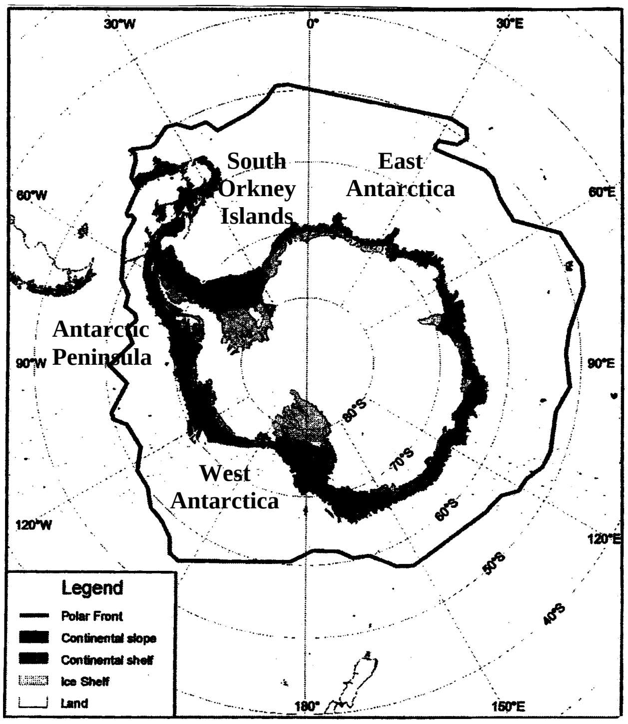

Fig. 1.1.1 Adapted from Clarke and Johnston (2003). Map of the Southern Ocean showing the mean position of the Polar Front (the Antarctic Convergence) and broad scale bathymetry around the Antarctic continent. Regions of East and West Antarctica and the Antarctic Peninsula are also indicated, including the South Orkney Islands.

Ice: Ice has a very important impact on the marine benthos (Dayton *et al.*, 1969), affecting daily light levels, salinity, temperature, sedimentation events and ocean currents. The influence of ice is variable. The impact of sea ice and small growlers are restricted to shallower water. However, large icebergs can influence the seabed $\leq$600m (Gutt and Starmans, 2001). Scouring on the continent transports huge quantities of land-based material from the continent onto the seabed as icebergs break-up, melt and deposit their load. These deposits tend to be poorly sorted and devoid of any river borne material. Small amounts of biological material, including diatomaceous elements and sponge spicules, and volcanic debris can also be present. The abrading of the coast can occur from the activity of fast ice, pack ice, brash ice, icebergs and bergy bits. Anchor ice, submerged ice attached or anchored to the bottom, is also important (Gutt, 2001). The literature suggests that approximately 5% of the Antarctic shelf is affected by grounded icebergs (Gutt, 2000), although locally this figure may be higher and depends on how the continental shelf is described. Some of the larger mobile icebergs are very effective at removing and displacing benthic flora and fauna as they plough large furrows along the seabed. These tabular ice masses often have 80% of their overall volume submerged and the results of Gutt and Starmans (2001) showed that iceberg scouring is among the five most significant disturbances that a large ecosystem on earth can experience. However, moderate ice scouring has also been suggested an agent for the considerable diversity we observe in the benthos and can be explained by the 'intermediate-disturbance hypothesis' (Huston, 1979; Barnes *et al*., 1996). The benthos can also be invaded by the presence of anchor ice, which forms as 2-5mm thick platelets directly on the ocean floor, and has the capacity to encase and trap benthic inhabitants, eventually removing them from the substratum (Picken, 1985). Dayton *et al*., (1970) and Dayton (1989) quantified the degree of disturbance caused by anchor ice in McMurdo Sound, reporting effects down to 33m depth, and suggested that this physical stress resulted in a vertical zonation of the benthos, (Gruzov and Pushkin, 1970; Shabica, 1972; Dayton, 1989). A piece of ice 2m^2 can carry 25kg of sediment, including animals such as the asteroid *Odontaster validus*, the nemertean *Parborlasia corrugatus*, the echinoid *Sterechinus neumayeri*, as well as isopods, fish and macroalgae. Anchor ice is therefore a factor, which can delimit the upper limit of the unique and generally rich benthic assemblage (Gutt, 2001).

Temperature: Temperatures in the Antarctic benthos are low and restricted over a narrow range around zero. The largest range in temperature is observed in surface waters and the shallow sub-littoral zone (4ºC to -1.9ºC), and is more marked at lower latitudes. Deeper zones are more conservative in temperature experiencing a range between –1.8ºC and 0.4ºC. Salinity and oxygen levels tend to be stable, however regions of ice formation and melting are exceptions to this general rule (Barry, 1988).

Seasonality: An intense seasonal signature in primary production contrasts the near-constant physical setting. The availability of food to benthic communities largely relies on the passage of pelagic primary production to the sea floor (Dell, 1972; Karl *et al*., 1991; Brockington, 2001), which can be highly variable. A single summer sedimentation event is common at high latitudes and is typical of polar ecosystems, compared to the bimodal episodes commonly observed in boreal waters (Caron *et al*., 2004). Annual vertical flux rates in the shallow-water nearshore Antarctic tend to vary between 2 and $50gCm^{-2}dy^{-1}$ during the winter and summer respectively (Cripps and Clarke, 1998). The transition of material between the pelagic to the benthic system is often recognised as the beginning of the benthic food web and a seasonal metabolic response by the sediment community follows (Karl *et al*., 1991; Graf, 1992). A proportion of the primary production is either recycled or remains suspended in the euphotic zone and is available to zooplankton grazers rather than being exported to the benthic community. However, any remaining cells that settle to the sea floor are then available firstly to the benthic suspension feeders, and then the remaining benthos (Gilbert, 1991a; Graf, 1992; Nedwell *et al*., 1993). A close coupling between benthic secondary production and vertical flux events has also been commented on in the seasonal environment of the deep sea (Tyler, 1988). There appears to be relationship between the processes in the water column and the biological processes on the seabed (Billett *et al*., 1983; Deuser, 1986).

In the nearshore, shallow environment the residence time of particulate matter in the water column is relatively short so that much material arrives at the seabed unaltered (Cripps and Clarke, 1998). Mincks *et al*., (2004) referred to the seasonal deposition of phytodetritus on the shelf of the West Antarctic Peninsula after the ice retreat, as a major food source for the shelf benthos and suggested that benthic detritivores could benefit from such a reserve all year round. The biochemical composition of the vertical flux depends upon its source and the various biogeochemical processes operating in the water column, including the effects of predation (Höglander *et al.*, 2004). Differences in species composition between the water column and sediment traps indicate large differences between species variability in settling (Cripps and Clarke, 1998; Höglander *et al.*, 2004).

Many studies have reviewed the frequency, content and impact of these sedimentation events. These occurrences have been associated with water mass exchange, the deposition of biological material after the break up of ice cover and/or bloom collapse and the release of fecal matter from zooplankton feeding on the derivatives of phytoplankton and ice algae (Palmisano *et al.*, 1985; Bathmann *et al.*, 1991). Sedimentation is thought to be the predominant loss of the spring bloom (Höglander *et al.*, 2004), after a period of increased storm activity and light deprivation (v. Bodungen *et al.*, 1986), and the transition of living cells to sinking, resting spores, which are then available to seed the next year's growth. Therefore, phytoplankton seeding patterns can have considerable effects on the transfer of biomass from pelagic to benthic subsystems (Bunt, 1964a) and the absolute biomass available to pelagic and benthic heterotrophs. The vertical flux can also show inter-annual variability associated with the differences in the intensity of annual autotrophic production (Cripps and Clarke, 1998). However, infaunal grazing and incidences of high bacterial turnover can sometimes mask the magnitude and importance of a post-summer sedimentation event (Gilbert, 1991b).

To conclude there is extreme seasonality in the Antarctic sediments, that is not temperature limited, but regulated by the seasonal availability of organic material. Inshore levels of benthic activity are comparable to those measured in temperate and tropical sediments (Nedwell *et al.*, 1993). The degree of coupling between the pelagic and benthic ecosystems may therefore be important depending on the oceanographic characteristics of a location and the feeding strategies of its inhabitants (Graf, 1992; Gutt *et al.*, 1998). This was emphasised by Dayton, (1990) who suggested that benthic communities are in fact organised around the nature, abundance and regularity of organic inputs, as well as the way in which they utilise these resources and the frequency with which they are disturbed.

The benthos is also a source of primary production, possessing large communities of microlagae, whose activity is seasonal depending on the levels of light that permeate to the seabed (Nedwell, 1989; Gilbert, 1991a, 1991b). Therefore, ice cover and the intensity and persistence of surface bloom events affect the waxing and waning of benthic blooms. These events are common in the austral spring after the ice retreats and before the summer bloom becomes properly established. As the pelagic bloom collapses and light levels increase in the water column, a second bloom can be observed in the benthos. Brêthes *et al.*, (1994) observed the reliance of the limpet *Nacella concinna* on the standing stock of microphytobenthos, compared to a low requirement for ice algae and/or phytoplankton standing stock. Similarly, *Laternula elliptica* was observed to benefit from benthic primary production and the advection of allochtonous food particles from the seabed by wind action (Ahn, 1997). Therefore, benthic production can also play an important part in the seasonal production cycle, especially in the seeding of the surface bloom through resuspension and the provision of food for filter feeders (Dayton and Oliver, 1977; Barry and Dayton, 1988; Dunbar *et al.*, 1989; Priddle *et al.*, 1995), other epifauna and infauna during the winter (Davenport, 1988; Gilbert, 1991a, 1991b).

1.2 Benthic Flora and Fauna

Origins: Antarctica formed at the centre of an ancient supercontinent called Gondwana, comprising Africa, South America, India, Australia and New Zealand. Scientists have now reconstructed a realistic chain of events documenting the passage on Gondwana through geological time. The final break in Gondwana occurred 25-35 million years ago between South America and Antarctica, at which point Antarctica reached its final polar position with widespread consequences (Livermore *et al.*, in press). This led to the opening of the Drake Passage and the surrounding of the continent in its entirety by water. The subsequent development of the Antarctic Circumpolar Current completed the climatic isolation of the continent and at this time the region began to correspondingly cool. Low temperatures encouraged the expansion of mountain glaciation to lower altitudes eventually producing the continental ice sheet, ice shelves and the full scale East Antarctic ice cap we recognise today. This occurrence also led to the inception of the Polar Front, an important (but not absolute) boundary to the physical passage of water from the

Antarctic into the three connecting ocean basins to the north and the migration of species and genetic material between hemispheres. The progressive isolation of the continent had important implications for Antarctic flora and fauna. In some instances extinction was inevitable as ice sheets ploughed across the continent. However, many marine species were able to adapt and accommodate change and soon became established, which resulted in the current high incidence of endemism (Arntz *et al*., 1994; Clarke and Johnston, 2003). White (1984) and Picken (1980) developed the concept that high levels of endemism resulted from the isolation of the Antarctic fauna after the circumpolar current system became properly established, and formed a hydrological barrier to easy dispersal.

Hypotheses discussing the origins of Antarctica's biology have been diverse suggesting colonization from the deep-sea and/or South America along the Scotia arc, and evolution *in situ* from a relict fauna to be likely (Lipps and Hickman, 1982). Clarke and Crame (1989) reviewed the origins of the Southern Ocean marine fauna and commented on the continuity with which shallow water habitats have been present over geological time. Geophysical evidence suggests that shallow waters have been accessible since the late Mesozoic possibly even longer, and on occasions were considerably more extensive than present. Fossil evidence dating from the Cretaceous/Tertiary suggests the existence of a diverse shallow water marine fauna with affinities with both the Tethys and Pacific basins, with the notable absence of two main groups: the decapods and teleost fish. Explanations for the absence of some groups are not yet forthcoming and it is unlikely that low temperatures have had any real part to play, as the teleost fish and decapods are well represented in the Arctic fauna. The exclusion of groups by geographical constraints is by no means an exhaustive explanation either, as all fauna would have been subject to the same barriers. Possibly habitat reduction from past intermittent ice events holds the key, encouraging the diversification of some groups into vacant ecological niches. Therefore, the apparent absence of some species and the success of others is more likely the effect of historical contingency (Clarke & Crame, 1992).

The extant fauna, is in many cases, highly diverse, reminiscent of numbers and diversities noted in any lower latitude location. However, the poor representation of some groups has given support to the theory of a latitudinal cline in species richness

(Clarke and Johnson, 2003). The reason for a cline in some species is not yet clear. It is unlikely that low temperature or the age of the Antarctic has played a pivotal role as many high diversity groups exist at the same temperature and unlike the lower diversity Arctic, Antarctica has evolved over longer time scales. However, this cline does appear striking in some animals, for example in molluscs. The occurrence of small size, poor calcification and the incidence of low ornamentation has been widely documented in cold water molluscs (Nicol, 1967). Clarke (1983) has suggested that this may reflect a high metabolic penalty of precipitating calcium carbonate from solution in seawater at low temperature and may explain the low numbers observed. However, other taxa with calcareous skeletons are diverse, such as echinoderms, brachiopods and pycnogonids, suggesting this explanation to not be exhaustive. Clarke and Crame (1989) suggested that reduction in temperature during the Cenozoic was an unlikely evolutionary barrier for most fauna. Such a barrier is now believed to have evolved *in situ*, and to have been present since at least the Late Cretaceous and possibly before. Therefore, the biological signatures we see today in the Antarctic are more likely to be a response to the intense seasonality, which characterises the continent, and not the low temperatures *per se* (Clarke, 1990). Several modern day groups comprise a large number of closely related species, which are almost certainly the product of adaptive radiations *in situ* over long time periods e.g. pycnogonids, gastropods, echinoderms, ascidians and notothenioid fish (Dell, 1972). The isolation of the Southern Ocean and the limited means for dispersal across the Polar Front lend more weight to the argument for the existence of a long established fauna with Cretaceous ancestry, and has eradicated the perception of the Antarctic marine habitat as an evolutionary backwater.

1.3 Antarctic Reproduction

During the 19th century Antarctic and Arctic taxa were thought to be very similar. This led to the idea of a global bi-polar fauna connected through the deep-sea via tropical submergence or reflecting the cold climates of the past (reviewed by Hedgpeth, 1957). Early expeditions also discovered brooding taxa in the sub-arctic and sub-antarctic giving rise to a classic reproductive paradigm, which was later given credence by Thorson (1950). Orton (1920) also received considerable support when he described a link between reproduction and temperature (reviewed by Giese and Pearse, 1974). The outcome of this relationship led many to believe that the constant conditions at the poles would predispose taxa to continuous aseasonal development. Many also thought that the metabolic and nutritional demands of reproductive taxa at the poles would be the same as warm water taxa, mainly owing to the acceptance at the time of metabolic cold adaptation and temperature compensation.

The reproductive characteristics originally assigned to Antarctic taxa were based on the knowledge that Antarctica exhibits a unique set of environmental conditions, namely a background of constant low temperatures contrasted against a highly seasonal food regime (Arntz *et al*., 1994). Such features, it was thought, include prolonged gametogenesis, deferred maturation, low fecundity and low reproductive condition (Clarke, 1987). These characteristics were said to be consistent with those of *K-strategists* (Clarke, 1979) and are commonly exhibited, at least in part, by a number of polar benthic invertebrates. Animals were also assumed to invest an appreciable amount of maternal energy in large egg development and therefore non-feeding larval patterns and a relatively high frequency of brood protection was presumed to be likely (Picken, 1985). Thorson (1950) observed this trend in a wide range of benthic invertebrates sampled from the Arctic and found larger egg sizes in these animals compared to their European, temperate neighbours. He concluded that 95% of all marine species of bottom invertebrate in the Arctic seemed to develop without a pelagic larva and suggested that this occurrence might be even more pronounced in the Antarctic. Most of Thorson’s work was based on studies of the Prosobranchia, a group he named the “barometer of the ecological conditions in the sea”. Thorson reconciled the gradual replacement of pelagic development with alternative strategies at the poles and in the deep-sea as a response to adverse abiotic and biotic conditions. This theory was reviewed by Mileikovsky (1971) who coined the term ‘Thorson’s Rule’ to explain a preference for non-pelagic development in larvae, which was modelled by Vance (1973). In general the eggs produced by the Antarctic benthos were thought to be larger than the spawn of comparable temperate species, and this represents another element incorporated into the evidence for another original theory of Thorson for a latitudinal cline in egg size (Clarke, 1992). However, the evidence for this trend is as yet equivocal. Egg size can also vary between females in a population. Larger females sometimes producing larger eggs can explain some of this variation (Emlet *et al*., 1987). It is inevitable that there is a trade-off between egg size and fecundity, in that individuals producing many eggs tend to favour an average of smaller sizes. However, it is likely that both phenotypic and environmental factors are influential.

Thorson's rule appeared plausible when the hazardous nature of the pelagic environment was considered. Chia (1968) argued that, in an unstable environment where the phytoplankton crop may fail, natural selection would favour those species, which evade life cycle stages in the plankton. Hardy (1960) suggested that the salinity fluctuations common in shallow water, where ice forms and melts, would adversely affect pelagic larvae, as would the storm activity notorious in the Southern Ocean (Ostregren, 1912). Similarly the precedence of direct development, and limited dispersal, conveniently explains the isolation of the Antarctic taxa and the large numbers of endemic fauna observed (White, 1977).

However, more recent studies have contradicted this major paradigm, presenting data confirming the presence of pelagic feeding larvae amongst several benthic taxa endemic to the Antarctic (Pearse *et al*., 1991: Berkman *et al*., 1991; Pearse, 1994), e.g. *Sterechinus neumayeri* (Pearse and Giese, 1966b), *Odontaster validus* (Pearse, 1965), *Parborlasia corrugatus* (Peck, 1993), *Nacella concinna* (Picken, 1979), *Liothyrella uva* (Peck and Robinson, 1994) and three large bivalves (*Laternula elliptica, Limatula hodgsoni and Adamussium colbecki*). This suggests that the views of Thorson describe a general tendency as opposed to a rule (Clarke, 1992). This was further exemplified through the work of Hain and Arnaud (1992), who used the size of early larval shells of molluscs from the Eastern Weddell Sea, reputed to be a better predictor of larval development mode than egg size, to review Thorson's data set. They concluded that out of 43 species of bivalve collected, 13 species reproduced via lecithotrophic larvae and 14 species by planktotrophic larvae. Brooding occurred in the 16 remaining Bivalvia species. A study by Pearse *et al*., (1991) looking at echinoderms agrees with the revisions of Thorson's work and validates the assertion that pelagic lecithotrophy and planktotrophy are common at southern polar latitudes and more important than once thought (Pearse *et al*., 1986).

Some of the more conspicuous echinoderms observed in McMurdo Sound by Pearse (1994) produce pelagic planktotrophic larvae; the asteroids *Odontaster validus*, *O. meridionalis* and *Porania antarctica* (Pearse and Bosch, 1986; Bosch, 1988, 1989), the echinoid *Sterechinus neumayeri* (Bosch *et al.*, 1987), and the ophiuroid *Ophionotus victoriae* (I. Bosch and R. Britton, unpublished observations of egg size, about 180µm diameter). These invertebrates are typical of the productive shallow water marine environment of Antarctica, which is frequently disturbed by anchor ice formation, and the correlation between pelagic development and high species abundance in the Antarctic has already been remarked upon by Clarke (1992) and reviewed by Mileikovsky (1971).

Pearse (1994) concluded that pelagic lecithotrophy predominates for all cold water localities, i.e. the deep sea, Arctic and Antarctic. Reasons for this are not completely forthcoming. However, the limited food supply does not appear to be the only important factor. It is more likely that other factors such as improved dispersal/colonisation capability, rate of development at low temperatures (Emlet *et al.,* 1987) or juvenile size at metamorphosis and habitat complexity, including potential larval predation may be more important (Ghiselin, 1987; Pearse *et al.*, 1991). The Antarctic benthos possesses very diverse and complex biotic assemblages, where larger eggs and the resulting advanced juveniles would have a competitive advantage (Pearse and Lockhart, 2004) and therefore this may explain the higher incidence of lecithotrophy.

However, some Antarctic groups do forego pelagic development and display a high incidence of brooding; this is especially true of cidaroid and spatangoid urchins and prosobranch gastropods, with the exception of *Nacella* (Picken, 1979). Pearse and Lockhart (2004) suggest that this is not an adaptation to low temperature and low larval food supply; instead they suggest that species-rich clades of brooders probably reflect enhanced speciation under exceptional Antarctic conditions, e.g. features such as the "species diversity pump" caused by glacial cycling encouraging speciation in isolated refugia (Clarke and Crame, 1992, 1997). Cidaroid urchins are an interesting exception to the modern day perceptions of Antarctic reproduction. This group comprises one or two clades that compose 80% of the regular urchin species in the Antarctic. One species almost exhibits bipolarity, extending from the Antarctic shelf through to the eastern Pacific to Alaska. Most species brood and reproduction appears aseasonal. Discoveries such as this have forced us to review the old paradigms and reconsider the ideology that the biodiversity patterns of marine invertebrates observed currently in the Antarctic are an adaptation to local environmental conditions. Instead, these patterns may be a result of several processes operating over different timescales. Poulin *et al*., (2002) has explained the unusually high proportion of brooders compared to broadcasters along the coastal Antarctic by describing a macroevolutionary trend resulting from differential extinction rates in development modes during adverse environmental conditions. They hypothesised the ecological dominance of broadcasters to be a result of processes operating at ecological timescales that are associated with the advantage of having a pelagic larvae under highly disturbed conditions (Poulin *et al*., 2002).

***Antarctic Reproductive Cycles*:** The degree to which reproductive traits are underpinned by the intense seasonality is variable among polar species (Arntz *et al*., 1994) and may be expressed by the adult and/or during the larval phase. A number of hypotheses have been proposed to explain why seasonal reproduction is an evolutionary stable strategy (ESS) for many marine organisms (Olive *et al*., 2000). Some hypotheses have been developed to derive a fitness advantage for seasonal reproduction from factors impacting on the success of the larval phase, such as constraining breeding to times when larval food is plentiful and fitness is maximised. It is therefore supposed that these times can be predicted from environmental signals providing a mechanism for the evolution of physiological responses that deliver larvae at the optimum time. A second group of hypotheses can be identified where the fitness component is related to the synchronous release of gametes rather than the timing of release. The selective advantage of seasonal reproduction, the so called ultimate factors, however, are potentially diverse and not well understood especially in the Antarctic. It has become accepted that a distinction can be made between these factors and proximate factors; the environmental inputs that are transduced by an organism allowing physiological responses to occur that organise a temporal sequence of cellular and physiological events culminating in seasonal reproduction.

Some Antarctic invertebrates do display strong seasonal oscillations in reproduction and growth, and tend to release their eggs/larvae/juveniles into the water column coincident in timing with the summer bloom period (Pearse, 1965; Arnaud, 1977). Many of these species have relatively rapid gametogenic cycles generally complete within one year, have diminutive size and show fast growth during nutritionally rich periods of the summer, and overwinter as slow development stages (Arntz *et al.*, 1992), e.g. the planktonic copepod *Calanoides acutus*. Some seasonal breeders do however express prolonged reproductive cycles, e.g. *Euphausia superba,* which exhibits a deferred gonad cycle (> 1yr) and cyclic growth, which is elevated during the productive summer period. A similar response occurs in the deep sea, where the availability of organic matter through sinking is thought to form a labile food source to drive reproductive growth (Tyler *et al.*, 1982; Tyler, 1986).

Some of the most abundant shallow water invertebrates in Antarctica produce pelagic feeding larvae and exhibit gametogenic cycles underpinned by a strong seasonal cyclicity. Despite producing pelagic feeding larvae, a number of these invertebrates consistently deliver their progeny into the water column out-of-phase with the annual summer bloom event, e.g. *Odontaster validus* has a seasonal breeding regime spawning in the austral winter (Pearse, 1965; Tyler *et al.*, 2003) and *Sterechinus neumayeri* spawns in the early austral spring (Pearse and Giese, 1966b). These echinoderms produce a feeding larva, which spends long periods in the plankton (Pearse and Bosch, 1986; Clarke, 1992; Stanwell-Smith *et al.*, 1999), mainly acting as a dispersive agent avoiding the hazards of Antarctic surface waters before developing feeding capabilities during the following bloom event (White, 1977). Bosch *et al.*, (1987) described the spawning cycle in *Sterechinus neumayeri* and concluded that a spring spawning period ensures larvae are in the plankton during the abbreviated summer bloom and are recruited as juveniles to the benthos when benthic chlorophyll a concentrations will be high.

These larvae appear to benefit from an alternative food source utilising dissolved organic material, bacteria and picoplankton, surviving independently of the micro-plankton cycle during a period when particulate material is diminished (Rivkin *et al.*, 1986; Pearse *et al.*, 1991). The incidence of bacterivory and osmotrophy is relatively high among the larvae of benthic invertebrates (Rivkin *et al.*, 1986; Olson *et al.*, 1987; Manahan, 1990; Rivkin, 1991) and Peck (1993) found that larvae of the nemertean *Parborlasia corrugtaus* were capable of feeding on particles less than 1μm. Bosch *et al.*, (1990) also showed that algae, bacteria and dissolved organic matter could all make significant contributions to the nutrition of larvae of the Antarctic starfish *Porania antarctica*.

Additionally the low temperatures observed in the Antarctic predispose feeding larvae to low metabolic rates and this may also contribute to larval survival during extended periods of apparent starvation (Olson *et al*., 1987; Hoegh-Guldberg and Manahan, 1995). A similar response is seen in temperate and tropical larvae on occasions when unsuitable conditions for settlement are experienced and metamorphosis is delayed extending the larval period in the water column. The larvae of the starfish *Mediaster aequalis* are lecithotropic and have insufficient maternal reserves to support a prolonged period in the plankton, consequently these larvae have to either down-regulate their metabolism or begin to uptake DOM (Bryan, 2004).

Spawning during a nutrient poor winter is also possible as some taxa possess specific storage organs, which store energy reserves for periods of limited food availability, e.g. the pyloric caeca of the starfish *Odontaster validus*. Alternatively, adults releasing their progeny during the austral winter may benefit from a reduced risk of predation (Clarke, 1992). Benthic suspension feeders are understood to be prevalent in the Antarctic and some cease feeding in the winter, making this a more rational time for pelagic larvae to be released. However, the traditional view of a long period of winter starvation may need to be revised as there are some Antarctic suspension feeders capable of feeding virtually all year round, only ceasing for 2-3 months around the middle of the austral winter (June-July) (Barnes and Clarke, 1995). The bryozoan *Arachnopusia inchoata* appears to feed continuously throughout the year (Barnes and Clarke, 1994) and therefore the threat of predation can remain even during the winter.

Seasonality of various types is common in Antarctic benthic invertebrates (reviewed by Clarke, 1988; Pearse *et al*., 1991; Brockington 2001). Orton's (1920) original perception that sea temperature is very important for synchronizing reproduction in marine invertebrates is of limited generality. His original perceptions were based on observing animals with mature gonads and associating these trends with definite temperatures and spawning. These events are not synonymous since in many species mature gonads are present long before spawning (Thorson, 1946). Factors with a more pronounced seasonality such as food availability and photoperiod are most probably more important, e.g. for *Odontaster validus* (Pearse, 1998). In contrast some taxa remain completely decoupled from the seasonal bloom event, reproducing either aseasonally with the capacity to broadcast propagules into the water column during the majority of the year (*Glyptonotus antarcticus*, Dearborn, 1967a; White, 1970) or by investing large amounts of energy into their eggs and displaying lecithotrophic development or viviparity (Meidlinger *et al*., 1998). However, there are exceptions for example in the amphipod *Orchomene plebs* that broods releasing juveniles seasonally during the austral summer bloom. The ascidian *Cnemidocarpa verrucosa* also reproduces seasonally during the winter via a lecithotrophic larva with protracted development (Sahade *et al*., 2004) possibly securing development during the summer phytoplankton bloom.

Levels of larval mortality are high in the water column (Strathmann, 1985). Therefore, environmental factors will be important in determining larval development mode and timing in the plankton, as well as determining benthic community structure (reviewed by Todd, 1998). Therefore, as in many other environments, the reproductive tempo of the Antarctic is controlled by the specific needs and constraints of species, and varies both among and between habitats.

Gametogenesis: The gametogenic development period of many invertebrates appears deferred in the Antarctic. Extended periods of oogenesis and spermatogenesis have been attributed to both the low temperature and low-level resource supply (Picken, 1985; Pearse, 1994). The ovary can exhibit two or even three annual cohorts of growing oocytes in the same animal at one time (Pearse *et al*., 1991) producing bimodal and sometimes tri-modal oocyte size distributions. These distributions arise after an overlap between the rapid growth of older oocytes, and a rapid replenishment of the supply of smaller oocytes to the oogonial pool. This pattern appears in a number of Antarctic seasonal breeders that have a period of gametogenic development between 18 and 24 months (Pearse 1965; Powell *et al.,* 2001; Brockington *et al.,* submitted). Bimodal oocyte distributions have been observed in taxa exhibiting both free-living and protected development strategies, e.g. *Adamussium colbecki* broadcasts planktotropic larvae (Pearse, 1965) whilst *Lissarca* *notorcadensis* broods embryos (Brey and Hain, 1992). This pattern has also been observed in *Odontaster validus* from McMurdo Sound (Pearse 1965), the sea urchin *Sterechinus neumayeri* (Pearse and Cameron 1991; Brockington *et al.,* submitted), the infaunal bivalve *Laternula elliptica* and the limpet *Nacella concinna* (Powell, 2001) as well as deep sea echinoids (Tyler & Gage 1984a, b). Other Antarctic taxa with long gametogenic development times include the brachiopod *Liothyrella uva* (Meidlinger *et al*., 1998), the octocoral *Ainigmaptilon antarcticum* (18-24 months) (Orejas *et al*., 2002) and the amphipods *Bovallia gigantea* (> 12 months) and *Kidderia subquadratum* (15-19 months) (reviewed by Clarke, 1988). The prolonged development cycles characteristic of many Antarctic taxa allow the storage of energy over two consecutive summers, interspersed with a single winter period of limited food. This adaptation may provide invertebrates with a way of overcoming the limited resource and restricted energy acquisition period of the winter, although the scallop *Adamussium colbecki* and the ascidian *Cnemidocarpa verrucosa* exhibit a discrete 12-month cycle (Tyler *et al*., 2003; Sahade *et al*., 2004).

Inter-annual Variation: Work on long-term reproductive cycles to date in marine environments worldwide is limited. Most studies are based on a grant or PhD duration of 2-3 years, which is inadequate to characterise variation between years, or even to evaluate seasonal variation accurately and identifying multi year trends is not possible. In Antarctica the situation is even worse. A few studies of reproductive cycles have been reported from Antarctic species using monthly samples over a 1-2 year period (Brockington *et al*., submitted; Tyler *et al*., 2003; Sahade *et al*., 2004). Some of these have shown considerable inter-annual variation in reproductive condition, e.g. the brachiopod *Liothyrella uva* (Meidlinger *et al*., 1998), the bivalve mollusc *Adamussium colbecki* (Chiantore *et al*., 2002) and the ascidian *Cnemidocarpa verucosa* (Sahade *et al*., 2004). However, despite inter-annual variability in reproductive condition being high, the timing of reproductive events shows remarkable consistency between years in many species. These data provide evidence of a plastic reproductive pattern in many Antarctic invertebrates, adapted to overcome the low level resource supply characteristic of the Antarctic continent and its unique environmental conditions. These patterns go some way to explain how some invertebrates with flexible ecologies and capabilities to accommodate inter-annual variation, have become dominant members of the Antarctic benthos.

However, this is not just an Antarctic phenomenon. Studies of many tropical and temperate invertebrate taxa have alluded to plastic reproductive patterns and year-year variations in reproductive characteristics, e.g. *Nepthys caeca* and *N. hombergi* (Oilve *et al*., 1985, 1997), *Strongylcentrotus droebachiensis* (Meidel and Scheibling, 1998) and *Pecten maximus* (Dorange *et al*., 1989).

Rate of Larval Development: Stanwell-Smith and colleagues (1999) studied the distribution, abundance and seasonality of marine invertebrate larvae in the maritime Antarctic at Signy Island (60º 43'S, 45º 36'W). The larval ecological diversity observed was comparable to the levels reported in temperate locations and higher than in the Arctic. However, larval abundances were comparatively low because of the long development period in the plankton and dilution over large spatial scales (Okubo, 1994). Shreeve and Peck (1995) also observed a broad diversity of 16 larval and juvenile types representing 7 invertebrate phyla in the Bellingshausen Sea, of which the early stages of nemerteans and echinoderms were most common.

Many studies have recorded the delayed rate at which embryonic development progresses in the Antarctic (Arntz *et al*., 1994), the most extreme example being the brooding gastropods, where some broods hatch after 30 months (Hain, 1991). In general, most studies reveal a reduction in development rate by x2-x20 for echinoderms (Bosch *et al*., 1987; Gutt, 1991; Stanwell-Smith and Peck, 1997) brachiopods (Peck *et al*., 2001) and nemerteans (Peck, 1993). *Sterechinus neumayeri* requires around 115 days to metamorphosize from a larval to juvenile phase at its ambient temperature (-1.5ºC), compared to the 60 days needed by its temperate Echinidae equivalent (10-16ºC), *Parechinus angulosus* (Bosch *et al*., 1987). Hoegh-Guldberg and Pearse (1995) observed that rates of Antarctic echinoderm embryonic development are close to their maximum for any given ambient temperature worldwide, and that temperature is the main factor affecting development rate. Delayed development has also been associated with the predisposition of some Antarctic taxa to produce large eggs (McLaren *et al*., 1969; Clarke, 1992). A larger egg can support a longer period of protected development increasing the likelihood of larval survival, suggesting the seasonal food supply, as well as temperature, as important in terms of development time (Clarke, 1982). This theory also suggests that long development times are necessary so that the young released are advanced and at a competitive advantage and are commonly in the water column when food supplies are plentiful. The Antarctic urchin *Sterechinus neumayeri* possesses eggs slightly larger than its temperate counterpart, however embryonic development is very much longer. Therefore, Hoegh-Guldberg and Pearse (1995) concluded that the evolution of developmental rates according to food availability has little support, and that instead temperature is most important. This describes the immediate thermodynamic effect of temperature on developmental processes and the increased sensitivity of these processes to low temperature.

Other studies have discussed the relevance of temperature and/or food availability in determining Antarctic larval development rates and have considered the energy required during deferred development (Marsh *et al*., 1999). Many Antarctic echinoderms have extended development periods in the plankton during which larvae are devoid of particulate food and must survive on reserves (Pearse *et al*., 1991; Shilling and Manahan, 1994). Marsh *et al*., (1999) concluded that the low level resource environment of the Antarctic does not pose problems for urchin larvae during development, mainly owing to low rates of larval metabolism which are principally driven by low temperature and the ability of larval feeding stages to acquire dissolved organic material (Rivkin, 1991). Therefore, it appears that Antarctic larvae have developed unique physiological mechanisms related to energy metabolism to survive at low temperatures with a diminished food supply.

1.4 Inter-annual Variation

Short-term environmental change occurs on monthly, seasonal and annual time scales, as well as varying spatially. Recent studies have alluded to the importance of change on more long-term scales. A well-known source of long-term oceanographic variation is the El Niño Southern Oscillation (ENSO), which refers to a connection between exceptionally warm sea temperatures in the tropical Pacific and atmospheric anomalies (Karoly, 1989). Inter-decadal and multi-decadal frequencies have also been observed in the climate variations of the northeast Pacific (Ware and Thomson, 2000). Air temperature records indicate that the climate of the northeast Pacific has oscillated at three dominant timescales over the last 400 years: the well documented 2-8 year ENSO timescale, a 20-40 year inter-decadal timescale and a 60-80 year multi-decadal timescale. These climatic variations can be felt over a broad scale even in the spatially distant deep sea (Smith *et al*., 2001; Ruhl and Smith, 2004). Anomalies on long-term time scales have also been described for the Antarctic on both inter-decadal and multi-decadal timescales (Murphy *et al*., 1995; White and Peterson, 1996; Turner *et al*., 2002; Turner, 2004).

The Southern Ocean is the only oceanic domain to encircle the globe completely and is the unifying link for exchanges of water masses between the world's major ocean basins. Therefore, this ocean is important for transmitting climate anomalies around the globe. In recent studies an inter-annual variation, with a cycle of 4-5 years, taking 8-10 years to encircle the pole, has been recorded. White and Peterson (1996) described these 4-5 year anomalies as an eastward propagating feature and referred to it as the Antarctic Circumpolar Wave (ACW) in which a dipole variation in sea ice extent moves round the Antarctic. The ACW influences sea surface temperature, wind-speed and sea-ice extent. Many studies have linked Antarctic and Southern Ocean climate variability with climate anomalies at lower latitudes, specifically the ENSO, as both signals propagate eastwards with a 3-5 year cyclicity (Peterson and White, 1998; White *et al*., 2001; Meredith *et al*., 2003; White and Annis, 2003; reviewed by Turner, 2004). A number of studies have described a connection between these two climatic anomalies. However, although the relationships between ENSO and pelagic ecosystems have received a considerable amount of attention at tropical and high northern latitudes, few studies of ENSO and related effects have been undertaken across the Southern Ocean and in the Antarctic coastal region.

Anomalies in sea surface temperature have been observed for a number of years in the North Atlantic Ocean (Dzhiganshin and Polonsky, 1995) and the Southern Ocean (Aoki, 1997). Inter-annual variation of Antarctic Ocean CO_2 uptake has been related to variations in nitrate, oxygen and dissolved inorganic carbon, which can partly be a result of the ACW, as the latter has a strong influence on Antarctic water masses (Louanchi and Hoppema, 2000; Louanchi *et al*., 2001).

Murphy *et al*., (1995) studied the long-term fast-ice record from the South Orkney Islands and commented upon the variability in sea ice extent, which appeared to show a clockwise circumpolar precession on a sub-decadal scale (7-9 years). Nihashi and Ohshima (2001) also reviewed the inter-annual variation in Antarctic Ocean sea-ice dynamics. The regional variability in sea ice extent appears to be linked to features of both the atmosphere and ocean systems and the overall configuration of sea ice around Antarctica.

There is a close coupling between sea ice conditions and ecosystem dynamics. The timing of the waxing and waning of sea ice determines the levels of light available for photosynthesis and the period during which production is viable. These effects can be felt across many trophic levels, including top predators. Croxall *et al*., (1988) showed that inter-annual variation occurred in sea bird and sea mammal populations of islands in the north Weddell Sea and Fraser *et al*., (1992) described changes in the dynamics of penguin populations on the Antarctic Peninsula. Both identified connections with sea-ice dynamics. Boltovskoy and Alder (1992) also observed inter-annual differences in the abundance of microzooplankon groups at a number of stations in the Weddell Sea and attributed regional enhancements of cells to the presence of the ice edge, which may vary its position with changing environmental conditions over inter-annual time scales.

Environmental inter-annual variation has significant implications for ecosystem function and the detection of short-term and long-term ecological change. Recent studies have described inter-annual variation in the reproductive seasonality and the reproductive condition of some marine invertebrates, e.g. in the gonad index of the green urchin *Strongylocentrotus droebachiensis* from Nova Scotia, Canada (Meidel and Scheibling, 1998), in the timing of spawning in Atlantic cod sampled off Newfoundland, Canada (Hutchings and Myers, 1994) and in a number of the Antarctic benthos including the brachiopod *Liothyrella uva* (Meidlinger *et al*., 1998), the limpet *Nacella concinna*, the bivalve *Laternula elliptica* (Powell, 2001) and the scallop *Adamussium colbecki* (Tyler *et al*., 2003). Chiantore *et al*., (2001) observed the degree to which inter-annual variation in reproduction varied between invertebrates with different feeding ecologies and concluded that the greatest year-year variation was exhibited in *A. colbecki*. Consequently, many studies have alluded to the role of the available ration in determining inter-annual disparity in reproductive traits. However, the integral role of other oceanographic parameters, which determine the strength and availability of the austral bloom are also important, e.g. ice and seawater temperature.

Another important aspect of reproductive success is recruitment. Dayton (1989) has described inter-annual variations in benthic invertebrate recruitment for the Antarctic. He observed inter-decadal differences in the recruitment of sponge populations to the shelf community of McMurdo Sound and attributed year-year differences to the dynamics of anchor ice. Stanwell-Smith and Barnes (1997) studied recruitment and growth on settlement panels at Signy Island, Antarctica and observed that successful recruitment levels of colonising taxa showed seasonal and inter-annual variation. Similar year-year variation in recruitment has been documented in the southeast Pacific, specifically for mussels and barnacles, and has been linked to the large-scale oceanographic effects of the 1997-1998 El Niño event (Navarette *et al*., 2002).

Therefore, inter-annual variation in the environment has important implications for biological processes and ecosystem dynamics, so that patterns in reproduction, recruitment and success vary over similar long-term time scales.

1.5 Project Aims

The primary aim of this study was to describe the long-term reproductive cycles of four species of Antarctic invertebrate (the ophiuroid *Ophionotus victoriae,* the asteroid *Odontaster validus,* the nemertean *Parborlasia corrugatus* and the holothurian *Heterocucumis steineni*) sampled from shallow water sites adjacent to the British Antarctic Survey's Rothera Research Station, Adelaide Island, West Antarctic Peninsula (67˚34 S 68˚08 W).

Reproductive success is a vital characteristic in species survival and evaluation of change in reproductive condition with time key to identifying vulnerable taxa. Characterising reproductive success with time is a major requirement in predicting species response to change and the early stages of species loss. This thesis describes the reproductive success of a selection of ecologically important Antarctic shallow water marine invertebrates over inter-annual time scales. Particular attention is paid to the reproductive cycles and reproductive condition of each invertebrate, and any intrinsic variability explained in terms of environmental oscillations and differences in species feeding ecology.

Additionally, aspects of invertebrate fertilisation kinetics will be investigated in both a temperate and polar starfish. A number of marine invertebrates, and especially echinoderms broadcast their gametes into the water column to await external fertilisation. Therefore, the successful fertilisation of these eggs and the adaptations undertaken to maximise potential fertilisation success, at the level of the gamete, individual, population and environment, are crucial elements of invertebrate reproductive fitness and survival. This thesis compares and contrasts the responses of a temperate and polar starfish, at the level of sperm and eggs, to variable environmental conditions in order to assess the effects of different environmental regimes on invertebrate fertilisation kinetics. The potential impacts of forecasted climate change are discussed in terms of gamete-specific characteristics and temperature sensitivity in the polar starfish.

Finally, the relative contribution of different bodily components to the biochemical profile of two polar invertebrates will also be described. The quality and quantity of food arriving at the seabed in the Antarctic has several effects on the composition and condition of tissues in marine invertebrates. It has a direct effect on the amount and composition of material channelled directly into the digestive tissues and diverted to storage, and it can have an indirect influence on the timing of reproduction and how energy is allocated to the gonad. This is important in the understanding of Antarctic reproductive cycles, many of which are underpinned by the intensely seasonal signature in primary production, and especially relevant when the potential impact of environmental change, in terms of seasonality is considered. This thesis describes the seasonal changes in the biochemical and proximate composition of reproductive, digestive and bodily tissues of two polar echinoderms. Gender-specific differences in tissue composition are discussed, and any patterns in the biochemical profile of the echinoderms described in terms of seasonality and differences in each species reproductive condition and ecology.

These data will be used to discuss the possible implications of future global change on species fitness and success, and the extreme sensitivity of the polar ecosystem used to emphasize the importance of Antarctic benthic invertebrates as highly sensitive and therefore, useful barometers for change.

Data also described in Grange *et al*., (2004) Long-term inter-annual cycles of the gametogenic ecology of the Antarctic brittle star *Ophionotus victoriae*. *Marine Ecology Progress Series*, **278**. 141-155. (Appendix Reprint)

2.1 Introduction

The waters round the Antarctic have low but stable background temperatures set against a highly seasonal food regime (Lipps and Hickman, 1982; Clarke *et al*., 1988; Dayton 1990; Clarke and Leakey, 1996). During the austral winter seawater temperatures drop to –1.9°C and the sea-surface is frozen, whereas summer water temperatures may rise to > 1.0°C. As a result of this stenothermal environment, energy input has a profound ecological effect. An intense seasonal signature in light and sea ice cover stimulates the very marked seasonality in surface primary production (Clarke, 1988), where the pulse of organic matter to the local seabed is temporally constrained.

Polar reproductive patterns typically have a long duration and gametogenic development is slow (Hedgpeth, 1970; Grange *et al*., 2004). It has been argued that it may not be the low temperatures *per se* that restrict Antarctic marine invertebrates, but the seasonal pattern in food availability (Clarke, 1982; Pearse *et al*., 1991). The latter has been assumed to be responsible for Antarctic spawning frequencies and magnitudes (Giese, 1959; Pearse, 1994). However, a more recent multivariate analysis indicates that temperature is the most important environmental factor controlling larval developmental rates (Hoegh-Guldberg and Pearse, 1995). Mortensen (1936) and Thorson (1950) proposed a high incidence of brood protection in Arctic invertebrates and Mileikovsky (1971) suggested this principle as a general rule and coined the term ‘Thorson’s rule’. Much of this was attributed to the paucity of planktonic food (Chia, 1974). However, the frequent occurrence of pelagic feeding and non-feeding reproductive patterns has more recently been documented in invertebrates from Antarctic latitudes (Table. 2.1.1) (Pearse, 1994; Pearse and Bosch, 1994). Thereafter, the current number of larval types in Antarctica has been shown to be high (Shreeve and Peck, 1995; Stanwell-Smith *et al*., 1999) in contrast to the earlier Arctic studies from the 1950s and 1960s.

Table. 2.1.1 Modified from Galley (2004). Reproductive periodicity and egg size (μm) in Antarctic shallow-water invertebrates. Length of oogenesis is referred to as Cycle; modes of development are listed as either AL = abbreviated lecithotrophy; B = brooding; L = lecithotrophic; P = planktotrophic. Periodicities are listed as either: A = aseasonal; S = seasonal.

Species	***Location***	***Depth (m)***	***Fecundity***	***Egg size (μm)***	***Mode***	***Periodicity***	***Cycle (month)***	***Reference***
Antarctic invertebrates								
ASTEROIDEA								
Neosmilaster georgianus	Anvers Island	2-15		2170	B	A		Bosch and Slattery, 1999
Diplasterias brucei	McMurdo	<33	300	2800	B	A		Bosch and Pearse, 1990
Notasterias armata	McMurdo	<33	50	3500	B	S		Bosch and Pearse, 1990
Acodontaster elongates	McMurdo	<33	3-4x10^3	540	L	S		Bosch and Pearse, 1990
Porania sp.	East Cape Evans	15-33	100-310	550	L	A		Bosch, 1989; Bosch and Pearse, 1990
Acodontaster hodgsoni	McMurdo	<33		550	L	S		Bosch and Pearse, 1990
Acodontaster conspicuus	McMurdo	<33	3-4x10^3	700	L	S		Bosch and Pearse, 1990
Bathybiaster loripes	McMurdo	<33		930	L	S		Bosch and Pearse, 1990
Psilaster charcoti	McMurdo	<33		950	L	S		Bosch and Pearse, 1990
Lophaster gaini	McMurdo	<33	3-4x10^3	1280	L	S		Bosch and Pearse, 1990
Perknaster fuscus	McMurdo	<33		1200	L	A		Pearse *et al*., 1985; Pearse *et al*., 1986
Odontaster validus	McMurdo	<10		150	P	S	<24	Pearse, 1965
Odontaster meridionalis	McMurdo	<33		190	P	S		Bosch and Pearse, 1990
Porania antarctica	New Harbor	15-33	3-4x10^4	550	P	S		Bosch, 1989; Bosch and Pearse, 1990
Psilaster charcoti	Antarctic Peninsula	~600m	10,000	578	L	A	18-24	Galley, 2004
HOLOTHUROIDEA								
Psolus dubiosus	Weddell Sea	189-840		1500	B	A		Gutt *et al*., 1992
Ekmocucumis steineni	Weddell Sea	189-840		1000	L	S		Gutt *et al*., 1992
Protelpidia murrayi	West Antarctic Peninsula	600	2000-35000	357	L	S	18-24	Galley, 2004
Peniagone sp	Antarctic Peninsula	600	5000	570	L	S	18-24	Galley, 2004
ECHINOIDEA								
Abatus nimrodi	McMurdo	shallow	30	1280	B	A		Pearse and McClintock, 1990
Abatus cordatus	Kerguelen	intertidal		1300	B	S	24	Magniez, 1983
Abatus shackletoni	McMurdo	shallow	30	1970	B	A		Pearse and

Species	***Location***	***Depth (m)***	***Fecundity***	***Egg size (μm)***	***Mode***	***Periodicity***	***Cycle (month)***	***Reference***
								McClintock, 1990
Sterechinus neumayeri	McMurdo Rothera	1-3 15-30		125 120	P P	S S	18-24 18-24	Pearse and Giese, 1966a; Brockington, 2001
Sterechinus antarcticus	West Antarctic Peninsula	600	12700	186	L	S	18-24	Galley, 2004
Ctenodicaris perrieri	West Antarctic Peninsula	600	1000	1050	B	A	18-24	Galley, 2004
Amphipneustes lorioli	West Antarctic Peninsula	600	200	850	B	A	18-24	Galley, 2004; Galley *et al*., 2005.
OHIUROIDEA								
Ophiosparte gigas				350	L			Pearse 1994
Ophionotus victoriae	Circumpolar (Rothera)	 15-20	140000 1-2x10^5	180 250 (max) 150 (max monthly average)	P P	S S	18-24 18-24	Pearse, 1994; Mortensen, 1936 Grange *et al*., 2004
Ophionotus hexactis	King Edward Cove	5-15m	Max 86 juveniles	200	B	S		Morison, 1979
BIVALVES								
Adamussium colbecki	Terra Nova Circumpolar (Rothera)	10-70 < 25		50	P P	S S	 12	Chiantore *et al*., (2002) Tyler *et al*., 2003
Lissarca notorcadensis	Weddell Sea, South Orkneys and South Shetland Shelves			1000-1350	B	S	18-24	Brey and Hain, 1992
Laternula elliptica	Rothera	15-20		240	L	S	18-24	Powell, 2001
Nacella concinna	Rothera	15-20		180-240	L	S	18-24	Powell, 2001
BRACHIOPODS								
Liothyrella uva	Signy Island	11	15-2000embryos	300	B/L	S	18-24	Meidlinger *et al*., 1998
ASCIDIAN								
Cnemidocarpa verrucosa	Potter Cove King George Island	< 30		200-250	L	S	12	Sahade *et al*., 2004
ANTHOZOAN								
Ainigmaptilon antarcticum	Eastern Weddell Sea	250-600	17000 oocytes 12 per polyp	700-900	AL	S	>12	Orejas *et al*., 2002

The benthic fauna of the Southern Ocean is now reasonably well known and generally diverse (Clarke and Johnston, 2003). The echinoderms are relatively well described and are particularly diverse in the Southern Ocean, often representing one of the most abundant and speciose macroinvertebrate groups in shallow Antarctic seas. High Antarctic shallow waters are often dominated by the Echinodermata, especially the echinoid *Sterechinus neumayeri*, the seastar *Odontaster validus* and the ophiuroid *Ophionotus victoriae* (McClintock *et al*., 1988; Brockington, 2001; Manjón-Cabeza and Ramos, 2003). Photographic surveys of the local sampling sites adjacent to Rothera Research Station revealed echinoderms to be the most speciose, the most common taxa being the urchin *S. neumayeri* and the sea cucumber *Heterocucumis steineni* (Bowden, in press). The substantial occurrence of echinoderms in extreme environments with highly seasonal food regimes signifies this phylum's ecological flexibility and energetic importance (McClintock, 1994). Consequently, echinoderms play a significant role in energy transfer in the Antarctic benthos.

The Southern Ocean benthic nemertean fauna are relatively well known (Clarke and Johnston, 2003). However, the overall diversity of nemertean worms is somewhat diminished in comparison to other taxa. The Southern Ocean is unusual in this respect as one of its largest species, *Parborlasia corrugatus*, is an extremely common and conspicuous member of the Antarctic shallow water benthos. This species exerts an important influence on the local community as it is a voracious predator and scavenger (Gibson, 1983, 1985).

An extensive series of studies have been published reviewing the reproductive ecology of echinoderms, and a few seminal papers have documented the reproductive habits of Antarctic taxa. Reproduction of ophiuroids has been reviewed most recently by Hendler (1991) and Byrne (1994), whilst the ecological specificities of Antarctic ophiuroids have been addressed by Dahm (1996). Brittle star spawning and reproductive patterns are extremely diverse (Hendler, 1991), and this plasticity has been coupled with variable environmental conditions (McGinley *et al*., 1987, Hadfield and Strathmann, 1996). However, the reproductive biology of high Antarctic ophiuroids is unknown except for remarks in major taxonomic works (Mortensen, 1936). *Ophionotus victoriae* (Bell, 1902) has a circumpolar distribution occurring throughout the high Antarctic in the Ross Sea and along the Antarctic Peninsula. This penta-radial ophiuroid often characterises macrobenthic assemblages within these regions and is an abundant shallow water invertebrate (Dahm, 1996;

Sáiz-Salinas *et al*., 1997, Arnaud *et al*., 1998). It inhabits a variety of substrata (level mud to gravel and rock bottoms) and ranges between 5 and 1266m depth (Madsen, 1967). *O. victoriae* is oviparous (Koehler, 1912) and Mortensen (1936) described the eggs as 'small and numerous', indicative of producing a typical ophiopluteus larva. The diet of *O. victoriae* is also very catholic possibly consuming over 50 prey species (Dearborn, 1977; Fratt and Dearborn, 1984: Dearborn and Edwards, 1985). Large *O. victoriae* even appear to be cannibalistic on small individuals of its own species. This suggests that ophiuroids have a high dietary plasticity allowing the exploitation of habitats where the seasonal availability of food is changeable and varied.

Chia and Walker (1991) have comprehensively reviewed the reproductive ecology of the asteroids. The reproductive characteristics of high Antarctic asteroids have received a relatively large amount of attention, mainly through the study of the common cushion star *Odontaster validus*. Most of this literature describes the McMurdo Sound (77°51 S, 166°40 E) population of the cushion star, although studies have also been done on individuals from Cape Evans (77º38 S, 166º24 E) (Pearse, 1965) and Signy Island in the maritime Antarctic (60º43 S, 45º36 W) (Stanwell-Smith and Clarke, 1998). *O. validus* (Koehler, 1912) is extremely abundant and distributed over a considerable area in most shallow water benthic sites that characterise the Antarctic continent (Dearborn, 1965; Arnaud, 1974; McClintock *et al*., 1988). This starfish occurs at sites in South Georgia (54˚ S), throughout the Southern Ocean and at an extreme southerly latitude in McMurdo Sound (78˚ S) (McClintock *et al*., 1988). Individuals range from the intertidal to 940m depth, but are most often observed at depths of ~15m down to 200m (Dearborn, 1977). *O. validus* displays an opportunistic feeding preference utilising a wide variety of available prey items by suspension feeding, grazing, scavenging and behaving as an active predator (Pearse, 1965). Feeding aggregations of this starfish have been recorded, several centimetres deep, on decaying organic material and Dayton *et al*., (1974) suggested that these starfish remove larval invertebrates during detrital feeding, possessing the capacity to regulate and control adult populations, e.g. regulate the sponge community by consuming the larval forms of sponge predators. Therefore, because of the numerical abundance of *O.validus* and the potential to exert considerable ecological influence on the benthic community, it is regarded to be of great ecological importance. This starfish reproduces annually during the austral winter, broadcasting eggs and sperm into the water column (Pearse, 1965, 1969). Pearse has also documented that *O. validus*, requires a prolonged 18-24 months for its development cycle, which he argued is also characteristic of many Antarctic invertebrate taxa (Pearse, 1994). Feeding larvae develop in the plankton, free-swimming over a prolonged period (Pearse, 1969; Stanwell-Smith and Peck, 1998) and they reportedly have the capacity to sequester dissolved organic material from the water column during the oligotrophic winter (Olson *et al*., 1987; Bosch *et al*., 1990).

The Holothuroidea have been described in a number of review articles (Hyman, 1955; Pawson, 1966). Holothurians are distributed widely in all seas and at all depths, and have adapted to a variety of different marine habitats (Pawson, 1966). However, this class has received limited attention compared to our knowledge of other echinoderm taxa, and this is especially true for Antarctic sea cucumbers, although they are recognised as being important members of the continental shelf and slope marine benthos, especially in sessile suspension feeding communities below the influence of ice scouring (Dayton *et al*., 1974; Gutt and Piepenburg, 1991). Various articles have also described the discovery of new holothurian species in the Weddell Sea (Gutt, 1990; Massin, 1992). A majority of the species found in the Antarctic belong to the dendochirotacean families, Psolidae and Cucumariidae (Dell, 1972). The relative success of these families within the Antarctic has been attributed to their capacity to reproduce via viviparity or brood protection (Gutt, 1991b). The species studied here was originally described as *Cucumaria antarcticus*. It has been latterly renamed as *Heterocucumis steineni* (Ludwig, 1898). The population and reproductive ecology of this invertebrate has received limited attention. *H. steineni* is known from the Antarctic, sub-Antarctic and the Antarctic islands (Gutt *et al*., 1992) and forms an abundant shallow-water population adjacent to Rothera Research Station off Adelaide Island to the west of the Antarctic Peninsula (Fraser *et al*., 2004). This holothurian is commonly observed with the posterior body half burrowed into the sediment. This plastic behaviour has been suggested as the main reason for it having the highest abundance among the Weddell Sea holothurians (Gutt, 1991a). Gutt *et al*., (1992) described seasonal differences in the reproductive habit and female fecundity of deep sea individuals of *H. steineni*. They also observed the possession of large yolky oocytes (0.3-1.0mm in diameter) forming multiple cohorts in the ovary and suggested a summer-spring spawning period. Current work has also described the seasonal variation in protein synthesis and metabolism in a shallow water population of *H. steineni* sampled adjacent to Rothera Research Station (Fraser *et al*., 2004).

A significant amount of work has been published describing the general morphology, ecology and characteristics of the Nemertea (Hyman, 1951; Gibson, 1994). The reproductive ecology has been previously described (Hyman, 1951). However, the reproductive biology of Antarctic nemerteans is relatively unknown except for *Parborlasia corrugatus* (McIntosh, 1876), a common member of the shallow-water marine benthos. This heteronemertean is conspicuous in the Antarctic mainly owing to its capacity to attain lengths in excess of a metre (Knox, 1970; Gibson, 1983). It has a circumpolar distribution and frequents depths from the intertidal down to 3000m (Gibson, 1983). *P. corrugatus* feeds on a variety of prey items and is regarded as a voracious predator and scavenger, capable of taking diatoms, sponges, anemones, polychaetes and fish (Dearborn, 1965). The ecological importance of this species is further exemplified by the apparent lack of predators capable of preying upon it (Dayton *et al*., 1974), despite being numerous and high in energy and nutrient content (Heine *et al*., 1991). *P. corrugatus* has been observed to secrete toxic compounds indigestible to most marine taxa, which is a common characteristic of the phylum (Kem, 1985). The reproductive and developmental biology of this species has been relatively well described. Larval pilidia have been observed for prolonged periods throughout the year in shallow Antarctic waters leading many to suggest an aseasonal quasi-continuous reproductive habit for the nemertean (Pearse *et al*., 1991; Shreeve and Peck, 1995; Stanwell-Smith *et al*., 1999). Peck (1993) reared the planktotrophic larvae over a period of 50-56days, and a series of sampling programmes have suggested the pilidia capable of at least a 150day existence in the plankton, a period in excess of the normal development times recorded for comparable temperate pilidia. The paucity of planktonic food during this time has convinced many that these larvae can assimilate and accumulate dissolved organic material from the water column (Peck, 1993). Rogers *et al*., (1998) have also looked at *P. corrugatus* population genetics in a population from the South Orkney Islands.

The Southern Ocean is characterised by environmental variation on a range of frequencies. One of the most recently described of these is the 'Antarctic Circumpolar Wave' (White and Peterson, 1996), in which a dipole variation in sea ice extent moves round the Antarctic. There is also considerable debate as to whether the waters round Antarctica are being affected by global warming (Vaughan *et al.*, 2001; Meredith and King, 2005). The stenothermal character of polar invertebrates may make them highly susceptible to temperature change, and they may be amongst the most vulnerable species worldwide to environmental modifications (Pörtner *et al.*, 1999; Peck and Conway, 2000; Peck, 2002: Pörtner, 2002). Reproductive success is a key characteristic in this respect and evaluation of change in reproductive condition with time key to identifying vulnerable taxa.

Work to date on long-term reproductive cycles in marine environments worldwide is limited. Most studies are based on a grant or PhD duration of 2-3 years, which is inadequate to characterise variation between years, or even to evaluate seasonal variation accurately and identifying multi-year trends is not possible. In Antarctica the situation is even worse. A few studies of reproductive cycles have been reported from Antarctic species using monthly samples over a 1-2 year period (Tyler *et al.*, 2003; Sahade *et al.*, 2004; Brockington *et al.*, submitted). Some of these have shown considerable inter-annual variation in reproductive condition, specifically gonad index which expresses gonad weight as a percentage of total body weight e.g. *Liothyrella uva* (Meidlinger *et al.*, 1998), *Adamussium colbecki* (Chiantore *et al.*, 2002) and the ascidian *Cnemidocarpa verucosa* (Sahade *et al.*, 2004). However, despite inter-annual variability in reproductive condition being high, the timing of reproductive events shows remarkable consistency between years in many species. These data provide evidence of a plastic reproductive pattern in many Antarctic invertebrates, adapted to overcome the low level resource supply characteristic of the Antarctic continent and its unique environmental conditions. These patterns go some way to explain how some invertebrates with flexible ecologies and capabilities to accommodate inter-annual variation, have become dominant members of the Antarctic benthos.

Owing to the limited duration and discontinuous sampling in previous invertebrate reproduction studies, this study is referred to as 'long-term'. In this context 'long-term' describes the collection of reproductive material over consecutive sampling months for a duration exceeding 1-2 years. In this case a 4-year study was undertaken. The reproductive condition of invertebrates can be defined in a number of ways. However, a simple and widely accepted indicator of reproductive condition is gonad index. This term is defined as the ratio of weight of the gonads to the total animal wet weight, multiplied by 100 to convert it to a percentage (Grant and Tyler, 1983), and was suitable for the purposes of this study.

The main aim of this study was to comment on the long-term reproductive cycles and reproductive condition of four shallow water Antarctic marine invertebrates during a 4-year study, (*Ophionotus victoriae*, *Odontaster validus*, *Parborlasia corrugatus* and *Heterocucumis steineni*). Where possible, comments were also made on the relationships between cycle characteristics and variability in the local physico-chemical environment. A final comment was made on the extent of inter-annual variation in the reproductive characteristics of each species and the role of each species trophic biology discussed.

2.2 Materials and Methods

Sampling

A long-term study was undertaken at the British Antarctic Survey's (BAS) Rothera Research station (Fig. 2.2.1) on Rothera Point, Adelaide Island to the west of the Antarctic Peninsula (67˚34 S 68˚08 W). Four shallow-water, abundant species of invertebrate were collected and their long-term gametogenic development analysed. These included the brittle star *Ophionotus victoriae*, the cushion star *Odontaster validus*, the nemertean *Parborlasia corrugatus* and the sea cucumber *Heterocucumis steineni* (Fig. 2.2.2). The sampling area was characterised by a predominantly cobble bottom with rocky boundaries, including a soft bottom area at Hanger Cove.

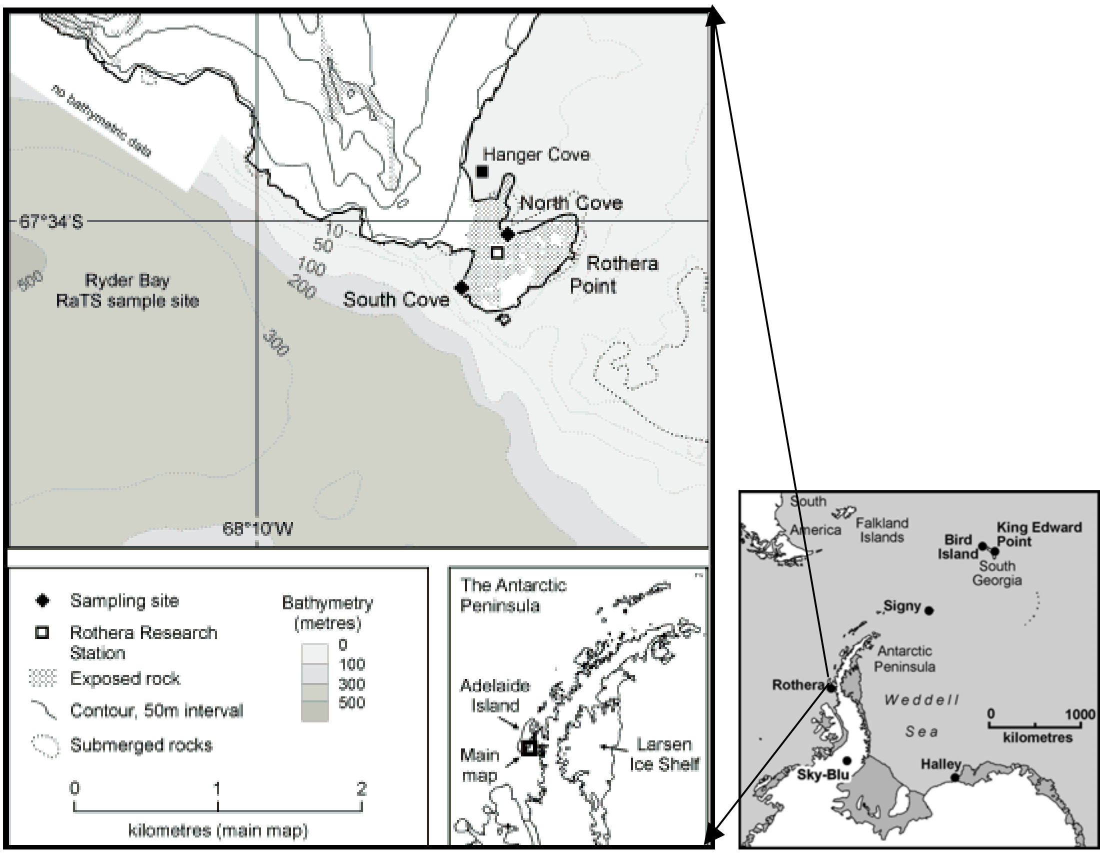

Fig. 2.2.1 The British Antarctic Survey Rothera Research Station and shallow water sampling sites.

Invertebrates were collected by SCUBA diver from 15-20m depth in North Cove, South Cove and Hanger Cove between July 1997 and January 2001 (Table. 2.2.1). A minimum of 10 adult individuals was collected each month, except on occasions when logistics problems and/or inclement weather precluded sample collection. Divers were instructed to collect large adults preferentially, where possible, to maximise the number of reproductively mature individuals sampled. Once collected, samples were preserved in 4% formal saline for transport and subsequent storage in the UK.

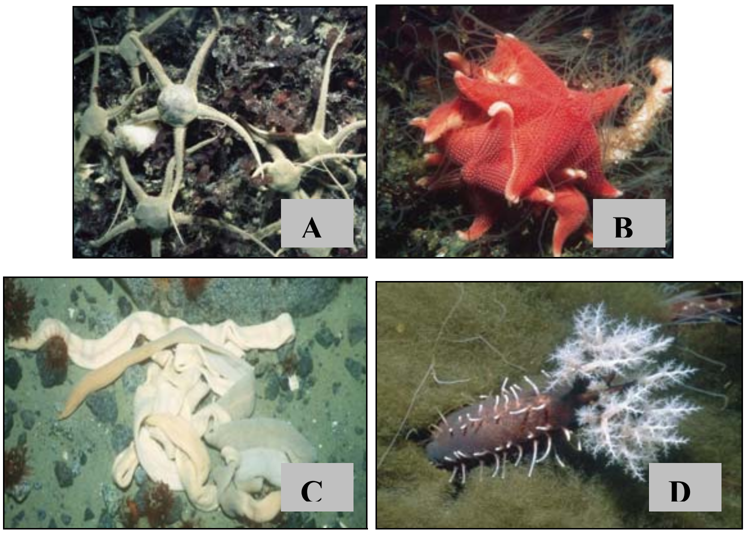

Fig. 2.2.2 The brittle star *Ophionotus victoriae* (A), the starfish *Odontaster validus* (B), the nemertean *Parborlasia corrugatus* (C) and the sea cucumber *Heterocucumis steineni* (D) respectively.

Table. 2.2.1 The monthly sampling regime for each invertebrate collected.

Species	**Months Sampled**	**Excluded Months**	**Sample Site**
Ophionotus victoriae	September 1997-December 2000 (38 months)	April and June 1999	North and Hanger Cove
Odontaster validus	July 1997-January 2001 (39 months)	October 1997, April, May and September 1999 and September 2000	South Cove
Parborlasia corrugatus	July 1997-November 2000 (39 months)	April and June 1999	Hanger Cove
Heterocucumis steineni	September 1997-January 2001 (39 months)	July and August 1999	South Cove

An environmental, long-term oceanographic monitoring programme has been maintained at Rothera (RaTS) by the BAS since 1997 and these data continue to be collected and archived today. Data from this archive was used to profile the environmental variability at Rothera during 1997 to 2001 and was provided by Professor Andrew Clarke. This programme compiles weekly recordings of temperature (by a SIS reversing thermometer at 15m depth), light (PAR mW/cm^2) and pigment concentrations (chlorophyll a and phaeophytin concentrations collected at 15m with NIO or Niskin sampling bottles) in 20, 5, 2 and 0.2µm size fractions from a CTD cast to a depth of 200m in Ryder Bay (Fig. 2.2.3). A Rothera sediment trap has also been situated on the south side of Trolval Island since November 1997 (Fig. 2.2.3). The trap is anchored to the bottom in 34m of water, and is suspended in mid water at 26m collecting particulate matter as it sinks through the water column. However, this programme has not provided a continuous record, and sampling was prevented in the winter of 2000 and in the aftermath of the Rothera fire in September 2001.

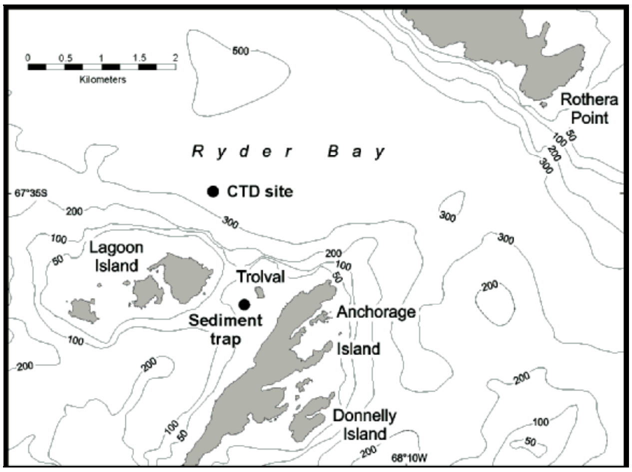

Fig. 2.2.3 The location of the Rothera sediment trap off Trolval Island and the CTD sampling site in Ryder Bay.

The invertebrates collected between July 1997 and January 2001 generated a large number of histological samples. A proportion of these samples have been processed by other workers and completed in accordance with the requirements of undergraduate and masters dissertations at the National Oceanography Centre, Southampton UK (Appendix Table. 1). The author (Laura Grange) processed the remaining samples, and a 4-year data set was completed for each of the four invertebrates studied. The author also reviewed and re-analysed all of the data generated by other workers to ensure continuity between samples and species comparisons. All processing was carried out using the same methodology and data analysis at the National Oceanography Centre, Southampton UK.

Biometry

The size (diameter or length) of each individual was measured (± 0.1mm) using vernier callipers, and where possible, the sex of individuals was determined during dissection. Gonads of each individual were removed and stored in a 70% propan-2-ol solution.

Ophionotus victoriae: The disc diameter of each individual was recorded (Fig. 2.2.4). This brittle star was pentamerous, where collections of numerous discrete gonads were aligned along the proximal part of each arm and were attached to the coelomic side of each of ten bursae (two bursa between each arm). The aboral surface of the disc was cut away and the gonads removed from two bursae using tweezers. Male *Ophionotus victoriae* possessed a red, sac-like gonad, whereas the ovaries were spherical and yellow (Fig. 2.2.4).

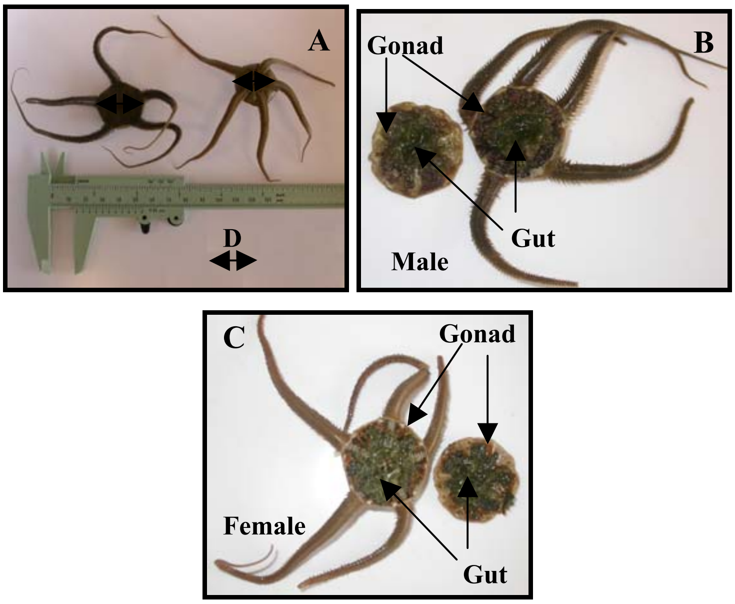

Fig. 2.2.4 *Ophionotus victoriae*. Disc diameter measurements showing the aboral and oral view (A), and male (B) and female (C) dissections identifying the gut and gonad tissue.

Odontaster validus: Two diameter measurements were recorded for each cushion star, ‘R’ denoting radial length and ‘r’ denoting body radius (Mortensen, 1927). The appearance of this seastar often varied depending on the volume of water retained inside each individual (Fig. 2.2.5). Each seastar had a pair of gonads in each arm, which were tuft-like or digitate (Fig. 2.2.5). A single pair was removed using tweezers and preserved. The gonad colour of individual starfish ranged from white to orange, and although the testes tended to be paler than ovary tissue (Pearse, 1965), sexes could only be confirmed by gamete identification. A pair of pyloric caeca, composed of a mass of glandular cells, was also removed and preserved. There were two pyloric caeca, or digestive glands, suspended in the coelom of each arm (Fig. 2.2.5).

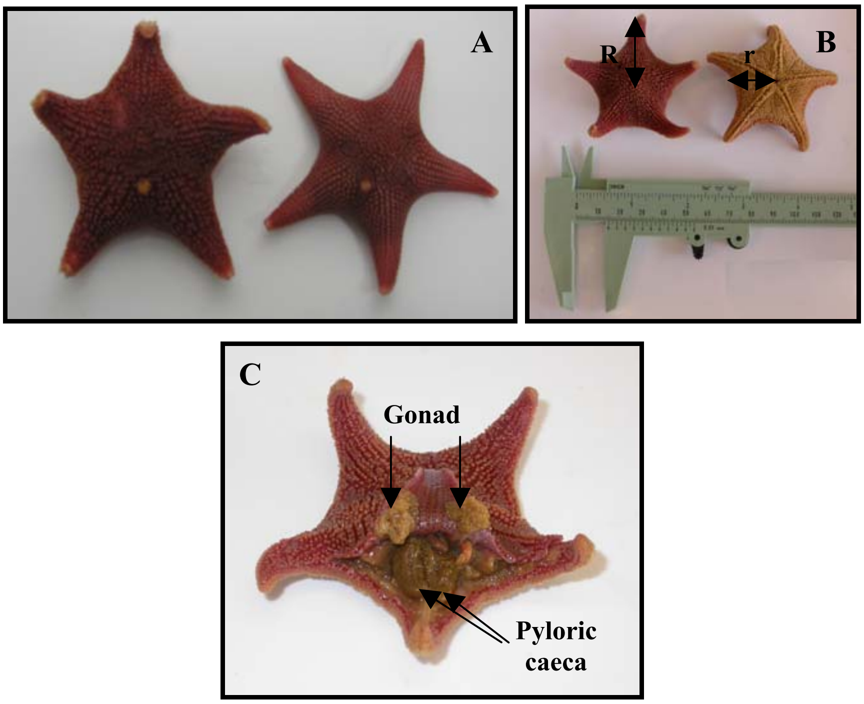

Fig. 2.2.5 *Odontaster validus*. Variation in body size caused by internal water content (A), measurements of radial length (R) and body radius (r) using an aboral and oral view (B) and a dissected individual describing the position and appearance of the gonad and pyloric caeca (C).

Parborlasia corrugatus and Heterocucumis steineni: The length of both the nemertean and sea cucumber (Fig. 2.2.6 and 2.2.7) were measured using a marked piece of string and were compared to a metre rule. The length of each nemertean was taken as the retracted length (Fig. 2.2.6) as opposed to an absolute measurement, as these nemerteans possess the ability to elongate and contract. Measurements were further complicated by the tendency of this large species to fragment. Handling stress and preservation caused many of the individuals to splinter into several pieces. The frequency with which nemerteans fragment is high (Coe, 1943; Riser, 1974), especially under stressful conditions. Another response to stress is to evert the proboscis, which is either lost or protrudes from the body wall at various points along the nemertean's length. Nemerteans are characterised by this eversible, muscular proboscis, which is contained within a dorsal fluid-filled chamber, the rhyncocoel (Gibson, 1994). Proboscis eversion or protrusion from the body wall was common in the sampled population and apparent in a small number of individuals in most months (Fig. 2.2.6). 16% of the sampled population everted the proboscis. There was a significant difference between the proportion of males and females presenting an everted proboscis (Mann Whitney, W = 955.5, P = 0.006) and 66% of individuals exhibiting an everted proboscis were male.

The nemertean reproductive system was simple. Gonads alternated with the intestinal diverticula to form a regular row along each side of the body. Gametes developed from stem cells that aggregated and became enclosed in an epithelium to form the gonad (Fig. 2.2.6). A piece of tissue ~5mm thick was dissected from each individual in cross section at a position approximately 1/3 of the animal's absolute length. This ensured a position sufficiently anterior of the foregut was sampled and would contain gonad. A gelatinous pink substance in the body cavity, identifiable as oocytes, was characteristic of fecund females, whereas a whitish substance was unique to gravid males.

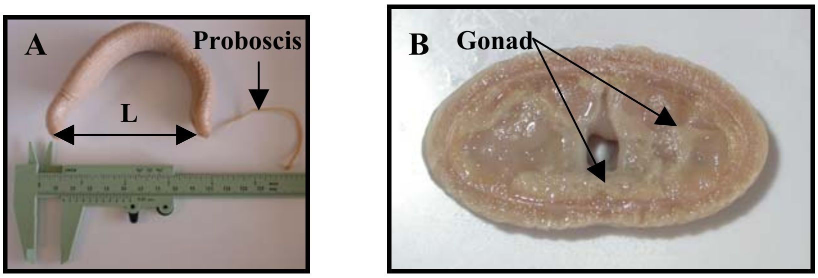

Fig. 2.2.6 *Parborlasia corrugatus*. Retracted length (mm) is measured and the everted proboscis labelled (A). A 5mm cross-section of the nemertean is labelled to identify regions of the gonad (B).

Heterocucumis steineni possessed a single gonad (Fig. 2.2.7), composed of a mass of tubules emanating from a gonad basis. The gonadal tubules were arranged as two tufts and a range of tubule sizes was observed, with the thinnest tubules being pale yellow, and the largest tubules being a darker orange containing large oocytes visible to the naked eye in females. The entire gonadal mass was removed from the body cavity for weighing and a subsample of gonad removed for histological preparation. Protrusions of the gonad were visible through the cloaca and body wall in several individuals and are common in many species under unfavourable conditions (Sedgwick, 1909; Pawson, 1966; Madsen and Hansen, 1994), perhaps caused by the stress of capture.

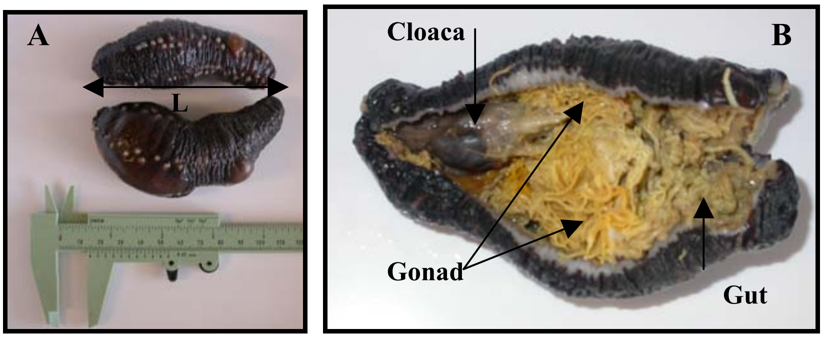

Fig. 2.2.7 *Heterocucumis steineni*. Animal length (mm) is measured (A) and an individual dissected to illustrate the position of the gonad (B). The gut and cloaca are also labelled.

Reproductive Condition

The wet weight (±0.01g) of each animal and individual gonad tissue was recorded for gonad index assessment. Gonad index was calculated in the same way for the starfish and the sea cucumber using total animal wet weight:

$\mathbf{GI = (GW*100)/BW}$ Where **GI** = Gonad Index (%) **GW** = Gonad mass (g) **BW** = Total animal mass (g)

However, this calculation was modified for the brittle star. Disc diameter and disc depth was used to calculate disc volume. Many individuals had part of or all of an arm missing, therefore a gross wet weight measure for gonad index was not possible and instead was calculated by the following formula:

$\mathbf{GI = (GW*100)/DV}$ Where **GI** = Gonad Index (%) **GW** = Gonad mass (g) **DV** = Disc Volume (mm^3).

Another simple measure of reproductive condition was also calculated using the gonad index data. The percentage change in the ovary and testis index was calculated between the most gravid, mature months and the months when individuals were spent. These data were compared across the study period and for each species, with the exception of *Parborlasia corrugatus*. It was not possible to calculate a reliable gonad index for the nemertean owing to there being no discrete gonad.

Histological Preparation

Each gonad sample was dehydrated in three two-hourly changes of 100% propan-2-ol then transferred to a clearing solution (Xylene) for 4-6 hours. The nemertean gonad samples were retained in xylene for a further 6-8 hours owing to the larger size and thickness of the tissue sections. All samples were impregnated in molten wax in a thermally controlled oven (70ºC) for a period of 12-15 hours, after which all samples were wax embedded. The liquid wax used to impregnate the nemertean samples was changed for fresh wax half way through the 12-15 hours oven time to remove any excess xylene solution, which appeared to leach from the samples and compromise the embedding process. All wax blocks were sectioned at 7µm on a standard microtome, where provision was made for duplicate/triplicate slides to be produced for each sample. The tissue sections were mounted onto slides and stained with Haemotoxylin ‘Z’ and Eosin (Cell Path UK). A DPX mountant was used to coverslip the slides.

Oocyte Image Analysis

Images of each slide were captured using the software Rainbow Runner (Jandell Scientific Software) and the oocyte feret diameter (µm) of at least 100 oocytes from each individual measured using SigmaScan Pro4 (Jandell Scientific Software). The feret diameter calculates the diameter of each oocyte assuming the best-fit spherical size of the oocyte. Only oocyte sections possessing a nucleus were measured. Previtellogenic oocytes were smaller with a darkly stained, basophilic nucleus or nucleolus, whereas vitellogenic oocytes were larger, coarse looking and eosinophilic (Hendler, 1991) (Fig. 2.2.8A). Not all oocyte sections yielded 100 measurable oocytes. This was mainly owed to either poor tissue processing, especially in the case of *Heterocucumis steineni*, or limited amounts of gonad material being available. A minimum of 40 oocytes was measured in these instances. Consequently, any females possessing < 40 measurable oocytes in the gonad sections were removed from any further analysis.

Staging Male Maturity

Males were described and staged based upon the appearance and characteristics of the testes. Female stages are also included in the figures below to illustrate the maturation process in the females collected (Fig. 2.2.8A, 2.2.9A, 2.2.10A and 2.2.11A). However, the female stages were not used in any further analysis as the oocyte size data generated much more useful information.

Five stages were identified in *Ophionotus victoriae*, specific to criteria distinguishing differences in the quantity and distribution of spermatozoa in the testes (Fig. 2.2.8B).

Stage I: The testes are small and contain no spermatozoa. However, spermatocytes may be visible as a thin layer toward the periphery of the gonad.

Stage II: Spermatozoa have rapidly increased in number, occurring in clusters but eventually radiating out into rows. Some spermatozoa also line the lumen of the testes, where the peripheral spermatocyte layer has visibly shrunk.

Stage III: The testes have increased in size and the spermatocyte layer has reduced further in thickness. Spermatozoa extend to the lumen in chains and spermiogenesis is rapid.

Stage IV: Spermatozoa occupy the entire lumen and have significantly increased in number explaining the increase in the size of the testes. Toward the end of this phase a whorl of spermatozoa develops, which arises from the remaining testicular mass, becomes separated and resides in the centre of the testes. The latter part of this stage precedes spawning.

Stage V: The testes are reduced in size and are similar in appearance to their immature stage I, specifically in terms of a thin spermatocyte layer. The lumen may also contain undischarged spermatozoa.

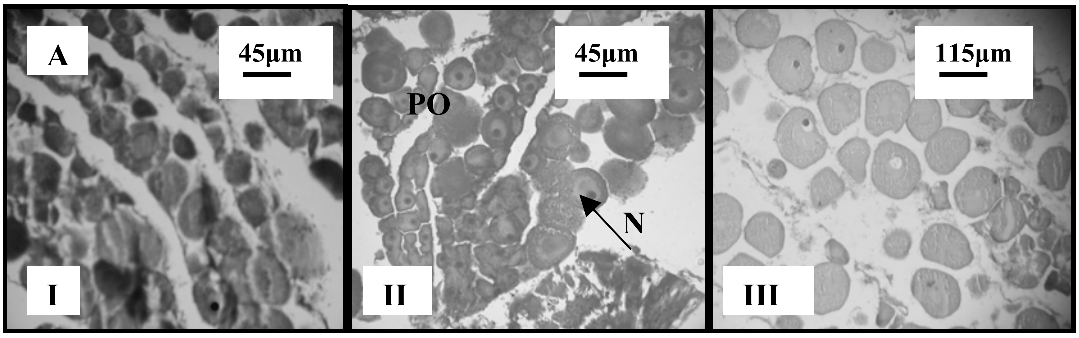

Fig. 2.2.8 *Ophionotus victoriae*. Female (A) and male (B) histological sections identifying progressive stages in gametogenic development. PO = previtellogenic oocyte, VO = vitellogenic oocyte, N = nucleus, BP = by-products, SC = spermatocytes, SG = spermatogonia and SZ = spermatozoa.

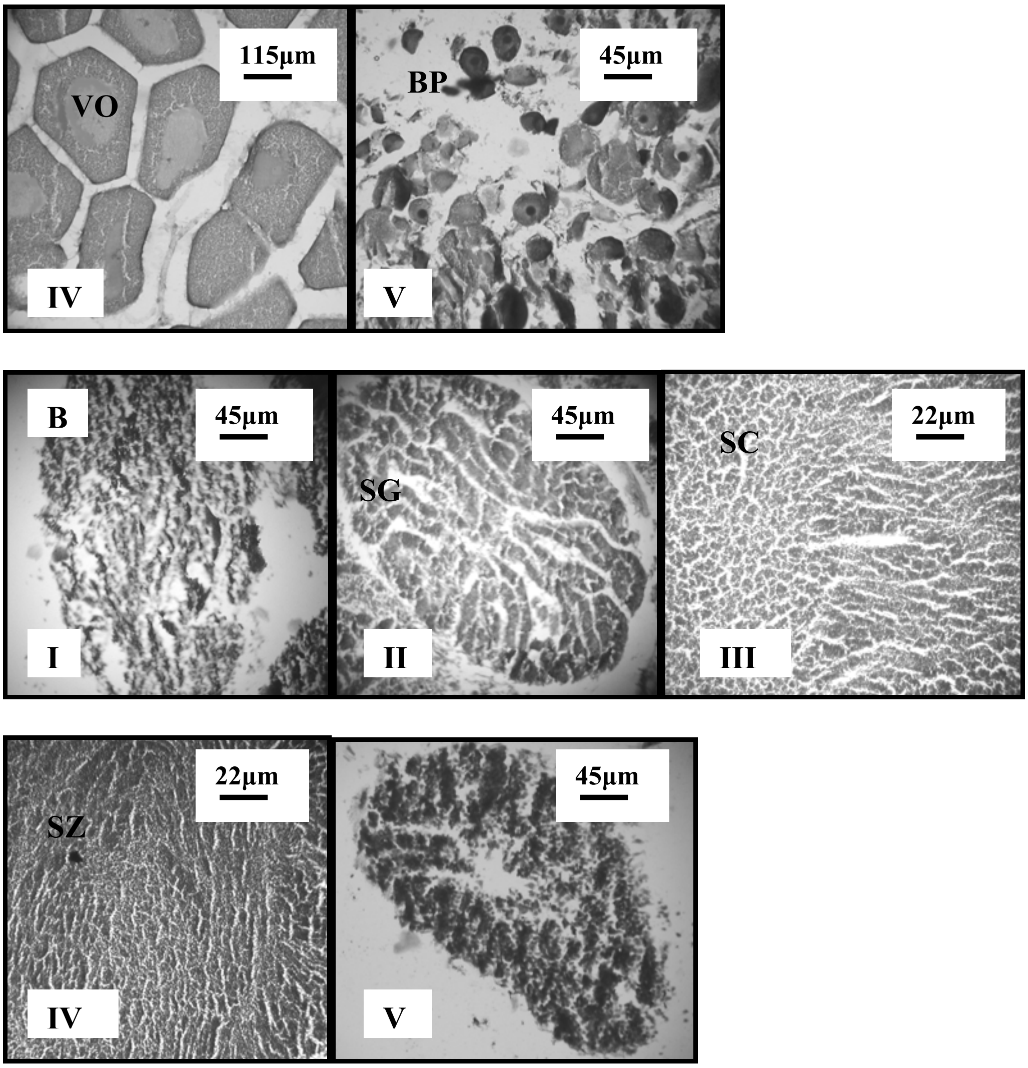

Fig. 2.2.8 contd *Ophionotus victoriae*. Female (A) and male (B) histological sections identifying progressive stages in gametogenic development. PO = previtellogenic oocyte, VO = vitellogenic oocyte, N = nucleus, BP = by-products, SC = spermatocytes, SG = spermatogonia and SZ = spermatozoa.

Male *Odontaster validus* were staged according to Pearse (1965). Pearse describes at least 5 histological stages, which are devised according to the relative amount and maturation stage of sperm in the testes (Fig. 2.2.9 B). A sixth stage (VI) describes the testes when spent.

Stage (I): Spermatogonia are present throughout the year but are especially abundant during August and December. Spermatogonia occur at the base of the epithelium and are characterised by a relatively large nucleus (4-6µm).

Stage (II): Spermatogonia give rise to spermatocytes, which have smaller nuclei (3-4µm) with dispersed, thread-like chromatin and are commonly seen during July and May, most notably between September and January.

Stage (III): Early spermatids are visible in January. Late spermatids are formed by the concentration of the chromatin and the loss of cytoplasm (April). The flagellum develops.

Stage (IV): Free in the central lumen of the testes are small (1.5µm), mature spermatozoa, each with an epical acrosome, a nucleus and a long flagellum (August).

Stage (V): Animals collected during July to September normally have large amounts of active sperm in the testes. Spawning occurs between August and October leaving a few lobes of residual sperm.

Stage (VI): Testes collected from mid-September to February are nearly devoid of active sperm and instead have a thick germinal epithelium.

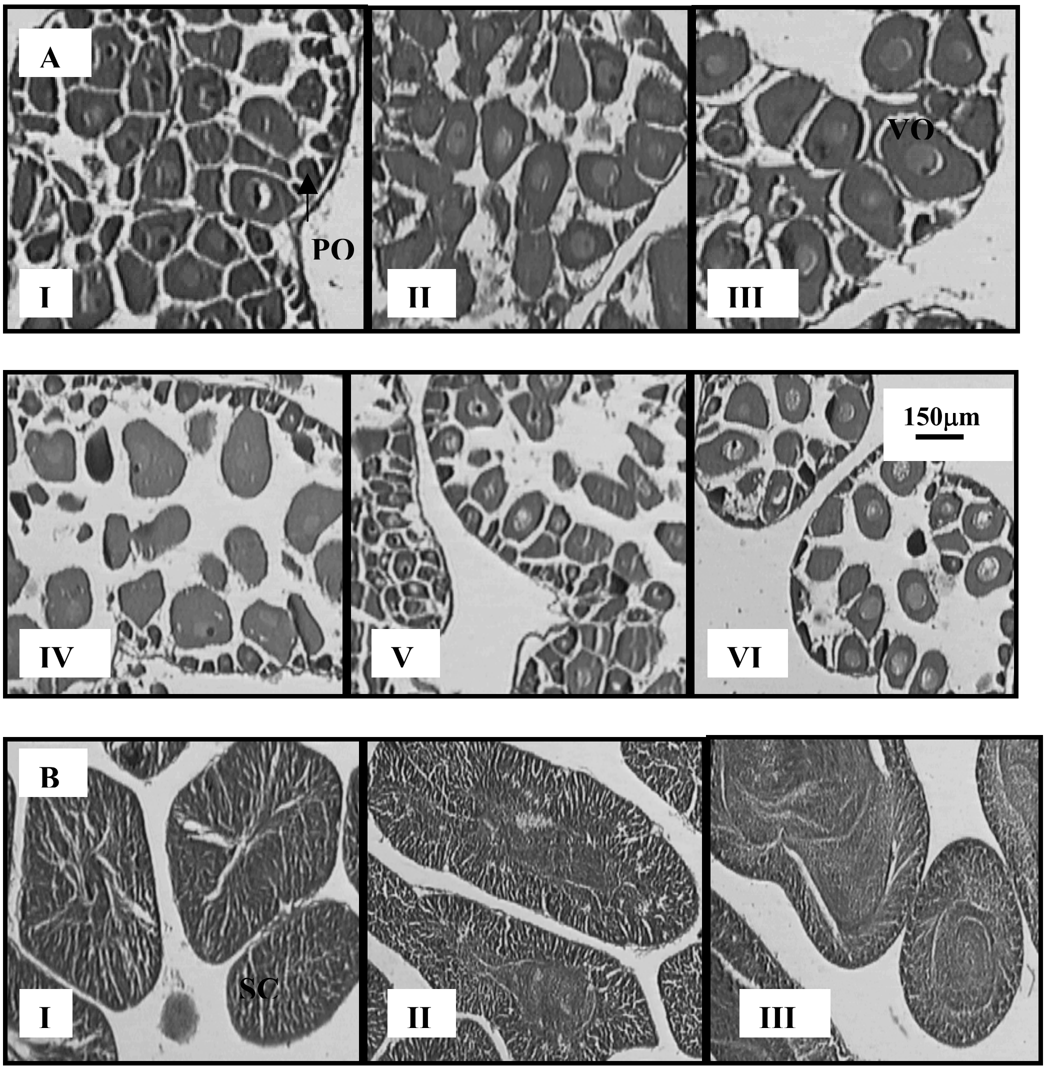

Fig. 2.2.9 *Odontaster validus*. Female (A) and male (B) histological sections identifying progressive stages in gametogenic development. PO = previtellogenic oocyte, VO = vitellogenic oocyte, SC = spermatocytes, SG = spermatogonia and SZ = spermatozoa.

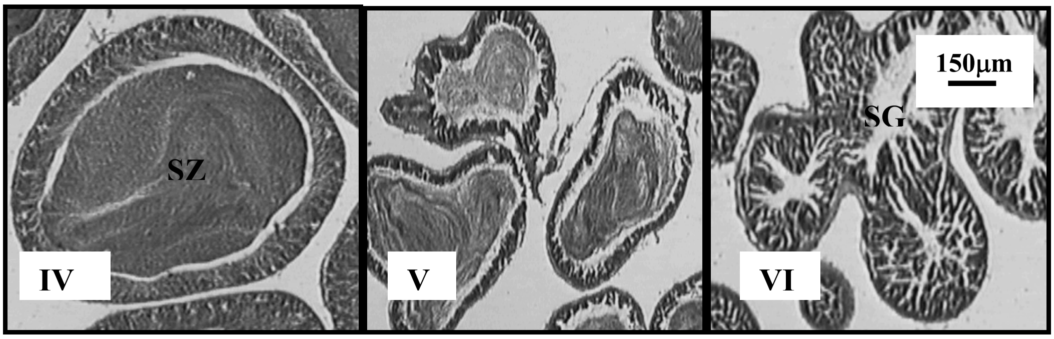

Fig. 2.2.9 contd *Odontaster validus*. Female (A) and male (B) histological sections identifying progressive stages in gametogenic development. PO = previtellogenic oocyte, VO = vitellogenic oocyte, SC = spermatocytes, SG = spermatogonia and SZ = spermatozoa.

Male *Heterocucumis steineni* were staged according to the broad descriptions illustrated in Foster and Hodgson (1995) and divided into 4 main maturity stages plus a spent stage (Fig. 2.2.10B):

Stage I: Gonad recovery post spawning: Any remaining spermatozoa are phagocytosed and removed from the testes as the tubules regress. The gonad is mainly composed of connective tissue and a thick epithelial layer.

Stage II: Initial growth: Gametogenesis resumes and the wall of the gonadal tubules begin to thicken. Early sperm stages are visible in the gonad tubules.

Stage III: Mature growth: Gametogenesis is at a mature stage and the tubules approach maximum diameter.

Stage IV: Ripe/Gravid male: Maximum tubule diameters are present in the testes and are packed with spermatozoa (40-90% tubule volume). Spaces may appear in the tubules during this period owed to partial spawning.

Stage V: Spent/Spawned: There is a rapid decrease in tubule diameters corresponding to the spawning of mature spermatozoa and individuals being spent. Spermatozoa content of the tubules drops to < 10%.

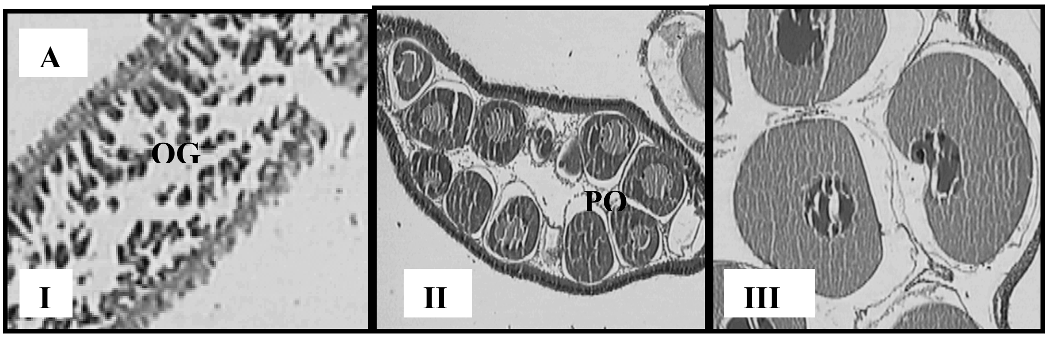

Fig. 2.2.10 *Heterocucumis steineni*. Female (A) and male (B) histological sections identifying progressive stages in gametogenic development. OG = oogonia, PO = previtellogenic oocyte, VO = vitellogenic oocyte, SG = spermatogonia and SZ = spermatozoa.

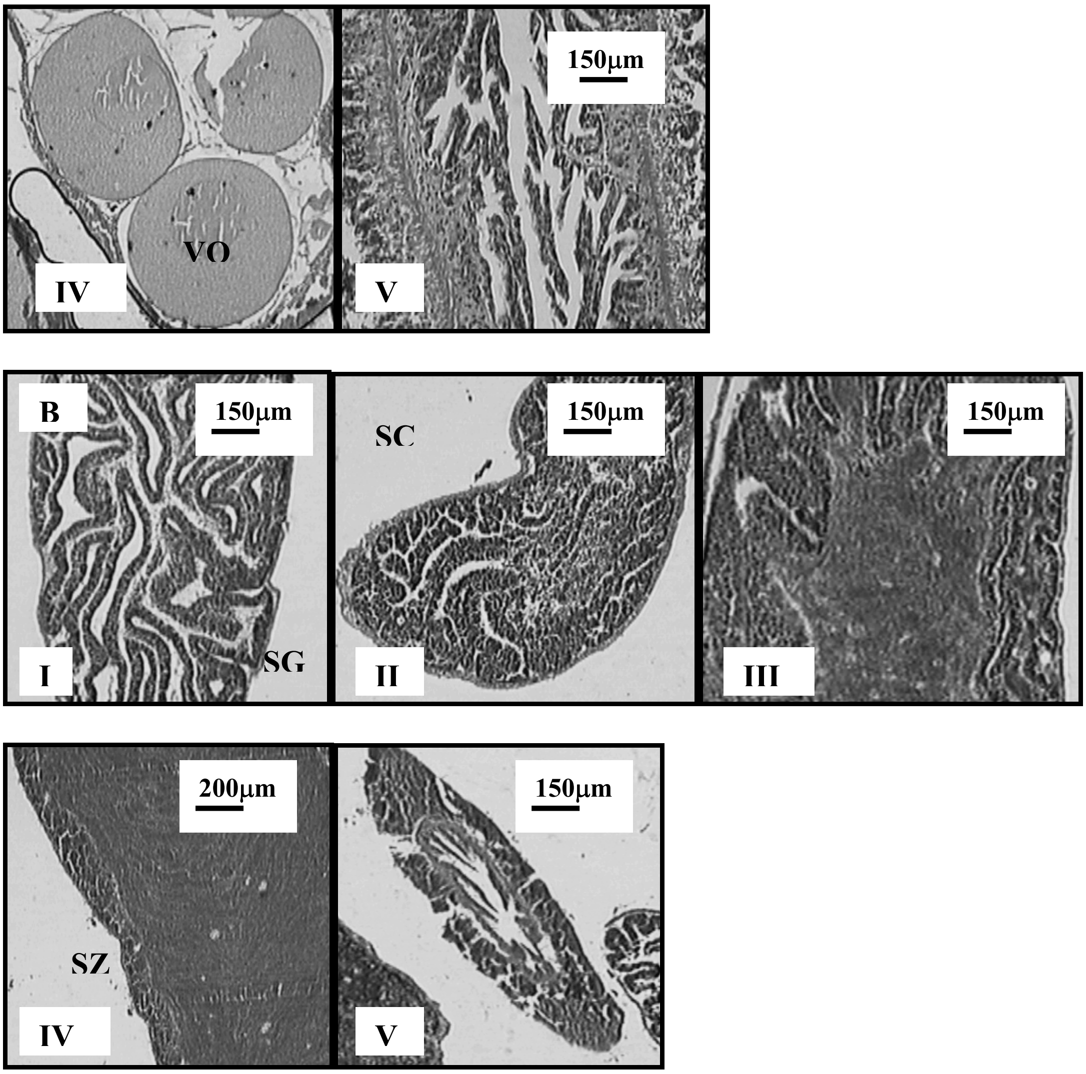

Fig. 2.2.10 contd *Heterocucumis steineni*. Female (A) and male (B) histological sections identifying progressive stages in gametogenic development. OG = oogonia, PO = previtellogenic oocyte, VO = vitellogenic oocyte, SG = spermatogonia and SZ = spermatozoa.

Because of the paucity of information both describing and reviewing gametogenesis in *Parborlasia corrugatus* and nemerteans in general, individuals were staged by comparison with observations of the other three species using simple comparisons between slides (Fig. 2.2.11B). Five maturity stages were recognised plus a spent stage (Stage VI) similar to that described for *Odontaster validus* (Pearse, 1965).

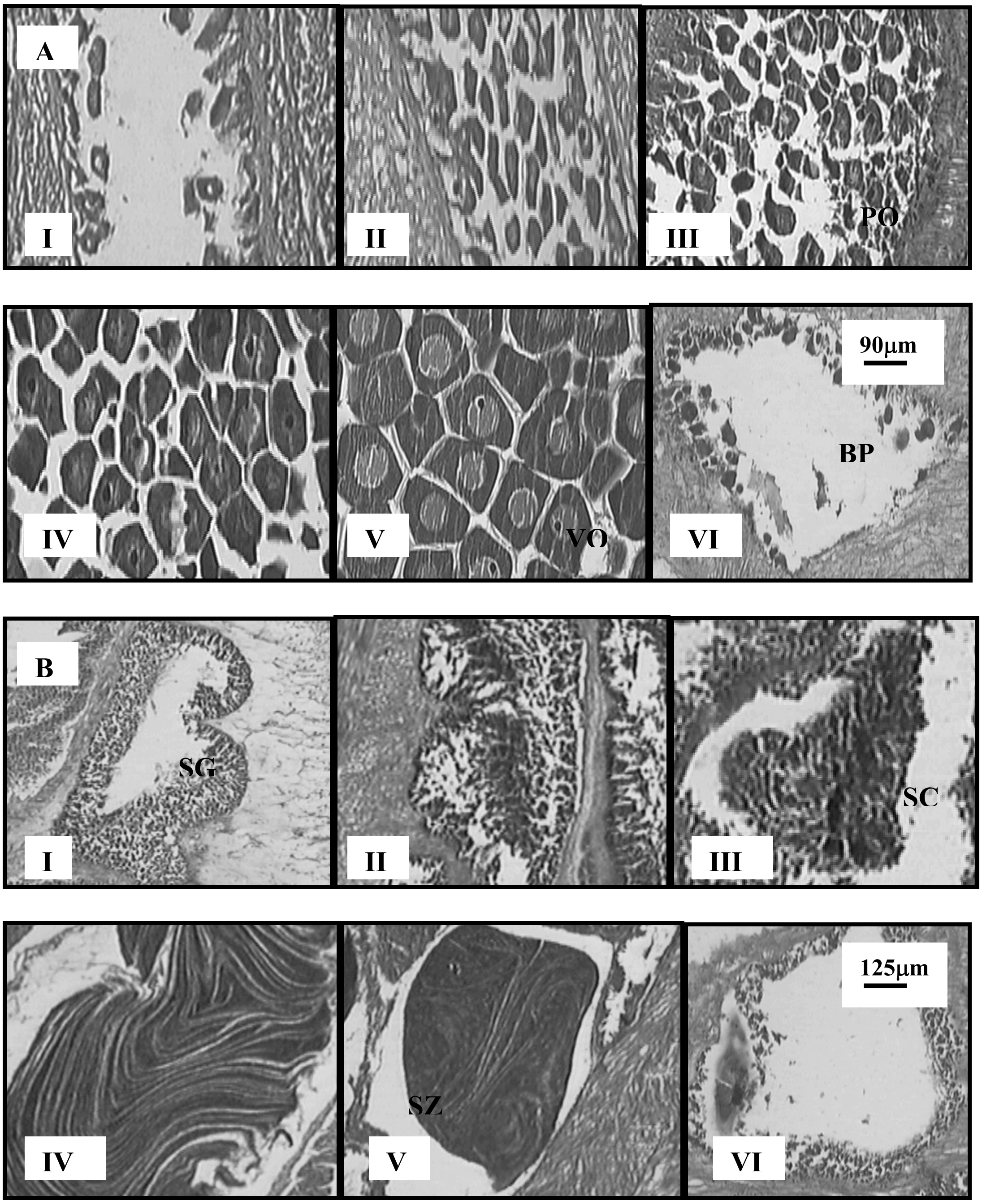

Fig. 2.2.11 *Parborlasia corrugatus*. Female (A) and male (B) histological sections identifying progressive stages in gametogenic development. PO = previtellogenic oocyte, VO = vitellogenic oocyte, SC = spermatocytes, SG = spermatogonia and SZ = spermatozoa.

Fecundity

Fecundity of *Ophionotus victoriae* was determined according to Sumida *et al*., (2000) from individuals collected during the months immediately before spawning. Individuals were dissected and all gonads counted. Three gonads were selected from each individual to act as one sample and two replicates. Images were captured from ovarian smear preparations using the software package Rainbow Runner and the gametes counted using SigmaScan Pro4. The number of vitellogenic oocytes was obtained as a measure of the actual instantaneous fecundity (egg number per gonad x gonad number) and the overall average egg number in each individual taken to produce an average fecundity for each year. The fecundity data were size-corrected to a standard female (standard female: average female disc diameter = 27mm):

*(Individual fecundity/Disc diameter) * Standard female disc diameter (27mm)*

A measure of reproductive output was calculated from the percentage decrease in gonad index over the spawning period of each sample year, and applied to the four yearly egg counts to determine the approximate number of eggs spawned.

Egg counts of the remaining species would have required serial sectioning of the gonad. Therefore owing to time and fiscal constraints, further species fecundity assessments were not undertaken.

Nutritional Analysis

The gut tissue from ten brittle star individuals of a similar-size from the austral winter, summer and spawning period from each sampling year (1997-2000) was dissected and weighed wet ($\pm$0.01g) to calculate gut index (GtI):

GtI = (GtW*100)/DV, where GtW = gut mass (g) and DV = disc volume (mm^3).

The pyloric caeca of the seastars were also weighed wet ($\pm$0.01g) and the pyloric caeca index (PI) calculated using total animal wet weight:

PI = (PW*100)/BW, where PW = pyloric caeca wet weight (g) and BW = total animal wet weight (g).

A simple measure of feeding effort was calculated using the percentage change in the pyloric caeca index for both males and females. This was determined from the months when animals appeared most satiated, with the largest proportion of stored material in the pyloric caeca, compared to months of relative starvation when the pyloric caeca were comparatively empty. These data were compared annually and seasonally over the sampling period.

Nutritional measurements were not possible for the nemertean or the sea cucumber. However, a number of nemertean individuals did display internal colouration particularly around the gut, exhibiting green and purple secretions possibly owed to dietary differences between individuals.

Statistical Analysis

Statistical techniques followed the methods of Fowler *et al*., (1998) and were carried out using Minitab 13.0. The majority of data were not normally distributed (according to the Anderson-Darling Normality test) and were tested using non-parametric statistical analyses (Appendix Table. 2). However, where appropriate parametric tests were used on any normally distributed data. The sex ratio of each of the sampled populations was assessed using the Chi-Square test. Comparisons in size and reproductive measurements were made between the individual sampling years and the sampling period as a whole (1997-2001). Kruskal-Wallis or One-Way ANOVA tests were used to identify any significant differences in animal size, organ indices and the oocyte data over the sampling period. Differences between individual sampling years were tested using the Mann Whitney non-parametric statistical test. The non-parametric Wilcoxon test for matched pairs was used to assess differences between the males and females collected. Correlative techniques were used to test the relationship between both biological and physical variables and where necessary data were ranked before being statistically analysed (Pearsons Product Moment Correlation and Regression analysis).

2.3 Results

Physical Environment

Annual ice cover in Rothera was variable during 1997-2000, with greatest coverage during the austral winter of 1997. The extent of ice cover was much reduced in the following years of the study and the occurrence of ice in the latter period was patchy in comparison with the extent observed in 1997. Temperatures ranged between -1.89˚C in June 1997 and +1.42˚C in February 1999 respectively, with an annual peak during January/February and a minimum during June/July each year (Fig. 2.3.1). The annual temperature cycle was closely tracked by the concentration of photosynthetic pigments in the waters around Rothera. Chlorophyll a concentrations ranged between 23mg m^{-3} in February 1998 and <0.03mg m^{-3} in September 1997. Maximum chlorophyll a concentrations varied between 1997-2000, with a lower peak in 1999 compared to 1998 and 2000. Phaeophytin pigment concentrations showed a similar pattern but were generally more consistent between years at the time of the bloom event. Concentrations peaked at 12.55mg m^{-3} in January 2000 and a minimum of 0.05mg m^{-3} was recorded in June 1998. Defined phytoplankton blooms were noticeable during the austral summers of 1998, 1999 and 2000: February 98 (23.28mg m^{-3}), February 99 (13.42mg m^{-3}), and a series of peaks between December 99 and February 2000 (23.71, 18.69, 20.96 and 20.04mg m^{-3}). The phytoplankton blooms generally lasted for 8-10 weeks. The annual light cycle, measured as averaged PAR data between 15 and 20m, ranged between 0 mW/cm^2 in January 1998 and 16.28 mW/cm^2 in December 1999. Light levels in 1998 also peaked during late November-early December (8.49 mW/cm^2). In some years the light climate peaked either side of the bloom event, before dipping during the intervening period. The sedimentation data were reliable in a comparative sense, highlighting the relative differences between years. Sedimentation varied markedly between 1997-2000, with a remarkable peak during the austral summer of 1998-1999 (550mg day^{-1}). Moderate flux levels were observed in the summers prior to and after this maximum event. The sedimentation events observed in 1998-1999 and 1999-2000 showed a wider coverage with periods of 240 days (September 98-May 99) and 180 days (November 99-May 00) respectively, compared to a much more limited period in 1997-1998 (91 days; November 97-February 98).

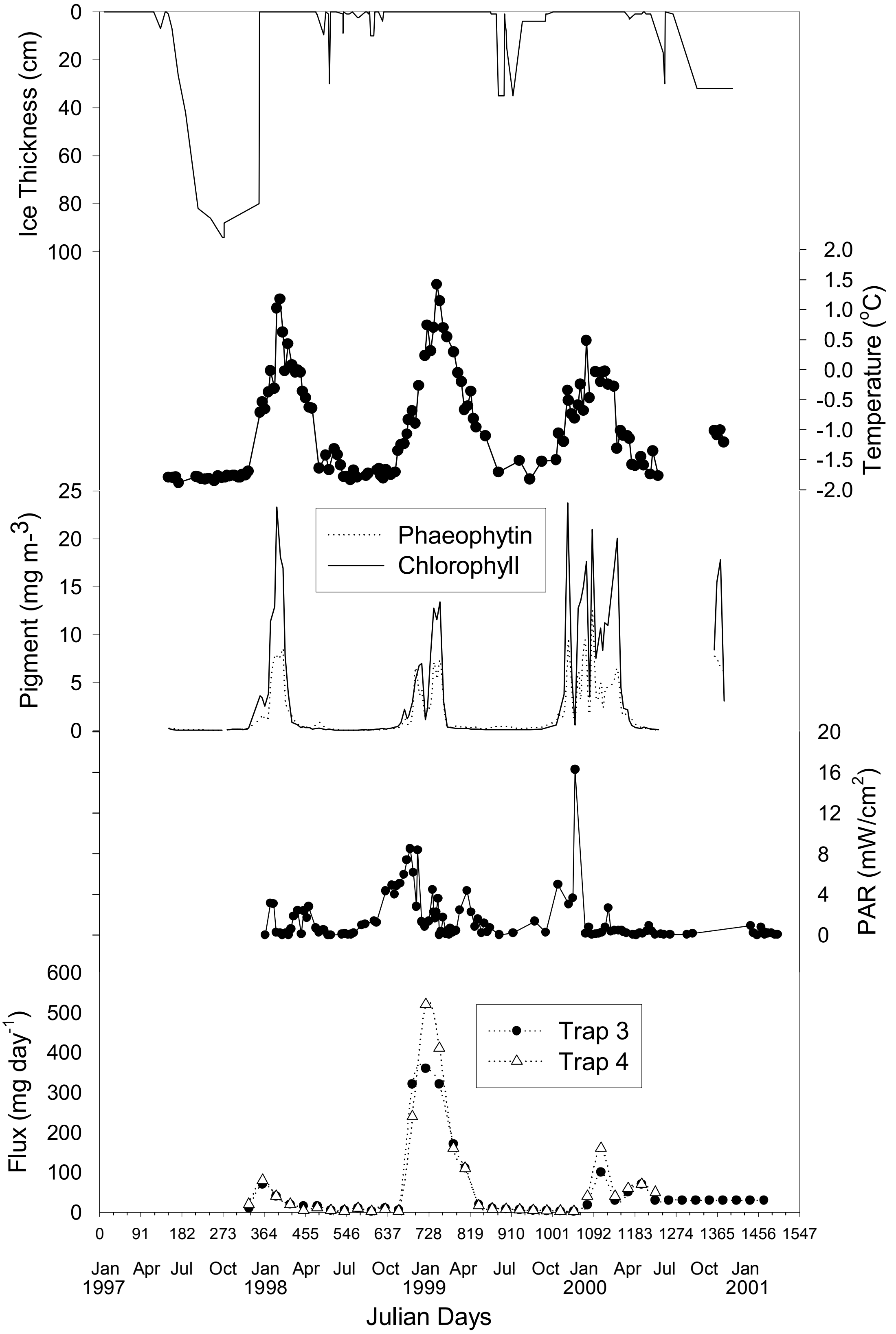

Fig. 2.3.1 Rothera Time Series Water Sampling Programme (RaTS) environmental data (1997-2001). Julian Day scale beginning from 1 = 1 January 1997. Data provided by A. Clarke.

Ophionotus victoriae

Sex Ratio and Disc Diameter

377 individuals were dissected of which 208 (55%) were male and 169 (45%) were female (Table. 2.3.1). The sex ratio of the sampled population differed only slightly from a ratio of 1:1 with a small bias toward males ($\chi2 = 4.04$, $P < 0.05$). The prevalence of males was significant in the smaller sample population collected during the austral summer of September 97-December 97 ($\chi2 = 6.43$, $P < 0.05$) and January 98-December 98 ($\chi2 = 6.13$, $P < 0.05$). However, the sex ratio was 1:1 in the samples collected in 1999 and 2000 ($\chi2 = 0.05$ and $\chi2 = 0.31$, N.S. respectively).

Table. 2.3.1 *Ophionotus victoriae*. Sex ratio of males and females collected between 1997 and the end of 2000 using a Chi Square statistical test.

Year	Male	Female	Total Individuals
1997 (Sep-Dec)	28	12	40
1998	73	46	119
1999	51	49	100
2000	56	62	118

Disc diameters ranged from 11.2mm to 38.1mm (mean = 26.46mm, Standard Deviation (SD) = 3.57, n = 378) (Fig. 2.3.2). The maximum disc diameter observed for both males (34.9mm) and females (38.1mm) exceeded the previous value reported for this species collected from the high Antarctic (32mm) (Dahm, 1999). The smallest individual in this study, an 11.2mm female had mature gonads, and was collected in October 2000. Therefore, the minimum size of this species at sexual maturity (in females) must be at least 11.2mm. The range of disc diameters between the male and female sample population did not differ significantly (Wilcoxon Test, $W+ = 295$ $W- = 446$, $N = 38$, $P = 0.278$). However, a one-way ANOVA of disc diameter confirmed a significant difference over the study period ($F = 5.21$, $P < 0.001$), with larger ophiuroids being collected toward the end of the study (Fig. 2.3.2). However, it is impossible to say whether this apparent increase in size is real or just a result of how different divers responded to instructions, especially as larger specimens were preferred to ensure reproductive maturity. Therefore, where necessary, some data are scaled to a standard individual to remove the affect of size.

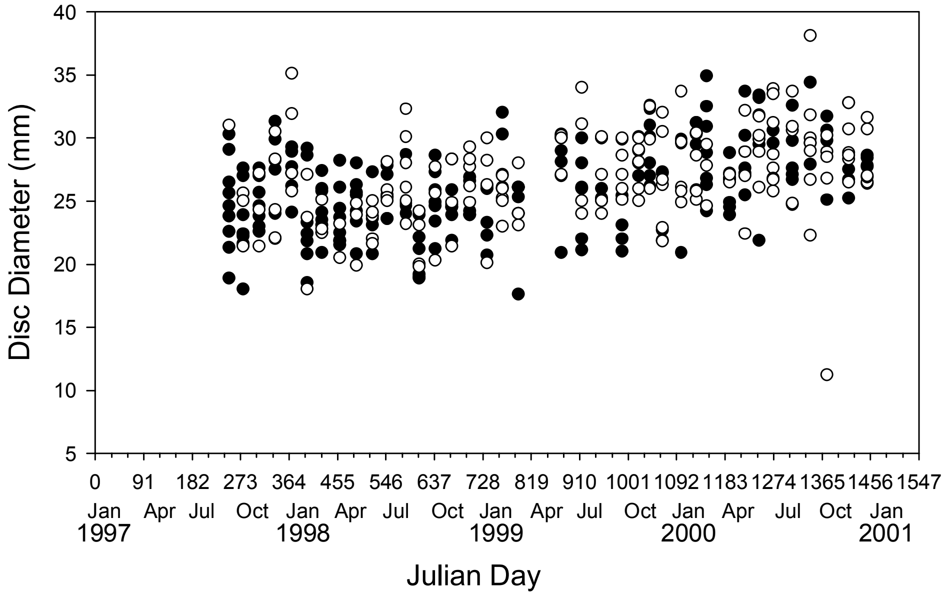

Fig. 2.3.2 *Ophionotus victoriae*. Individual male (●) and female (○) disc diameter values for animals sampled on a monthly basis between September 1997 and December 2000. The larger range in disc diameter was recorded for female *Ophionotus victoriae* with a minimum disc diameter of 11.2mm and a maximum of 38.1mm (mean = 27.0, SD = 3.67, n = 169). Male disc diameter ranged from 17.6mm to a maximum of 34.9mm (mean = 26.05, SD = 3.47, n = 208).

Fecundity

The maximum average actual fecundity observed was 248,000 eggs per ripe individual in 1999 (Fig. 2.3.3).

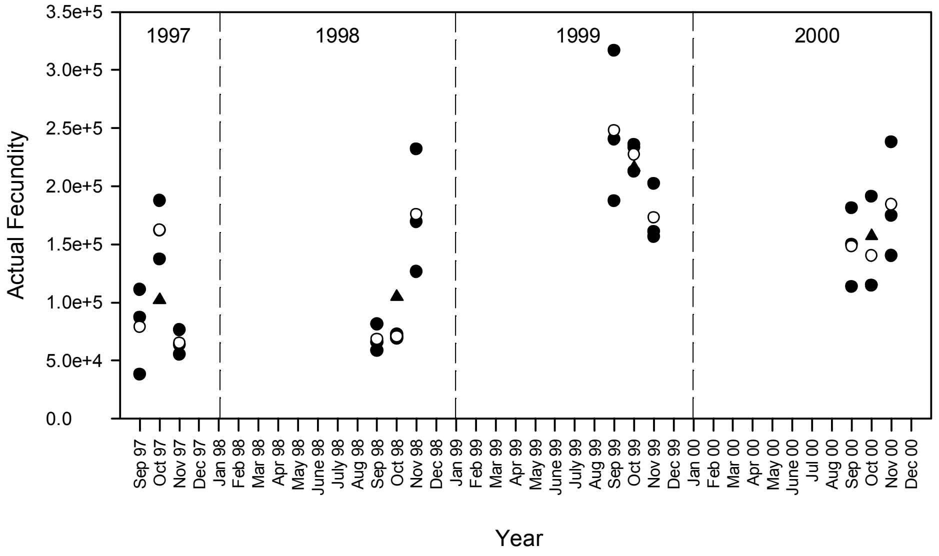

Fig. 2.3.3 *Ophionotus victoriae*. Fecundity data for females sampled between 1997 and 2000. Egg number per gonad (●), average egg number per individual (○) and average egg number per year (▲).

However, mean fecundity varied from 100,000 eggs per individual during the ripest months of 1997 and 1998 and 220,000 during the late austral spring of 1999. The average fecundity of *O. victoriae* in 2000 was 160,000 eggs.

There was significant variability within actual fecundity (Fig. 2.3.3) over the four spawning periods (One-Way ANOVA, F = 10.04, P < 0.001). Differences were also observed when fecundities were compared between individual years (Table. 2.3.2). A Tukey's test for pairwise comparisons identified the largest differences between fecundities in 1997 and 1999 and 1998 and 1999. In contrast there was no significant difference between the fecundities observed in 1997 and 1998.

Table. 2.3.2 *Ophionotus victoriae*. Comparisons in actual fecundity and overall gonad index between individual years (1997-2001). The variation in fecundity and gonad index of individual ophiuroids was tested between years. A 2-sample T-test or a non-parametric Mann Whitney test was used depending on if the data were normally distributed (Appendix Table. 2). A significant difference was indicated by a P < 0.05. A test for equal variance was also undertaken taken when a 2-sample T-test was employed and the results included in the assumptions of the test (Appendix Table. 3).

Year Comparison	Test and Test Statistic	P value	DF
1997-1998 Fecundity	2-sample T-test, T value = -0.11	0.911	16
1997-1999 Fecundity	2-sample T-test, T value = -4.84	< 0.001	16
1997-2000 Fecundity	2-sample T-test, T value = -2.51	0.023	16
1998-1999 Fecundity	2-sample T-test, T value = -4.32	0.001	16
1998-2000 Fecundity	2-sample T-test, T value = -2.15	0.047	16
1999-2000 Fecundity	2- sample T-test, T value = 2.72	0.015	16
1997-1999 GI	Mann Whitney, W = 1749.0	< 0.001	
1997-2000 GI	Mann Whitney, W = 2034.0	< 0.001	
1998-1999 GI	Mann Whitney, W = 10033.0	< 0.001	
1998-2000 GI	Mann Whitney, W = 11088.0	< 0.001	

The number of gonads was high and variable (80-240) and fecundity was disc diameter dependent ($r^2 = 0.725$, $P < 0.05$) (Fig. 2.3.4).

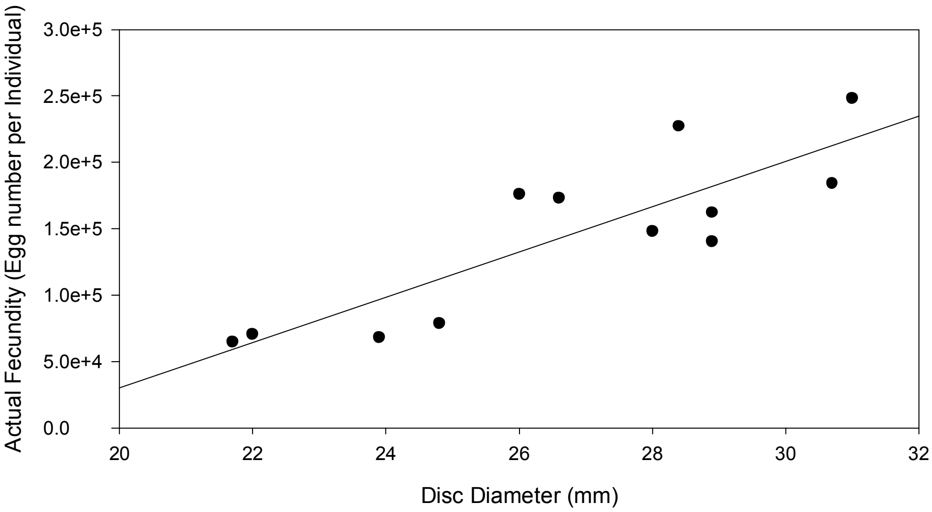

Fig. 2.3.4 *Ophionotus victoriae*. Correlation between individual actual fecundity and individual disc diameter (±0.01mm). A linear regression was used and the variables were significantly correlated (r^2 = 0.725, $P < 0.05$).

Actual fecundity was also scaled on a per millimetre basis and calculated for a standard female (average size) of 27mm disc diameter (Fig. 2.3.5). Although the calculation of a proper scaling co-efficient would have been preferable, the limited data available prevented the calculation of such a relationship and slope. For a standard female (27mm disc diameter) fecundity was highest in 1999 (~2.0×10^5 ± 37570 eggs), with a more modest accumulation of eggs in 1997 and 1998 (1.1×10^5 ± 42096 and 1.2×10^5 ± 57963 eggs). A standard female in 2000 produced approximately 1.5×10^5 ± 36786 eggs. Comparisons between Figure 2.3.1, Figure 2.3.3 and Figure 2.3.5 suggest that the annual pattern in fecundity between 1997-2000 was uncoupled with the preceding chlorophyll maxima in the waters around Rothera, but was closely coupled with the magnitude of the preceding benthic sedimentation event (8-9 months previous).

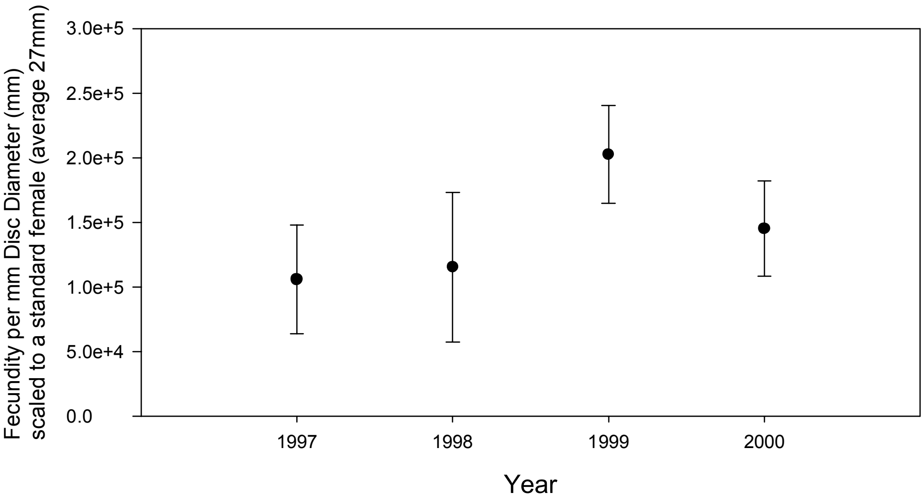

Fig. 2.3.5 *Ophionotus victoriae*. Annual fecundity data calculated per millimetre disc diameter for a standard female (27mm disc diameter). The overall mean fecundity (●) is plotted and the error bars represent ± SD (N = 3).

Reproductive Condition

The gonad index showed significant inter-annual variation (Fig. 2.3.6). The testis index ranged from 0.001 in October 1998 to 1.9 in September 1999 (mean = 0.2, SD= 0.30, n = 208), whereas the ovary index ranged from 0.01 in January 1999 to 1.6 in October 2000 (mean = 0.2, SD = 0.25, n = 169). Four spawning events were apparent from the data, which were similar in their timing during November-December of each year, but differed markedly in magnitude between years. In November 1997 spawning was limited (13% decrease), as no significant change in gonad index was evident. A moderate peak in the index occurred a year later in 1998 (0.5) with a spawning event evident in December, indicated by a 90% reduction in gonad index. Gonad index was high in 1999 (1.9) and 2000 (1.6), where a percentage decrease of 96% and 89% was observed in gonad index respectively.

Inter-annual variation in reproductive condition was synchronous between the male and females (Wilcoxon Test, W+ = 471 W- = 232, N = 37, P = 0.073). However, there was a significant difference in the overall gonad index during the sampling period (Kruskal-Wallis, H = 65.80, P < 0.001). There was no significant difference between the gonad indices of 1997 and 1998 (Mann Whitney, W = 3290.0, P = 0.722) or between 1999 and 2000 (Mann Whitney, W = 11781.0, P = 0.074). However, significant differences were found between 1997 and 1999, 1997 and 2000, 1998 and 1999 and 1998 and 2000 (Table. 2.3.2). The trend in gonad index was similar to the variation in actual fecundity during the study period. Comparisons between Figure 2.3.1, 2.3.3 and 2.3.6 show that cycles in both reproductive characteristics were not strictly in phase with the annual sedimentation event at Rothera, although the patterns in all three characteristics were similar, e.g. a high fecundity followed a good nutritional season and a reduced fecundity occurred after a season of low surface flux.

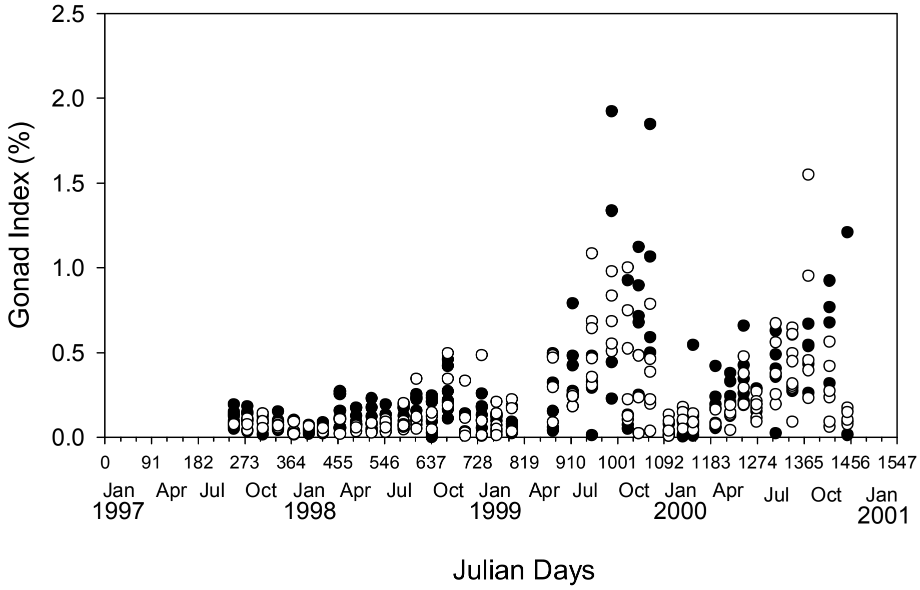

Fig. 2.3.6 *Ophionotus victoriae*. Individual male (●) and female (○) gonad index values for animals sampled on a monthly basis between September 1997 and December 2000.

The reproductive output calculations allowed an estimate of the number of eggs spawned. According to the 13%, 90%, 96% and 89% decrease in gonad index over the spawning periods of 1997, 1998, 1999 and 2000 respectively, spawning was a magnitude greater in 1999 ($2x10^5$ eggs) and 2000 ($1.4x10^5$ eggs) compared with the fecundity in 1997 (Table. 2.3.3). A smaller number of eggs were released in 1998 ($9.4x10^4$ eggs), and the lowest spawning event was recorded in 1997 ($1.3x10^4$ eggs).

Table. 2.3.3 *Ophionotus victoriae*. Reproductive output is presented as the number of spawned eggs in individual years (1997 to 2001), calculated from the percentage decrease in female gonad index each year and average egg number produced each year by gravid females (egg counts were averaged from 3 females, and the number of eggs quantified from three ovaries in each female).

Year	Gravid Fecundity	Percentage Decrease (%)	Post-spawned Fecundity	Spawned Eggs
1997	1.02E+5	12.5	8.92E + 4	1.27E + 4
1998	1.05E+5	90	1.05E + 4	9.44E + 4
1999	2.16E+5	96	8.64E + 3	2.07E + 5
2000	1.57E+5	88.9	1.75E + 4	1.40E + 5

Oocyte Feret Diameter

There was a marked intra- and inter-annual variation in mean oocyte size (oocyte feret diameter) between 1997-2000 (Kruskal-Wallis, H = 511.99, P < 0.001) (Fig. 2.3.7).

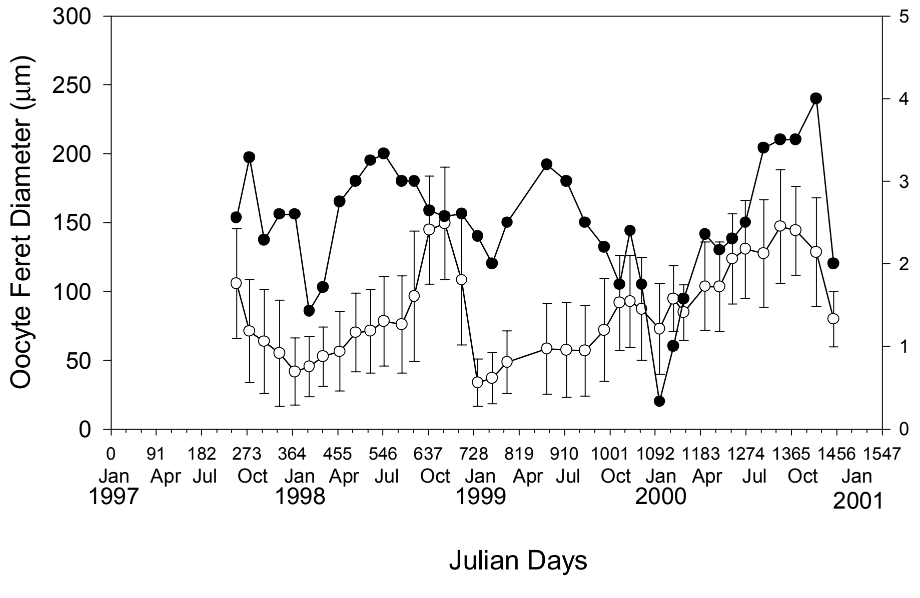

Fig. 2.3.7 *Ophionotus victoriae*. Mean oocyte feret diameter (○) sampled on a monthly basis between September 1997 and December 2000. The overall mean oocyte size is plotted and the error bars represent ± SD. Average male maturity stage is plotted (●) and male maturity stage V is plotted as zero to convey the testes as being spent.

Four spawning events were identified. The first was moderate and marked the release of oocytes in the late austral spring/ early summer of 1997, where oocyte sizes decreased from an average of 116µm (SD = 40.1, n = 100) in September 1997 to 42µm (SD = 24.4, n = 509) in January 1998. The maximum average oocyte size was observed in November 1998 (mean = 149µm, SD = 41.1, n = 341) followed by a large spawning event and a reduction in average oocyte size to 34µm (SD = 17.0, n = 615) in January 1999. This decrease in size translated to a significant loss in gamete volume consistent with the decrease in gonad index. The average oocyte size increased during 1999 but only reached an average of 93µm (SD = 33.5, n = 467) in November 1999. Spawning followed this period, but only reduced the average oocyte size to 73µm (SD = 32.7, n = 464) in January 2000. Average oocyte size increased to 128µm (SD = 39.6, n = 681) in November 2000 and declined until the end of the study period, consistent in timing with the other three spawning events.

The proportion of previtellogenic and vitellogenic oocytes differed significantly between months sampled (Mann Whitney, W = 996.0, P < 0.0001). The changeable pattern in oocyte maturity stages during each year suggests that this invertebrate is a seasonal reproducer, which is also illustrated in the pattern of oocyte size classes and the proportion of oocytes within each size category throughout the sampling period (Fig. 2.3.8). The largest vitellogenic oocytes predominated in the ovaries of gravid females during the early austral spring- early austral summer and are noticeably absent during late summer and early winter, when the oocyte pool was composed of previtellogenic generations of oocytes.

The reproductive cycle had a long-term component in addition to the seasonal signature described above (Fig 2.3.8). This figure shows overlapping generations of oocytes. The first generation of pre-vitellogenic oocytes has started developing before spawning of the preceding generation, which is especially clear in oocyte frequency data for October/November 1997. These data illustrate a bimodal distribution of oocyte sizes showing the presence of two generations of oocytes and an 18-24 month gametogenic cycle. All of the individual oocyte size distribution histograms, for every female ophiuroid collected, are contained in Appendix Figure 1.

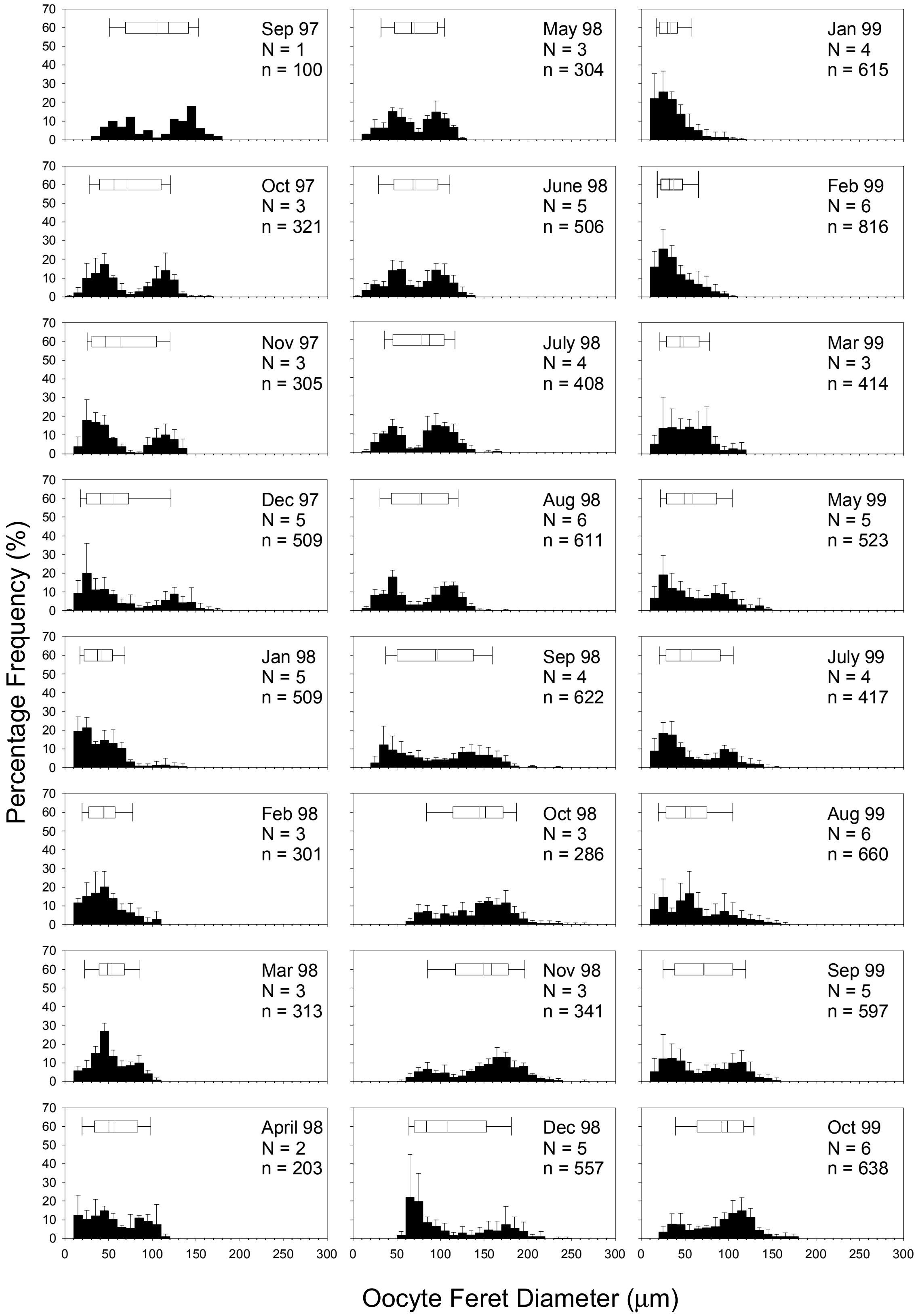

Fig. 2.3.8 *Ophionotus victoriae.* Pooled oocyte feret diameter histograms (± SD), where N = number of females, n = number of eggs counted (1997-2000) and monthly box plots covering the oocyte feret diameter range (the left and right hand limits of the box represent the 25th and 75th percentiles; box whiskers represent the 10th and 90th percentiles; the median (-) and mean (-) of each oocyte range is also shown).

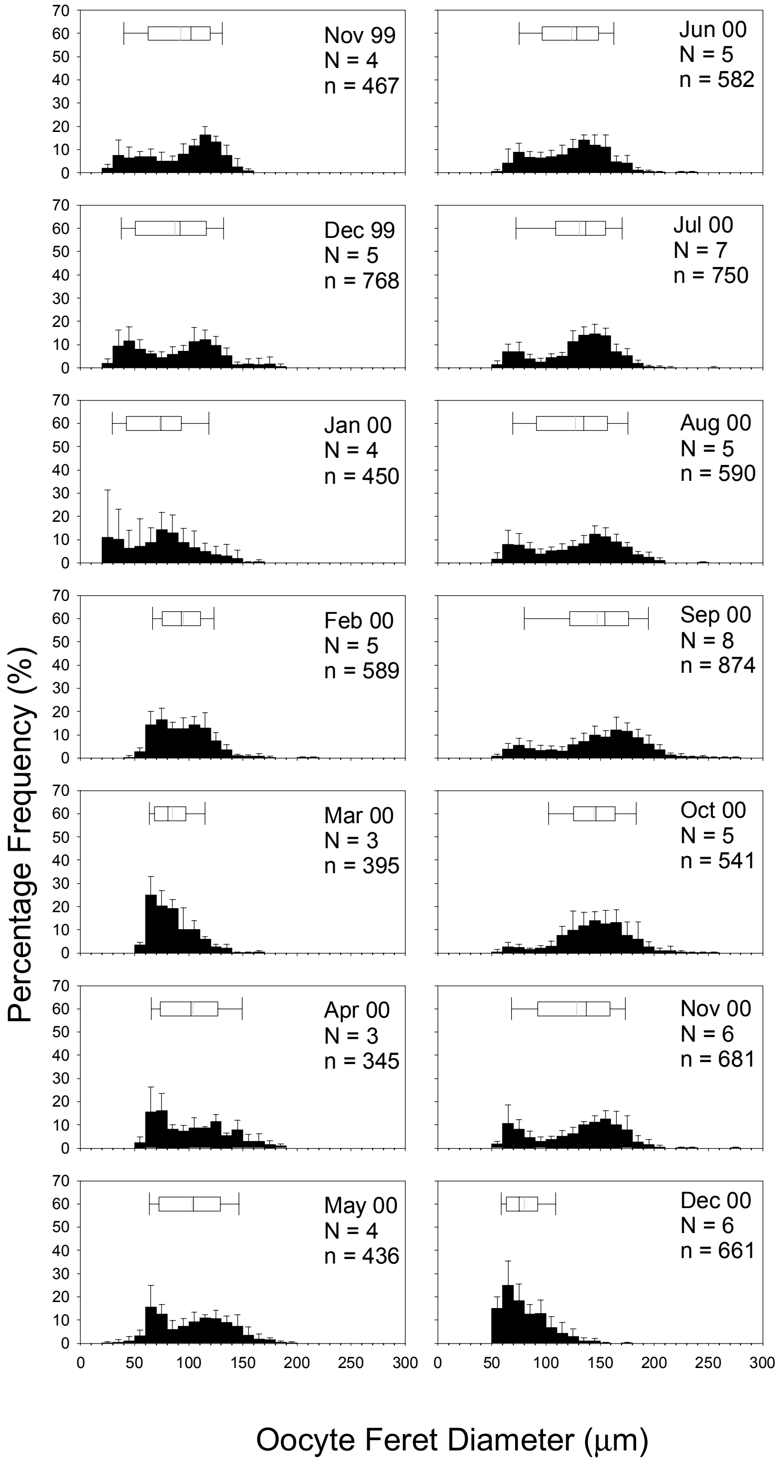

Fig. 2.3.8 contd *Ophionotus victoriae.* Pooled oocyte feret diameter histograms (± SD), where N = number of females, n = number of eggs counted (1997-2000) and monthly box plots covering the oocyte feret diameter range (the left and right hand limits of the box represent the 25th and 75th percentiles; box whiskers represent the 10th and 90th percentiles; the median (-) and mean (-) of each oocyte range is also shown).

In *O. victoriae* both oocyte number and average oocyte size changed each year emphasising the large inter-annual variation in the reproductive ecology of this animal.

Male Maturity

The Male Maturity Index ranged between being spent and being at an immature gametogenic stage (I-II) during the austral summer of each year (Fig 2.3.7). The latter was pronounced in January 2000 when the testes were spent. The more mature stages (III-IV) were found as early as the late austral winter up until the spawning season. Male and female spawning was synchronous.

Reproductive Cycle

Mean egg size was the only character that proved to be normally distributed (Anderson Darling Normality, A-squared = 0.521, P = 0.172). Therefore, these data were ranked prior to performing correlations. Some environmental and biological factors appeared correlated (Table. 2.3.4). Temperature was the only environmental factor that had a significant correlation with the mean maximum oocyte size (Pearsons Product Correlation, -0.409, P = 0.018), especially when the effect of chlorophyll was accounted for (Pearsons Product Correlation, -0.520, P = 0.002). The relationship between the mean maximum oocyte size and all the environmental factors combined (temperature and pigments) proved significant (r = 5.84, P = 0.006). This was also the case for gonad index (r = 4.97, P = 0.039).

Table. 2.3.4 Correlations between reproductive (average gonad index and average oocyte size) and environmental variables (chlorophyll, phaeophytin and temperature) between 1997 and the end of 2000. All data were ranked prior to analysis and tested using Pearson's Product Moment Correlation. A P < 0.05 indicated that variables were significantly correlated.

Correlated Variable	Test Statistic	P-value
Chlorophyll and Phaeophytin	0.910	P < 0.001
Temperature and Chlorophyll	0.747	P < 0.001
Temperature and Phaeophytin	0.695	P < 0.001
Gonad Index and Egg Size	0.500	P = 0.003

Nutritional condition

Some inter-annual variation was evident in the gut index (Fig 2.3.9) and followed a similar trend to gonad index over the three-year sampling period, having two low-value years (1997-1998) and two higher-value years (1999-2000). There was a lag of approximately three months between the peak gut index and peak gonad index. This was especially evident during 1999 and 2000. A marked decline occurred in the gut index during the spawning period of 1999 and 2000. Patterns in these data suggest an almost monotonic increase in gut index over the sampling period and this may reflect an underlying long-term component (~4 years). These patterns are not in phase with the cycle in the gonad or the annual sedimentation events at Rothera.

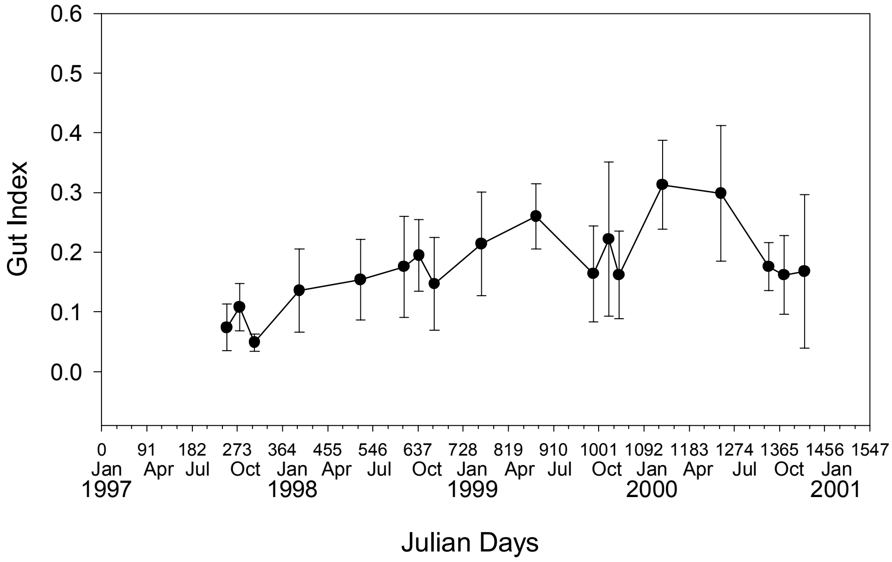

Fig. 2.3.9 *Ophionotus victoriae.* Monthly gut index values for October 1997 to December 2000. The overall mean gut index is plotted (●) and the error bars represent ± SD (N = 10).

An interesting discovery was observed in three brittle star individuals. Each exhibited a small polycheate within the disc, within the digestive tissue. A single specimen was sent to the Natural History Museum for identification. Polychaetes have been studied which live inside the guts of sea urchins, however most are commensal living on the exterior of their host when associated with echinoderms (G Patterson, pers. comm.). Unfortunately, the preserved state of the specimen was poor and little information was forthcoming, although the specimen was classed as Nepthyidae (G Patterson, pers. comm.).

Odontaster validus

Sex ratio and Size Characteristics

407 individuals were sampled of which 222 (54.5%) were male and 182 (44.7%) were female. 3 (0.7%) individuals collected during 1998 had both male and female characteristics and were classed as hermaphrodites (Table. 2.3.5). The sex ratio of the sampled population was slightly biased toward males ($\chi 2 = 3.84$, $P < 0.05$). This was not true for all years (1997 $\chi 2 = 0.076$, 1998 $\chi 2 = 0.195$ and 1999 $\chi 2 = 0.134$, N.S.) except 2000, where males were more numerous ($\chi 2 = 8.377$, $P < 0.01$).

Table. 2.3.5 *Odontaster validus*. Sex ratio of males and females collected between 1997 and the beginning of 2001 using a Chi Square statistical test.

Year	Male	Female	Total Individuals
1997 (Jul-Dec)	32	34	66
1998	69	64	133 (+ 3 hermaphrodites)
1999	35	32	67
2000 (Jan-Jan 2001)	86	52	138

Animal wet weight ranged from 5.92g to 37.83g (mean = 15.92g, SD = 5.46, n = 407) (Fig 2.3.10).

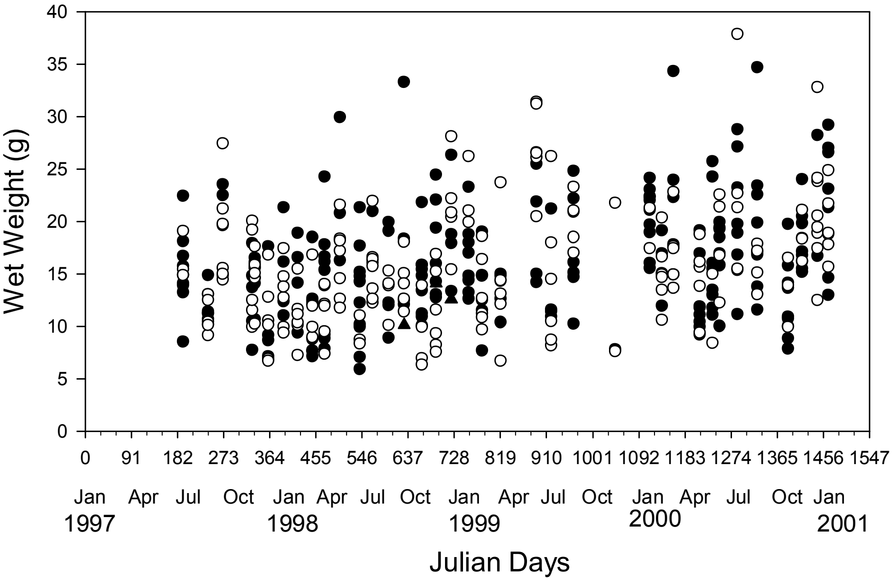

Fig. 2.3.10 *Odontaster validus*. Individual male (●), female (○) and hermaphrodite (▲) wet weight values (±0.01g) sampled on a monthly basis between July 1997 and January 2001. Female wet weight ranged between 6.37g and 37.83g (mean = 15.64g, SD = 5.54, n = 182) and male wet weight ranged between 5.92g and 34.70g (mean = 16.19g, SD = 5.41, n = 222).

The smallest individual collected during the study was male (5.92g). This animal was collected during June 1998 and was reproductively mature. The smallest female (6.37g), was collected during October 1998 was mature. The range in animal wet weight between the males and females sampled did not significantly differ (Wilcoxon Test, W+ = 432 W- = 271, N = 37, P = 0.228). The wet weight variation was also examined between years. Some years were significantly different from others (Table. 2.3.6).

Table. 2.3.6 *Odontaster validus*. Comparisons in wet weight (±0.01g), radial length (R ±0.01mm) and body radius (r ±0.01mm) between individual years (1997-2001). The variation in wet weight, radial length and body radius of individual starfish was tested between years. A 2-sample T-test or a non-parametric Mann Whitney test was used depending on if the data were normally distributed (Appendix Table. 2). A significant difference was indicated by a $P < 0.05$. A test for equal variance was also undertaken when a 2-sample T-test was employed and the results included in the assumptions of the test (Appendix Table. 4).

Characteristic	Year	Test Statistic	P-Value	DF
Animal Wet Weight	1997-98	W = 6784.0	P = 0.828	
	1997-99	W = 3940.5	P = 0.030	
	1997-00	W = 4885.0	P < 0.001	
	1998-99	W = 12836.5	P = 0.009	
	1998-00	W = 14968.0	P < 0.001	
	1999-00	W = 6220.0	P = 0.088	
Radial Length	1997-98	T = 3.21	P = 0.002	200
	1997-99	T = 2.58	P = 0.011	118
	1997-00	T = 6.35	P < 0.001	160
	1998-99	T = -0.03	P = 0.975	201
	1998-00	T = 3.22	P = 0.001	272
	1999-00	T = 2.50	P = 0.013	203
Body Radius	1997-98	W = 7384.0	P = 0.079	
	1997-99	W = 4412.5	P = 0.968	
	1997-00	W = 8784.5	P < 0.001	
	1998-99	W = 13267.5	P = 0.125	
	1998-00	W = 21628.5	P < 0.001	
	1999-00	W = 8673.5	P < 0.001	

Radial length (‘R’) ranged from 19.00mm to 47.00mm (mean = 34.43mm, SD = 5.54, n = 407 Fig. 2.3.11) and body radius (‘r’) ranged from 10.00mm to 25.00mm (mean = 16.20mm, SD = 2.65, n = 407 Fig. 2.3.12). The range in radial length and body radius between the males and females sampled did not significantly differ (R = Wilcoxon Test, W+ = 420 W- = 246, N = 36, P = 0.174, r = Wilcoxon Test, W+ = 384.5 W- = 281.5, N = 36, P = 0.423). The variation between both measurements was also recorded between years, and again some years were significantly different from others (Table. 2.3.6).

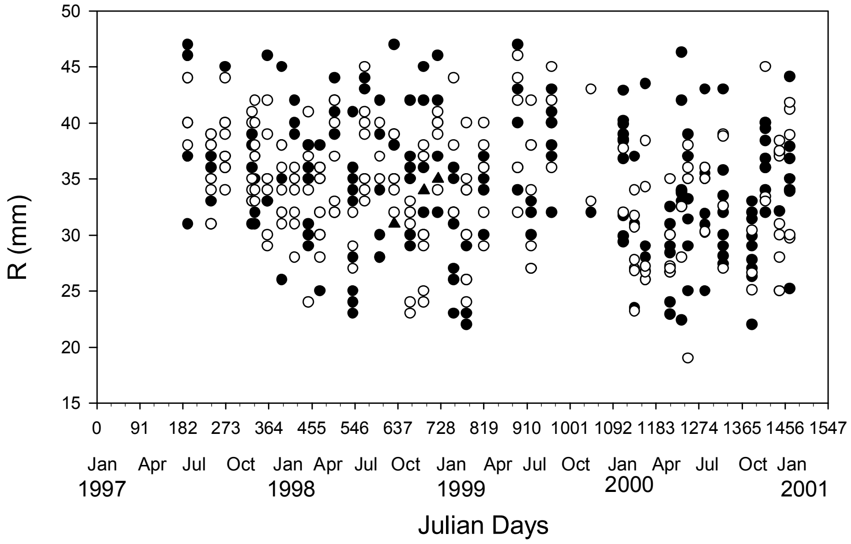

Fig. 2.3.11 *Odontaster validus*. Individual male (●), female (○) and hermaphrodite (▲) radial length values (R ±0.01mm) sampled on a monthly basis between July 1997 and January 2001.

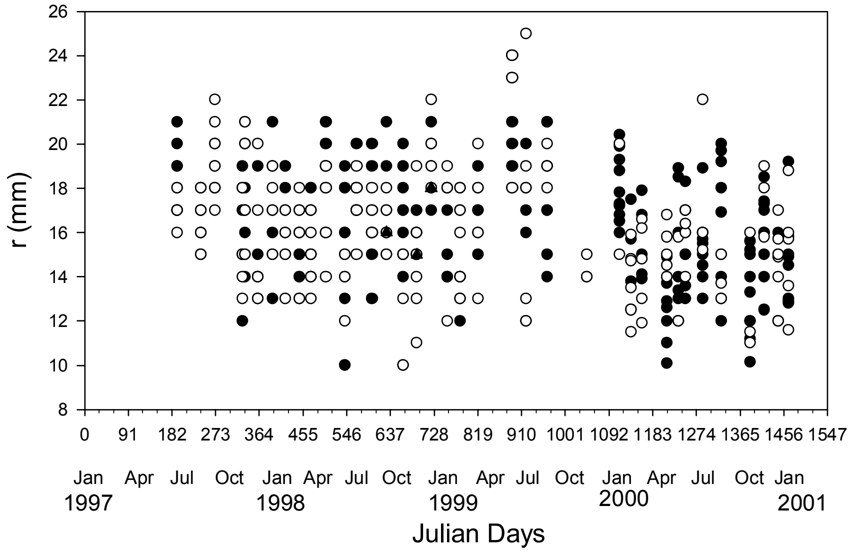

Fig. 2.3.12 *Odontaster validus*. Individual male (●), female (○) and hermaphrodite (▲) body radius values (r ±0.01mm) sampled on a monthly basis between July 1997 and January 2001.

All size variables were significantly correlated (Table. 2.3.7 and Fig. 2.3.13) and there was significant variation in all the size variables over the sampling period (Table. 2.3.8).

Table. 2.3.7 *Odontaster validus*. Correlations between animal wet weight (±0.01g), radial length (R ±0.01mm) and body radius (r ±0.01mm) collected between 1997 and early 2001. All data were ranked and tested using Pearson's Product Moment Correlation. A $P < 0.05$ indicated that variables were significantly correlated.

Comparison	**Test Statistic**	**P-Value**
Animal Wet Weight v R	0.585	$P < 0.001$
Animal Wet Weight v r	0.588	$P < 0.001$
R v r	0.705	$P < 0.001$

Table 2.3.8 *Odontaster validus*. Comparisons in wet weight (±0.01g), radial length (R ±0.01mm) and body radius (r ±0.01mm) over the entire study period (1997-2001). The wet weight, radial length and body radius of individual starfish were compared. A One-way ANOVA or Kruskal-Wallis non-parametric analysis was used depending on if the data were normally distributed and a significant difference was indicated by a $P < 0.05$.

Characteristic	**Test Statistic**	**P-Value**
Animal Wet Weight	$H = 39.68$	$P < 0.001$
R	$F = 5.15$	$P < 0.001$
r	$H = 39.46$	$P < 0.001$

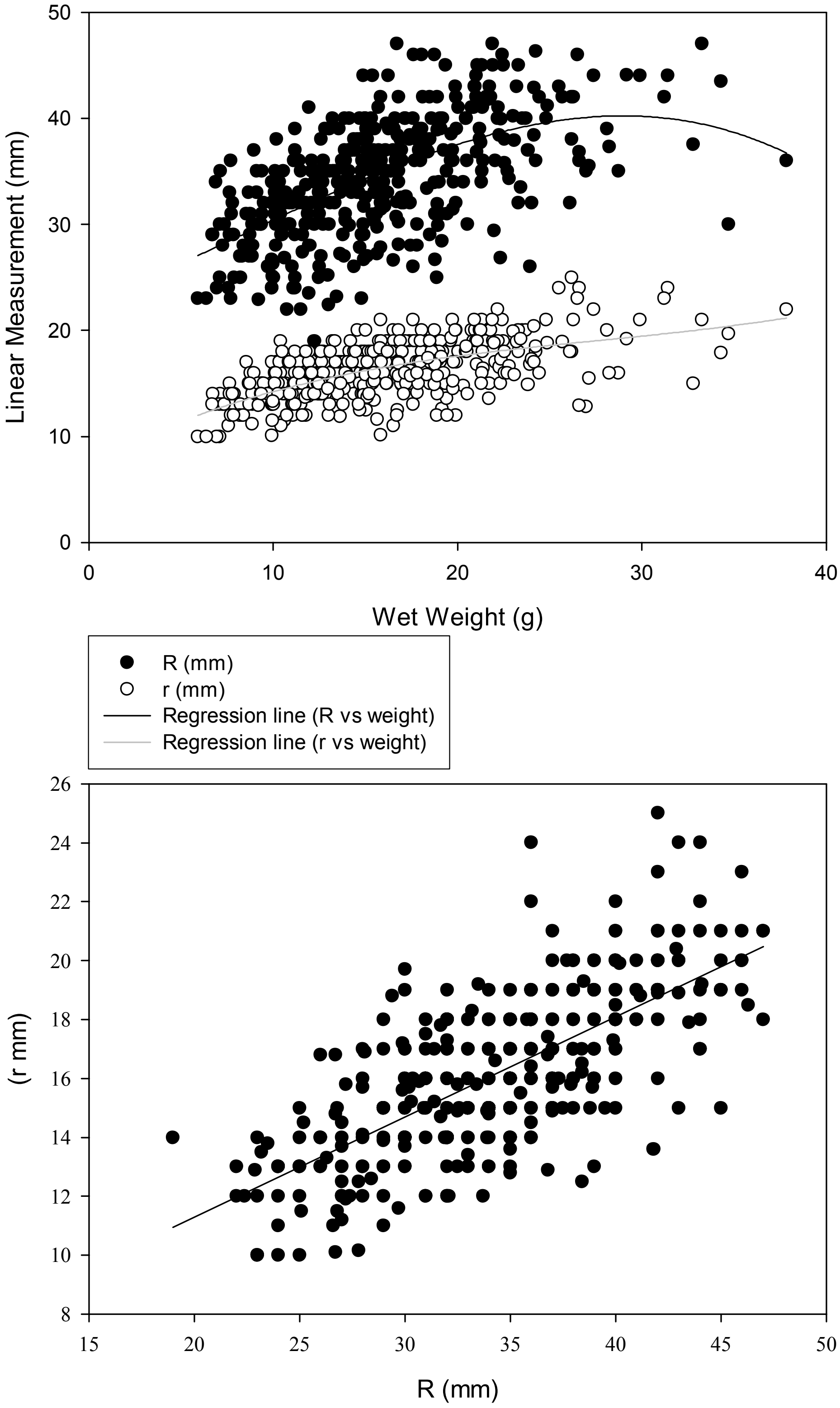

Fig. 2.3.13 *Odontaster validus*. Non-liner (cubic) regression between radial length (●) and body radius (○) (±0.01mm) with wet weight (±0.01g) (R = 0.589 and 0.619 respectively, P < 0.0001) and linear regression between body radius and radial length. (R = 0.711, P < 0.0001).

Reproductive condition

The gonad index did show evidence of inter-annual variation (Fig. 2.3.14). The testis index ranged from extreme values of 0.4 to 19.1 recorded in December 2000 and January 1999 respectively (mean = 6.3, SD = 4.05, n = 222), whereas the ovary index ranged from extreme values of 0.31 to 20.34 obtained for individuals collected in October 1998 and April 2000 respectively (mean = 5.5, SD = 3.28, n = 182).

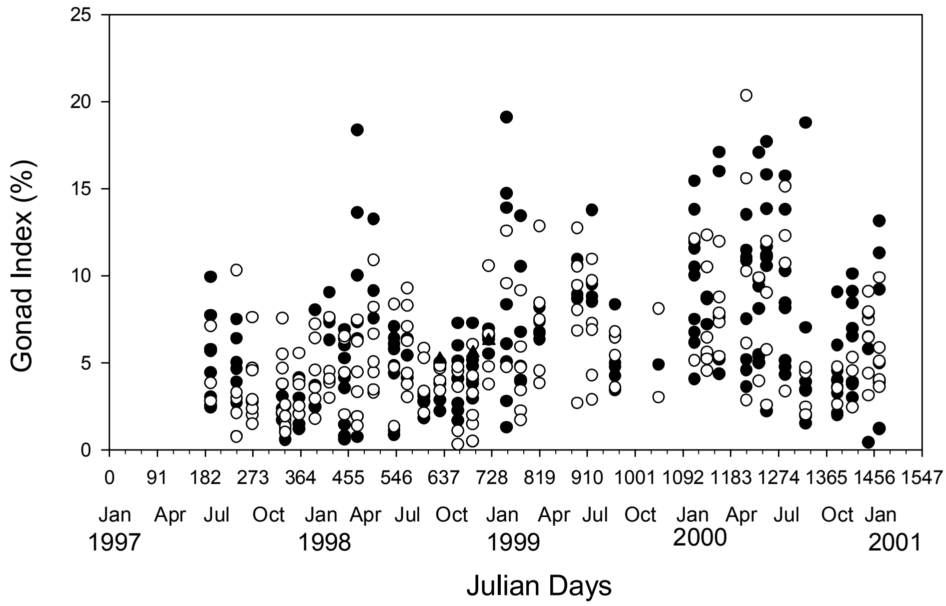

Fig. 2.3.14 *Odontaster validus*. Individual male (●), female (○) and hermaphrodite (▲) gonad index values sampled on a monthly basis between July 1997 and January 2001.

Four spawning events were evident from the data and were consistent in their timing between July and late November each year. However, these events varied in magnitude with the largest percentage decrease in the female gonad during 1997 and 2000 (62% and 61% respectively), and the largest decreases occurring in the testes during 1997 and 1998 (78% and 73% respectively). Both males and females experienced the smallest event during 1999 (52% decrease and 34% decrease respectively) and moderate spawning episodes were recorded during the remaining years (Table. 2.3.9).

Table. 2.3.9 *Odontaster validus*. Reproductive condition is presented as the percentage decrease in the male and female gonad index between years (1997-2001), calculated from the average gonad index before (fecund) and after spawning (spent).

Year	Spawning	Fecund GI Mean	Spent GI Mean	%Decrease
Female				
1997	July-Dec	4.580	1.742	61.96
1998	July-Nov	6.010	2.908	51.62
1999	June-Nov	8.371	5.553	33.66
2000	July-Nov	10.362	4.088	60.55
Male				
1997	July-Dec	5.469	1.196	78.13
1998	July-Nov	9.972	2.731	72.62
1999	June-Nov	10.127	4.878	51.83
2000	July-Nov	11.738	4.276	63.57

Male and female reproductive condition did not differ significantly over the sampling period (Wilcoxon Test, W+ = 462 W- = 241, N = 37, P = 0.097). However, there was a significant difference in overall animal gonad index over the 3.5-year study (Kruskal Wallis H = 75.66, P < 0.001). This was also true for comparisons between the individual years sampled, except when comparing the variation in gonad index between 1999 and 2000 (Mann Whitney W = 6971.5, P = 0.861) (Table. 2.3.10).

Table. 2.3.10 *Odontaster validus*. Comparisons in overall gonad index between individual years. The variation in gonad index of individual starfish was tested between years (1997-2001). A Mann Whitney non-parametric analysis was employed and a P < 0.05 indicated a significant difference in overall gonad index between years.

Year	Test Statistic	P-Value
1997-98	W = 5285.5	P < 0.001
1997-99	W = 2967.0	P < 0.001
1997-00	W = 3997.5	P < 0.001
1998-99	W = 11919.0	P < 0.001
1998-00	W = 15288.5	P < 0.001
1999-00	W = 6971.5	P = 0.861

Energy Investment and Storage

The pyloric caeca index (PC index) did show evidence of strong seasonal variation (Fig. 2.3.15). Male PC index ranged from extreme values 2.6 to 36.7 in December 1997 and August 1998 respectively, whereas female PC index ranged from 3.5 to 32.7 obtained for individuals collected in February 1999 and December 1998 respectively.

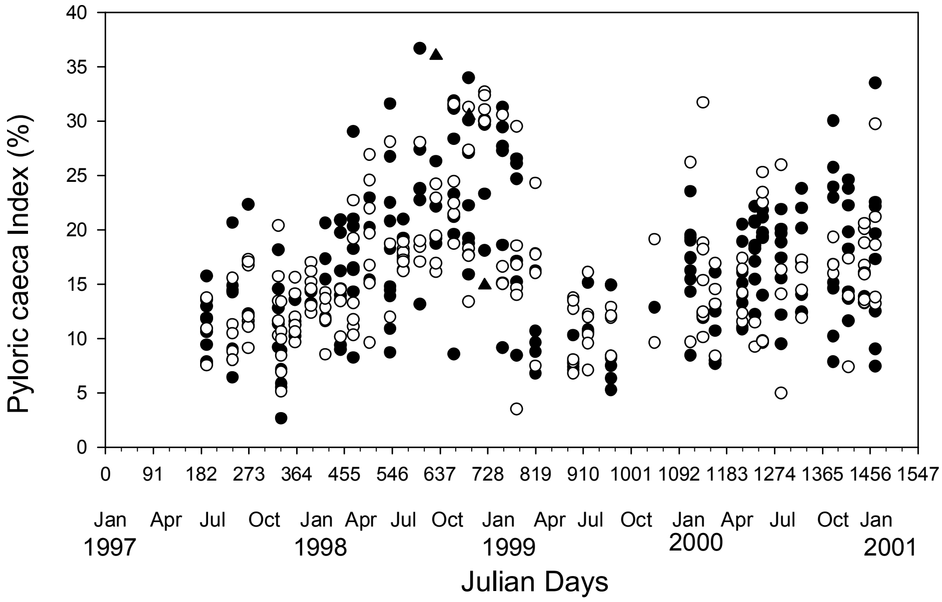

Fig. 2.3.15 *Odontaster validus*. Individual male (●), female (○) and hermaphrodite (▲) pyloric caeca index values sampled on a monthly basis between July 1997 and January 2001.

This index was not as cyclical as the pattern observed in the gonad, although a significant removal of material from the digestive tissues was evident during 1999. This episode followed a period of investment in the pyloric caeca, which spanned nearly two years from the beginning of the study (July 1997) until January 1999. This period preceded an appreciable reduction in the PC index, which continued until the July 1999. The pattern observed in the pyloric caeca was reminiscent of a two-year cycle and contrasted with the annual spawning signature evident in the gonad index. The male and female PC index showed similar losses in investment in 1999 (68% and 64% respectively). Investment during the following year may be building up to a re-occurrence of this event. However, a further year's data would be necessary to confirm this. Nevertheless, based on the current data I would expect the event in 2001 to be of a lesser magnitude owing to the lower values recorded during 1999 compared to 1998.

There was no significant difference between the male and female PC index during the 3.5-year study (Wilcoxon Test, W+ = 396 W- = 307, N = 37, P = 0.507). However, similar to the variability in gonad index, there was a significant difference in the PC index over the entire sampling period (Kruskal Wallis H = 85.36, P <

0.001). This was also true when index variability between the individual sampling years was compared, with the exception of the comparison between 1997 and 1999 (Mann Whitney W = 4055.0, P = 0.099) (Table. 2.3.11).

Table. 2.3.11 *Odontaster validus*. Comparisons in overall pyloric caeca index between individual years. The variation in pyloric caeca index of individual starfish was tested between years (1997-2001). A Mann Whitney non-parametric analysis was employed and a $P < 0.05$ indicated a significant difference in overall pyloric caeca index between years.

Year	Test Statistic	P-Value
1997-98	W = 3486.0	P < 0.001
1997-99	W = 4055.0	P = 0.099
1997-00	W = 4254.0	P < 0.001
1998-99	W = 16064	P < 0.001
1998-00	W = 21121.5	P < 0.001
1999-00	W = 5523.0	P < 0.001

The pyloric caeca and gonad indices observed during the 3.5-year study were correlated (Pearsons Product Correlation, 0.246, P < 0.001).

Oocyte Feret Diameter

There was a significant inter-annual variation in mean oocyte size during the 3.5-year study (One Way ANOVA F = 4.92, P = 0.006). The mean oocyte sizes recorded in 2000 differed most from the mean value for all observations, having generally larger egg sizes. The largest differences in mean oocyte size were recorded between 1997 and 2000, and 1998 and 2000 (Tukey's test for pairwise comparisons). However, this variability was much less marked than the variation in oocyte size recorded for *Ophionotus victoriae* (Grange *et al.*, 2004). The maximum oocyte size of 183µm was observed for one individual in July 2000 and the maximum average oocyte size of 117µm was observed in May 2000.

Four spawning events were identified characterising this animal as an annual reproducer (Fig. 2.3.16). The timing of each spawning event was consistent between years, releasing larger oocytes during the austral winter (July-November).

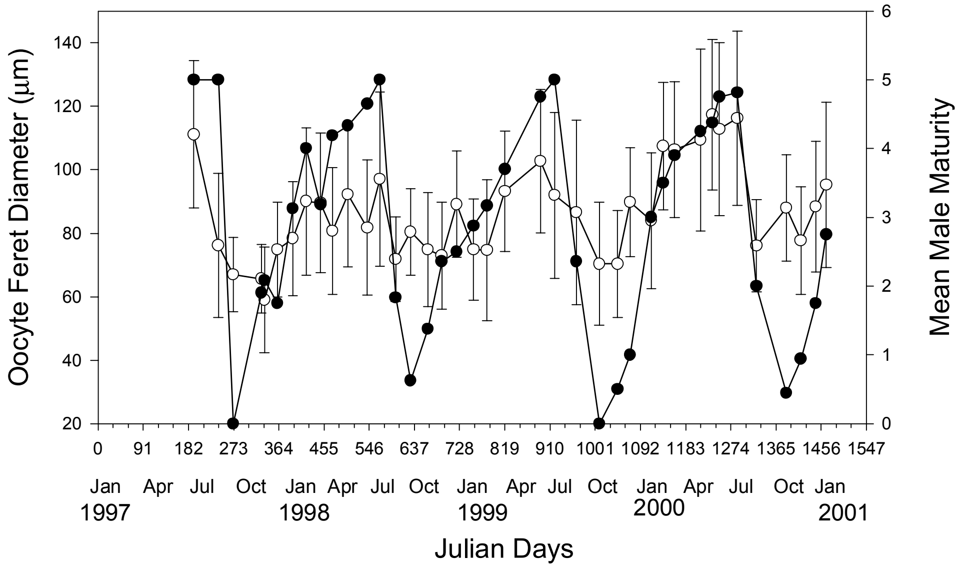

Fig. 2.3.16 *Odontaster validus*. Mean oocyte feret diameter (○) sampled on a monthly basis between July 1997 and January 2001. The overall mean oocyte size is plotted and the error bars represent ± SD. Average male maturity stage is plotted (●) and male maturity stage VI is plotted as zero to convey the testes as being spent.

These patterns in egg size, and the apparent decrease in the proportion of large oocytes in the ovary, coincided with reductions in gonad mass. The first spawning event spanned the months from July 1997 to the beginning of December, where mean oocyte size reduced from 111µm (SD = 23.16, n = 304) to 59µm (SD = 16.66, n = 604). This period coincided with the greatest percentage decrease in the ovary index (62%) of the whole study. The second episode was similar in timing between July 1998 and November 1998, but saw a less marked reduction in oocyte size from 97µm (SD = 27.45, n = 734) to 73µm (SD = 16.77, n = 620), and also a smaller reduction in the gonad index of 52%. A similar trend was observed in the following year with the proportion of larger oocytes (mean = 103µm, SD = 22.62, n = 661) decreasing during the austral winter (mean = 70µm, SD = 16.87, n = 642). The smallest reduction in gonad index occurred during spawning in 1999 (34%). Patterns in egg size in 2000 were reminiscent of 1997. A larger mean oocyte size of 116µm (SD = 27.51, n = 437) decreased to 78µm (SD = 16.91, n = 349) from July to November. A similarly large reduction in gonad index was also recorded (61%).

A significant difference between the proportions of oocyte stages in the ovary was evident over the whole of the sampling period (Mann Whitney, W = 2226.0, P <

0.0001). The proportion of previtellogenic and mature vitellogenic oocytes differed significantly between months sampled. The largest oocytes were unique to fecund females during the austral winter, and although present within the ovary during the majority of the sampling period, composed a lower percentage of the oocyte pool during the late austral spring and austral summer (Fig. 2.3.17). This pattern was repeated annually.

The oocyte size distributions did not clearly show a long-term component in the reproductive cycle of *O. validus* (Fig. 2.3.17). The majority of the oocyte histograms exhibited a single cohort generation in the ovary at any one time and a reproductive tempo of ~12 months. This was illustrated well in September 1997, September 1998 and November 1999 and was generally common throughout the study. This is unusual considering the substantial amount of evidence presented by Pearse (1965) for bimodality from McMurdo Sound and Cape Evans populations of the same seastar suggesting *O.validus* to possess a long-term gametogenic cycle (18-24 months). There was little evidence of bi- or tri-modal oocyte size classes from this study, however the oocyte histograms constructed for August 1999, April 2000 and January 2001 possibly suggest the simultaneous existence of overlapping generations of cohorts in the ovary. All of the individual oocyte size distribution histograms, for every female asteroid collected, are contained in Appendix Figure 2.

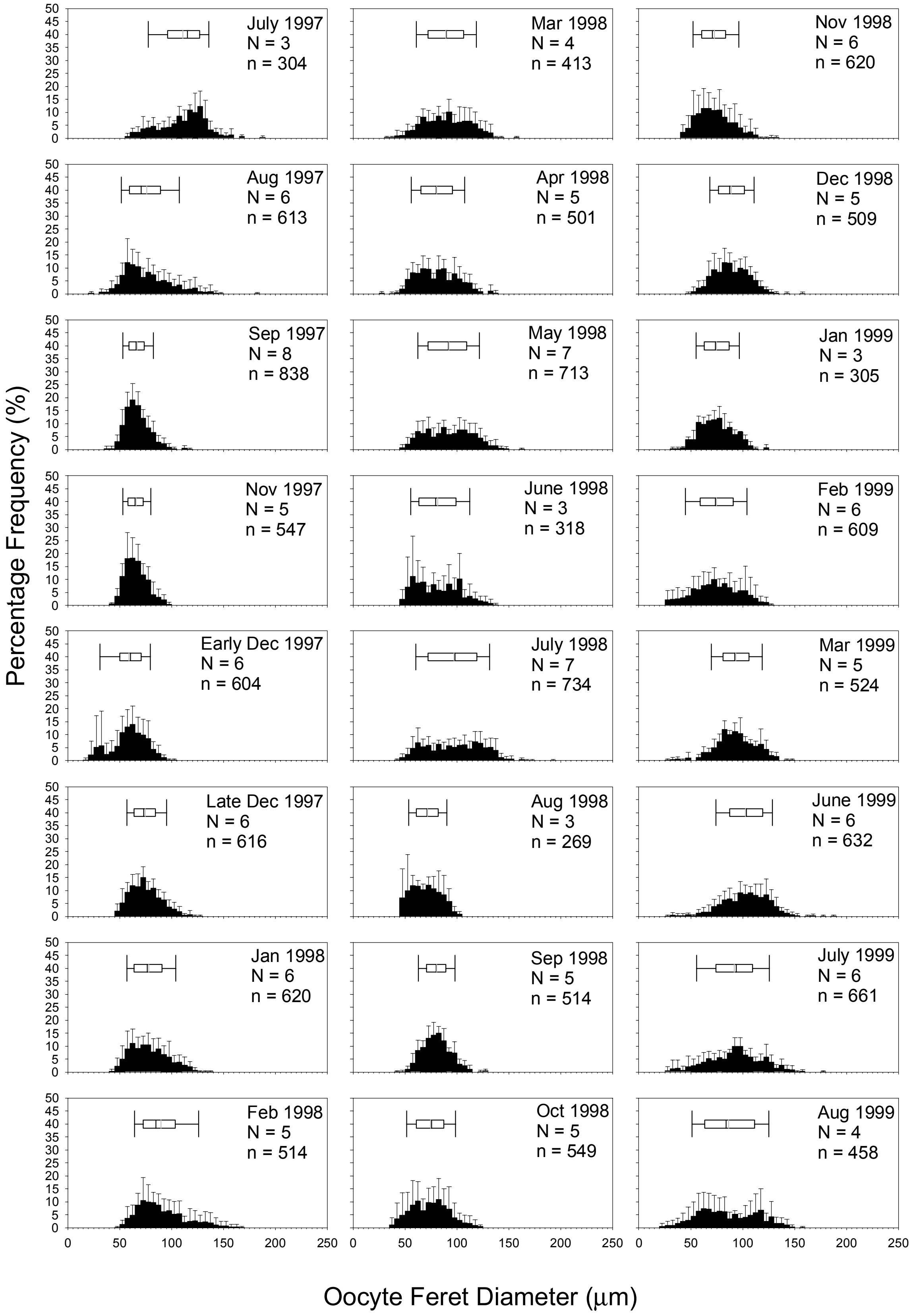

Fig. 2.3.17 *Odontaster validus*. Pooled oocyte feret diameter histograms (± SD), where N = number of females, n = number of eggs counted (1997-2001) and monthly box plots covering the oocyte feret diameter range (the left and right hand limits of the box represent the 25th and 75th percentiles; box

whiskers represent the 10th and 90th percentiles; the median (-) and mean ( ) of each oocyte range is also shown).

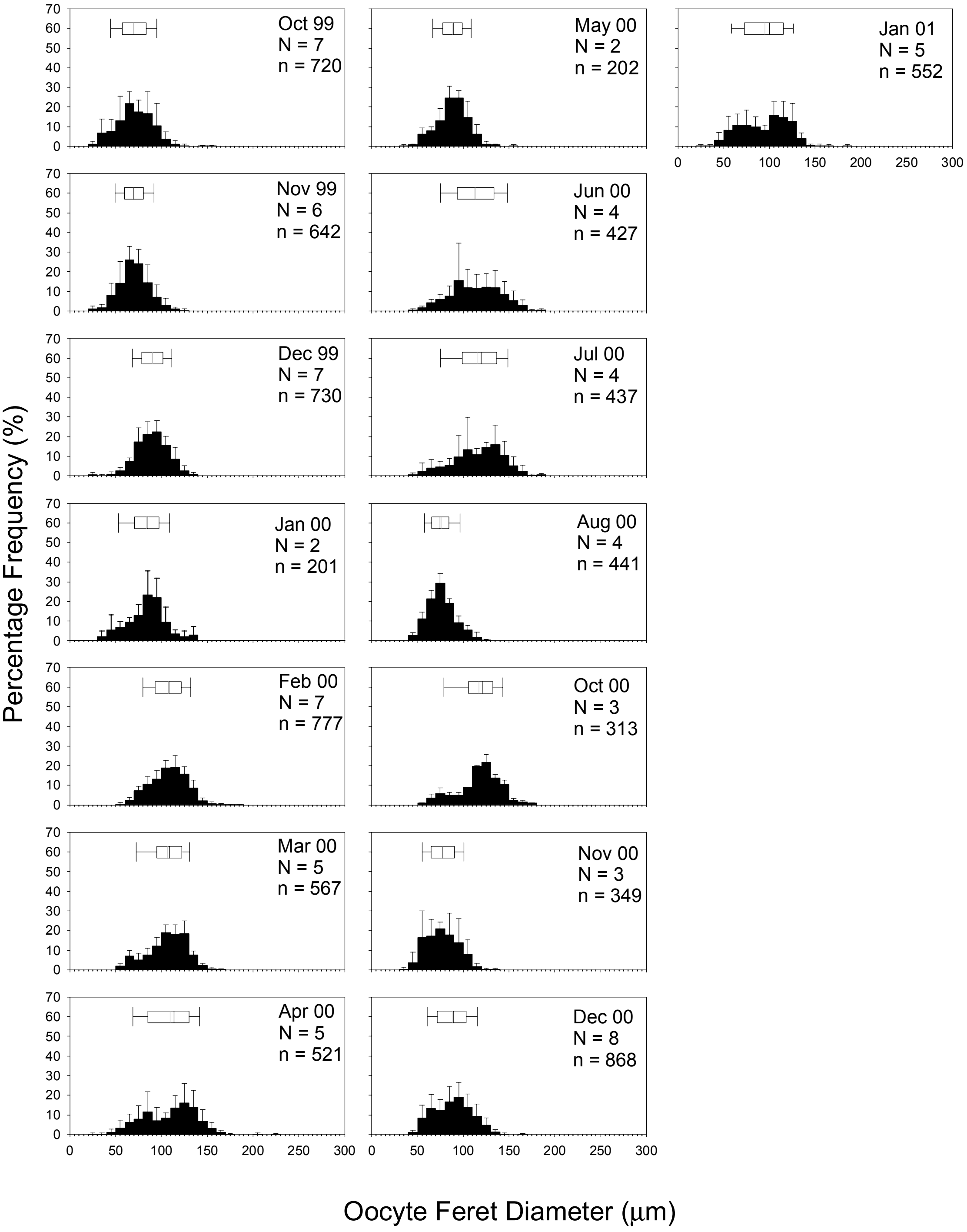

Fig. 2.3.17 contd *Odontaster validus*. Pooled oocyte feret diameter histograms (± SD), where N = number of females, n = number of eggs counted (1997-2001) and monthly box plots covering the oocyte feret diameter range (the left and right hand limits of the box represent the 25th and 75th percentiles; box whiskers represent the 10th and 90th percentiles; the median (-) and mean ( ) of each oocyte range is also shown).

O. validus varied in both oocyte characteristics and reproductive condition each year emphasising the existence of inter-annual variation in the reproductive ecology of this seastar.

Male Maturity

There was a clear seasonal cycle in male spawning, which was consistent over the 3.5-year study and comparable with the timing of female episodes (Fig. 2.3.16). Spawning was synchronous between individual male and female *O. validus*. The male maturity index ranged between being spent and being at an immature spermatogenic stage (I-II) during the austral winter (July-September) in each year. Significant amounts of active sperm (IV-V) were observed in the testes as early as February and were prevalent from May-June.

Parborlasia corrugatus

Sex ratio and Size Characteristics

Over the 3.5 years a total of 467 individual nemerteans were sampled of which 59% (274) were male and 41% (193) were female (Table. 2.3.12) and the sex ratio differed from 1:1 ($\chi2 = 14.051$, $P < 0.01$). This pattern was not consistent for all years, but males were more common in the 1999 and 2000 samples ($\chi2 = 6.434$, $P < 0.05$ and $\chi2 = 11.762$, $P < 0.01$ respectively). Male and female numbers were not significantly different in 1997 and 1998 ($\chi2 = 0.032$, N.S. and $\chi2 = 0.878$, N.S. respectively).

Table. 2.3.12 *Parborlasia corrugatus*. Sex ratio of males and females collected between 1997 and the end of 2000 using a Chi Square statistical test.

Year	Male	Female	Total Individuals
1997 (July-Dec)	32	31	63
1998	75	64	139
1999	75	47	122
2000 (Jan-Nov)	92	51	143

The smallest individual was 150mm retracted length, whereas the largest was 550mm (Fig. 2.3.18) (mean = 309mm, SD = 68.63, n = 467).

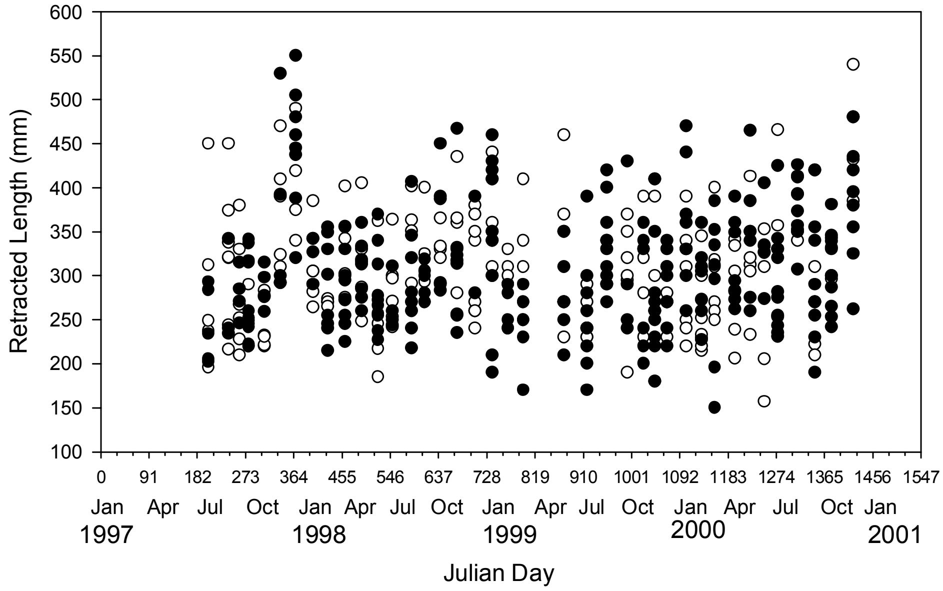

Fig. 2.3.18 *Parborlasia corrugatus.* Individual male (●) and female (○) retracted length values (±0.01mm) sampled on a monthly basis between July 1997 and November 2000. Female size varied from 157 to 540mm retracted length (mean = 310mm, SD = 66.46, n = 193) compared to 150 to 550mm recorded for males (mean = 308mm, SD = 70.22, n = 274).

There was no significant difference in retracted length between males and females (Wilcoxon Test, W+ = 345.5 W- = 434.5, N = 39, P = 0.539). There were however, significant differences in the length of individuals between years (Table. 2.3.13), with the exception of 1997 and 1999 and 1998 and 2000. A Kruskal-Wallis test confirmed a significant difference in the retracted length of individuals over the study period (H = 15.84, P < 0.001). Some individuals had fragmented, and a single male collected in June 2000 provided an example of a posteriorly bifid nemertean. Gibson and Winsor (1980) collected a similar individual during March 1970 from 20-27m depth from a trawl area 63°30'15''W longitude along latitude 64°49'13''S. Gibson and Winsor (1980) concluded that the unusual body form was the result of aberrant regeneration subsequent to the animal receiving a deep injury.

Table. 2.3.13 *Parborlasia corrugatus*. Comparisons in retracted length (±0.01mm) between individual years. The variation in retracted length of individual nemerteans was tested between years (1997-2000). A Mann Whitney non-parametric analysis was employed and a $P < 0.05$ indicated a significant difference in retracted length between years.

Year	Test Statistic	P-Value
1997-1998	W = 5629.0	P = 0.004
1997-1999	W = 5550.5	P = 0.372
1997-2000	W = 5287.0	P = 0.002
1998-1999	W = 19616.5	P = 0.021
1998-2000	W = 19238.0	P = 0.530
1999-2000	W = 14522.5	P = 0.006

The total animal wet weight was also recorded for the nemerteans sampled, with the exception of the months between December 1998 and January 2000 (Fig. 2.3.19). This subsample was analysed by B. Caswell who did not to record animal wet weight. The common occurrence of fragmentation in this species when stressed renders any firm conclusions about weight compromised, and therefore no statistical manipulation was performed on these data. However, some general comments are made. Animal wet weight ranged between 25.45g (March 2000) and 155.25g (December 1997) (mean = 66.74g, SD = 21.36, n = 320). Female wet weight was recorded between 26.77g to 155.25g (mean = 67.28g, SD = 24.90, n = 132), and male wet weight ranged between extreme values of 25.45g and 133.20g (mean = 66.36, SD = 18.53, n = 188).

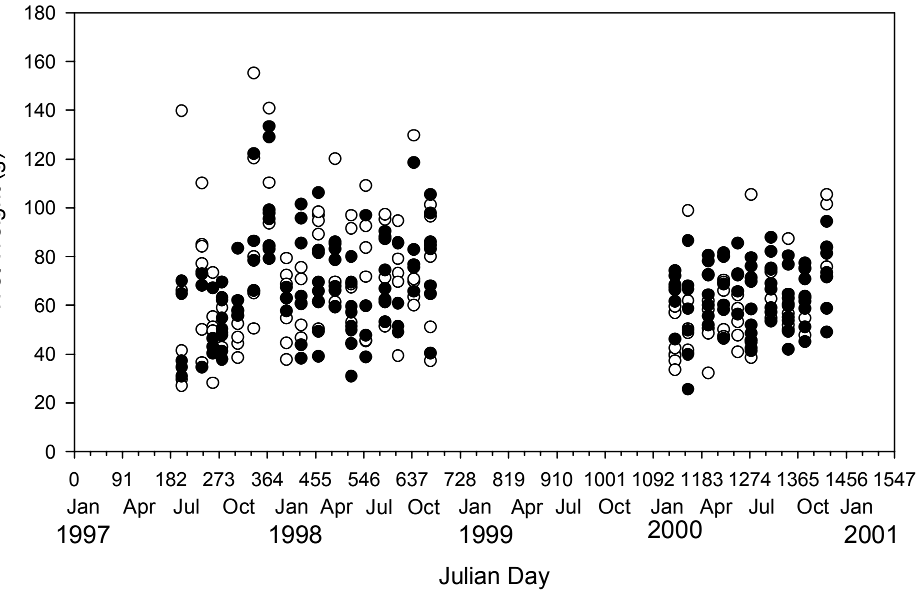

Fig. 2.3.19 *Parborlasia corrugatus*. Individual male (●) and female (○) wet weight values (±0.01g) sampled on a monthly basis between July 1997 and November 2000.

Oocyte Feret Diameter

There was no evidence of any significant inter-annual variation in mean oocyte size during the study period (One Way ANOVA, F = 0.71, P = 0.553). The maximum oocyte size (137μm) was recorded in a female collected in October 1997 and a maximum average oocyte size of 79μm was recorded in October 2000.

The oocyte size distribution data suggested the occurrence of three separate spawning events between July 1997-November 2000 (Fig. 2.3.20).

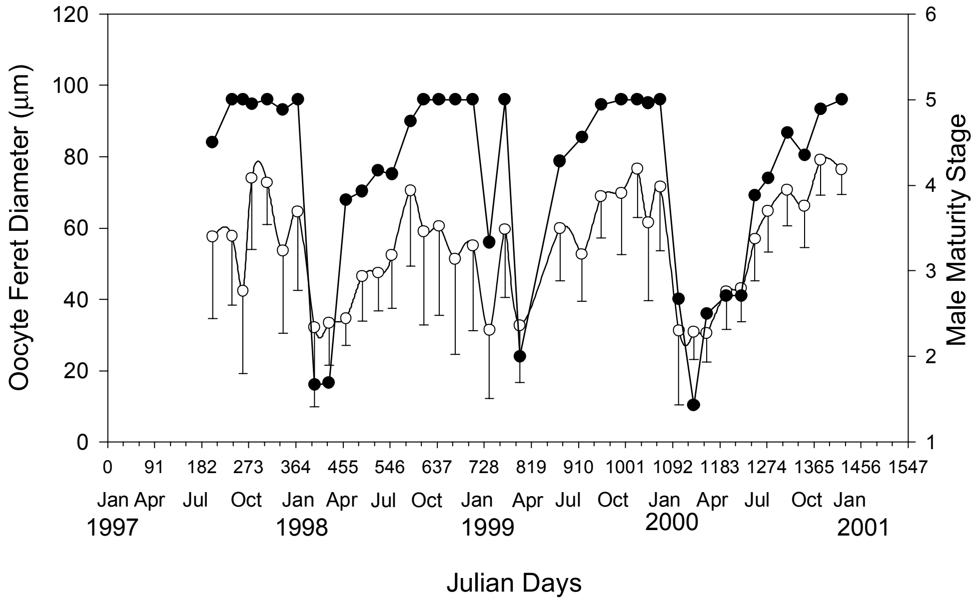

Fig. 2.3.20 *Parborlasia corrugatus*. Mean oocyte feret diameter (○) sampled on a monthly basis between July 1997 and November 2000. The overall mean oocyte size is plotted and the error bars represent ± SD. Only the negative tail of each error bar is plotted so overlap between data points is minimised. Average male maturity stage is plotted (●) and male maturity stage VI is plotted as zero to convey the testes as being spent.

These events were consistent in timing between each sample year occurring in the mid-austral summer and were signified by a reduction in the proportion of large oocytes from the ovaries of gravid females. The first female spawning event possibly spanned the end of 1997 and the beginning of 1998, starting as early as November 1997, and becoming properly established by January 1998. This event was marked by a reduction in oocyte size from an average 73μm in November 1997 to 32μm in February 1998. The spawning period in 1998 was less clear in females than in 1997. This may well be a product of the large variation between females within the months spanning August 1998 to December 1998. However, a pronounced reduction in oocyte size occurred between December 1998 and January 1999 and this probably represents the second spawning event of the study period (55µm and 31µm respectively). The third and final spawning event was more obvious and described a reduction in average oocyte size from 72µm to 31µm between December 1999 and February 2000. Average oocyte size began to increase after this point up until the final month of sampling and presumably pre-empts a fourth spawning event at the beginning of 2001. The consistency of the post-spawning oocyte size is remarkable. *P. corrugatus* therefore exhibits a seasonal spawning pattern, consistent in timing between consecutive years. The proportion of maturing oocytes in the ovary differed significantly over the study period and confirms this seasonality (Mann Whitney, W = 1938.0, P < 0.001). Large vitellogenic oocytes were present throughout each sampling year (Fig. 2.3.21), however they were more common during the late austral winter to the mid-austral summer. The prolonged occurrence of vitellogenic oocytes in the ovary of fecund females was most evident in 1998, however spawning was complete by the following February. This period of the late austral summer also signified an increase in the proportion of small, pre-vitellogenic oocytes (0-50µm).

The oogenic cycle exhibited by *P. corrugatus* took 15-16 months (Fig. 2.3.21). Shedding of large mature oocytes occurred during the mid-summer of each sampling year, and a corresponding increase in the proportion of small oocytes followed. However, there was a short overlap between the initiation of this new generation and the loss of the mature cohort, which is best illustrated by the bi-modal oocyte distributions between September 1997-January 1998, August 1998-December 1998 and September 1999-December 1999. The remaining months exhibited normal oocyte size distributions characteristic of a species with an annual oogenic cycle. All of the individual oocyte size distribution histograms, for every female nemertean collected, are contained in Appendix Figure 3.

Male Maturity

Three separate spawning events were clear in the male gonad and were perhaps more marked than the progressive loss of mature oocytes from the ovary (Fig. 2.3.20). Male individuals were at a mature stage of gametogenic development (IV-V) throughout the majority of the year, only exhibiting exceptions (I-III) during and immediately after each spawning event. The loss of active sperm from the testes was coincident with the reduction of large oocytes from the oocyte size distribution plots (Fig. 2.3.21). Therefore, male and female spawning was synchronous and consistent in timing during the mid-austral summer of each sampling year.

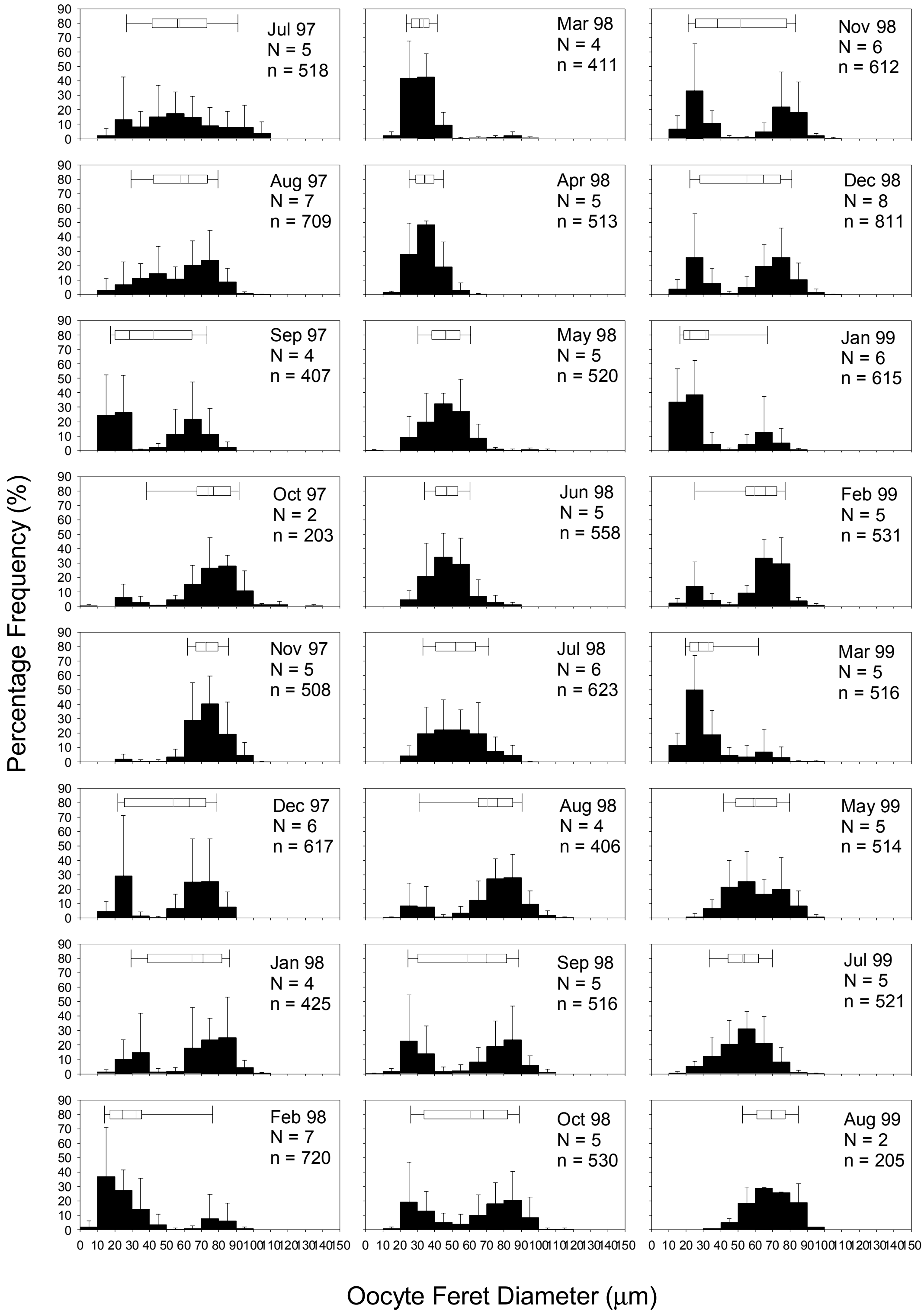

Fig. 2.3.21 *Parborlasia corrugatus*. Pooled oocyte feret diameter histograms (± SD), where N = number of females, n = number of eggs counted (1997-2000) and monthly box plots covering the oocyte feret diameter range (the left and right hand limits of the box represent the 25th and 75th percentiles; box whiskers represent the 10th and 90th percentiles; the median (-) and mean ( ) of each oocyte range is also shown).

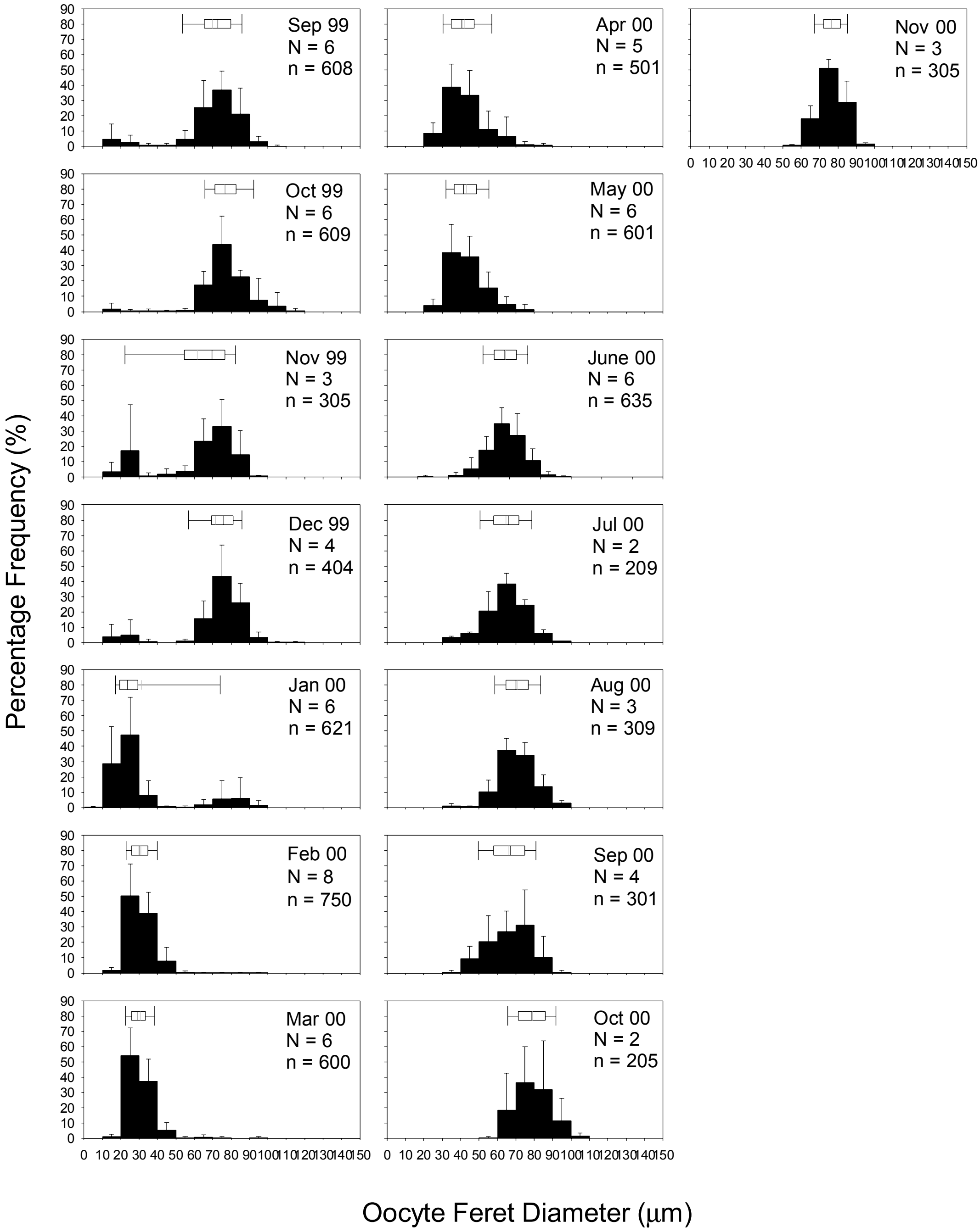

Fig. 2.3.21 contd *Parborlasia corrugatus*. Pooled oocyte feret diameter histograms (± SD), where N = number of females, n = number of eggs counted (1997-2000) and monthly box plots covering the oocyte feret diameter range (the left and right hand limits of the box represent the 25th and 75th percentiles; box whiskers represent the 10th and 90th percentiles; the median (-) and mean (-) of each oocyte range is also shown).

Heterocucumis steineni

Sex ratio and Size Characteristics

429 individuals were sampled, where 54% were male (230) and 46% were female (199) and the sex ratio did not significantly differ from 1:1 ($\chi 2$ = 2.242, N.S). The sex ratio was also 1:1 for each individual sampling year (1997 $\chi 2$ = 0.039, 1998 $\chi 2$ = 0.264 and 2000 $\chi 2$ = 0.016, N.S.), with the exception of 1999 (Table. 2.3.14). Significantly more males were sampled in 1999 ($\chi 2$ = 5.743, $P < 0.05$).

Table. 2.3.14 *Heterocucumis steineni*. Sex ratio of males and females collected between 1997 and early 2001 using a Chi Square statistical test.

Year	Male	Female	Total Individuals
1997 (Sep-Dec)	26	25	51
1998	73	67	140
1999	67	42	109
2000 (Jan 00-Jan01)	64	65	129

The smallest individual (43.2mm) was collected in November 1999 and the largest (196.0mm) was collected in February 2000 (mean length = 89.83mm, SD = 23.40, n = 421 Fig. 2.3.22).

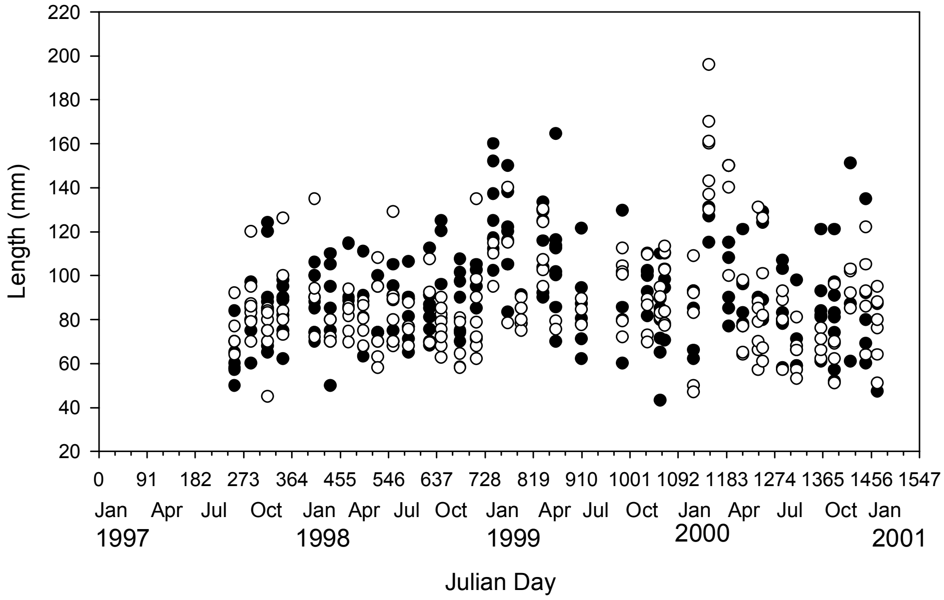

Fig. 2.3.22 *Heterocucumis steineni*. Individual male (●) and female (○) length values (±0.01mm) sampled on a monthly basis between September 1997 and January 2001. Female length ranged between 45.0mm and 196.0mm (mean = 87.6mm, SD = 23.9, n = 194) and male length ranged between 43.2mm and 164.6mm (mean = 91.8, SD = 22.8, n = 227).

Individual length differed between some sampling years (Table. 2.3.15). However, male and female length did not vary significantly over the study period (Wilcoxon Test, W+ = 496 W- = 245, N = 38, P = 0.070).

Table. 2.3.15 *Heterocucumis steineni*. Comparisons in wet weight (±0.01g) and length (±0.01mm) between individual years. The variation in wet weight and length of individual holothurians was tested between years (1997-2001). A Mann Whitney non-parametric analysis was employed and a P < 0.05 indicated a significant difference in length between years.

Characteristic	**Year**	**Test Statistic**	**P-value**
Length	1997-1998	W = 4213.0	P = 0.136
	1997-1999	W = 2728.0	P < 0.0001
	1997-2000	W = 4058.0	P = 0.077
	1998-1999	W = 13060.0	P < 0.0001
	1998-2000	W = 16825.0	P = 0.444
	1999-2000	W = 14825.5	P = 0.001
Wet Weight	1997-1998	W = 3454.0	P = 0.0001
	1997-1999	W = 2608.5	P < 0.0001
	1997-2000	W = 3671.5	P = 0.003
	1998-1999	W = 14438.0	P = 0.004
	1998-2000	W = 17967.0	P = 0.267
	1999-2000	W = 14935.0	P = 0.0003

Animal wet weight also varied significantly over the sampling period (Kruskal Wallis, H = 34.53, P < 0.001). The smallest individual (18.88g) was collected in December 1997 and the largest (171.90g) was collected in February 2000 (mean wet weight= 64.90g, SD = 24.88, n = 421 Fig. 2.3.23). Animal wet weight differed between each sampling year (Table. 2.3.15) and there was a significant difference between male and female wet weight during the study period (Wilcoxon Test, W+ = 568 W- = 173, N = 38, P = 0.004).

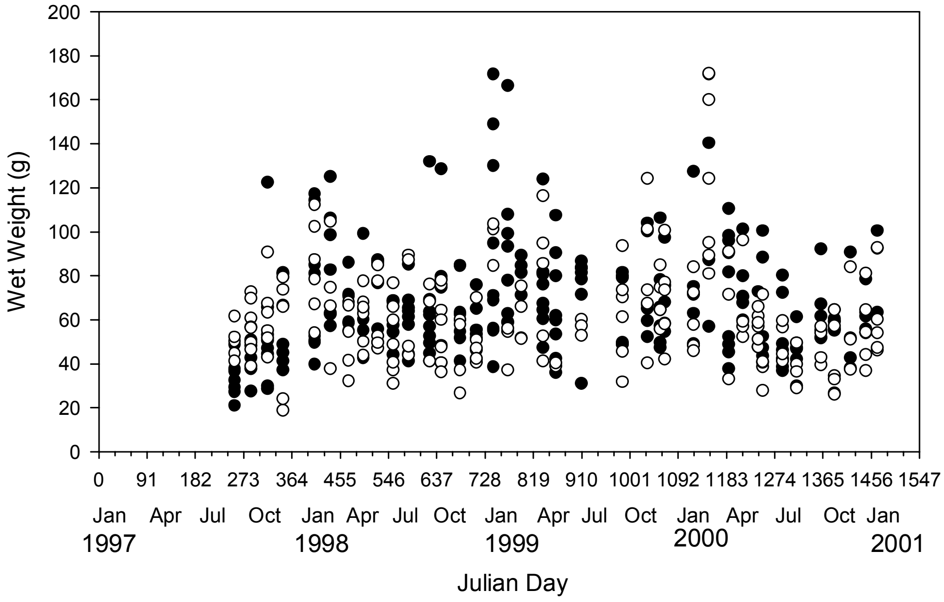

Fig. 2.3.23 *Heterocucumis steineni*. Individual male (●) and female (○) wet weight values (±0.01g) sampled on a monthly basis between July 1997 and January 2001. Female wet weight ranged from 18.9g to 171.9g (mean = 61.9g, SD = 24.2, n = 194) and males between 20.9g and 171.6g (mean = 67.4g, SD = 25.2, n = 227).

Both size measurements were significantly correlated with each other and gonad index (Table. 2.3.16). A Kruskal-Wallis test in both sea cucumber length and wet weight confirmed a significant variation in these variables over time between 1997-2001 (Table. 2.3.17).

Table. 2.3.16 *Heterocucumis steineni*. Correlations between animal wet weight (±0.01g), length (±0.01mm) and gonad index collected between 1997 and early 2001. All data were ranked and tested using Pearson's Product Moment Correlation. A P < 0.05 indicated that variables were significantly correlated.

Comparison	Test Statistic	P-Value
Wet Weight vs Length	0.524	P < 0.001
Wet Weight vs Gonad Index	-0.1.58	P = 0.001
Length vs Gonad Index	-0.141	P = 0.004

Table 2.3.17 *Heterocucumis steineni.* Comparisons in wet weight (±0.01g) and length (R ±0.01mm) over the entire study period (1997-2001). The wet weight and length of individual holothurians were compared. A Kruskal-Wallis non-parametric analysis was used and a significant difference was indicated by a $P < 0.05$.

Characteristic	'H' Test Statistic	P-Value
Wet Weight	34.53	$P < 0.001$
Length	35.32	$P < 0.001$

Reproductive Condition

There was inter-annual variation in gonad index over the study period (Fig. 2.3.24). The lowest overall gonad index (3.8) was recorded in July 2000 and the highest (85.1) was recorded in August 2000 (mean = 25.0, SD = 9.55, n = 421). This variation was much less marked than the patterns observed in the brittle star.

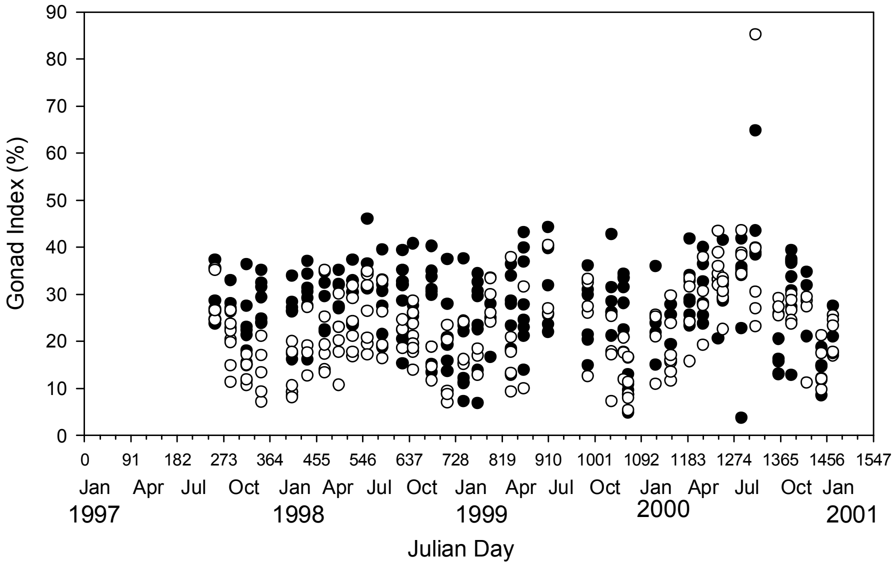

Fig. 2.3.24 *Heterocucumis steineni.* Individual male (●) and female (○) gonad index values sampled on a monthly basis between July 1997 and January 2001.

Four spawning events were evident from the cycles in gonad index and all occurred during the austral winter (July-August). However, the spawning events differed in magnitude, with the largest reproductive investment being recorded in 1999 and 2000 for both males and females (69% and 63% loss by females respectively and 66% and 69% loss by males respectively). Inter-annual variation in reproductive condition was clear from comparisons between individual sampling years and was better illustrated by the percentage loss from the gonad between gravid and spent periods (Table. 2.3.18).

Table. 2.3.18 *Heterocucumis steineni*. Reproductive condition is presented as the percentage decrease in the male and female gonad index between years (1997-2001), calculated from the average gonad index before (fecund) and after spawning (spent).

Year	Spawning	Fecund GI Mean	Spent GI Mean	%Decrease
Female				
1997	Sep-Feb	29.70	13.57	54.30
1998	July-Dec	26.35	15.76	40.20
1999	June-Dec	31.06	9.73	68.68
2000	Aug-Dec	38.72	14.41	62.78
Male				
1997	Sep-Feb	28.66	22.97	19.87
1998	July-Dec	35.17	22.77	35.26
1999	June-Dec	32.42	10.90	66.38
2000	Aug-Dec	45.05	13.76	69.45

Male and female gonad index did vary significantly over the sampling period (Wilcoxon Test, W+ = 615 W- = 126, N = 38, $P < 0.001$). Female gonad index ranged from extreme values of 3.8 to 85.1 (mean = 22.4, SD = 9.59, n = 194), and males ranged from 3.8 to 64.8 (mean = 27.2, SD = 8.96, n = 227). The variation in individual gonad index was significant over the study period (One-Way ANOVA, F = 5.25, P = 0.001), and some of the individual years were also significantly different from each other (Table. 2.3.19). The largest difference in gonad index was recorded between 1997 and 2000, and 1999 and 2000 using Tukey's test for pairwise comparisons.

Table. 2.3.19 *Heterocucumis steineni*. Comparisons in overall gonad index between individual years. The variation in gonad index of individual holothurians was tested between years (1997-2001). A 2-sample T-test was employed and a $P < 0.05$ indicated a significant difference in overall gonad index between years. A test for equal variance was also undertaken and the results included in the assumptions of the test (Appendix Table. 5). The 2-sample T-test was repeated (1997-1999, 1997-2000 and 1998-2000) assuming equal and unequal variance (*) when the test for equal variance returned P values both greater than and less than $P = 0.05$.

Year	Test Statistic	P-Value	DF
1997-1998	T = -1.05	P = 0.296	181
1997-1999*	T = -0.11	P = 0.914	120
1997-1999	T = -0.10	P = 0.920	188
1997-2000*	T = -3.05	P = 0.003	126
1997-2000	T = -2.65	P = 0.009	178
1998-1999	T = 1.08	P = 0.279	239
1998-2000*	T = -2.48	P = 0.014	241
1998-2000	T = -2.49	P = 0.013	259
1999-2000	T = -3.16	P = 0.002	236

Oocyte Feret Diameter

There was no significant inter-annual variation in the average oocyte feret diameter over the sampling period (One-Way ANOVA, F = 2.79, P = 0.055). The maximum oocyte size for an individual was 568µm and was recorded in November 1999. Female data were pooled and the average oocyte size recorded for each month studied. The average oocyte size was greatest during July 1999 (365µm).

A proportion of these data were originally analysed as part of a separate undergraduate and masters dissertation project (Appendix Table. 1). The reproductive data generated for collections between April 1998 and December 1999 were flawed, owing to poor sample processing and an inadequate interpretation of these data. It was not possible to repeat the reproductive analyses on the original gonad tissue, because the original samples had not been labelled or separately bagged and therefore, individual tissues were not identifiable. Consequently, I had to re-section the original tissue and produce new slides, which I later calibrated and photographed. Previous sectioning meant that a limited number of sections could be cut from the original wax blocks and a correspondingly low number of eggs were captured (< 40 oocytes). Therefore, only a small number of females, or on occasions a single female, per sampling month could be re-analysed and the oocyte data used. Therefore, firm conclusions concerning the April 1998-December 1999 reproductive data were compromised. However, broad patterns could be inferred, bearing the sampling limitations in mind.

Two spawning events were discernable from the oocyte size data and each occurred in the austral winter (June-July) (Fig. 2.3.25). These events were each marked by a reduction in oocyte size and the shedding of large mature oocytes from fecund females.

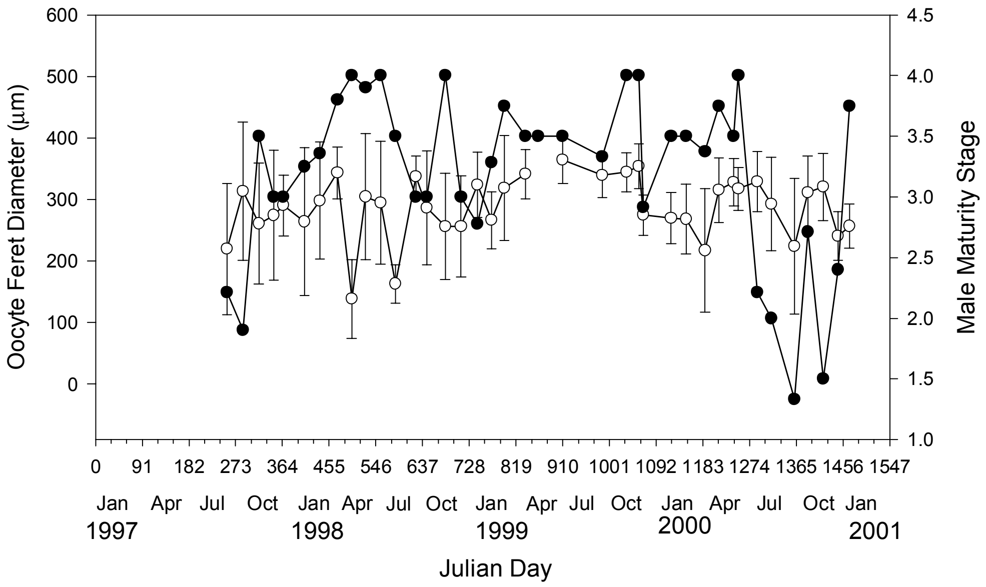

Fig. 2.3.25 *Heterocucumis steineni.* Mean oocyte feret diameter (○) sampled on a monthly basis between July 1997 and January 2001. The overall mean oocyte size is plotted and the error bars represent ± SD. Average male maturity stage is plotted (●) and male maturity stage V is plotted as zero to convey the testes as being spent.

The spawning period in 1997 could not be obtained from the oocyte data, because sampling only started in September 1997 and spawning probably occurred before sampling started for this study. The oocyte cycle in 1998 was not clear, however there is some suggestion that spawning occurred during June-August, when the average oocyte size fell from 305µm to 163µm. The apparent loss of a significant proportion of large oocytes between April and May 1998 was not attributed to spawning. The May 1998 histogram represents only a single female's oocyte size distribution and the small sample size probably accounted for this anomaly. A clear spawning episode was not apparent from the oocyte data in 1999 and poor sample preparation and small sample sizes probably prevented the spawning cycle from being identified. The 2000 spawning event was more apparent, and the average oocyte size decreased from 329µm in July 2000 to 224µm in September.

The proportion of previtellogenic and vitellogenic oocytes differed significantly between months sampled (Mann Whitney, $W = 2268.0$, $P < 0.0001$). The largest proportion of mature oocytes was present in the ovary during June-July (Fig. 2.3.26). However, this pattern was only clearly recorded in 1997 and 1999. Patterns in 1997 and possibly 2000 suggested the removal of large vitellogenic oocytes from the ovary during the austral winter. However, the occurrence of a young cohort generation was apparent 2-3 months prior to the spawning period and persisted during the shedding of mature gametes. Therefore, there was a degree of overlap between the shedding of the mature generation and the introduction of new cohort to the oocyte pool.

A long-term component in the gametogenic cycle of *Heterocucumis steineni* was not clear from the oocyte size distribution data (Fig. 2.3.26). The majority of the oocyte histograms exhibited uni-modal oocyte size classes and a single cohort of oocytes in the ovary at any one time, e.g. April, 1998, June 1999, January 2000 and May 2000. However, a bimodal oocyte distribution was suggested in June 1998, July 1998, March 2000 and August 2000 and described the simultaneous co-existence of two cohorts in the ovary. The distributions of the oocyte data in other months were not so clear and many were constructed from only one or two females possibly accounting for this anomaly, e.g. August 1998, September 1998 and December 1999. Many of the oocyte distributions were also broad encompassing a wide range of oocyte size classes, where the frequencies recorded for each class were similar. Therefore, any clear distribution patterns were difficult to discern especially in 1998-1999. All of the individual oocyte size distribution histograms, for every female holothurian collected, are contained in Appendix Figure 4.

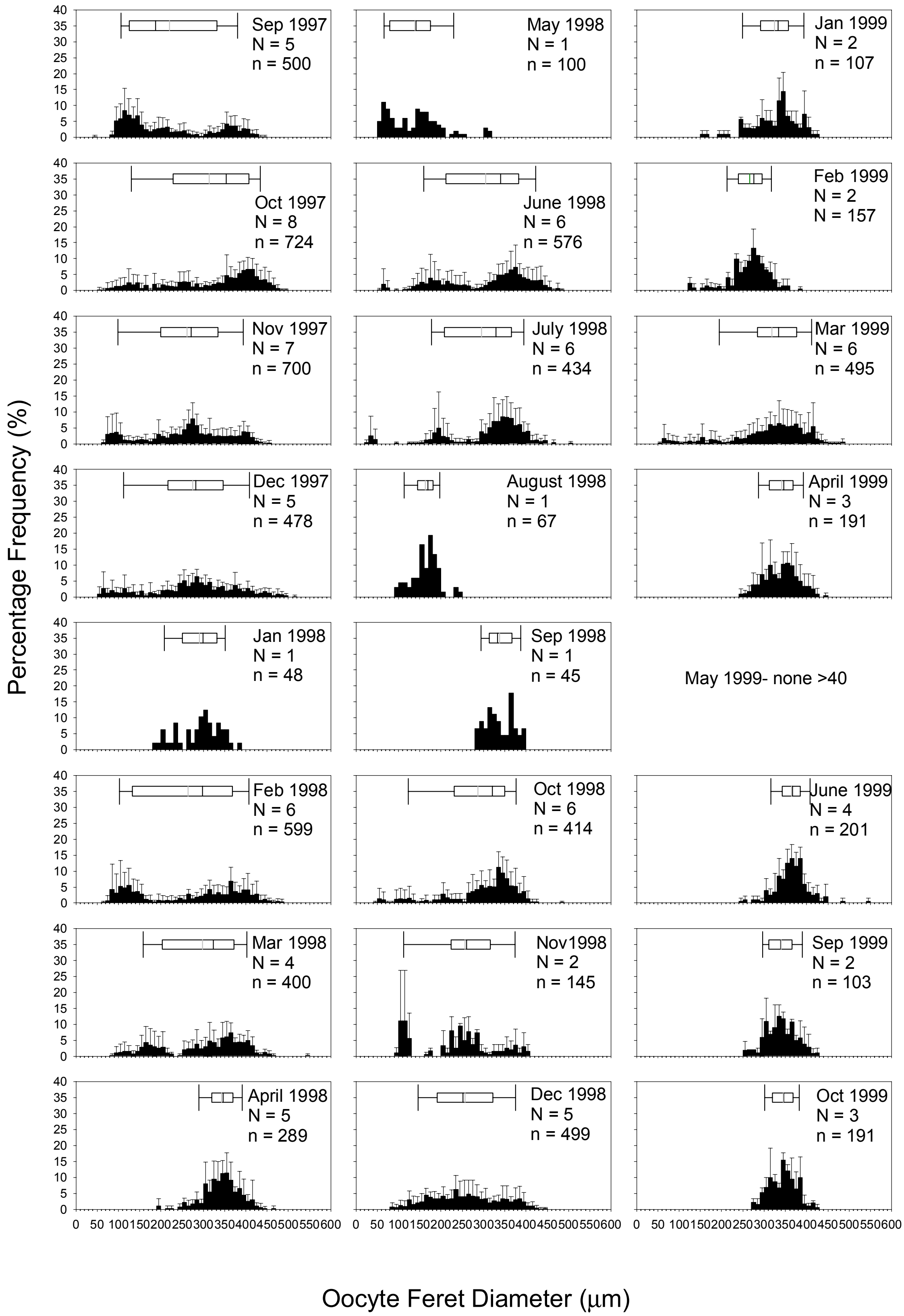

Fig. 2.3.26 *Heterocucumis steineni.* Pooled oocyte feret diameter histograms (± SD), where N = number of females, n = number of eggs counted (1997-2001) and monthly box plots covering the oocyte feret diameter range (the left and right hand limits of the box represent the 25th and 75th percentiles; box whiskers represent the 10th and 90th percentiles; the median (-) and mean (-) of each oocyte range is also shown).

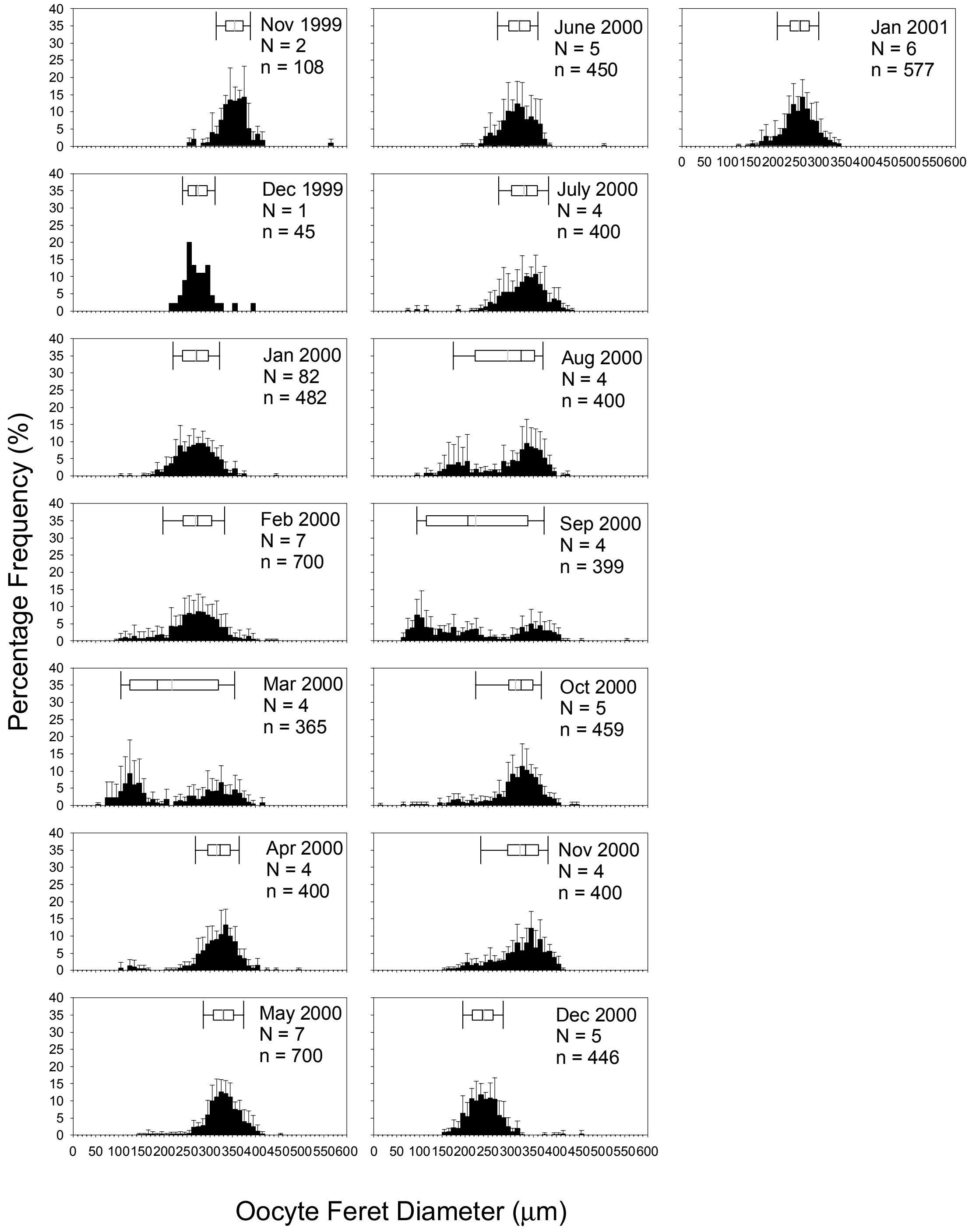

Fig. 2.3.26 contd *Heterocucumis steineni*. Pooled oocyte feret diameter histograms (± SD), where N = number of females, n = number of eggs counted (1997-2001) and monthly box plots covering the oocyte feret diameter range (the left and right hand limits of the box represent the 25th and 75th percentiles; box whiskers represent the 10th and 90th percentiles; the median (-) and mean (-) of each oocyte range is also shown).

Male Maturity

Three separate spawning events were also evident in the male maturity data (Fig 2.2.25). Mature males (IV) possessed active sperm during the austral winter. A notable decrease in male maturity occurred in the austral winter of 1998 and 2000. Immature males were sampled during the late austral spring-mid austral summer in 1997 and 2000 after spawning. The apparent lack of immature males in 1999 was most probably a facet of the small sample number and poor sample preservation mentioned in the earlier section. This may also explain the variable signal observed in the male maturity index during most of 1999. Male and female spawning was synchronous and consistent in timing each year.

Experimental Constraints

- These data were generated by both this thesis and the work of previous undergraduate and masters dissertations. Therefore, each data set was re-calibrated and re-measured to ensure consistency between data analyses and each study. Unfortunately, the methods employed by some previous workers were not of a high enough standard and this affected the reliability of some of the data. This was especially apparent for the 1998-1999 data for *Heterocucumis steineni*. In this species the oocyte data covering this period was seriously flawed and any means of re-calibrating the data set impossible. New sections had to be prepared from the original wax blocks, which had already been sectioned in a previous study. New blocks could not be made using the original gonad tissue, owing to its poor preservation and lack of labelling. Therefore, new sections often contained a limited number of eggs per female (< 40 eggs). Females with < 40 eggs were not included in the monthly oocyte distributions and this meant some months were only represented by one or two females.
- Monthly field collections of each species were not always possible. This resulted in some months not being sampled and gaps in each data set. This was due to logistical difficulties hindering collections, e.g. poor weather during the austral winter. All species samples collected during 2001 and early 2002 were lost on 29th September 2001 during a fire at the Bonner Laboratory facility on Rothera Research Station, which prevented a longer time series

from being analysed. The fire completely destroyed the laboratory and the station’s scuba diving facility, preventing sampling for a number of months and destroying all stored samples in the laboratory. However, sampling recommenced in early 2002.

2.4 Discussion

Ophionotus victoriae

Sex Ratio and Disc Diameter

Ophionotus victoriae is dioecious, with a sex ratio that varied between samples from different years with a slight bias toward males. In dioecious ophiuroids the sex ratio is usually close to unity (Cuenot, 1988; Hendler, 1991). Deviation from unity may occur as a result of differential recruitment, growth, mortality or sampling bias (Vevers, 1953; Skjaeveland, 1973). Size selective bias for larger individuals by divers cannot explain the marginal male prevalence in *O. victoriae,* as there was no significant difference in the size of the male and female ophiuroids. Therefore, an ecological explanation may be more reliable, e.g. individual aggregations or recruitment/mortality factors. More general reproductive reviews suggest that most brittle stars are dioecious, although many hermaphroditic specimens have been observed, and these invariably brood their young (Byrne, 1994). Byrne (1991) described broodcare in a Florida population of the Caribbean ophiuroid *Ophionereis olivacea*, which is incidentally also a protandric hermaphrodite. This is a common feature amongst some Caribbean ophiuroids (Hendler, 1979), although the majority of viviparous ophiuroids that have been described are coldwater or deepwater forms (Mortensen, 1936).

The smallest individual *Ophionotus victoriae* examined was 11.2mm disc diameter, and this individual was already developing gonads, suggesting early maturity in this species. The congeneric *O. hexactis* is similar, becoming sexually mature at 7 to 10mm, and capable of brooding at 12mm disc diameter (Morison, 1979). The upper size range is similar in both species reaching a maximum diameter of 39mm (pers obs and Morison, 1979). Dahm and Brey (1998) used an iterative approach to correct size-at-age data for specimens of *O. victoriae*, where the largest individual examined was 22 years old with a disc diameter of 32.5mm. The smallest individual recorded in the present study would have been approximately 8-9 years old according to this method. Fell (1966) discussed the age at which ophiuroids reach their maximum size and maturity, and suggested the average maximum age of any ophiuroid to be around

15 years. However, we now recognise polar species live significantly longer than this.

Reproductive Condition

All individuals had developing gonads. The gonad index increased throughout the austral winter and spring, peaking during October and November before decreasing in December. A number of reproductive studies have identified discrete spawning cycles in ophiuroids (Hendler, 1991). However, prolonged breeding seasons and continuous reproduction are common in tropical brittle stars (Mladenov, 1983). The cyclic pattern recorded in the current study displayed considerable inter-annual variation between consecutive years. During 1997 and 1998 the gonad index was low whilst in the following years (1999 and 2000) the index was significantly higher. Similarly, the actual fecundity recorded for *O. victoriae*, which although variable, remained relatively high, ranging from ~10^4 eggs in 1997 to ~2.0×10^5 eggs in 1999. Pearse (1994) described a similar fecundity in this brittle star ~ 1.4×10^5 from McMurdo Sound. A direct relationship between reproductive condition and planktonic chlorophyll production could not be established. However, a connection between the sedimentation events at Rothera and ophiuroid reproductive characteristics was suggested. The magnitude and duration of each annual sedimentation event may play an important role in the following year's reproductive success. Patterns of ice cover and thickness probably modulate sedimentation, which then underpin reproductive condition in the following year.

Oocyte Frequencies

This study is the first to examine the temporal variation in oocyte size frequencies of *Ophionotus victoriae*. Pearse (1994) reported a maximum oocyte size of 180µm for this brittle star, which compares with the average maximum oocyte size frequency of 170-190µm in the current study. This relatively small oocyte size, coupled with the high fecundity of this species, indicates planktotrophy. Most ophiuroid larvae proceed through a feeding ophiopluteus stage, characteristic of planktotrophic species (Byrne, 1994). This is in contrast to the old brooding paradigm referred to by Mileikovsky (1971) as Thorson's rule. However, Fell (1966) recognised an increase in the prevalence of viviparity in the southern oceans (Antarctic, South America and New Zealand) and most deep sea species appear to reproduce directly, where indirect development appears to be rare except for within a few truly deep-sea species, e.g. *Ophiura ljungmani* (Tyler, 1980), *Ophiocten gracilis* (Sumida *et al*., 2000) and *Ophiocten hastatum* (Gage *et al*., 2004). However, although most Antarctic larvae are pelagic lecithotrophs, some of the most abundant polar invertebrates produce planktotrophic larvae, and this trait is exhibited in 20-25% of polar echinoderms, many of which appear numerically abundant in shallow water sites, including the asteroids *Odontaster validus*, *O. meridionalis* and *Porania antarctica* (Pearse and Bosch, 1986; Bosch, 1988, 1989) and the echinoid *Sterechinus neumayeri* (Bosch *et al*., 1987).

The genus *Ophionotus* comprises two species, *Ophionotus victoriae* and *Ophionotus hexactis*. The ecology of these species differs significantly. *O. hexactis* is a hexamerous ophiuroid, similar in size to its sibling species and with an overlapping distribution around South Georgia (Mortensen, 1936; Fell, 1966). However, a large dichotomy is exhibited between the reproductive habits of these two species. *O. hexactis* is a subantarctic brittle star with intraovarian broodcare. Its eggs develop to sizes around 200µm. Morison (1979) recorded variable numbers of embryos (between 16 and 24) in the ovary at any one time and the juveniles attain disc diameters of 8mm, developing arms with a series of segments before birth (Turner and Dearborn, 1979). Eckelbarger (1994) commented upon the enigma of discovering two sympatric species with different reproductive strategies under the same environmental conditions. He suggested that this intraspecific difference could not be habitat driven, but may be caused by differences in the vitellogenic mechanisms and the methods by which nutrients are distributed during oogenesis.

The largest oocytes observed in the current study were around 250µm. These egg sizes are somewhat larger than those described by Hendler (1975) as being typical of a planktotrophic development pattern in tropical and temperate ophiuroids (70-90 µm). Egg sizes in several groups do get larger towards the poles (Clarke, 1979, 1992). This cline in egg size may partially account for the more extreme oocyte sizes observed. Alternatively, this may suggest a tendency towards a more adaptive development regime in *Ophionotus victoriae*. Larger egg sizes have been associated with a move to facultative planktotrophy associated with selection for larger juveniles, a shorter time for metamorphosis, a higher efficiency of fertilisation and insurance against fluctuations in phytoplankton abundance (Wray, 1995). The incidence of facultative planktotrophy has been reported as present among some Antarctic ophiuroids (Hendler, 1975) and may explain the extreme oocyte sizes recorded. Intermediate egg sizes and an abbreviated developmental phase intermediate in character between a direct and indirect development strategy have also been previously described in some deep-sea ophiuroids (Tyler, 1980).

Average oocyte size also varied inter-annually. Egg sizes in echinoderms vary not only between populations, location and time, but also within the single spawn of a single female and between females (Emlet *et al.*, 1987). Seemingly, scientists working on January examples of *O. victoriae* in Terra Nova Bay, Ross Sea recorded egg sizes twice as large as those recorded in this study (M Chiantore, pers. comm.). Lawrence (1991) associated such variability with a change in nutritional status and or/condition of the female and may be an adaptive response to unpredictable environments (Travis, 1984; Kaplan and Cooper, 1984; Sibly *et al.*, 1987). Scientific consensus is that ‘good’ years, in terms of resource availability, favour the production of a large number of small eggs, whereas a ‘poor’ year tends to see a few large eggs being produced (reviewed by George *et al.*, 1991). Planktotrophic species are more affected by changes in food ration, especially when resources are scarce (McEdward and Miner 2003). Therefore, fecundity-time models tell us that when food is scarce fitness is low for small-egg strategies and increases with increasing egg size, whereas at higher food levels there is very high reproductive success when smaller egg sizes are favoured. *Ophionotus victoriae* appears to have a flexible strategy for egg size.

The patterns exhibited in Figure 2.3.1 and Figure 2.3.7 suggest that the resource input that arrives at the sea-bed by sedimentation and precedes the following reproductive event at Rothera drives the reproductive characteristics in this brittle star. Temperature was the only other environmental variable to correlate significantly with oocyte size. Benoît and Pepin (1999) attributed this apparent effect to the way in which temperature interacts with maternal contributions to egg size, which are themselves partly ration dependent.

Gametogenic Cycle

There were two distinct cohorts of oocytes maturing in the ovary at any one time throughout the study period. This pattern appears in a number of Antarctic seasonal breeders that have a period of gametogenic development between 18 and 24 months (Pearse, 1965; Powell *et al*., 2001, Brockington *et al*., submitted). This pattern has been observed in *Odontaster validus* from McMurdo Sound populations (Pearse, 1965) and has also been recorded in *Sterechinus neumayeri* (Brockington, 2001; Pearse and Cameron, 1991), in the infaunal bivalve *Laternula elliptica* and in the limpet *Nacella concinna* (Powell, 2001), as well as in deep sea echinoids (Tyler and Gage, 1984b). Other Antarctic taxa with long gametogenic development times include the brachiopod *Liothyrella uva* (Meidlinger *et al*., 1998), the octocoral *Ainigmaptilon antarcticum* (18-24 months) (Orejas *et al*., 2002), the amphipod *Bonallia gigantea* (> 12 months) and the bivalve *Kidderia subquadratum* (15-19 months) (reviewed by Clarke, 1988), although the scallop *Adamussium colbecki* and the ascidian *Cnemidocarpa verrucosa* exhibit a discrete 12-month cycle (Tyler *et al*., 2003; Sahade *et al*., 2004). The prolonged development cycles characteristic of many Antarctic taxa allow the storage of energy over two consecutive summers, interspersed with a single winter period of limited food. This adaptation may provide invertebrates with a way of overcoming the limited resource and restricted energy acquisition period of the winter.

These data suggest oocyte development is progressive, developing slowly at first and increasing rapidly prior to spawning when chlorophyll levels in the water column are increasing and much of the sedimented organics have been assimilated.

Reproductive and Spawning Cycles

There was no significant difference between the seasonal pattern of the gonad index of the males and females studied indicating synchrony of reproduction. Reproductive synchrony between male and female invertebrates has been documented before in the Antarctic, e.g. *Odontaster validus* (Pearse, 1965) and *Adamussium colbecki* (Tyler *et al*., 2003), and in the deep sea (Tyler, 1988; Eckelbarger and Watling, 1995). Synchronisation of spawning in these invertebrates may involve physical and biological cues from the environment (Himmelman, 1975; Starr *et al*., 1990), where endogenous rhythms, such as biological clocks, may also be involved. Soong *et al*.,

(2005) discussed the possible roles of pheremones in inducing spawning in brittle stars and emphasised the importance of gametes being available when members of the opposite sex of the same species are in the vicinity. This behaviour in *Ophionotus victoriae* probably describes a mechanism by which reproductive synchrony is encouraged and gamete wastage is minimised.

Spawning occurred annually during November and December. A similar spawning occurred in the circumpolar Antarctic echinoid *S. neumayeri*, where Brockington (2001) documented spawning between November and January during 1997 and 1998 in the North Cove population of this species. Populations of the limpet *Nacella concinna* from the same location have also been observed to spawn during the early austral summer (Powell, 2001).

The development rate of Antarctic echinoderm larvae is 2-5 times slower than development in comparable temperate echinoderms (Bosch *et al.*, 1987; Stanwell-Smith and Peck, 1998). Therefore, the ophioplutei probably remain in the plankton for an extended period with the additional advantage of occupying the water column during the high summer production period. However, no great number of ophioplutei was recovered by Stanwell-Smith *et al.*, (1999) during plankton sampling in the shallow-water habitats at Signy in the maritime Antarctic. The behavioural habits of these larvae are poorly described, although demersal larvae have been described for some other Antarctic taxa (Pearse, 1994). Recent work confirms the presence of ophiuroid larvae, albeit in very low numbers, during December in the shallow water habitats adjacent to Rothera Research Station and early recruits were observed settling in January (D Bowden, pers. comm.). The timing of the spawning period may also confer additional benefits for this Antarctic brittle star, by providing slightly warmer water conditions in an environment where development is characteristically slow and allowing a sufficient time period for development to proceed and allow larval settlement under optimal conditions in terms of local food abundance and/or predation pressure (Stanwell-Smith and Peck, 1998).

Nutritional condition

The strong seasonality of the water column chlorophyll biomass at Rothera has implications for benthic feeding rates both for suspension feeders, via vertical flux, and for generalists and deposit feeders dependent on fixation and remineralisation

(Brockington *et al.*, 2001). This habit has been exhibited well within the genus *Ophiura* in the Bristol Channel (Tyler, 1977). The availability of food limited gonad production of *O. albida* and has been recognised as an important factor governing gonad production in most echinoderms (Holland, 1967; Gonor, 1973). However, our data indicate that the bloom itself is not the most important factor for *O. victoriae*, because sedimentation appears to have a stronger role. Kellogg and Kellogg (1982) referred to the acquisition of sediment by *Ophionotus victoriae* and reviewed its implications on sediment reworking. The small but evident peak in gut index during September and October in 1998 may be attributed to the break up of winter fast ice in Ryder Bay and the initiation of a benthic bloom, a feature observed previously at Signy Island (Gilbert, 1991a), and exploited by the echinoid *S. neumayeri* (Brockington *et al.*, 2001). The significant decline in the gut index during September-November 1999 and 2000 may have been caused by a diversion of energy into reproductive condition. Ophiuroids may find it difficult to feed when gravid owing to the space occupied by the gonads in the disc. Thorson (1953) also commented on a “loading up” before spawning and the swelling of ophiuroid gonads, totally reducing the lumen of the stomach and preventing brittle stars from feeding.

The almost monotonic increase in gut index over the sampling period suggests the possibility of a long-term component in the gut index cycle. The patterns in gut index are not strictly in phase with the annual cycle in gonad index or sedimentation so no direct link can be argued from the current data. However, the largest index occurred in 2000 following a moderate flux event, after the largest sedimentation event, which occurred in the previous year (1999). This may reflect a combined effect between the 1999 and 2000 flux events and a larger availability of material at the seabed during 1999 and 2000. Owing to the magnitude of the flux in 1999 material may have persisted over a prolonged time period, being processed slowly and remaining in the sediment over a long duration, available for consumption alongside the flux event in 2000. Smaller flux events seen in the preceding years may be processed more rapidly producing smaller peaks in gut index. However, it is difficult to say if this cycle is intrinsic or driven by some external environmental variable, especially as the cycle appears to be at least 4 years in duration and ideally additional years data would be required before any firm conclusions could be made.

Odontaster validus

Sex ratio and Size (Radial length 'R' and Body radius 'r')

The sex ratio of the sampled population was slightly biased toward males, mainly owing to the inflated number of males collected in the last year of study. However, a 1:1 sex ratio was observed in three out of the four-year study period. Of the 407 individuals dissected 3 were hermaphrodites. Pearse (1965) described a single hermaphrodite from sampling sites in the high Antarctic and this specimen was the largest individual collected (25.7g eviscerated weight from Cape Evans 77˚38'S, 166˚24'E). The population sampled by Pearse totalled 349 individuals (182 males and 167 females) and had a sex ratio of 1:1. Pearse (1965) compared populations from McMurdo Sound (77˚51S, 166˚40'E) and Cape Evans. Individuals from McMurdo Sound were smaller than those from Cape Evans (largest animal collected 18.4g eviscerated weight). One gram of eviscerated weight equates to 2-3g wet weight (Pearse, 1965). The maximum size of individuals recovered from McMurdo Sound and Cape Evans corresponded to a wet weight of 36.8-55.2g and 51.4-77.1g respectively. The maximum size observed in an individual from the current study was a 37.83g female. Therefore, the starfish collected from shallow water sites adjacent to Adelaide Island, on the West Antarctic Peninsula are more comparable in size to the McMurdo Sound population. The variation in body water content recorded by Pearse was similar to the observations made in this study. Stanwell-Smith and Clarke (1998) also studied a shallow water population of *O. validus* at Signy Island in the maritime Antarctic (60˚43'S, 45˚36'W). Weights were calculated for starfish in the range of 5.7g to 41.8g wet mass (mean = 19.92 ± 0.46). This range in wet mass is comparable to the weights recorded in the current study (5.92 to 37.83g). 221 individuals were sexed (120 females, 95 males and 6 hermaphrodites), and as in the aforementioned study by Pearse (1965), a 1:1 male and female sex ratio was observed. Stanwell-Smith and Clarke (1998) also referred to a moderate (38%) number of the population being parasitised by the ascothoracid barnacle *Dendrogaster antarctica*. However, no incidences of parasitism were observed in the current study.

Reproductive Condition

The gonad index exhibited a significant degree of inter-annual variation. Spawning occurred each year between July and November over the 3.5 years of this study. Pearse (1965, 1969) suggested that *Odontaster validus* is capable of spawning from May to mid-September, where the actual onset and duration of spawning varies slightly between sites. Stanwell-Smith and Clarke (1998) observed a similar winter spawning period in this seastar between April and June at Signy Island, and also found it could be artificially induced to spawn during May and June. Despite the timing of spawning events being comparable between sites, the magnitude of gametes released varies both spatially and temporally, suggesting that inter-annual variation in reproductive condition is not just a phenomenon isolated to the shallow water populations of *O. validus* adjacent to the Peninsula. Brockington (2001) observed large differences in reproductive output in the Rothera population of the Antarctic urchin *Sterechinus neumayeri* over short distances, which he attributed to food supply.

Pearse (1965) observed a clear dichotomy between the gonads of individuals sampled from McMurdo Sound and Cape Evans. The Cape Evans population had gonads twice as large as those collected from McMurdo and correspondingly produced more gametes during spawning. He attributed these trends to a better quality food ration available at Cape Evans. Only a single population of this seastar was investigated in the present study. Relatively, larger spawning years were observed during 1997, 1998 and 2000 compared to 1999. The chlorophyll levels recorded in the plankton at Rothera during 1999 were also lower in comparison to the other years studied. Although *O. validus* is somewhat decoupled from the summer phytoplankton bloom because it is a scavenger and predator, the increased availability of dead and decaying material and possible prey species at this time, may go some may to explain the trends observed in gonad index and reproductive condition. However, the degree of inter-annual variation evident in *O. validus* was reduced in comparison to the marked variation in the gonad index of *Ophionotus victoriae* over the same time period. The more conservative response of *Odontaster validus* is probably a consequence of its opportunistic feeding habit, capable of utilising many prey items through scavenging and behaving as an active predator, as well as removing plankton and detrital material from the surrounding water column and benthos (Pearse, 1965). The detritivorous habit of the brittle star may result it its reproductive biology being more heavily affected by annual sedimentation events, which were extremely seasonal and variable during the study period. The comparative study of Chiantore *et al*., (2002) described the reproductive ecology of *O. validus* from Terra Nova Bay over a 2-year period and compared the degree of inter-annual variation observed with an Antarctic generalist, *Sterechinus neumayeri* and the suspension feeding Antarctic scallop, *Adamussium colbecki*. They recorded strong inter-annual differences between years in the scallop, but failed to record any differences of a comparable magnitude in the other species. These patterns were attributed to the inter-annual differences in food availability and the success of *O. validus* attributed to a 2-year oogenic cycle, coupled with an opportunistic feeding behaviour, expressed in both the starfish and sea urchin. Stanwell-Smith and Clarke (1998) also recorded inter-annual variability in the gonad index of this starfish at Signy Island over a 2-year study period. However, with only two year's data available for comparison, the study allowed no firm conclusions to be made about the main factors affecting the reproductive cycle. This was made further complicated by the large amount of variation recorded between individual gonad index, which was also noted in the present study, especially during the spawning period.

No significant difference was observed between the male and female gonad index during the present study. There was also no significant difference observed between the male and female population studied at Signy Island (Stanwell-Smith and Clarke, 1998).

Energy Investment and Storage

A significant degree of inter-annual variation was evident in both the male and female pyloric caeca index during the study period and a long-term cycle was also observed in these tissues. Patterns in the pyloric caeca index suggested that an annual cycle, clearly exhibited by gonad index in *O. validus,* was not reciprocated in the pyloric caeca. It is difficult to determine whether this long-term component is intrinsic to the nutrient storage cycle in *O. validus*, or whether an external environmental variable is responsible. A study would have to be extended over a much longer time scale to ascertain whether this pattern is intrinsic and replicated on a 2-yearly basis, or is merely an anomaly of the time period studied. However, many have remarked upon the slow rate at which life in the Antarctic progresses, where prolonged growth, gametogenic and development cycles are considered characteristic of many invertebrates (Pearse, 1994). The importance of this underlying long-term component in the cycle in the pyloric caeca cannot be reliably concluded from a ~4 year study period, and additional years would be necessary to identify the main factor driving this long-term cycle.

The population in the present study exhibited increasing pyloric caeca index values during July 1997 until January 1999. A large reduction in the pyloric caeca index followed this period of investment, indicative of starvation or at least a time of reduced feeding during the following winter. Levels only began to increase again in the following July. The period of investment in the pyloric caeca coincided with a period of investment in the gonads over the austral summer. Pearse (1965) recorded a similar parallel investment pattern in the Cape Evans population of *O. validus*. This population exhibited over a 50% increase in the pyloric caeca index between January and April, where the proximate composition of lipid, carbohydrate and protein also increased over the same time period. Pearse (1965) attributed these trends to the nutritionally-rich environment providing resources both directly to gametogenic growth and the pyloric caeca to be stored in reserve. A similar parallel summer growth period was also recorded in the starfish population at Signy Island (Stanwell-Smith and Clarke, 1998). A relationship between the accumulation of nutrient reserves in the pyloric caeca and gametogenesis has also been suggested by studies on *Asterias rubens*, on *Pisaster ochraceus* and *P. brevispinus* (Chia and Walker, 1991). However, in these asteroid species the size of the pyloric caeca varies reciprocally over the year with the size of the gonads. Identifying such links in long-lived slow growing species with extended gametogenic cycles that live in extremely seasonal environments might be expected to be more difficult. Previous studies of asteroids have found that seasonal changes in the gonad index are common, but not always inversely correlated with a decline in the pyloric caeca index (Chia and Walker, 1991). The prolonged nature of the cycle exhibited in the present study is most probably driven by similar factors that determine the extended ecological cycles expressed in many Antarctic asteroids.

Oocyte Feret Diameter

There was a significant difference in mean oocyte size over the sampling period of the current study. The range in echinoderm oocyte sizes can vary greatly between individual females and within a population (Emlet *et al.*, 1987). The maximum average oocyte size was recorded as 117µm in the current study. The largest oocyte size recorded was 183µm. Pearse (1965) recorded a similar maximum egg size of 170µm. This seastar produces a planktotrophic larva (Pearse, 1994), which is similar to several other conspicuous Antarctic echinoderms, e.g. *Odontaster meridionalis, Porania antarctica* (Pearse and Bosch, 1986; Bosch, 1988, 1989) and the echinoid *Sterechinus neumayeri* (Bosch *et al.*, 1987). Pelagic planktotrophy is also expressed in three other asteroid species known in McMurdo Sound (Bosch and Pearse, 1990).

The current study revealed a ~12 month period of oocyte growth in *O. validus*. A series of monthly oocyte size distributions exhibited mainly uni-modal egg size classes during the study period, which is somewhat counterintuitive if the conclusions of previous studies are considered. Pearse (1965) recorded an 18-24 month oocyte cycle in *O. validus*, with overlapping annual generations of oocytes in both populations from McMurdo Sound and Cape Evans. Pearse (1965) estimated the frequency of different sizes of oocytes in ovarian sections by counting and measuring at random the diameters of 50 oocytes per animal and only used oocytes sectioned through the nucleus. He constructed oocyte frequency polygons by dividing the size range of oocytes between 0-150µm at 17.8µm intervals and averaged the percentage of each oocyte size group. A similar technique was used in this study, however 100 oocytes not 50, sectioned through the nucleus, were selected at random and measured. An oocyte range of 0-250µm was divided first into 25 groups, at 10µm intervals, and then into 50 groups, at 5µm intervals and the percentage of each size group averaged and a size distribution histogram constructed so as to identify bi- or tri-modal cohorts. However, the frequency polygons constructed by Pearse (1965) represented a single female from either McMurdo Sound or Cape Evans, whereas the histograms constructed in the current study represented the monthly distributions of oocyte sizes averaged and pooled for all the females collected in each month. These histograms are contained in Appendix Figure 2 and the majority have a uni-modal distribution similar to the monthly oocyte size classes. The similarity between the methods used to display the oocyte data suggests the differences between these studies are not method driven. Furthermore, the many similarities in the reproductive characteristics of these two populations, such as the timing of spawning in the seastar and the release of planktotrophic larvae, would suggest that the period of gametogenic maturation should be similar. The prevalence of a prolonged development period (18-24 month) among some invertebrates has been attributed to the extreme seasonality characteristic of the Antarctic and the harsh winter conditions, allowing individuals to store energy over two consecutive summers, interspersed with a single period of limited food. Rothera differs by almost 11 degrees of latitude from McMurdo Sound and Cape Evans. Individuals at the more extreme southerly latitude experience harsher winters, a strongly seasonal photoperiod, a longer duration of winter fast ice and a greatly reduced seasonal variation in seawater temperatures, which will inevitably influence reproductive ecology. Differences in larval development time between populations of seastars have also been observed between Signy and McMurdo Sound and were recorded by Stanwell-Smith (1997), emphasising the fact that spatial heterogeneity in environmental factors can result in biological differences throughout the Southern Ocean. Therefore, subtle differences in the salient features of the environment between the two localities may in part account for this anomaly. It may be that there is a relationship between the duration of oogenesis, the duration of primary production hence latitude, and the observation of bi-modal or uni-modal oocyte size distributions. That is, if oogenesis requires a certain energy input, it may be that this is achieved in one summer at the latitude of Rothera, but spread over consecutive summers at McMurdo Sound. This would give a bi-modal oocyte distribution at the higher latitude sites because mature oocytes initiated in the previous summer would be present at the same time as the newly-forming oocytes in the present summer. However, in the absence of a longer study it is difficult to say whether the 12 month gametogenic cycle suggested by the current study is a real characteristic of the Rothera Point population of *Odontaster validus*.

Both 12 month and extended gametogenic development periods have been observed in a number of Antarctic invertebrates, including *Parborlasia corrugatus* (15-16 months this study), *Adamussium colbecki* (12 months Tyler *et al*., 2003), *Sterechinus neumayeri* (14-24 months Brockington, 2001) and *Ophionotus victoriae* (18-24 months Grange *et al.*, 2004). Similar long-term cycles have been recorded in the brooding asteroid *Lepasterias hexactis* (Chia, 1964) and in the echinoid *Stronglycentrotus purpuratus* (Holland, 1964). This is in contrast to the less than or slightly more than 1 year required for primary oocyte growth in many species of Asteriidae, Ophidiastridae and Astropectinidae (Pearse, 1965). Oocyte cycles are therefore extended in many cold-water echinoderms x2-x3 times over temperate species.

Male Maturity

There was a clear seasonal cycle in male gonad index, consistent in timing between consecutive years of the study period and coincident with the female episodes of gamete release. Male and female gametogenesis also proceeded in synchrony in the population of *O. validus* at McMurdo Sound and Cape Evans (Pearse, 1965). 5 spermatogenic stages were observed in the current study and were recognisable from the histological gonad preparations according to Pearse (1965).

Gametogenic Cycle and Larval Release

The reproductive periodicities of *O. validus* in populations at McMurdo Sound (Pearse, 1965), the Balleny Islands (67ºS) and Robertson Bay (71ºS) (Pearse, 1966) agree with the periodicity described in the population collected during the current study from South Cove, Rothera. These studies indicate reproductive synchrony in this species over much of its circumcontinental Antarctic distribution. These locations differ quite markedly in the duration of the photoperiod and the range of winter temperatures, suggesting that the gametogenic cycle in this seastar is unlikely to be under direct photoperiodic or temperature control. However, the onset of summer production appears very well defined throughout Antarctic waters, and most probably acts in some way to synchronise reproduction ensuring the release of eggs into the water column within sufficient time to develop into competent feeding larvae, capable of utilising the elevated food levels experienced during the following austral summer. Figure 2.3.17 shows a larger proportion of vitellogenic oocytes in the ovary from April onwards, suggesting *O.validus* may process the most recent bloom into larvae ready for release into the water column in the austral winter.

The cycle in female and male gonad index, female oocyte size classes and male maturity index all indicate a marked seasonality in the reproductive habit of *O.*

validus. A reduction in gonad mass, coincident with a synchronous shedding of large, mature oocytes from the ovary and active sperm from the testis occurred during the austral winter of each year in the current study. These data confirm that *O. validus* reproduces via a feeding planktotrophic larva released into the water column in the early winter (Stanwell-Smith and Clarke, 1998). Recent work by Bowden (D Bowden, pers. comm.) recorded high numbers of asteroid larvae (gastrula and bipinnaria) in the plankton during August-November during 2001 and 2002 from shallow water sites adjacent to Rothera Research Station (Fig. 2.4.1). However, larvae released during this time period are subject to an environment devoid and depauperate in available planktonic food. Rivkin *et al*., (1986) produced evidence that asteroid larvae could feed on bacteria, a behaviour also exhibited in other Antarctic invertebrate larvae (Peck, 1993). Pearse *et al*., (1991) found that bacterial ingestion was of little importance to *O. validus*, although the ability of larvae to sequester dissolved organic material and to depress their metabolic rate to a low level, decreases the importance of food abundance as a selection criterion for this invertebrate. The low temperatures observed in the Antarctic predispose many feeding larvae to low metabolic rates and this contributes to larval survival during extended periods of apparent starvation (Olson *et al*., 1987; Brockington, 2001; Peck and Prothero-Thomas, 2002).

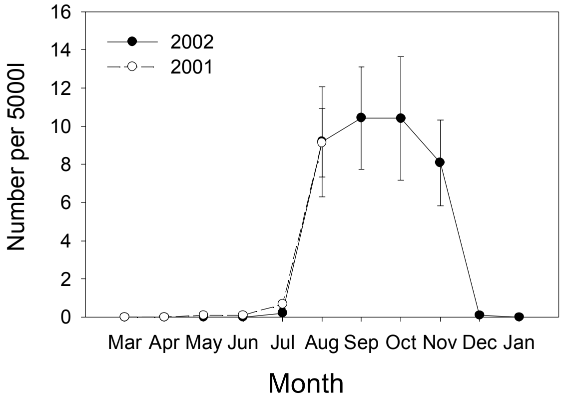

Fig. 2.4.1 Larval abundance for asteroids (gastrulae/bipinnaria) during 2001-2002 from shallow water sites adjacent to Rothera Research Station, Adelaide Island, West Antarctic Peninsula (D Bowden, pers. comm.).

Both Pearse (1965) and Stanwell-Smith and Clarke (1998) suggested the possible benefits associated with winter spawning. Embryonic development in *O. validus* is very slow, and nearly 2 months are required for the bipinnaria larva to develop from a fertilised egg (Pearse, 1965, 1969; Peck and Prothero-Thomas, 2002). If development proceeded at the same slow rate 7-9 months would be required until an advanced larval stage was attained (Pearse, 1965; Bosch, 1988; Hoegh-Guldberg and Manahan, 1994; Shilling and Manahan, 1994). Therefore, advanced larvae should appear in the water column at the time of high summer production and would be able to take full advantage of the superior summer food conditions. Early *O. validus* recruits were observed settling on settlement plates as early as December, but mainly during January, when planktonic and benthic food levels were increasing (D Bowden, pers. comm.). Spawning events in advance of the summer production period also occur in other Antarctic invertebrates with planktotrophic larvae, e.g. *Sterechinus neumayeri* and *Ophionotus victoriae,* which spawn in the early austral spring (Brockington, 2001; Grange *et al*., 2004). Some adult members of the Antarctic benthos are also able to reproduce during a nutrient-poor winter, as they possess energy reserves laid down in specific storage organs for periods of limited food availability. The data from the current study confirm this role of the pyloric caeca in the starfish *O. validus*. Alternatively, adults may benefit from the release of their progeny during the austral winter, when predation upon pelagic larvae is minimal (Clarke, 1988, 1992). Benthic suspension feeders are prevalent in the Antarctic and commonly cease feeding in the winter (A Clarke, pers. comm.), making this a more rational time for pelagic larvae to be released. However, advantages and disadvantages can be associated with both a winter and summer spawning pattern (Stanwell-Smith and Peck, 1998). The relationship between temperature and development rate and temperature and embryonic mortality are likely to be important in the spawning pattern and field occurrence of larvae, e.g. in *O. meridionalis* where the number of non-viable embryos increase with increasing temperature and *Sterechinus neumayeri* where eggs are optimally viable only within a strict temperature window (-0.2 to +1.7˚C, Stanwell-Smith and Peck, 1998). However, others factors such as egg quality, which should be associated with previous adult nutrition and benthic predation/food availability during the settlement phase may also be important.

Bowden (D Bowden, pers.comm.) has shown that many vagile species at Rothera Point, regardless of development type, recruit to the benthos in the summer and has suggested that this is because the juveniles are dependent on benthic food restricted to the summer: benthic algal films and deposited phytoplankton for grazers and juveniles of prey species for predators. This theory may explain why many benthic Antarctic invertebrates, including *Ophionotus victoriae* and *Odontaster validus*, spawn some months prior to the austral summer.

Parborlasia corrugatus

Sex ratio and Size Characteristics

P. corrugatus is a heteronemertean in the class Anopla belonging to the phylum Rhynchocoela. This species is dioecious. Most nemertean species are oviparous and possess separate sexes (Hyman, 1951; Riser, 1974; Gibson, 1994). However, hermaphroditism has been observed in *Prostoma, Prosorhochmus* and some species of *Argonemertes* (Gibson, 1994). The study population deviated from a 1:1 sex ratio, with a disproportionate number of male individuals being collected during the last two years of the study. Individuals were randomly collected and gender was not distinguishable from the external morphology, so it is unlikely this anomaly resulted from sampling bias. The specimens in the current study ranged between 157 and 550mm in retracted length and there was no difference between male and female length. Nemerteans are characteristically elongate and are often capable of considerable elongation and contraction (Gibson, 1994). Species of nemertean range in length between a few millimetres (*Carcinonemertes, Oerstedia, Tetrastemma*) up to 30m (*Lineus longissimus*). However, the majority are usually recorded between 20-30cm and the size range between male and female individuals is usually approximately the same (Riser, 1974). Therefore, *Parborlasia corrugatus* exhibits a good example of polar gigantism, capable of growing to a length in excess of 1m (Knox, 1970) and a wet mass of 140g (Heine *et al.*, 1991). The individuals collected by Heine *et al.*, (1991) were all weighed > 25g wet mass. The nemerteans sampled in the present study ranged between 24.45-155.25g wet mass (mean = 66.74g $\underline{+}$ 21.36 SD). These data were comparable to the average measurements (68.7g wet mass) made by Heine *et al.*, (1991). Many examples of the marine benthos attain large sizes in the Antarctic, and this appears to be facilitated by oxygen availability (Chapelle and Peck, 1999, 2004), or more specifically the amount of oxygen available to cross respiratory surfaces, which increases with decreasing temperature.

Oocyte Feret Diameter

A maximum oocyte size of 137µm was recorded in the present study. Peck (1993) used air-shock stimuli to induce spawning in an aquarium-reared population of *P. corrugatus*. These individuals spawned vast numbers of spherical eggs of 136µm into seawater. Smaller egg sizes have been observed in other previously described nemerteans, e.g. *Micrura caeca* mature eggs are 90µm and *M. akkeshiensis* are 100µm (Peck, 1993). There was no significant variation in average oocyte size over the sampling period of the current study. However, inter-annual differences were observed in the size of both the brittle star and starfish oocytes recorded over the same time period. These data support the release of planktotrophic pilidia larvae observed by Peck (1993).

Three distinct spawning events were evident in this nemertean and were signified by the loss of a significant proportion of large, mature oocytes from the ovary. These events occurred annually during the austral summer and were consistent in timing between consecutive years of the study period. The description of a seasonal spawning period from the current study contradicts the assumptions of previous work on this nemertean. Larval pilidia have been observed for prolonged periods throughout the year in shallow Antarctic waters leading many to suggest an aseasonal quasi-continuous reproductive habit for *P. corrugatus* (Pearse *et al*., 1991; Shreeve and Peck, 1995; Stanwell-Smith *et al*., 1999). However, Pearse and Giese (1966a) did observe spawned-out specimens of *P. corrugatus* in McMurdo Sound, Antarctica in November and December, indicative of an early austral summer spawner. The scavenging and predatory behaviour exhibited in *P.corrugatus* further supports a de-coupling of this nemertean from the extreme seasonality characteristic of the Antarctic (Dayton, 1965). While the oocyte size distributions produced in this study do exhibit an annual spawning period during December-February, large oocytes were present in the gonad throughout most of the year. These data illustrate a 15-16 month oogenic cycle in the ovary of *P. corrugatus*, which is reduced in comparison to the prolonged gametogenic development cycle (18-24 months) exhibited in many Antarctic invertebrates. This cycle is similar to the gametogenic development exhibited by the deep sea echinoid *Echinus affinis* from the Rockall Trough, northeast Atlantic Ocean (Tyler and Gage, 1984a) and shorter oogenic cycles have been observed in the Antarctic before. Tyler *et al*., (2003) reported a reproductive habit more pectinid (12 month) in character than Antarctic in the scallop *Adamussium colbecki* and the ascidian *Cnemidocarpa verrucosa* also displays a discrete 12-month reproductive cycle (Sahade *et al*., 2004).

Male Maturity

The release of active sperm from the testes coincided with the loss of mature oocytes from the ovary confirming an annual spawning period in this nemertean and a synchronous reproductive habit between the males and females sampled during the present study. Despite their being discrete spawning periods in male *P. corrugatus*, mature sperm were apparent in the testes throughout most of the year. Most nemerteans exhibit seasonal reproduction, even though this may extend over 3-4 months (Gibson, 1994). The presence of active sperm and vitellogenic oocytes in the gonad of *P. corrugatus* throughout the present study suggests that this nemertean has the potential to spawn frequently and/or over a prolonged period. *Malacobdella grossa* is the only nemertean in the British Isles to reproduce throughout the year, although superimposed upon the annual rhythm are peaks in activity, which can be correlated with the availability of planktonic food (Gibson, 1968). Such a reproductive habit may explain the patterns observed in the current population of *P. corrugatus*, which exhibited a tendency to spawn actively during the austral summer when levels of planktonic food in the water column are increasing.

Reproductive cycle and Larval Development

Male and female spawning was synchronous in the current study. Little is known about the natural reproductive habits of nemerteans, although it is known that in some species mature worms associate together and spawn synchronously, e.g. *Lineus rubber* (Gibson, 1994). Synchronous spawning may also occur without individual contact, apparently through chemical stimulation (Hyman, 1951). Alternatively the male can crawl across the female broadcasting sperm or in many species several worms enclose themselves in a mucous sheath within which the sex cells are discharged.

The small oocyte sizes recorded in the current study and observations of reared larval pilidia by Peck (1993) support planktotrophic larval development in *P. corrugatus*.

This is a common trait in the development of heteronemerteans (Gibson, 1994). Asexual reproduction as a regular reproductive adaptation is rare amongst nemerteans. The development stages observed in the embryos of *P. corrugatus* are similar to those previously described for some temperate water nemerteans (Peck, 1993). However, a broad dichotomy exists between the developmental times of temperate and polar pilidia. The pilidium stage in temperate species is reached 120-190 hours post-fertilisation, which is much sooner than in *P. corrugatus* (600-700 hours) (Peck, 1993). The persistence of pilidia larvae in Antarctic shallow waters has also suggested that the larvae have a long planktonic phase (Shreeve and Peck, 1995). Delayed development times have been described in a number of Antarctic invertebrates. Embryos and larvae of Antarctic echinoderms have been shown to develop 2-5 times slower than comparable temperate species (Bosch *et al.*, 1987). Development in *P. corrugatus* proceeds 2.5-20 times slower than comparable temperate species (Peck, 1993). The prolonged development time exhibited in Antarctic nemertean larvae lends little support to the theory of temperature compensation in cold water species (Peck, 1993) and also suggests alternative feeding adaptation must be prevalent amongst Antarctic larvae if individuals are to survive the depauperate austral winter. Recent work by Bowden (D Bowden, pers. comm.) observed large numbers of pilidia larvae in the water column adjacent to Rothera Research Station during 2001 and 2002 in the early austral winter (Fig. 2.4.2).

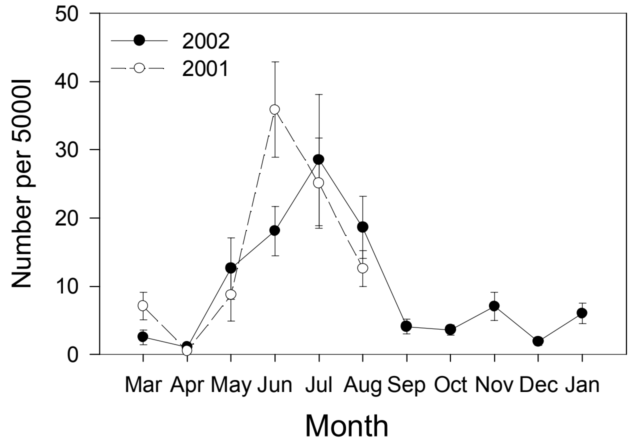

Fig. 2.4.2 Larval abundance for nemerteans (pilidia) during 2001-2002 from shallow water sites adjacent to Rothera Research Station, Adelaide Island, West Antarctic Peninsula (D Bowden, pers. comm.).

Peck (1993) observed the feeding behaviour of larval pilidia and confirmed the capability of *P. corrugatus* to utilise a wide variety of prey items, including bacteria and cell fragments < 1µm in diameter, as well as microalgae. Early recruits were observed settling during December-March, when summer production levels were high (D Bowden pers. comm.).

Heterocucumis steineni

Sex ratio and Size Characteristics

Heterocucumis steineni collected in the current study were separately sexed. A male to female sex ratio of 1:1 was observed and is a common characteristic of gonochoric holothurians (Smiley *et al.*, 1991). An exception is observed in *Thyonepsolus nutriens* (Wootton, 1949) which has a female:male sex ratio of 14:1. Deviation from a 1:1 ratio is common in parasitic and commensal species, but the majority of Holothuroidea are dioecious (Hyman, 1955). However, there are some hermaphrodite species in the cucumarids, synaptids and some within the aspidochirotes. This study recorded a range in individual size between 43.2mm and 196.0mm in length (mean = 89.83mm, SD = 23.40). A comparison of sea cucumber length between individuals and between different species is subjective as these echinoderms commonly retract (Hyman, 1955). A wet mass measure is also flawed owing to many individuals retaining water internally. The individuals weighed recorded a range between the extreme values of 18.88g and 171.90g wet mass (mean = 64.90g, SD = 24.88). The females and males did not significantly differ in length, however animal wet weight did differ sexually. Gutt *et al.*, (1992) studied a southeastern Weddell Sea population of *Heterocucumis steineni.* They quantified the 'gutted weight' of each individual by removing the intestine and gonad and then weighing the whole animal wet mass. Therefore, the range in individual gutted wet weight recorded is not comparable (< 10g) to the total body wet mass recorded in this study, although it was the case that no significant difference was observed between the mean gutted weights of the male and female population. The largest individuals in the current study were female and observed during February, which may reflect increased feeding activities during high summer production levels. Barnes and Clarke (1995) observed a distinct seasonality in the feeding activity of many suspension feeding Antarctic invertebrates, coincident with high summer production levels. Antarctic holothurians represent an abundant and influential member of the Antarctic benthic suspension feeding community (Dayton *et al*., 1974).

Reproductive Condition

A limited degree of variation in male and female gonad index between the sampling years was recorded in the present study. This holothurian is a filter feeder and therefore I would expect to have seen a large signal in gonad index in response to the seasonal variation in phytoplankton production over the sampling period. However, these data should be considered with some caution owing to the method used to calculate gonad index. The gonad mass of each individual was expressed over total animal wet weight to ascertain a measure of the gonad index. Animal wet weight may vary quite considerably between individuals, owing to the changeable volume of water retained internally. Therefore, these data should be interpreted carefully. There was a significant difference between the male and female gonad index recorded during the current study, although the average indices observed by Gutt *et al*., (1992) were similar between the males and females studied from the southeastern Weddell Sea.

Four distinct spawning events were evident in the loss of mass from the ovary and testis of individuals during the present study and were coincident in timing during each consecutive austral winter. The majority of the Holothuroidea display an annual reproductive cycle (Smiley *et al*., 1991). The largest investment in reproductive condition occurred during 1999 and 2000, which saw the greatest loss of mass from both the male and female gonad. A similar response was described in the reproductive condition of the brittle star *Ophionotus victoriae* over the same time period (Grange *et al*., 2004). The reproductive cycle of this Antarctic ophiuroid has been connected with the sedimentation patterns at Rothera (Grange *et al*., 2004) and may also be important in the reproductive activities of *H. steineni*. Gutt *et al*., (1992) found no direct correlation between the reproductive period of *H. steineni* and summer production. However, the strong seasonality of the water column chlorophyll biomass recorded at Rothera is likely to have important implications for benthic feeding rates both for suspension feeders, via vertical flux, and also for generalists and deposit feeders depending on fixation and remineralisation rates

(Brockington *et al*., 2001). Therefore, this may explain the comparable patterns in the reproductive condition of these two Antarctic echinoderms.

Gutt *et al*., (1992) suggested an alternative spawning pattern in *H. steineni* releasing gametes into the water during the austral summer. This contrasts with the winter spawning pattern described in the present study. Differences in the biological characteristics between populations of the same species have been attributed to spatial heterogeneity and differences in environmental variables (Stanwell-Smith, 1997). The individuals sampled by Gutt *et al*., (1992) were collected from the southeastern Weddell Sea between 200 and 800m, in contrast to the individuals sampled in this study from a shallow-water (15-20m), coastal site adjacent to Adelaide Island, to the west of the Antarctic Peninsula. Gutt *et al*., (1992) also suggested a delayed or prolonged spawning to be possible depending on the locality of the individuals studied. The area studied by Gutt *et al*., (1992) experiences a large degree of variation in sea ice extent from one year to the next (Zwally *et al*., 1983), and an area of open water larger than the yearly average was evident during the sampling year, suggesting an important part of the annual flux of organic material to the sea bed would have occurred early. These extreme conditions may have been responsible for the observed timing of the holothurian reproductive period, but without further years of data or a complete year being sampled, it is impossible to confirm if this was the case. This may explain the disparity in the timing of reproduction between the two sites. Furthermore, Gutt *et al*., (1992) only carried out sampling on two single occasions providing a snap shot in time of the conditions present in the southeastern Weddell Sea within a single year, whereas the current study encompassed almost a 4-year monthly sampling programme. It is therefore difficult to make any firm comparisons between their study and the current study, which comments on inter-annual variation in the cycles of gametogenic development in the Antarctic holothurian.

Gutt *et al*., (1992) also studied the reproductive biology of *Psolus dubiosus* from the southeastern Weddell Sea and observed a different reproductive habit to that described for *H. steineni*. *P. dubiosus* possessed larger oocytes and a lower fecundity, reproducing aseasonally via brood protection. Gutt *et al*., (1992) suggested since *H. steineni* has a more northern limit of distribution than *P. dubiosus*, the difference in evolutionary development is in accordance with Thorson’s rule. Direct development has also been previously described in a number of deep sea holothurians (Tyler *et al.*, 1985, 1994; Gutt, 1991b reviewed by Smiley *et al*, 1991).

Oocyte Feret Diameter

There was no significant variation in egg size during the current study period. The maximum egg size observed was 568µm and the maximum average egg size was recorded during July 1999 (365µm). These egg sizes correspond to a release of large, yolky oocytes into the water column, which develop through a lecithotrophic larva (Smiley *et al.*, 1991). A large number of larval types are now recognised in the Antarctic, many of which have been described as possessing a lecithotrophic life history (Pearse *et al.*, 1991). Gutt *et al.*, (1992) confirmed the presence of large, yolky oocytes in the ovary of *H. steineni* and the same lecithotrophic development. However, they observed oocytes between 0.3-1.0mm, which exceed the maximum oocyte size recorded in the current study. However, deep sea holothurians are predisposed to larger egg sizes (Smiley *et al.*, 1991). Similar maximum egg sizes to the one recorded in the current study have been reported previously for the Dendrochirotida, e.g. *Cucumaria frondosa* 575µm, *C. miniata* 550µm and *C. piperata* 525µm (Smiley *et al.*, 1991).

The specimens examined in this study did appear to possess distinct cohorts of unbranched tubules containing different stages of oocytes. This satisfies one of the assumptions made in the formulation of the “Tubule Recruitment Model” described by Smiley, (1988) for *Parastichopus californicus* (as *Stichopus californicus*). The position and arrangement of the gonadal tubules along the gonad basis was not studied, and therefore the assumption that oocyte maturity and tubule diameter progresses from anterior to posterior can neither be substantiated or disproved. However in contrast, Gutt *et al.*, (1992) reported the presence of oocytes of all developmental stages in single gonadal tubules in *H. steineni*. Geographical differences in the patterns of gonad development have been described for a number of holothurians (Sewell *et al.*, 1997) and may explain the differences between the current study and observations made by Gutt *et al.*, (1992). The model also assumes that spent tubules are resorbed and that oogensis takes ~12 months. However, phagocyctosis was rarely observed in the current study and many polar invertebrates have prolonged gametogenic development cycles, although this was not overly clear in this holothurian (18-24 months Pearse, 1965; Brockington, 2001; Grange *et al.*, 2004). Without further investigation it is not possible to state whether *H. steineni* conforms to the tubule recruitment model or not, although Smiley *et al.*, (1991) did suggest that the model may not be applicable to Antarctic or deep sea species, which was also found to be the case by Tyler *et al.*, (1994). Sewell *et al.*, (1997) concluded that the recruitment model may only be applied to a small number of holothurians and most appropriate in the aspidochirote families Stichopodidae and Holthuriidae. However, it is not valid for many other aspidochirotids. The discovery that gonad development varies in holothurians as a function of taxonomic position, geographical location, and habitat, and even within individuals in the same location, suggests that the model does not have a wide applicability within the holothurians.

The shedding of mature oocytes from the ovary indicated a distinct annual spawning event during the austral winter for *H. steineni*. Gutt *et al.*, (1992) observed a lack of mature oocytes in females sampled during the austral autumn, contrasting the winter spawning habit described in the current study. However, the current data should be interpreted with some caution. A number of the monthly samples collected had to be re-analysed and owing to limitations of the data, only a small number of individuals or a single female could be taken as representative of each month and used in the reproductive analyses.

A long-term 18-24 month cycle in gametogenic development was not clearly shown in the oocyte size distributions, in fact many exhibited either uni-modal size classes or extremely broad oocyte size distributions. However, Gutt *et al.*, (1992) did describe the presence of two overlapping oocyte generations in the ovary of *H. steineni* and observed the presence of large, yolky oocytes forming 1-3 cohorts in the ovary at any one time. The differences between these two studies can probably be explained by the limitations in the data described above and is further complicated as histological analysis of the holothurian gonad is also problematic. The gonad is composed of numerous tubules of different diameters and an equal cross section of all of these tubules needs to be processed through histology if a true representation of gametogenic maturity is to be attained. The large number of gonadal tubules makes it difficult to attain a true representation of the proportion of different oocyte size classes in the ovary at any one time and may partially account for the oocyte distributions described. Many other Antarctic invertebrate taxa exhibit prolonged oogenesis cycles (18-24months), including a number of shallow water Antarctic echinoderms, e.g. *Odontaster validus* (Pearse, 1965), *Sterechinus neumayeri* (Brockington, 2001) and *Ophionotus victoriae* (Grange *et al*., 2004).

Male Maturity

Annual spawning events were more clearly indicated in the male maturity data. Male *H. steineni* possessed active sperm during the austral winter. Large proportions of active sperm were lost during this period of the current study, which was consistent in timing between consecutive years and coincident with the spawning events in the female population sampled. Synchrony between the reproductive signals of male and female invertebrates has been reported in the Antarctic before (Pearse *et al*., 1991; Brockington *et al*, 2001; Grange *et al*., 2004). Synchronous spawning within the Holothuroidea has been suggested by the aggregating of individuals into assemblages (Smiley *et al*., 1991) and has the advantage of increasing the chances of successful external fertilisation.

Reproductive Cycle and Larval Development

The cycle in gonad index, male maturity index and female oocyte size all suggest a winter spawning pattern in this shallow-water population of *H. steineni*. The release of large yolky oocytes into the water column supports a lecithotrophic life history in this holothurian. Broadcasters with direct development have been described before within the Holothuroidea (Smiley *et al*., 1991). However, the majority of these have been described as possessing summer or spring spawning patterns (February-May) and have been concentrated in the cold temperate waters of the northern oceans. Despite this, Boolootian’s (1966) analysis of the reproductive cycle of *Thyone briareus* showed winter spawning. This species belongs to the Dendrochirotida, to which *H. steineni* also belongs. The timing of direct-developing holothurian reproductive events has been associated with the food requirements of the juveniles upon settling (Smiley *et al*., 1991). Recent work by Bowden (D Bowden pers. comm.) has confirmed the presence of early holothurian recruits on settlement plates in February/March. He also occasionally observed holothurian doliolarias throughout the austral winter, which may suggest the capacity of these larvae to overwinter. The decoupling of these larvae from high summer production levels and the availability of planktonic food in the water column is largely a result of their lecithotrophic life history. Consequently these larvae utilise internal nutrient reserves to sustain energy levels during the depauperate winter, settling during the following summer when food levels are increasing. Many Antarctic echinoderm larvae exhibit adaptive feeding patterns to survive the nutrient-poor winter, including the uptake of dissolved organic material, e.g. *Odontaster validus* (Olson *et al.*, 1987) *Parborlasia corrugatus* (Peck, 1993) and *Cucumaria ferrari* (McClintock *et al.*, 1994). A prolonged over-wintering development cycle has been previously described for Antarctic echinoderm larvae, most notably within the starfish *Odontaster validus* (Pearse, 1969; Bosch *et al.*, 1987).

Inter-annual Variation

Recent evidence suggests inter-annual cycles to be important not only within Antarctic invertebrates, but within marine communities worldwide. The El Nino Southern Ocean Oscillation (ENSO) modifies the physico-chemical and biological signatures of the Pacific Ocean every few years (Navarrete *et al.*, 2002; Wong *et al.*, 2002) and the effect may extend to the Southern Ocean (Dayton, 1989; Meredith *et al.*, 2004). A similar forcing has been recently identified in the Antarctic as the ‘Antarctic Circumpolar Wave’ characterised by a 4-5 year cycle influencing both biological and environmental parameters (White and Peterson, 1996).

The potential of further global climate change has important implications for the western Antarctic Peninsula, which has undergone the most rapid warming of any region in the Southern Hemisphere (King, 1994; King and Harangozo, 1998; Meredith and King, 2005), with mean annual temperatures increasing by more than 2.5ºC over the last fifty years. Antarctic taxa are regarded as very sensitive barometers for change. The combination of very limited functional scopes, with slow rates of adaptation and restricted dispersal capacities makes Antarctic invertebrates the most temperature sensitive fauna on Earth and are likely to be amongst the most vulnerable species worldwide to environmental modifications (Peck *et al.*, 2004). The functional ecology of a selection of sensitive and ecologically important Antarctic marine invertebrates has been investigated. All have upper experimental temperatures at or below 10ºC, and the most stenothermal, the brachiopod *Liothyrella uva*, the infaunal bivalve *Laternula elliptica* and the scallop *Adamussium colbecki*, experience fatalities at 4ºC. A broad range of invertebrates experience anaerobic metabolism between 2-6ºC and their basic biological functions appear to be inhibited between 2-8ºC. These data suggest that the majority of taxa now known in the Antarctic would be adversely affected by any future rise in sea temperature that could elevate current summer temperatures 1-2ºC above their annual mean. Reproductive success is a vital characteristic in species survival and evaluation of change in reproductive condition with time key to identifying vulnerable taxa. Characterising reproductive success with time is a major requirement in predicting species response to change and the early stages of species loss.

A large diversity in the reproductive patterns of four shallow-water invertebrates was observed in the current study. Three of the four invertebrates were broadcast spawners and possessed indirect development via a planktotrophic larva (the brittle star *Ophionotus victoriae*, the seastar *Odontaster validus* and the nemertean *Parborlasia corrugatus*). However, the timing of spawning differed amongst these invertebrates during the late austral spring, the austral winter and during the late austral summer respectively. The sea cucumber *Heterocucumis steineni* was also a broadcast spawner releasing eggs during the austral winter, but possessed large, yolky eggs typical of lecithotrophic development.

This study presents further evidence of inter-annual variation in reproduction in the Antarctic. A significant degree of inter-annual variation was evident in the reproductive ecology of the detritivore, *Ophionotus victoriae*, which may have been driven by the highly seasonal sedimentation events at Rothera. The amount of inter-annual variation exhibited in the reproductive ecology of *Odontaster validus,* a scavenger, was reduced in comparison, and lowest in the predatory nemertean *P. corrugatus*. Chiantore *et al.,* (2002) observed a very obvious dichotomy between the inter-annual variation in reproduction in the scallop *Adamussium colbecki*, a filter-feeder, and two other Antarctic opportunists, capable of utilising a number of prey items. They attributed these differences to the trophic biology of the invertebrates and the significant degree of coupling between cycles in Antarctic seasonality and suspension feeders. The strong seasonality of water column chlorophyll in the

Antarctic has an important influence on filter feeders, via vertical flux, and detritivores, dependent on fixation and remineralisation, and probably drives the patterns observed in the current study and the previous investigation by Chiantore *et al.*, (2002). Assuming these assertions are correct, I would have expected to have seen a large signal in the gonad index and gametogenic cycle of the only filter-feeder studied, *Heterocucumis steineni,* in response to the seasonality in phytoplankton production levels over the study period. However, evidence for inter-annual variation in the reproduction of the sea cucumber was lacking, although the limitations of the data described previously probably account for this anomaly. Therefore, the trophic position and reliance of Antarctic invertebrates on the seasonal water column production appears to be important in both reproductive ecology and inter-annual variation in gametogenic development.

I believe there were 3 main aspects to the reproductive ecology of these invertebrates: (1) The timing of each spawning event was the same between consecutive years (1997-2000) and between males and females (2) the oogenic cycle underlying the annual seasonal cycle was prolonged in *Ophionotus victoriae* (18-24 months), although this typically Antarctic characteristic was not clear in the other echinoderms studied (*Odontaster validus* and *Heterocucumis steineni*), and a discrete 15-16 month oogenic cycle was observed in *Parborlasia corrugatus* (3) inter-annual variation in reproductive condition was observed, and appeared to be influenced by the trophic position of each invertebrate. A combination of all or some of these patterns may be an adaptation to the strong seasonality and low level resource supply characteristic of the Antarctic. The plasticity of these species and their ability to reproduce despite dramatic inter-annual variation in food supply and the variability in the physical components of the environment, may explain why each is a dominant member of the Antarctic benthos and a useful barometer for change.

3.1 Introduction

A number of marine invertebrates, and especially echinoderms broadcast their gametes into the water column to await external fertilisation (Giese and Kanatani, 1987). The successful fertilisation of these eggs is a crucial element of invertebrate reproductive success and can contribute to the level of recruitment to the parent population and the viability of the future generation of propagules. In as early as 1938 Mortensen recognised the discrepancy between the number of eggs produced and the number of offspring settling, attributing this inconsistency to gamete wastage and fertilisation failures. An appreciation of the factors controlling the fertilisation process is essential, if the circumstances causing fertilisation success and failure are to be fully understood.

These factors are numerous and varied, and include the concentration of sperm available to fertilise eggs, the density, behaviour and distribution of the spawning population, the longevity of gametes and the physical traits of eggs and sperm. The situation is made further complicated by the effects of external physical parameters on the fertilisation processes, including temperature and salinity effects, and the hydrodynamic flow conditions experienced within the immediate environment. The factors able to mediate fertilisation success were categorised into gamete, individual, population and environmental specific factors by Levitan (1995) and are summarised in Table. 3.1.1. The relative importance of each factor is equivocal and probably depends on species and habitat/environment-specific characteristics.

Table. 3.1.1 Factors influencing fertilisation success (adapted from Levitan, 1995 and Powell, 2001).

Gamete	**Individual**	**Population**	**Environmental**
1. Sperm *Morphology* *Behaviour* *Velocity* *Longevity* 2. Egg *Size* *Jelly Coat* *Chemotaxis* *Sperm receptors* 3. General *Age* *Compatibility*	1. Behaviour *Aggregation* *Synchrony* *Spawning* 2. Posture *Spawning rate* 3. Morphology *Size* *Reproductive output* 4. Age 5. Energy Allocation	1. Density 2. Size 3. Distribution 4. Size Structure 5. Age Structure 6. Sex Ratio	1. Topographical Complexity 2. Flow *Advective Velocity* *Turbulence* 3. Water depth 4. Water Quality *Temperature* *Salinity* *pH* 5. Nutrients (Harrison and Ward, 2001) 6. Toxic algae (Granmo *et al.*, 1988; Caldwell *et al.*, 2002) 7. Pollution (Krause, 1994).

A number of studies have investigated the subject of fertilisation success in marine invertebrates using both laboratory and field-based techniques, and in some instances examples of *in situ* natural spawning success have been observed and quantified.

The vast majority of these studies have utilised temperate and tropical invertebrates (reviewed by Levitan, 1995; Levitan and Sewell, 1998). These studies have included a wide array of molluscan species, some commercially important, polychaetes annelids, coral, ascidians, bryozoans and fish (Table. 3.1.2).

Table. 3.1.2 Fertilisation studies using a number of temperate and tropical marine invertebrates.

Species	**Reference**
Molluscs	
Haliotis tuberculata (abalone)	Baker and Tyler, 2001
Chlamys bifrons (scallop)	Styan and Butler, 2000
C. asperrima (scallop)	Styan and Butler, 2000
Cerastoderma edule (mussel)	André and Lindegarth 1995
Mytilus edulis (mussel)	Levy and Couturier, 1996
Polychaetes	
Sabellaria cementarium	Thomas, 1994a
Phragmatopoma californica	Thomas, 1994a
Galeolaria caespitose	Kupriyanova and Havenhand, 2002
Coral	
Pseudoplexaura porosa	Lasker *et al.,* 1996; Coma and Lasker, 1997ab
Plexaura kuna	
Ascidians	Lasker *et al.,* 1996; Coma and Lasker, 1997ab
Ciona intestinalis	
Pyura stolonifera	
Bryozoans	Jantzen and Havenhand, 2001
Celleporella hyaline	Marshall *et al.,* 2000
Fish	
Clupea pallasi	Yund and McCartney, 1994; Manriquez *et al.,* 2001
Gadus morhua	Griffin *et al.,* 1998
	Litvak and Trippel, 1998

On many occasions echinoderms, and especially echinoids have been regarded as useful models for free-spawning invertebrates in fertilisation kinetics studies (Table. 3.1.3).

Table. 3.1.3 Echinoid fertilisation studies.

Species	**Reference**
Paracentrotus lividus	Vogel *et al.,* 1982
Strongylocentrotus droebachiensis	Pennington, 1985; Meidel and Yund, 2001; Epel, 1991
S. franciscanus	Levitan *et al.,* 1991
S. purpuratus	Mead and Denny, 1995
Echinometra lacunter	Sewell and Young, 1999

Sewell and Levitan (1992) also reported on the fertilisation success of the dendrochirotid sea cucumber *Cucumaria miniata* during a natural spawning event and variable spawning rates have been demonstrated for several other holothurians

(Babcock *et al.,* 1992). In addition asteroids have received considerable attention. Dramatic increases in the number of individuals in populations of the crown-of-thorns starfish *Acanthaster planci* have had devastating effects on coral reefs worldwide (Moran, 1986), and may be specifically relevant to fertilisation success, which has encouraged the comprehensive study of this asteroid (Babcock *et al.*, 1994; Benzie and Dixon, 1994; Benzie *et al.*, 1994). A number of other seastars have also been investigated and have had aspects of their fertilisation ecology described (*Luidia clathrata* Hintz and Lawrence, 1994; *Lepasterias polaris* Hamel and Mercier, 1995; *Asterias rubens* Williams and Bentley, 2002; *Coscinasterias muricata* Babcock *et al.,* 2000; *Oreaster reticulatus* Metaxas *et al.,* 2002).

Studies have also researched the substances controlling oocyte maturation and spawning in echinoderms, specifically starfish (Kanatani and Shirai, 1971; Kanatani, 1975; Kanatani and Nagahama, 1983; Shirai, 1991). The system that operates in starfish involves a peptide hormone in the nervous system, a gonad-stimulating substance (GSS), which triggers the gonad to produce a maturation-inducing substance (MIS), an inducer of oocyte maturation and spawning (Kanatani, 1975). This system has also been observed in sea cucumbers (Shirai, 1991) and involves the substance 1-methyladenine which is also commonly dissolved in distilled water and injected intracoelomically into asteroids to induce spawning artificially (Bosch and Pearse, 1990). External cues have also been identified as important triggers that act to initiate and sometimes synchronise spawning success in asteroids (temperature: Minchin, 1987; photoperiod: Pearse and Eernisse, 1986; food and lunar/tidal cycling (Boolootian, 1966 and reviewed by Chia and Walker, 1991).

In both laboratory and field observational studies, female fertilisation success ranges between 0-100% (Levitan, 1995). Many field experiments have exhibited high fertilisation levels, but success is variable and rarely reaches 100%. This is mainly a result of rapid dilution of gametes in the water column and subsequent sperm limitation (Levitan and Petersen, 1995). This situation is exacerbated in moderate-high flow conditions, which can severely limit the number of eggs successfully fertilised (Pennington, 1985), especially in regions like the surf zone where fertilisation success has been reported to be as low as zero (Denny and Shibata, 1989). Therefore, invertebrates have developed spawning adaptations to counter these detrimental effects, such as aggregative behaviour and synchronous spawning (Pennington, 1985; Babcock *et al.*, 1992; Levitan *et al.*, 1992; Sewell and Levitan, 1992; Coma and Lasker, 1997ab), which have been demonstrated for crinoids (Kubota, 1981), holothurians (McEuen, 1988; Sewell and Levitan, 1992), asteroids (Minchin, 1987), ophiuroids (Hendler and Meyer, 1982; Run *et al.*, 1988), echinoids (Randall *et al.*, 1964; Minchin, 1992) and in the deep sea (Young *et al.*, 1992). Highly synchronised seasonal spawning has been suggested to also have the advantage of swamping predators or preventing specialised predator feeding (Olive *et al*., 2000). Furthermore, Levitan (1988) observed poor fertilisation success in the sea urchin *Diadema antillarum* when spawning episodes were asynchronous and sporadic, and only involved a few sparse individuals. Soong *et al*., (2005) discussed the importance of inductive mechanisms in inducing males and females of the same species to spawn whilst in close proximity. In echinoderms both sexes have been observed to release gametes first, although more species have been reported to exhibit male-first spawning. However, the relative importance of sperm limitation or sperm competition in a species probably determines the order by which male and female echinoderms spawn, e.g. the high selective pressure of sperm limitation in some species will result in females spawning first, closely followed by males, whereas sperm competition may encourage the early release of sperm, occasionally some time prior to the appearance of eggs in the water column. However, in some species, e.g. the starfish *Asterias rubens*, induction is bi-directional where the gametes of either sex induce the opposite sex to spawn (Soong *et al*., 2005). Therefore, the possible role of pheromones in ensuring fertilisation success may also be important in some species of echinoderm. Other adaptations have been gamete-specific and have included adaptations in both sperm and egg morphology, common in many marine invertebrates (Table. 3.1.4).

Table. 3.1.4 Gamete-specific adaptations to maximise fertilisation success.

Adaptation	Reference
Sperm	
Morphology	Eckelbarger *et al.*, 1989abc
Velocity	Kupriyanova and Havenhand, 2002)
Egg	
Size	Levitan, 1993, 1996, 1998; Podolsky and Strathmann, 1996; Marshall *et al.*, 2000
Jelly Coat	Podolsky, 2002
Surface Receptors	Rosati, 1995
Other	
Chemotaxis	Miller, 1982, 1985, 1989; Bolton and Havenhand, 1996
Gamete Longevity	Manriquez *et al.*, 2001; Meidel and Yund, 2001; Williams and Bentley, 2002; Johnson and Yund, 2004

The release of gametes in viscous fluids also delays the rate at which gametes disperse and are diluted (Levitan, 1995). The influence of egg concentration is often recorded as comparatively negligible and only has a significant influence when egg concentration is high, which only ever strictly applies to the first few moments after female spawning (Levitan, 1995).

There are many examples of free-spawning broadcast spawners amongst Antarctic polar invertebrates that develop into pelagic larvae in the water column at temperatures between –1.8 and 2ºC (Clarke, 1992; Pearse, 1994; Stanwell-Smith *et al.*,1999). Many of these invertebrates also exhibit spawning adaptations including aggregative behaviour, observed in the stacks of *Nacella concinna* (Picken and Allan, 1983) and synchronous spawning, demonstrated for the brittle star *Ophionotus victoriae* (Grange *et al.,* 2004). However, the fertilisation dynamics of polar invertebrates have received limited attention except for a small number of species-specific studies (Powell *et al.*, 2001).

Antarctic invertebrates have evolved over millennial time timescales and have become closely adapted to the low temperatures that characterise the marine environment (Clarke and Crame, 1989). These low temperatures affect the physical attributes of the marine environment increasing water viscosity and density, gas solubility and decreasing pH. Temperature changes have a great effect on sperm and specifically sperm motility, as well as directly affecting fertilisation success (Greenwood and Bennett, 1981). Viscosity also has a marked influence on the motion and movement of small microscopic cells (Podolsky and Emlet, 1993). 40% of the decrease in swimming speed of sand dollar larvae, *Dendraster excentricus*, over a 10-degree drop in temperature, was accounted for by increases in viscosity. Viscosity can also affect other biological processes such as suspension feeding, accounting for over half the reduction in larval feeding rate at lower temperatures (Podolsky, 1994). Therefore, the high viscosities inherent at low temperatures, most probably affect the path and velocity at which sperm swim. However, although these attributes are known to characterise Antarctic waters, their effect and influence on invertebrate fertilisation success remain largely unknown.

Antarctic invertebrates are also stenothermal and experience a very narrow range of environmental parameters within which they can function efficiently (Peck *et al.*, 2004). The thermal resistance of cells, such as male and female gametes, tends to be species-specific and is often correlated with the degree of thermophily of the species concerned (Andronikov, 1975). This has important implications for the distribution and potential survival of Antarctic invertebrates, especially if the predicted global ocean temperature rise of 1 to 2ºC over the next century is realised.

Studies of the fertilisation ecology of Antarctic shallow-water invertebrates are rare. The fertilisation ecology of the Antarctic shallow-water bivalve *Laternula elliptica* and the common Antarctic limpet *Nacella concinna* contrasts with the patterns observed in comparable temperate and tropical species (Powell *et al.*, 2001). These invertebrates necessitate sperm concentrations 1-2 orders of magnitude larger than their comparable temperate counterparts to achieve optimal fertilisation rates. These sperm are also long-lived, and the gametes are stenothermal and stenohaline. These species also employ spawning adaptations to maximise fertilisation success, such as aggregative behaviour, spawning under ice and during low flow conditions. The differences in fertilisation patterns between temperate and Antarctic conspecifics are most probably species and environment driven, and comparisons between these two regions are important if we are to understand how fertilisation kinetics are driven in different locations, and how animals will respond to environmental modifications at the gamete level.

The current study investigated the fertilisation kinetics of a temperate starfish *Marthasterias glacialis* and an ecologically similar seastar from the Antarctic, *Odontaster validus.* These species are ideal for fertilisation studies as they are both abundant and conspicuous members of the benthos and are free-spawners. They differ dramatically in distribution and there is a broad dichotomy between their functional limits and the environmental conditions they experience.

Marthasterias glacialis, the spiny starfish, has a broad range and occurs from the Northern extremity of Iceland to South Africa, including the Mediterranean (Nichols and Barker, 1984; Minchin, 1987). It is common in Irish Waters and in the English Channel, where Plymouth Sound is towards the eastern edge of its range. Individuals in Plymouth Sound experience a broad range in temperature between 5-18˚C and salinities of 32. The fertilisation kinetics of this asteroid have not been investigated, however the reproductive ecology has received some attention. According to Mortensen (1927) and Lönning (1976) spawning takes place in the summer. Nichols and Barker (1984) observed spawning in Plymouth Sound, England, during May-June and Minchin (1987) recorded spawning in the Mulroy Bay, Ireland population during July and August in 1978.

Odontaster validus is ubiquitous to the Antarctic and is extremely abundant. This starfish can be observed at sites in South Georgia (54˚S), throughout the Southern Ocean and at an extreme southerly latitude in McMurdo Sound (78˚S) (McClintock *et al.,* 1988). It is a conspicuous member of the shallow-water benthos experiencing temperatures between –1.9 and 2˚C and a stable background of typical marine salinities (Clarke and Leakey, 1996). The reproductive ecology of *O. validus* has received considerable attention (Pearse, 1965; 1969) and it produces a planktotrophic larva during the austral winter (Pearse, 1969; Pearse *et al.,* 1991). Stanwell-Smith and Clarke (1998) studied spawning competence in the starfish and found individuals could be induced to spawn during May and June. However, the factors affecting fertilisation success in this invertebrate have yet to be studied in any detail.

The main aim of this study was to assess the affects of several factors on fertilisation success in both *Marthasterias glacialis* and *Odontaster validus*: sperm dilution, temperature, salinity, gamete longevity, contact time between gametes, viscosity and egg jelly characteristics and sperm motility. Secondly, efforts were made to compare and contrast the fertilisation success of both starfish under controlled experimental conditions, manipulating a single variable whilst the ambient conditions were maintained constant. A final comment was made on the broadscale implications of these fertilisation traits in respect of forecasted climatic change and predicted environmental warming in the Antarctic.

3.2 Materials and Methods

Sampling

The temperate *Marthasterias glacialis* was SCUBA diver collected from 15-20m depth just east of Knap Shoal, (50°19.57S 4°09.55W) in Plymouth Sound (Fig. 3.2.1), during March 2003 and March 2004. Live individuals were retained in collection bags and suspended from the marina pontoon for a short period after collection. The samples were then transported back to the aquarium at the National Oceanography Centre, Southampton (NOC) in two large cool boxes, using seawater from the Sound and freezer packs to maintain a cool temperature inside each container (Fig. 3.3.2). The sampled individuals were kept in three 1m deep tanks in a circulating natural seawater system and were maintained at ambient seawater temperature (~12-13˚C). Individuals were fed with a diet of live mussels (*Mytilus edulis*) and occasionally small pieces of fish. Experimental trials were undertaken during April-mid May to coincide with the published spawning period in the natural populations of this starfish.

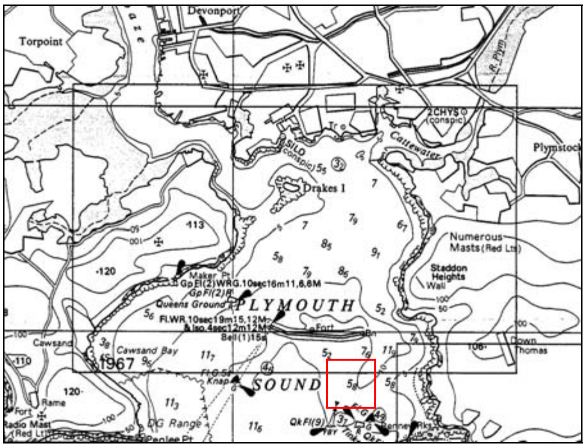

Fig. 3.2.1 *Marthasterias glacialis*. Starfish sampling site at Knap Shoal (-), Plymouth Sound, UK (50°19.57S 4°09.55W).

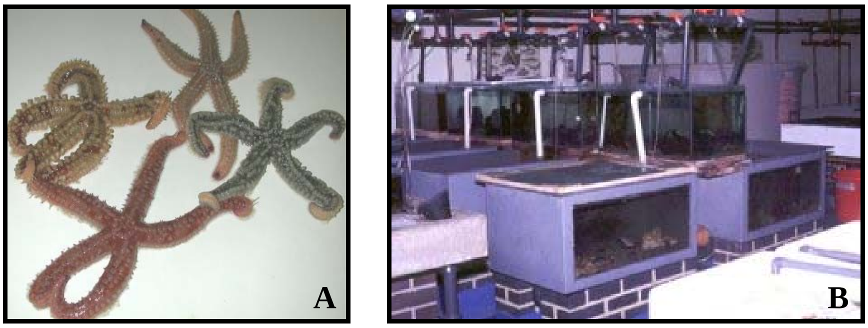

Fig. 3.2.2 *Marthasterias glacialis*. A collection of average sized individuals (A) and the NOC flow through seawater aquarium (B).

The Antarctic *Odontaster validus* was SCUBA diver collected from 15-20m depth in South Cove, Rothera Point, Adelaide Island (67˚34 S 68˚08 W). Collections were undertaken during the late summer field seasons of March 2003 and March 2004. Starfish were transferred directly to the Bonner Laboratory aquarium facility in a bucket of water to minimise handling and thermal stress. Live individuals were maintained on station in the temperature controlled, flow-through seawater aquarium, adjacent to South Cove (Fig. 3.2.3). Animals were transported back to the UK aboard the RRS Ernest Shackleton in a thermally controlled transport aquarium, housed in a large refrigeration container. They were then transferred to the British Antarctic Survey Headquarters in Cambridge and kept in 1m deep tanks in a purpose-built aquarium. This semi-closed recirculating system was housed in a constant temperature room, where a refrigeration system helped the air temperature to remain at 0°C ± 2ºC. This in turn maintained the seawater temperature at 0.5ºC ± 0.5ºC. Animals were fed a varied diet of fish and crustaceans. Experimental trials were conducted in July-late August to coincide with the natural spawning period of *O. validus* during the austral winter.

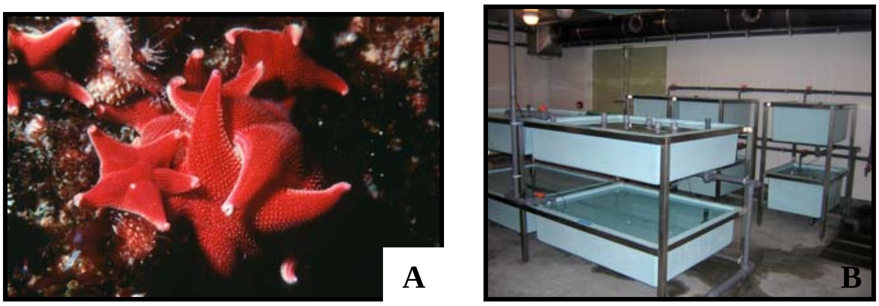

Fig. 3.2.3 *Odontaster validus*. A photograph of a natural assemblage of *O. validus* (A) and the Bonner Laboratory Aquarium Facility (B) housed at the BAS Rothera Research Station.

A similar methodology was adopted in the experiments on both starfish species and wherever possible trials were repeated three times. Each experiment involved the use of a single male and female pairing. Different individuals were used for each trial.

Sperm Dilution

Individual starfish (n = 6) were contained in separate aquaria with 1-2L of seawater. Experiments were undertaken at the normal physiological temperature of each species and maintained constant. In the case of *Odontaster validus* the aquaria were placed in a thermally controlled experimental room adjacent to the main aquarium, where experimental seawater temperatures were maintained between 0.5ºC ± 0.5ºC (Fig. 3.2.4). Male and female starfish could not be distinguished from the external morphology of either species. Therefore, individuals were chosen at random, although where possible larger starfish were preferred to ensure the majority of individuals selected would be reproductively mature. Each starfish was weighed wet (±0.01g) on an electronic balance and measured using vernier callipers (±0.01mm). The radial length (R) and body radius (r) were measured (Chapter 2.2). Each starfish was injected intracoelomically with a dose of refrigerated (~4ºC) 1-methyladenine 99% (1×10^{-4}M 0.015g in 100ml distilled water) (Sigma Chemicals) using a sterile needle to artificially induce spawning (3-4ml for *Marthasterias rubens* and 1-3ml for *Odontaster validus*). Each animal was injected at the top of each arm, into the coelom (Bosch and Pearse, 1990), and a different needle was used for each individual to avoid any contamination between individuals.

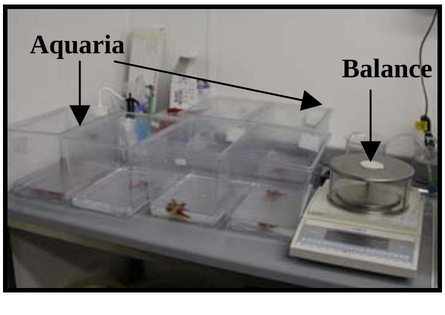

Fig. 3.2.4 A typical aquarium set-up, consisting of an electronic balance and 6 small aquaria used to house individual starfish.

A series of 30x 30ml experimental vials were set up in triplicate (3 replicates) and provision was also made for 3 control vials. All glassware was sterile before use. A

9ml sample of seawater was transferred into each experimental vial and each control vial using a micropipettor (Volac). The time necessary for each individual to spawn was invariably different, although approximately 20-30 minutes was usual. Males tended to spawn before females in both species. In both species males spawned thick, milky-white packets of sperm from the gonopores on their aboral surfaces (Fig. 3.2.5). Females released eggs from the gonopores, which were negatively buoyant and settled upon the aquaria floor. Eggs spawned by female *M. glacialis* were pink in colour, whereas female *O. validus* released orange eggs (Fig. 3.2.5). The viability of both male and female gametes was checked under a compound microscope using a well slide. Checks ensured eggs were spherical and smooth and sperm were motile and active. Experimental trials were abandoned and repeated where possible, if this was not the case.

N.B. An aqueous yellow secretion was sometimes visible from the oral surface of starfish after administering the 1-methyladenine injection. These were stomach secretions and were a secondary effect of the administered chemical, most probably caused by spontaneous contractions of the gut (A Marsh, pers. comm.).

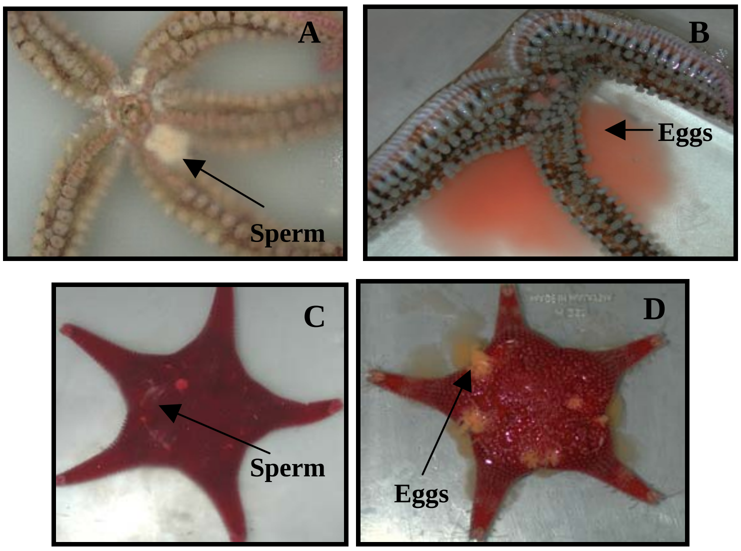

Fig. 3.2.5 *Marthasterias glacialis* and *Odontaster validus*. Typical signs of male and female spawning (A = *M.glacialis* male, B = *M. glacialis* female, C = *O. validus* male and D = *O.validus* female).

A sterile Pasteur pipette was used to siphon concentrated sperm from the gonopores and surrounding seawater of the most active male and was deposited into a sterile vial. 1ml of the dry sperm was transferred using a micropipettor into the first three replicate vials and agitated to ensure thorough mixing. This produced a 10^{-1} dilution of the initial sperm concentrate, after which the pipette tip was changed. A 1ml subsample of 10^{-1} sperm dilution was transferred into the second set of vials and this process was repeated for all replicate 10^{-1} sperm solutions, producing a sperm dilution of 10^{-2} in strength in the second set of experimental vials. The same protocol was followed between all remaining vials creating a stepwise serial dilution in sperm concentration from 10^{-1} to 10^{-10}. A final 1ml subsample was removed from the last set of experimental vials and discarded.

A sterile Pasteur pipette was used to transfer eggs from the most fecund female and deposited into a sterile glass receptacle. Subsamples of eggs were then placed in a 1L beaker of seawater to produce an optimum egg solution (100-200 eggs ml^{-1}). One ml of optimum egg solution was transferred into each control vial (eggs plus seawater only) and each experimental vial, taking care not to contaminate the pipette tip with the contained sperm dilution. Each vial was agitated to ensure thorough mixing and left to incubate over 1-2 hours. Each solution was then fixed using 4% buffered formalin. These samples allowed the optimum sperm concentration to be calculated.

The most active male and female were then returned to the aquarium and held separate from the remaining sample population to ensure that they were not re-sampled, and to reduce the chance of spontaneous spawning of other individuals in their vicinity.

Any remaining starfish not selected for the sperm dilution experiment, that had successfully spawned, were allowed to spawn for an hour before being returned to the aquarium. The 1L sperm and egg suspensions of these individuals were fixed using 4% buffered formalin. These samples were used to quantify gamete release in both species of starfish.

Experiments conducted were designed to test the effects of several control variables on fertilisation success. Each trial necessitated the collection of 6 starfish and their separation into individual plastic aquaria containing 1-2L seawater. An injection of 1-methyladenine was administered intracoelomically to each starfish, after individuals were weighed wet ($\underline{+}$0.01g) on an electronic balance and measured using vernier callipers ($\underline{+}$0.01mm) (R and r). Individuals were then allowed to spawn, and dry sperm and eggs were collected from the most active male and fecund female respectively. Optimum gamete concentrations (obtained from previous trials) were prepared and used to test the effects of separate variables on fertilisation success (1-3x10^5 sperm ml^{-1} *Marthasterias glacialis,* 1-3x10^6 sperm ml^{-1} *Odontaster validus* and 100-200 eggs ml^{-1} for both species). Mixed sperm and egg solutions were left to develop under specific conditions over 1-2 hours before being fixed using 4% buffered formalin. Any remaining starfish that had spawned, but were not selected for the individual trials, were allowed to spawn over the period an hour. Individuals were then returned to the aquarium and the remaining 1L sperm and egg solutions were fixed using 4% buffered formalin and used to quantify male and female gamete release in both starfish.

Temperature

A temperature gradient was set up between two Haake thermo-circulators using two aluminium thermo-gradient blocks and a series of tube connectors (Fig. 3.2.6). The temperature range required was set according to the normal functional limits of each species and the circulators were programmed to reach both high and low extremes outside the normal scope of each starfish (5-30°C for *Marthasterias glacialis* and -1.8-13°C for *Odontaster validus*). On occasions when this experiment was carried out at the BAS, the equipment was set up adjacent to the thermally-controlled aquarium in a refrigerated experimental room. A series of sterile 30ml experimental vials were positioned in triplicate at intervals along the thermo-gradient blocks. A micropipettor was used to distribute 8ml seawater into each vial and allowed to acclimate for 1 to 2 hours. The seawater temperature in each vial was checked using an aquarium probe and recorded.

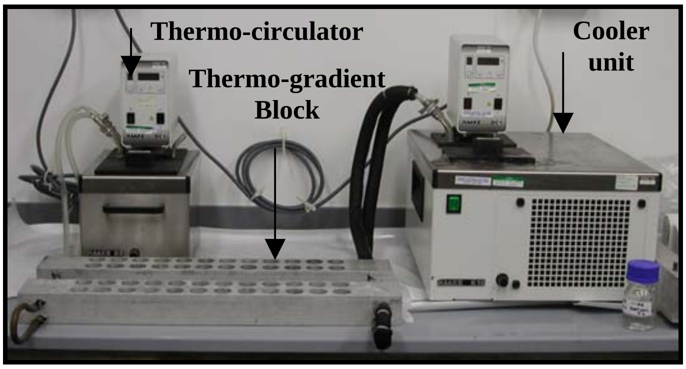

Fig. 3.2.6 A typical temperature trial experimental set-up, consisting of two aluminium thermogradient blocks and two Haake thermocirculators including a cooler unit.

One ml optimum sperm solution was placed in each experimental vial along the thermogradient blocks and allowed to acclimate to the *in situ* temperature for 30-minutes. A 1ml subsample of the optimum egg solution was then transferred into each experimental vial, with care not to contaminate the pipette tip with the contained sperm solution. Each vial was agitated to ensure thorough mixing and left to incubate for 1 to 2 hours. A control experiment was also set up at ambient temperature, using three replicate samples of 9ml seawater and 1ml optimum egg solution.

Salinity

The effect of salinity on fertilisation success was assessed. Eight sterile 30ml experimental vials were set up in triplicate and filled with 10ml serial dilutions of seawater. Progressively larger quantities of freshwater were used to produce a gradient in salinity between the 8 sets of experimental vials:

1. 10ml seawater = 33
2. 9.5ml seawater + 0.5ml freshwater = 32
3. 9.0ml seawater + 1.0ml freshwater = 31
4. 8.5ml seawater + 1.5ml freshwater = 30
5. 8.0ml seawater + 2.0ml freshwater = 29
6. 7.5ml seawater + 2.5ml freshwater = 28
7. 7.0ml seawater + 3.0ml freshwater = 27
8. 6.5ml seawater + 3.5ml freshwater = 26

These salinities were checked with a PINPOINT salinity monitor (American Marine USA Inc), which uses electrical conductivity to measure salinity. A micropipettor was used to deliver 1ml optimum sperm concentration to each experimental vial. Each sperm solution was permitted 30 minutes for the sperm to acclimate to the *in situ* salinity. A 1ml subsample of optimum egg solution was then added to each vial and the contents were thoroughly mixed. Three replicate seawater and optimum egg solutions (without sperm) were set up in addition as controls.

Gamete Age

The effect of gamete age on fertilisation success was assessed. 18 sterile 30ml experimental vials were set up containing 8ml of seawater. Optimum sperm and egg solutions were kept aerated over long periods. Gametes were aged according to the following protocol: 0mins (time of spawning), 30mins, 1hour, 1h30mins, 2hours and 2h30mins, and where possible some trials were extended at generally 4 to 12 hourly intervals to cover a 48hour period. Separate micropipettors were used to deliver 1ml optimum sperm and egg solution into the experimental vials at these set time intervals. A triplicate control experiment was included using 9ml seawater and 1ml optimum egg solution. Time constraints only allowed aged sperm and egg to be tested. Ideally both the effects of fresh eggs and aged sperm, and fresh sperm and aged eggs would have also been investigated had time constraints not been an issue.

Contact time between gametes

60 sterile 60ml experimental vials were set up and filled with 8ml seawater (6 replicates for 10 experimental trials). A straining apparatus was made using 20ml Nitex syringes and a 48µm mesh (Fig. 3.2.7), according to Baker (2001). 30 Nitex mesh strainers were inserted into three of the six replicate vials for each of the 10 experimental trials, for the easy removal of eggs from each sperm solution. Separate micropipettors were used to transfer 1ml optimum gamete solution into each experimental vial containing a Nitex mesh strainer. The strainers were carefully removed at set time intervals from each vial individually and the mesh washed in a 1L beaker of seawater This action was repeated several times to ensure the removal of any residual sperm from both the syringe and the retained eggs, and guaranteed any fertilisation observed to be the product of interactions between male and female gametes during the set contact-time intervals: 5secs, 30secs, 1 min, 2 min, 5 min, 10 min, 30 min, 1 hour, 2 hours and 4 hours, timed using a stopwatch. After thorough washing the Nitex strainers were inserted into the remaining experimental vials, possessing only eggs retained on the 48µm mesh. Three replicate control vials containing only eggs and seawater were also set up.

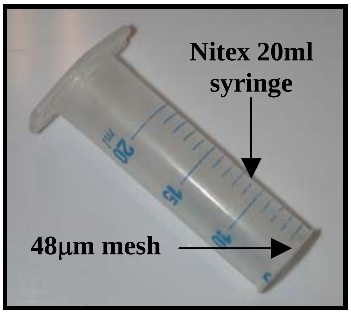

Fig. 3.2.7 An example of a 20ml Nitex mesh strainer used to separate sperm and eggs after specified contact times.

Viscosity

Three different chemical agents were used to alter seawater viscosity artificially; glycerol, methylcellulose and polyvinylpyrollidone (PVP) (Sigma Chemicals). Each solution was prepared with progressively larger quantities of each chemical and the resultant solutions were agitated using an electronic stirrer until they were homogenous. The viscosity of each solution was measured using a Gilmont Falling Ball Viscometer (Glass Tube #1). The viscometer was assembled and filled with solution taking care to avoid air bubbles. The rate at which two high precision balls of stainless steel and tantalum progressed down the tube was recorded and repeated in triplicate using a stopwatch. The type of ball chosen depended on the viscosity of the solution and the fall rate of each ball. Measurements were reproducible from 0.2-1% depending upon the time of descent. The viscosity was calculated as follows:

$$\mu = K(pf - p)t$$

where, μ = viscosity in centipoises (cp)

pf = density of ball (gms ml^{-1})

80.2 for stainless steel

16.6 for tantalum

p = density of liquid (gms/ml)

t = time of descent (minutes)

K = viscometer constant (for a Glass tube #1 K = 0.3). The viscometer standard is obtained by measuring the time of descent for a standard liquid.

Five different seawater solutions of each agent were tested in triplicate (x3 replicate experimental vials, Table. 3.2.1).

Table. 3.2.1 Separate experimental trials were designed using increasing concentrations of three chemical agents used to artificially alter seawater viscosity. Viscosity was calculated using a Falling Ball Viscometer and quantified by the passage of a weighted ball along the viscometer's length. Viscosity A = seawater viscosity maintained at 12ºC ± 0.5ºC, B = seawater viscosity maintained at 0.5ºC ± 0.5ºC.

Solution	Chemical Agent	High Precision Ball	Viscosity A (cp)	Viscosity B (cp)
SW1	Seawater only	Stainless Steel	1.27	1.45
SW2 PVP	0.14g 100ml SW	Stainless Steel	1.48	1.62
SW3 PVP	0.28g 100ml SW	Stainless Steel	1.77	1.96
SW4 PVP	0.56g 100ml SW	Stainless Steel	2.35	2.58
SW5 PVP	1.12g 100ml SW	Tantalum	4.24	5.21
SW6 PVP	2.24g 100ml SW	Tantalum	9.23	10.08
SW2 Methylcellulose	0.14g 100ml SW	Stainless Steel	N/A	1.76
SW3 Methylcellulose	0.28g 100ml SW	Stainless Steel	N/A	2.05
SW4 Methylcellulose	0.56g 100ml SW	Stainless Steel	N/A	3.09
SW5 Methylcellulose	1.12g 100ml SW	Tantalum	N/A	6.54
SW6 Methylcellulose	5.24g 100ml SW	Tantalum	N/A	16.02
SW2 Gylcerol	5ml 100ml SW	Stainless Steel	N/A	1.61
SW3 Gylcerol	10ml 100ml SW	Stainless Steel	N/A	1.80
SW4 Gylcerol	15ml 100ml SW	Stainless Steel	N/A	2.08
SW5 Gylcerol	20ml 100ml SW	Tantalum	N/A	2.67
SW6 Gylcerol	30ml 100ml SW	Tantalum	N/A	3.21

8ml of each solution was placed into three replicate sterile 30ml experimental vials (48 vials total) and 3 control vials were also set up containing only eggs and seawater. Different pipette tips were used to dispense each solution to avoid cross contamination between dilutions and between agents.

A micropipettor was used to dispense 1ml optimum sperm solution into each experimental vial and 30 minutes allowed for sperm to acclimate to the *in situ* seawater viscosity. 1ml optimum egg solution was added to each vial and agitated to ensure thorough mixing.

N.B. The effect of viscosity on fertilisation success was assessed for *Marthasterias glacialis* and *Odontaster validus* using PVP-seawater solutions only. The poor spawning response of *O. validus* prevented an extensive investigation from being undertaken and consequently only a single PVP treatment was tested. Experiments on the effect of viscosity on fertilisation success were therefore transferred to the infaunal Antarctic bivalve *Laternula elliptica*, using serial dilutions of PVP with seawater, and two other chemical agents. This invertebrate could be reliably strip-spawned and had a greater potential of producing successful experimental trials.

Egg Jelly

The jelly coats were separated from starfish eggs following the protocol for removing mucopolysaccharides from samples for later analysis, according to Adam Marsh (A Marsh, pers. comm.). Glacial acetic acid was used to adjust the ph of a 100ml volume of seawater to 5.2. A 10ml subsample of this solution was pipetted into 3 replicate 30ml experimental vials containing Nitex mesh strainers. Another 6 vials were filled with 10ml normal seawater. A 1ml subsample of optimum egg solution was pipetted into the 3 vials containing acidic seawater and 3 of the vials containing normal seawater. The acidic solutions were gently mixed and placed in a cool box over ice for approximately 5 minutes. The Nitex mesh strainers were removed from each vial individually and washed carefully in a 1L beaker of seawater. Each Nitex strainer was then inserted into a new 60ml vial containing 10ml normal seawater. A 1ml subsample of optimum sperm solution was pipetted into each experimental vial containing the strained eggs and the vials containing eggs in normal seawater.

Fecundity

Individual *Odontaster validus* were held in separate aquaria and induced to spawn using injections of 1-methyladenine. Each starfish was weighed wet (±0.01g) and measured using vernier callipers (±0.01mm). Only female starfish were required. Therefore, any males were returned to the aquarium and any spare aquaria were emptied. Individuals were allowed to spawn into 1L seawater over an extended period until all visual signs of spawning had stopped. All 1L samples were fixed using 4% buffered formalin and used to quantify female fecundity.

Fertilisation and Gamete Counts

100 eggs were counted from each experimental trial and were quantified using a Sedgewick Rafter counting cell. Each count was repeated in triplicate. The percentage of fertilised and non-fertilised eggs was quantified to indicate fertilisation success. Fertilised eggs were identified by the presence of a fertilisation membrane. Instances of normal and abnormal fertilisation, including polyspermy, were also

recorded. The presence of multiple sperm attached to the jelly coat of an egg was used to indicate polyspermy (Fig. 3.2.8).

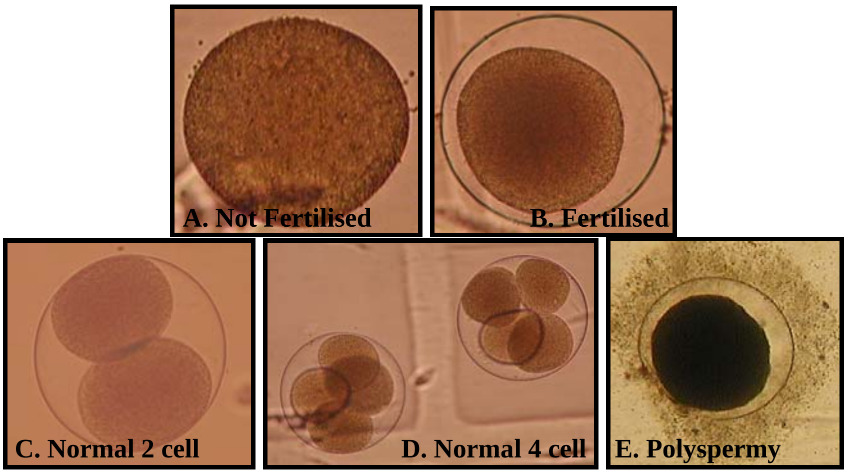

Fig. 3.2.8 Fertilisation was scored by the absence (A) or presence of a fertilisation membrane (B), and normal cell cleavages (C and D). Polyspermy was also recorded and regarded as abnormal (E).

Individual female fecundity and gamete release were measured in 3 replicate 1ml samples using a Sedgewick Rafter counting cell and the number of eggs in 1L of seawater calculated. Checks were made to ensure optimum egg solutions were accurate (100-200 eggs ml^{-1}). 100 eggs from each control were also counted and the occurrence of any fertilised eggs recorded. Any experiments where controls had a fertilisation rate >1% were discarded and, where possible repeated.

Individual male gamete release and concentrated sperm counts were accomplished using two methods. A haemocytometer counting cell was used to quantify the amount of sperm per ml^{-1} in three replicate counts of the 2003 samples of both starfish species. The 2004 samples of both species were quantified using a Coulter Multisizer II. Three replicate 500µl samples of each solution were analysed and the number of sperm quantified on a 1.9-20.0µm scale for particle size. Solutions were continually agitated to ensure thorough mixing using the in-built stirrer apparatus and the 500µl subsamples were counted within a 100µm tube. Serial dilutions of each sample had to be prepared using a standard control solution (isoton) to ensure accurate counts. A control (isoton) count was also undertaken in triplicate and recorded as a background count, which was subtracted from all other sperm counts. The concentration of sperm ml^{-1} calculated by both methods was converted into the number of sperm in 1L seawater. A 1ml subsample of dry sperm from both *Marthasterias glacialis* and *Odontaster validus* was quantified and compared between the two methods to ensure that similar concentrations of sperm were being detected. This test was undertaken for a single male of each species in 2003 using the haemocytometer and in 2004 using the Coulter Multisizer II, and 3 replicate counts of each dry sperm sample were tested (Table. 3.2.2).

Table. 3.2.2 Dry sperm concentrations (ml^{-1}) from individual males in 2003, using the haemocytometer counting cell, and in 2004 using the Coulter Multisizer II. Data are presented as overall means and ±SD from 3 replicate counts.

Haemocytometer	**Mean**	**SD**	**Min**	**Max**
Marthasterias glacialis	2.10E+08	2.10E+07	1.92E+08	2.33E+08
Odontaster validus	3.18E+08	3.13E+07	2.98E+08	3.54E+08
Coulter Multisizer	**Mean**	**SD**	**Min**	**Max**
Marthasterias glacialis	1.70E+08	5.71E+06	1.65E+08	1.76E+08
Odontaster validus	3.88E+08	1.23E+07	3.74E+08	3.96E+08

Competence to spawn

Individual *Odontaster validus* were SCUBA diver collected from 15-20m in South Cove, adjacent to Rothera Research Station during the summer and winter field season of 2003-2004 (December 2003-August 2004). Individuals were maintained in the Bonner Laboratory's flow-through aquarium facility. Monthly collections of individuals (10-15 starfish) were separated into individual aquaria and held in a thermally controlled experimental room adjacent to the main aquarium. Individuals were weighed wet (±0.01g) and measured using vernier callipers (±0.01mm) (R and r). Injections of 1-methyladenine were administered to induce spawning. The spawning competence of each individual starfish was assessed as the ability to release eggs or sperm. The starfish often contorted their body shape, curled up and raised themselves onto the tips of each arm. This behaviour was similar to the spawning posture often exhibited by natural populations of starfish. A lack of spawning was also noted.

Sperm Swimming Speed

The poor spawning response of individual *Odontaster validus* necessitated a more reliable Antarctic invertebrate to be selected and used in the sperm swimming trials.

The infaunal bivalve *Laternula elliptica* and the nemertean *Parborlasia corrugatus* were chosen and could be reliably strip spawned.

Experimental trials were implemented in the facilities of Rothera Research Station and the British Antarctic Survey, Cambridge aquarium during the Antarctic summer field season of 2003-2004 and during summer 2004 in the UK respectively. The infaunal bivalve *Laternula elliptica* and the nemertean *Parborlasia corrugatus* were SCUBA diver collected from 15-20m in Hanger Cove to the north of Rothera Research Station. Individuals were maintained in the Bonner Laboratory aquarium facility. A collection of these animals was also transported to the BAS headquarters on the RRS Ernest Shackleton in a purpose built container aquarium. The effects of temperature and viscosity on sperm swimming speed were studied. Individuals were strip spawned and a single male was required for each experimental trial. A single incision was made along the shell skirt to cut the adductor muscle of the hermaphrodite bivalve *Laternula elliptica*. The two shell valves were prized apart to allow access to the gonad (Fig. 3.2.9). A scalpel was used to make a small incision along the posterior section of the gonad. Care was taken not to puncture the gonad in an anterior position, to avoid eggs being released. Concentrated sperm was siphoned from the gonad using a sterile Pasteur pipette and deposited into a 30ml vial. Animal wet weight (±0.01g), shell length, width and height were also measured using vernier callipers (±0.01mm) (Fig. 3.2.9). Individual *Parborlasia corrugatus* were also strip spawned, however separate sexes could not be distinguished by external morphology. Therefore, dissections had to be made to find a suitable male specimen and any female individuals were discarded. A long incision was made down the length of each nemertean at a position sufficiently posterior to the mouth (1/3 animal's length) to avoid the foregut. Packets of sperm were visible running laterally down the length of each male within the epithelium of the body wall and could be pierced using a sterile Pasteur pipette (Fig. 3.2.9). Concentrated sperm was also found free in the body cavity. Dry sperm was siphoned from each nemertean using a sterile Pasteur pipette and deposited into a 30ml vial. Individuals were weighed wet (±0.01g) and their retracted length (±0.01 mm) was also recorded (Fig. 3.2.9).

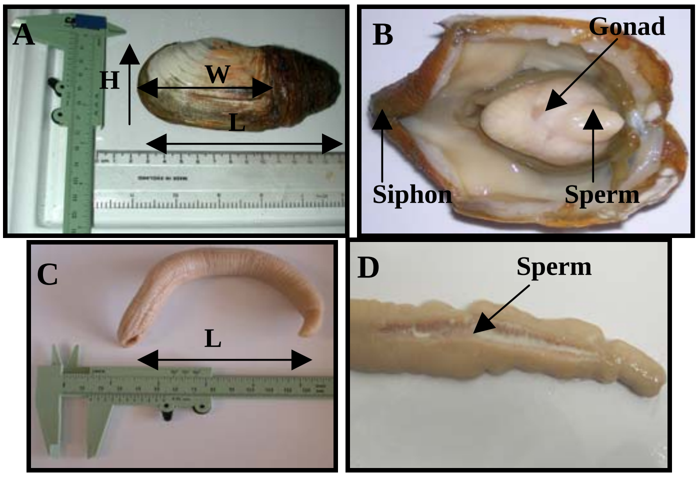

Fig. 3.2.9 *Laternula elliptica* (A and B) and *Parborlasia corrugatus* (C and D). Individuals were weighed wet, measured and concentrated sperm was removed by strip spawning. L = length (±0.01mm), W = width (±0.01mm) and H = height (±0.01mm).

The effects of temperature were assessed along a temperature gradient set up between two Haake Thermo-circulators using coupled aluminium thermo-gradient blocks. The thermo-circulators were filled with 50% ethanol and programmed between the ambient winter seawater temperature of –1.9ºC and an upper limit outside the normal scope of Antarctic invertebrates (~6°C). After temperatures across the blocks had equilibrated the temperature of each vial was checked and recorded. Sterile 30ml experimental vials were positioned at set intervals along the temperature gradient blocks. The concentrated sperm was diluted in seawater to a concentration of 10^{-1}. Two ml of the sperm solution was distributed between each vial and allowed 30 minutes to acclimatise to the *in situ* temperature. Small subsamples of sperm were taken from each vial and transferred separately onto a standard microscope slide, and viewed under a compound microscope. A Peltier cold stage apparatus was mounted onto the microscope and connected to a microcontroller to modify accurately the temperature of each slide and the microenvironment of sperm whilst being viewed (Fig. 3.2.10). A Nikon Digital camera was mounted onto the microscope and used to capture 40 seconds of video footage at each temperature. The temperature of the Peltier apparatus was modified between experimental trials to match the exact temperature recorded in each vial along the thermogradient blocks.

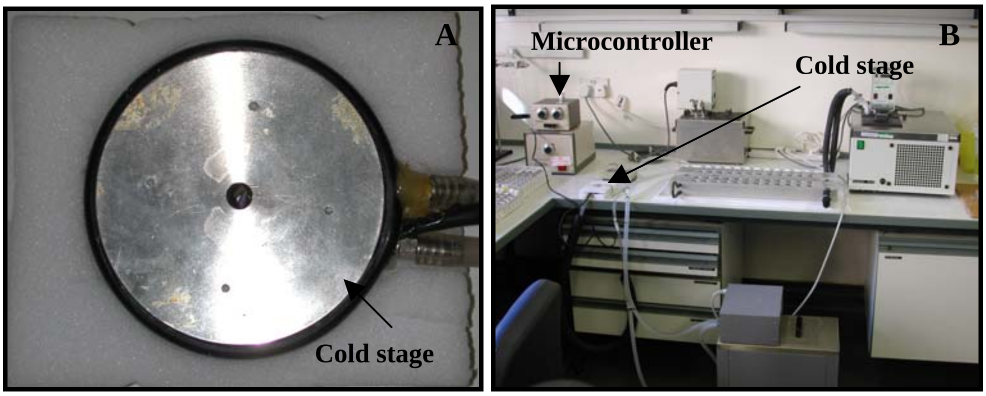

Fig. 3.2.10 The Peltier Cold Stage (A) and a typical experimental set-up (B) used to monitor sperm swimming speed.

In viscosity trials using glycerol, methylcellulose and polyvinylpyrollidone (PVP) solutions in seawater, 5 dilutions of each agent were prepared in 8ml triplicates, plus a final seawater control (Table. 3.2.1). Viscosity was measured using a Gilmont Falling Ball Viscometer. A 1ml subsample of concentrated sperm was pipetted into each solution and agitated to ensure thorough mixing. The sperm solutions were placed in the thermogradient block, which had been set to 0.5 ± 0.5°C and left over a 30 minute period to acclimatise to the *in situ* conditions. Small subsamples of sperm were taken from each vial and transferred individually onto a standard microscope slide, and viewed under a compound microscope. The Peltier cold stage apparatus was used to maintain each slide at a constant temperature (0.5 ± 0.5°C). A Nikon digital camera was mounted onto the microscope and used to capture 40 seconds of video footage of sperm at each viscosity.

The video footage from each trial was viewed using QuickTime Viewer. The rate of sperm swimming was recorded by hand, frame by frame, and calibrated for 3 replicate sperm and the data compared between trials. The path travelled by individual sperm was traced from the screen over a measured number of seconds and calibrated to a magnification of x100. The concentration of dry sperm collected from *Laternula elliptica* and *Parborlasia corrugatus* during each trial was also quantified.

Statistical Manipulation

All data were tested using the Anderson-Darling test for normality (Appendix Table. 6-10). The effects of different variables on fertilisation success were investigated separately. If any interesting patterns in the effect of individual variables on fertilisation success were apparent graphically, those variables were statistically tested. The effect of viscosity on fertilisation success was tested for *Marthasterias glacialis*, *Odontaster validus* and *Laternula elliptica*. A 2-sample T-test was used to test for a significant difference in fertilisation success between high and low viscosities. The effect of removing the jelly coat from the eggs of *O. validus* was also assessed using a 2-sample T-test. On occasions where the 2-sample T-test was used a test for equal variance was also undertaken on the data, and the results of this analysis included in the assumptions of the T-test.

The relationships between animal wet weight (±0.01g) and size (R and r ±0.01mm), and female fecundity and male and female gamete release were tested using parametric and non-parametric correlations. Decisions on which test to use were taken following analyses to see if the data were normally distributed.

The competence of spawning in *O. validus* was also statistically tested. The difference between the wet weight (±0.01g) and size (R and r ±0.01mm) of the competent and non-responsive individuals was determined using a Mann Whitney test. The same non-parametric analysis was used to test for a significant difference in the wet weight (±0.01g) and size (R and r ±0.01mm) of both the competent males and females with the wet weight (±0.01g) and size (R and r ±0.01mm) of the total number of starfish injected.

All statistical tests were undertaken using Minitab 13.0 and all data were plotted using Sigma Plot 8.0 (SPSS Software).

3.3 Results

Sperm Dilution *Marthasterias glacialis* (Fig. 3.3.1)

An optimum sperm concentration was calculated between 1.70x10^5-3.79x10^5 sperm ml^{-1}. 100% fertilisation success was observed at these concentrations. However, large numbers of eggs ($\geq$80%) were being successfully fertilised over a broad range of sperm concentrations (10^6-10^3 sperm ml^{-1}). Fertilisation success was greatly reduced at sperm concentrations $\leq$ $10^2 ml^{-1}$ (~15%-0%). The frequency of abnormal development was considerable at sperm concentrations $\geq$ $10^6 ml^{-1}$ and in all cases was attributed to polyspermy.

Sperm Dilution *Odontaster validus* (Fig. 3.3.2)

The optimum sperm concentration was a magnitude greater in the polar starfish compared to its temperate counterpart. Fertilisation success was optimum at sperm concentrations between 1.66x10^6-3.18x10^6 sperm ml^{-1}. Fertilisation success varied between experimental trials (80-100%), but was consistently high at concentrations between 10^6-10^5 sperm ml^{-1}. Polyspermy was common above these concentrations. Fertilisation success decreased at concentrations $\leq$ $10^4 ml^{-1}$. A large proportion of eggs remained unfertilised at these low concentrations of sperm. However, the decline in the number of eggs successfully fertilised was more dramatic in *M. glacialis*, often dropping to near 0% below concentrations of $10^2 ml^{-1}$. Moderate fertilisation success (20-40%) was still apparent in *O. validus* at these concentrations.

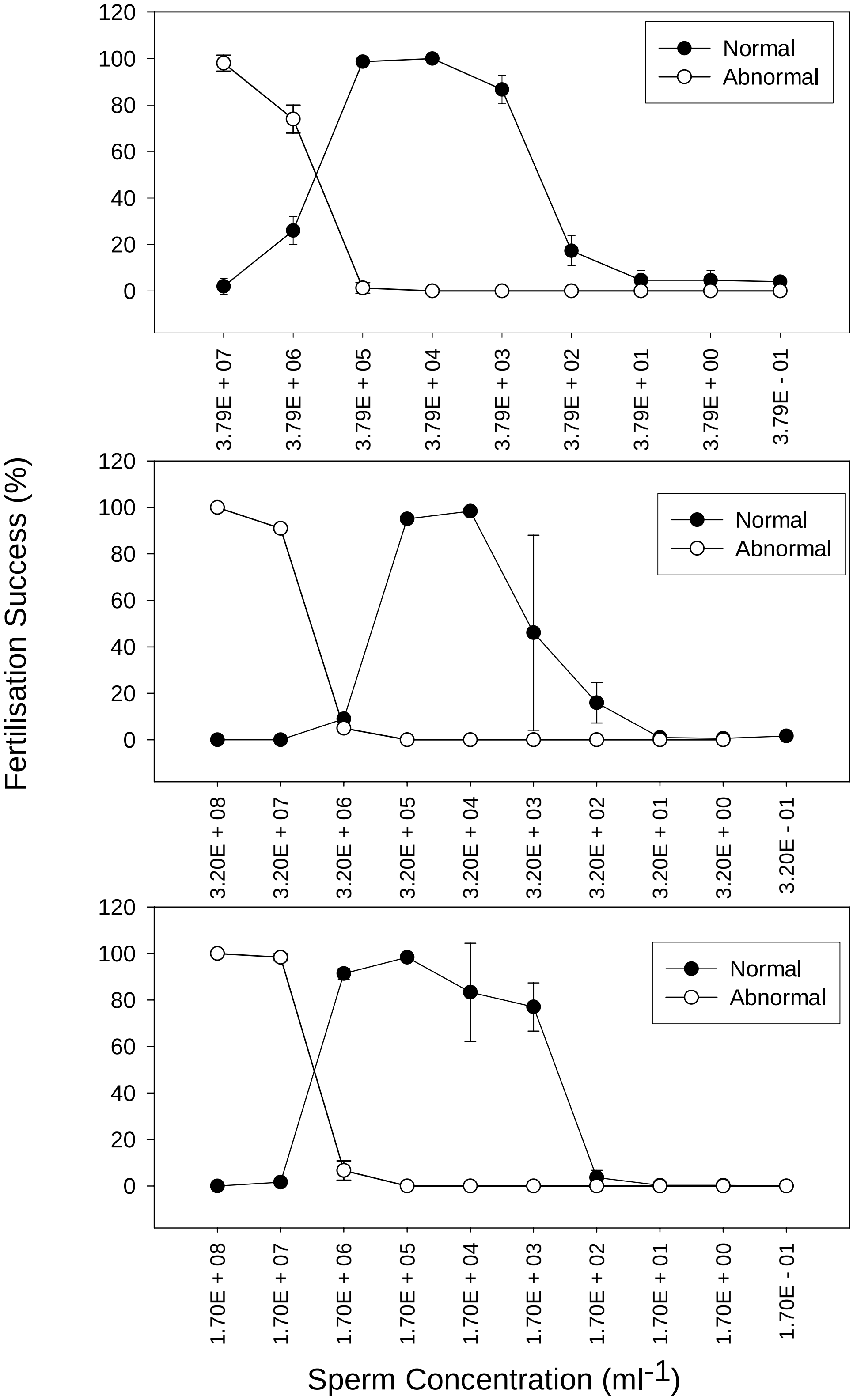

Fig. 3.3.1 *Marthasterias glacialis*. The effect of sperm dilution on fertilisation success. This experiment was repeated on three separate occasions using one male and one female starfish. A different male-female paring was used during each trial. Data are presented as overall means + SD of three replicate egg counts. Data are based on normal, abnormal and unfertilised egg counts. Only the normal and abnormal fertilisation data are plotted. Temperature was maintained at 12˚C + 0.5˚C.

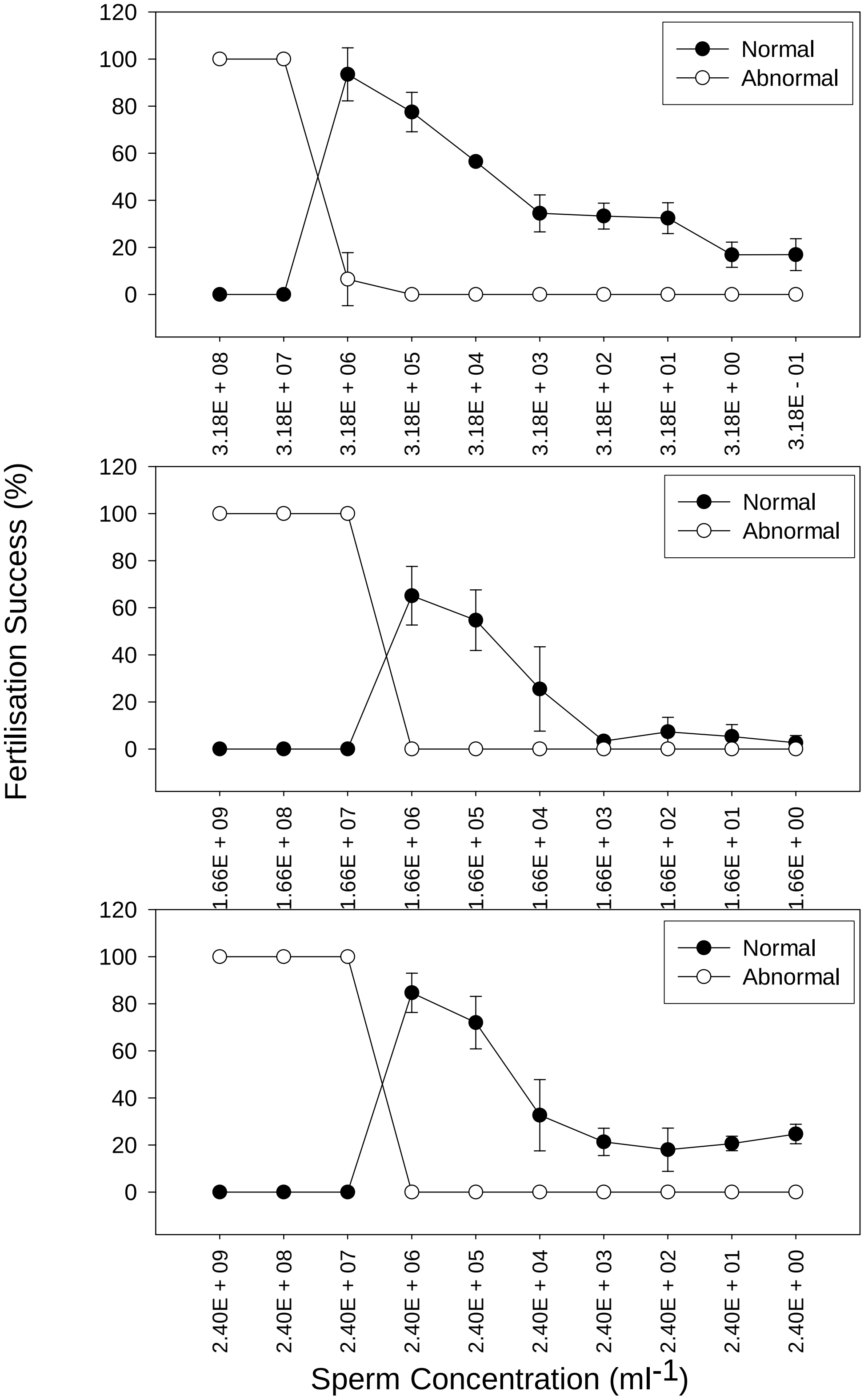

Fig. 3.3.2 *Odontaster validus*. The effect of sperm dilution on fertilisation success. This experiment was repeated on three separate occasions using one male and one female starfish. A different male-female paring was used during each trial. Data are presented as overall means ± SD of three replicate egg counts. Data are based on normal, abnormal and unfertilised egg counts. Only the normal and abnormal fertilisation data are plotted. Temperature was maintained at 0.5˚C ± 0.5˚C.

Temperature *Marthasterias glacialis* (Fig. 3.3.3)

A large proportion of eggs (>60%) were fertilised by sperm acclimated to temperatures between 8-19°C. Maximum fertilisation success (90%) was observed at 12.5ºC. Lower fertilisation success was observed under conditions outside this temperature window. A decline in fertilisation occurred at temperatures > 20.0ºC and no eggs were fertilised at temperatures above 25ºC. The number of fertilised eggs also decreased at temperatures < 7ºC, with ~40% success achieved at 5.5ºC.

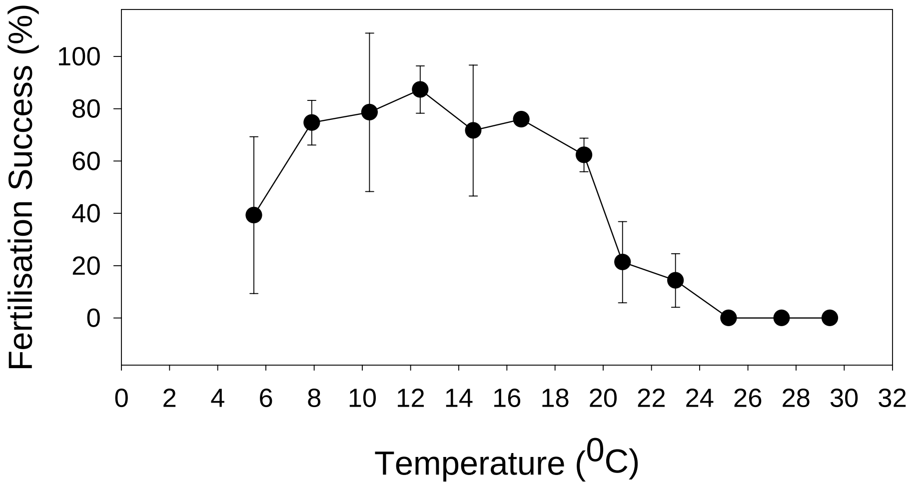

Fig. 3.3.3 *Marthasterias glacialis*. The effect of temperature on fertilisation success. The plot represents the fertilisation success between a single male and female. Data are presented as overall means ± SD of three replicate egg counts. Data are based on normal fertilisation and unfertilised egg counts, although the unfertilised egg counts are not plotted. No abnormal fertilisation was observed.

Temperature *Odontaster validus* (Fig. 3.3.4)

The highest level of fertilisation success recorded varied between replicates and between each trial (20-100%). However, all three experimental trials revealed a similar pattern. Fertilisation success was generally impaired at temperatures > 4ºC and maximum success was achieved at -1ºC. The proportion of fertilised eggs was greatly reduced at temperatures ≥ 6ºC. No eggs were normally fertilised at temperatures above this point and the frequency of abnormal development increased above 4ºC. Temperature was more restrictive on *O. validus*, limiting successful fertilisation to a narrow temperature window (-1ºC to +4ºC).

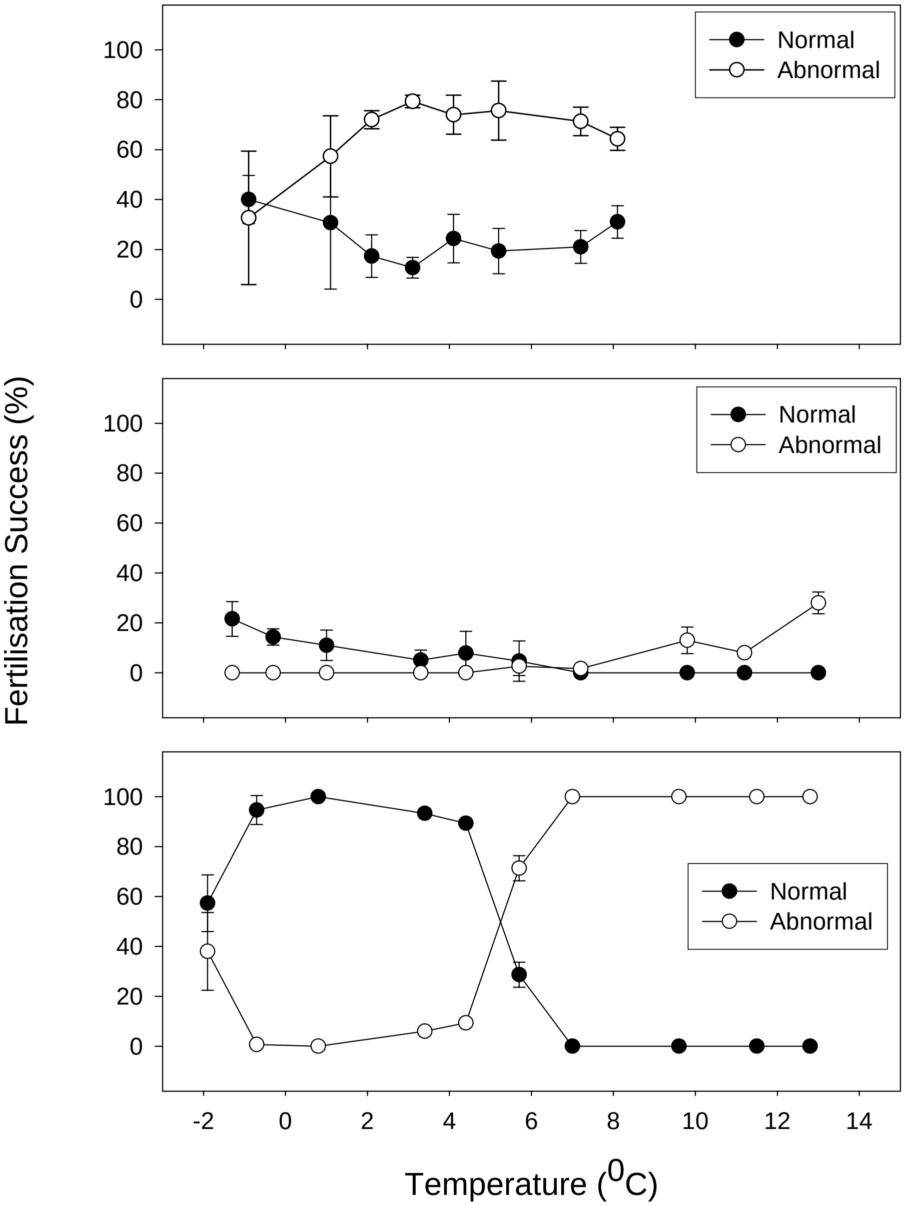

Fig. 3.3.4 *Odontaster validus*. The effect of temperature on fertilisation success. This experiment was repeated on three separate occasions using one male and one female starfish. A different male-female paring was used during each trial. The first trial was only undertaken between –1ºC and 8ºC. Future trials were extended to 13ºC. Data are presented as overall means ± SD of three replicate egg counts. Data are based on normal, abnormal and unfertilised egg counts, although the unfertilised egg counts are not plotted.

Salinity *Marthasterias glacialis* (Fig. 3.3.5)

In each experimental trial fertilisation success decreased consistently with decreasing salinity. The number of eggs fertilised declined to a low level below a salinity of 24. A consistent increase in the proportion of abnormal egg development was also exhibited. Extremely low and zero levels of fertilisation success were common at salinities at and below 24. Maximum fertilisation success was observed in normal seawater conditions (33-34).

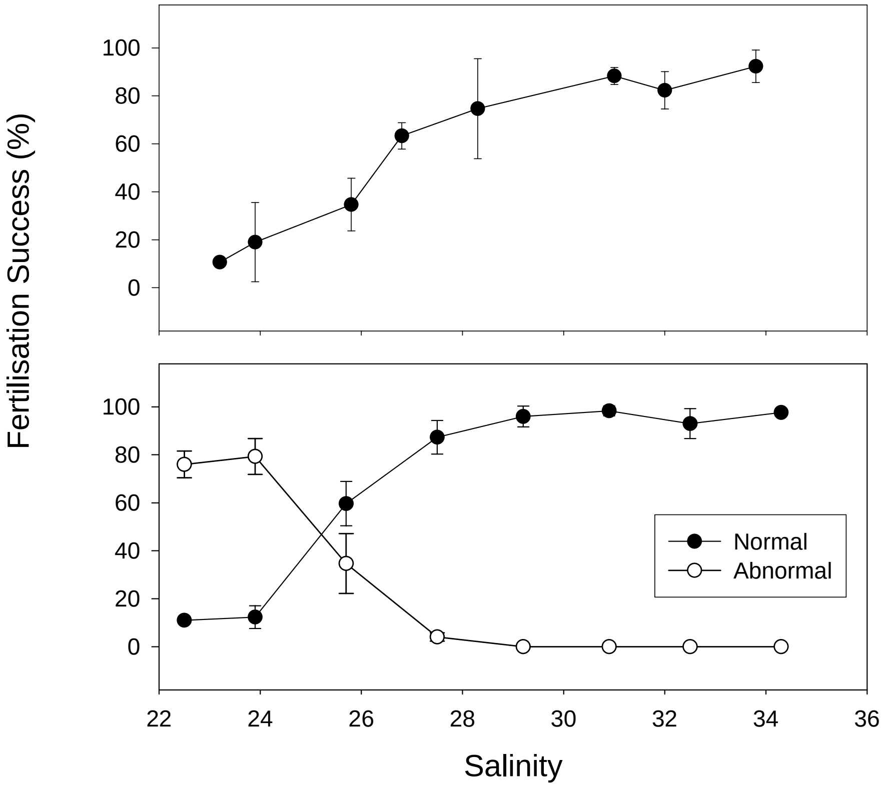

Fig. 3.3.5 *Marthasterias glacialis*. The effect of salinity on fertilisation success. This experiment was repeated on two separate occasions using one male and one female starfish. A different male-female paring was used during each trial. Data are presented as overall means ± SD of three replicate egg counts. Data are based on normal, abnormal and unfertilised egg counts, although the unfertilised egg counts are not plotted. No abnormal fertilisation was apparent in the first trial. Temperature was maintained at 12˚C ± 0.5˚C.

Salinity *Odontaster validus* (Fig. 3.3.6)

The highest level of fertilisation success was variable between each experimental trial (40-100%), but a clear pattern between salinity and the number of eggs fertilised was observed.

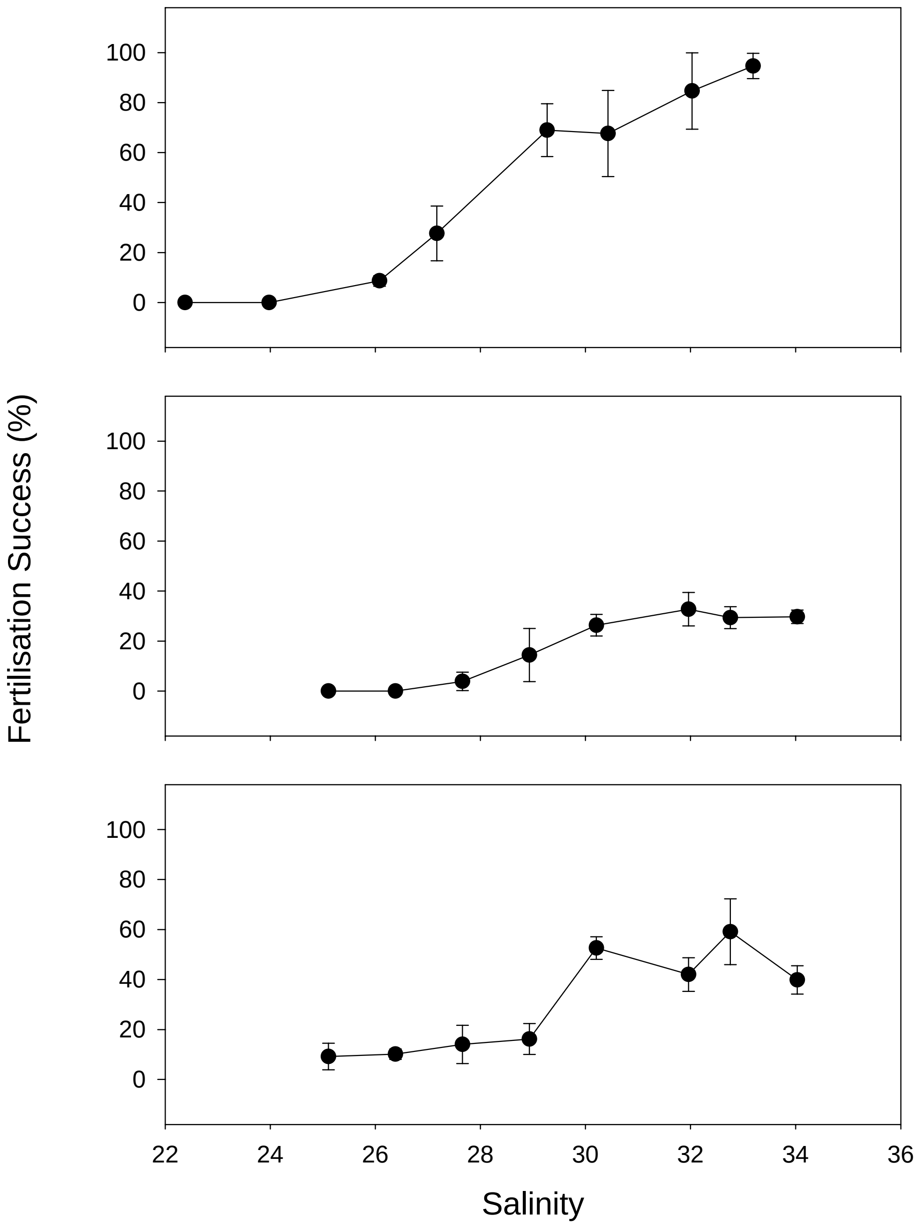

Fig. 3.3.6 *Odontaster validus*. The effect of salinity on fertilisation success. This experiment was repeated on three separate occasions using one male and one female starfish. A different male-female paring was used during each trial. Data are presented as overall means ± SD of three replicate egg counts. Data are based on normal and unfertilised egg counts. No abnormal fertilisation was observed. Temperature was maintained at 0.5˚C ± 0.5˚C.

Fertilisation success decreased with decreasing salinity. There was a considerable decline in the number of eggs fertilised at salinities below 30. Low and zero levels of fertilisation success were observed at salinities below 28. Maximum fertilisation success was recorded in normal seawater conditions (33-34).

Gamete Age *Marthasterias glacialis* (Fig. 3.3.7)

Fertilisation success varied between experimental trials (40-100%), but the effect of gamete age was consistent.

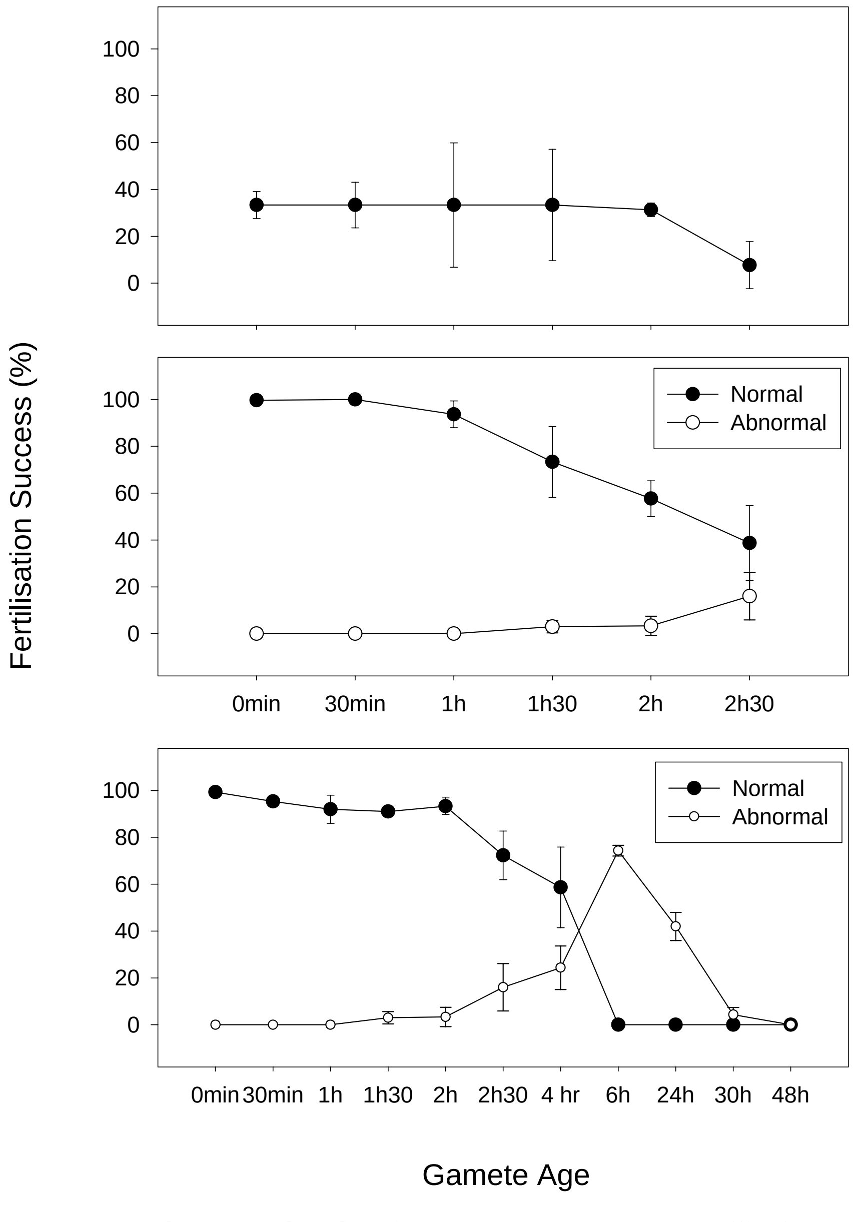

Fig. 3.3.7 *Marthasterias glacialis*. The effect of gamete age on fertilisation success. This experiment was repeated on three separate occasions using one male and one female starfish. A different male-female paring was used during each trial. Data are presented as overall means ± SD of three replicate egg counts. Data are based on normal, abnormal and unfertilised egg counts, although the unfertilised egg counts are not plotted. No abnormal fertilisation was apparent in the first trial. Temperature was maintained at 12°C ± 0.5°C.

High levels of fertilisation success were observed when gametes were aged from 0 (just spawned) to 2 hours. Interactions between gametes of a greater age resulted in a lower number of fertilised eggs. This was especially apparent after eggs and sperm were aged for more than 4 hours before being mixed. Abnormal cleavages increased after 2 hours of aging and accounted for all interactions after 6 hours.

Gamete Age *Odontaster validus* (Fig. 3.3.8)

Gamete age had an important and replicable effect on fertilisation success, but maximum fertilisation success varied between experimental trials (40-80%).

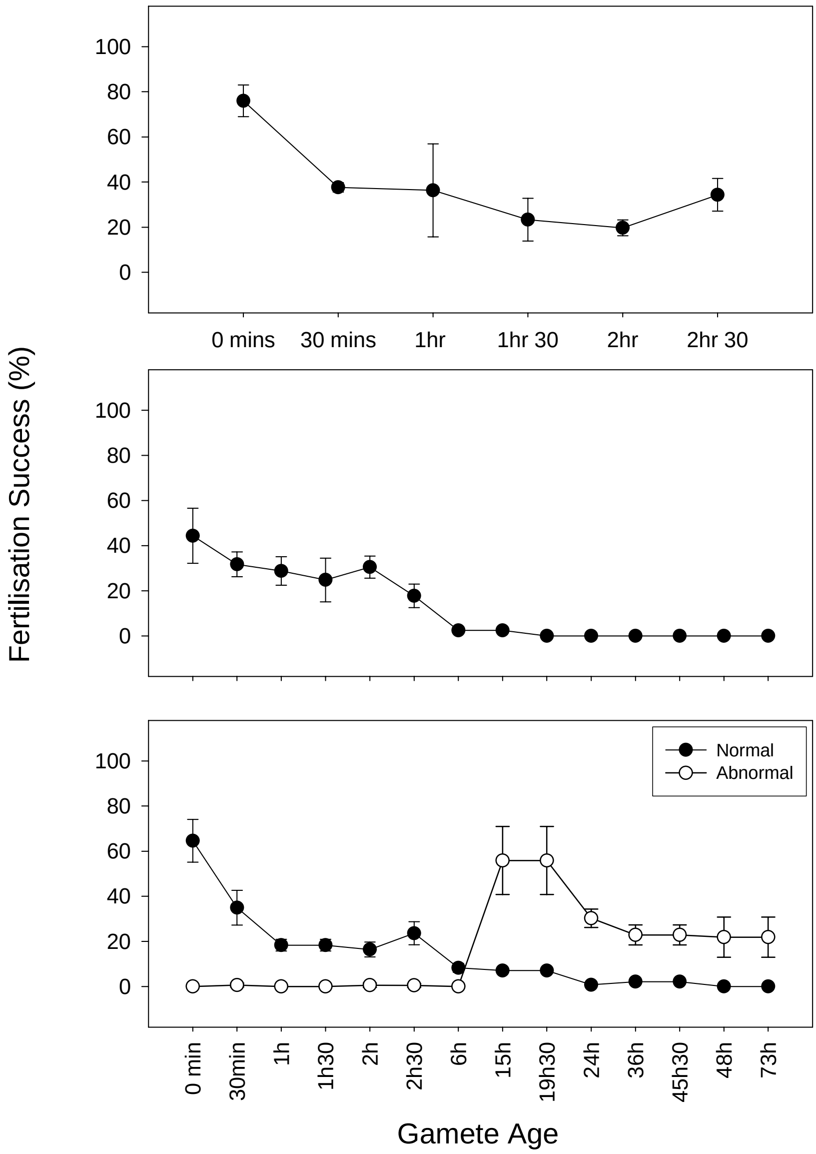

Fig. 3.3.8 *Odontaster validus*. The effect of gamete age on fertilisation success. This experiment was repeated on three separate occasions using one male and one female starfish. A different male-female paring was used during each trial. Data are presented as overall means ± SD of three replicate egg counts. Data are based on normal, abnormal and unfertilised egg counts, although the unfertilised egg counts are not plotted. No abnormal development was apparent in the first two trials. Temperature was maintained at 0.5˚C ± 0.5˚C.

The majority of eggs were fertilised using recently spawned gametes. A steady decline in fertilisation success was evident with gamete age. Fertilisation was still possible, albeit at a low level, between gametes aged ≥ 6 hours and 24 hours. Low levels of fertilisation success were coincident with an increase in the number of abnormal embryos produced. A large proportion of eggs were not fertilised by sperm aged in excess of 24hours.

Contact Time between Egg and Sperm *Marthasterias glacialis* (Fig. 3.3.9)

Maximum fertilisation success was low in this trial (40%). No successful sperm-egg interactions were recorded before 5 minutes of contact had elapsed between gametes. After this point, fertilisation success generally increased with sperm-egg contact time. Maximum success was recorded after 2hrs, although a similar number of successful interactions were observed between gametes exposed to contact times between 5mins and 2hours.

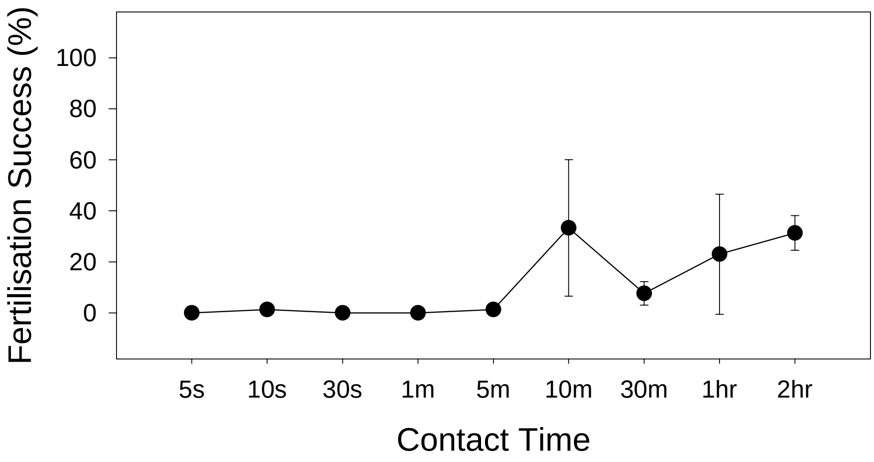

Fig 3.3.9 *Marthasterias glacialis*. The effect of egg and sperm contact time on fertilisation success. This plot represents the fertilisation success between a single male and female. Data are presented as overall means of three replicate egg counts ± SD. Data are based on normal fertilisation and unfertilised egg counts. No abnormal fertilisation was observed. Temperature was maintained at 12˚C ± 0.5˚C.

Contact Time between Egg and Sperm *Odontaster validus* (Fig. 3.3.10)

Number of successful sperm and egg interactions increased with contact time between gametes, at least over the early stages of the trials. A large proportion of eggs (~70%) was fertilised after only limited contact between egg and sperm (5 seconds-1 minute). Maximum fertilisation success (100%) was observed after a 5 minute contact period between gametes. Prolonged contact times above 5 minutes accrued no obvious benefits to fertilisation success.

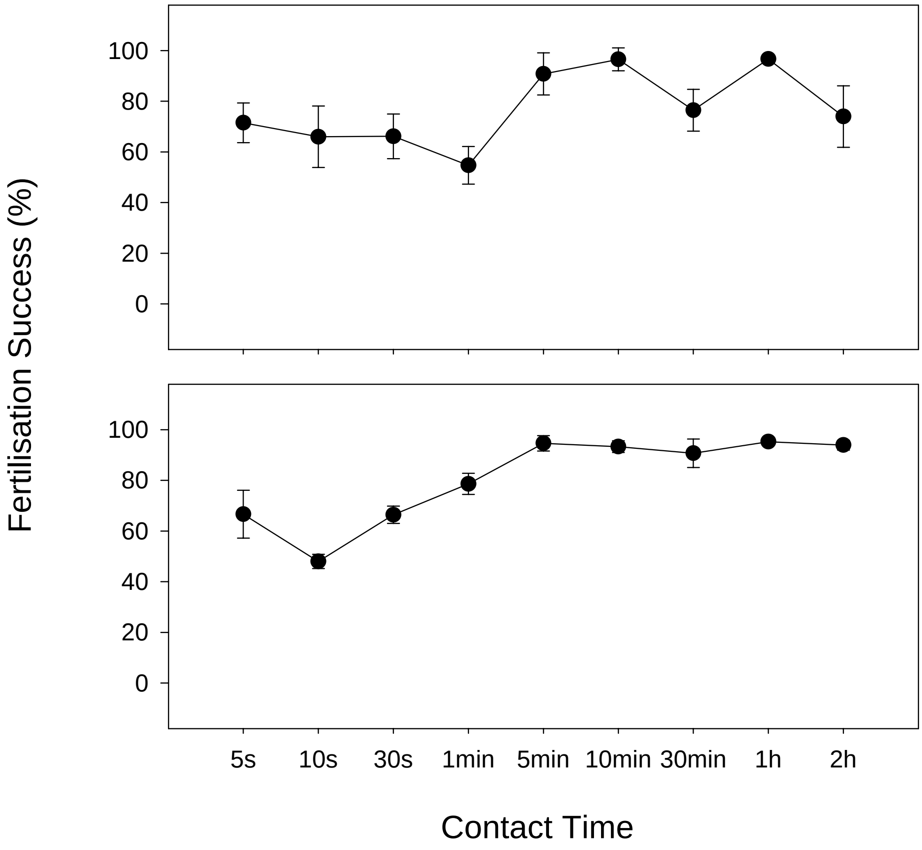

Fig. 3.3.10 *Odontaster validus*. The effect of egg and sperm contact time on fertilisation success. This experiment was repeated on two separate occasions using one male and one female starfish. A different male-female paring was used during each trial. Data are presented as overall means ± SD of three replicate egg counts. Data are based on normal fertilisation and unfertilised egg counts, although the unfertilised egg counts are not plotted. No abnormal fertilisation was observed. Temperature was maintained at 0.5˚C ± 0.5˚C.

Viscosity *Marthasterias glacialis* (Fig. 3.3.11)

The effect of seawater viscosity on fertilisation success was assessed using increasing quantities of PVP. Fertilisation success decreased with increasing seawater viscosity. A rapid decline in the number of fertilised eggs resulting in normal egg development was observed in seawater solutions with a viscosity of > 3.5cp. Very low egg numbers were fertilised at seawater viscosities > 4.0cp. There was a significant difference between the number of eggs fertilised above and below a seawater viscosity of 4.0cp (Mann Whitney W = 540.0, P < 0.001). A consistent increase in abnormal egg development was observed with increasing seawater viscosity.

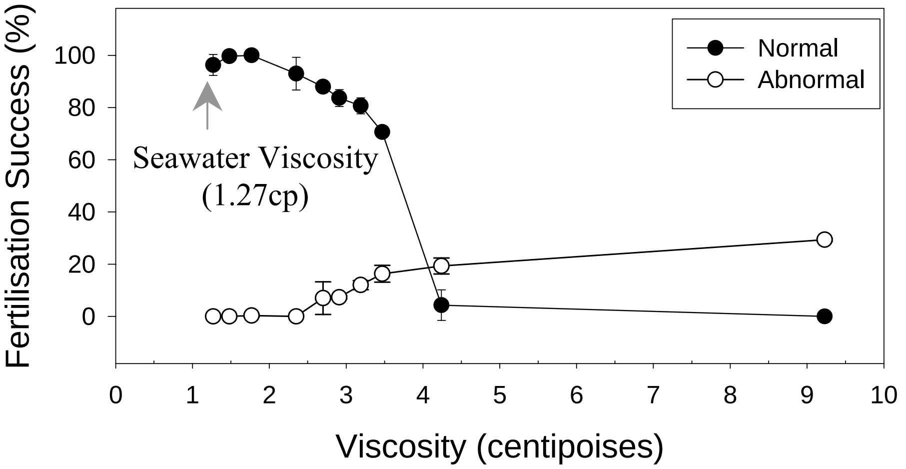

Fig. 3.3.11 *Marthasterias glacialis*. The effect of seawater viscosity on fertilisation success. This plot represents the fertilisation success between a single male and female. Data are presented as overall means ± SD of three replicate egg counts. Data are based on normal, abnormal and unfertilised egg counts, although the unfertilised egg counts are not plotted. Temperature was maintained at 12˚C ± 0.5˚C.

Viscosity *Odontaster validus* (Fig. 3.3.12)

Preliminary work suggested a negative effect of increased viscosity on fertilisation success. This initial trial used PVP to increase the viscosity of seawater artificially. There was a significant difference between the levels of success recorded in natural seawater and in a PVP-seawater solution (2 sample T-test with equal variance (Table. 3.3.1) T value = 8.29, DF = 4, P = 0.001). A number of attempts were made to repeat this experiment using *Odontaster validus*. However, competence to spawn amongst the sample population was low. Therefore, *Laternula elliptica* was used instead.

Table. 3.3.1 *Odontaster validus*. Test for equal variance in fertilisation success between normal seawater and PVP-seawater conditions.

Test	Test Statistic	P-value
F-Test	1.79	0.717
Levene's Test	0.11	0.759

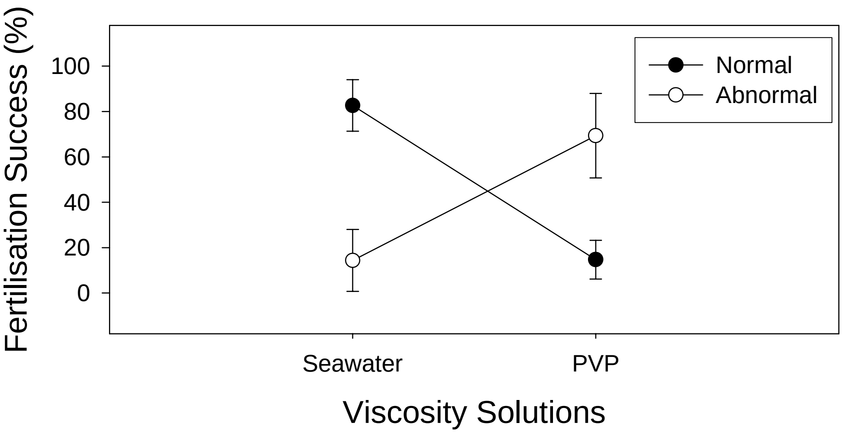

Fig. 3.3.12 *Odontaster validus*. The effect of seawater viscosity on fertilisation success. Data are presented as overall means ± SD from three replicate egg counts. Data are based on normal, abnormal and unfertilised egg counts, although the unfertilised egg counts are not plotted. Temperature was maintained at 0.5˚C ± 0.5˚C.

Viscosity *Laternula elliptica* (Fig. 3.3.13)

Experiments tested the effects of three separate chemical agents, including PVP (glycerol and methylcellulose) on the fertilisation success of the infaunal Antarctic bivalve, *Laternula elliptica*. *L. elliptica* was strip spawned and the sperm and egg were siphoned from separate regions of the gonad to avoid mixing and premature fertilisation. A general trend between increasing seawater viscosity and decreasing fertilisation success was evident in each trial. Maximum fertilisation success was observed under normal seawater conditions. The frequency of normal egg development decreased with even small increments in viscosity and low levels of success were recorded at viscosities ≥ 4.0cp. A similar trend was observed both in the temperate starfish and between chemical agents.

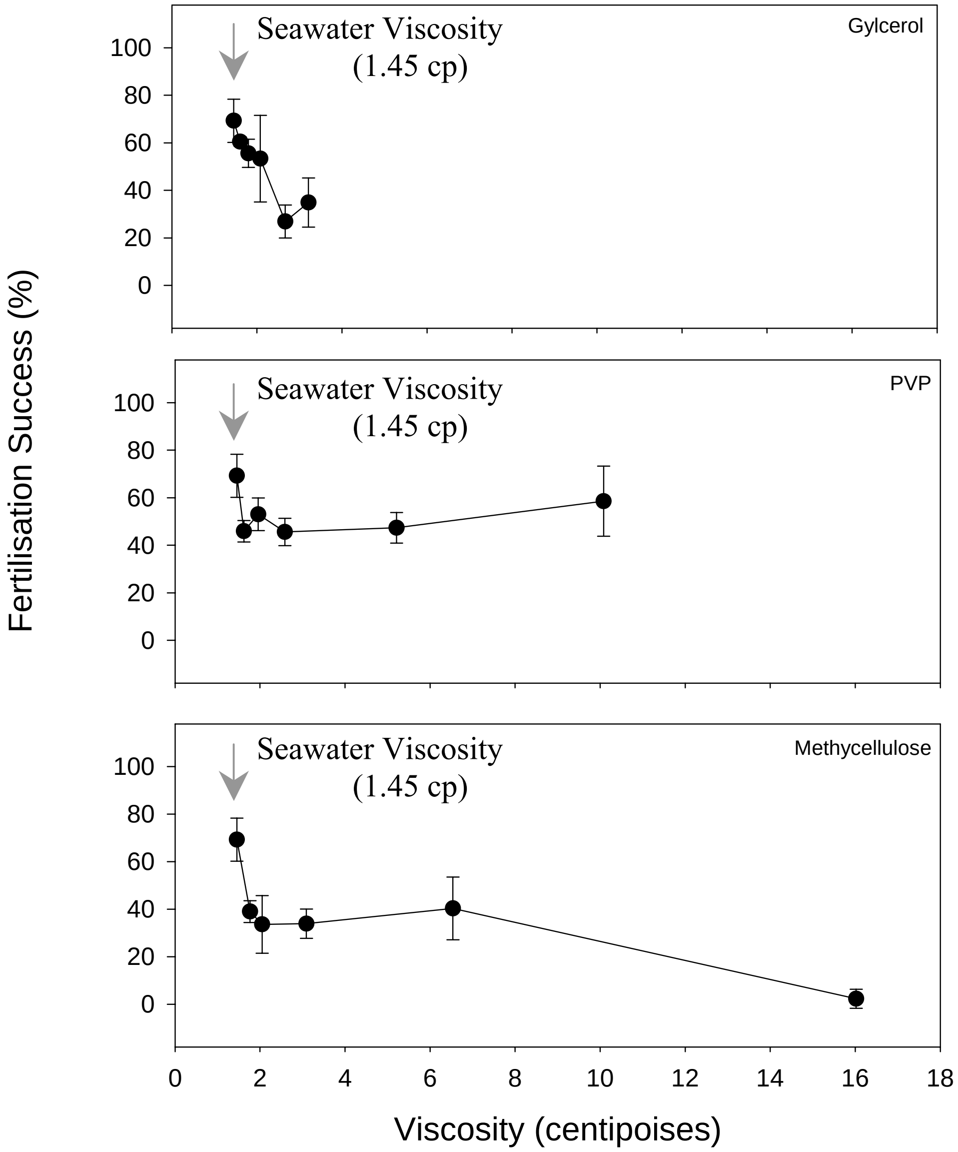

Fig. 3.3.12 *Laternula elliptica*. The effect of seawater viscosity on fertilisation success. This plot represents the fertilisation success between a single male and female. Data are presented as overall means ± SD of three replicate egg counts. Data are based on normal fertilisation and unfertilised egg counts, although the unfertilised egg counts are not plotted. No abnormal fertilisation was observed. Temperature was maintained at 0.5˚C ± 0.5˚C.

A significant difference in fertilisation success was recorded between seawater viscosities above and below 4.0cp, when methylcellulose was used to alter seawater viscosity artificially (2-sample T-test with equal variance (Table. 3.3.2) T value = -2.39, DF = 16, P = 0.030), although there was no significant difference between the effects of seawater viscosities above and below 4.0cp on fertilisation success, when PVP was used (2-sample T-test with equal variance (Table. 3.3.2) T value = -0.09, DF = 16, P = 0.930). Further increases in seawater viscosity had little influence on fertilisation success, with the exception of zero success after sperm were acclimated to the most viscous seawater solution (16.0cp using methylcellulose). Seawater viscosity was altered less by the additions of glycerol (maximum = 3.5cp). However, a significant difference in success was recorded between seawater viscosities above and below 2.0cp using glycerol (2-sample T-test with equal variance (Table. 3.3.2) T-value = -5.48, DF = 16, P <0.001).

Table. 3.3.2 *Laternula elliptica*. Test for equal variance in fertilisation success between normal seawater conditions and enhanced seawater viscosities using PVP, glycerol and methylcellulose.

Test	Test Statistic	P-value
Glycerol		
F-Test	0.66	0.678
Levene's Test	0.00	0.950
PVP		
F-Test	1.06	0.868
Levene's Test	0.12	0.732
Methylcellulose		
F-Test	1.76	0.406
Levene's Test	1.56	0.229

Egg Jelly *Odontaster validus* (Fig. 3.3.14)

Fertilisation success was optimal under normal seawater and egg conditions in *Odontaster validus* (100%). The removal of the jelly coat from starfish eggs did significantly affect fertilisation success (2-Sample T test with equal variance (Table. 3.3.3) T value = 5.00, DF = 4, P = 0.038), and reduced the number of successful sperm-egg interactions recorded from ~100% to ~80%.

Table. 3.3.3 *Odontaster validus.* Test for equal variance in fertilisation success between normal egg conditions and the removal of the jelly coat.

Test	Test Statistic	P-value
F-Test	0.16	0.273
Levene's Test	0.40	0.564

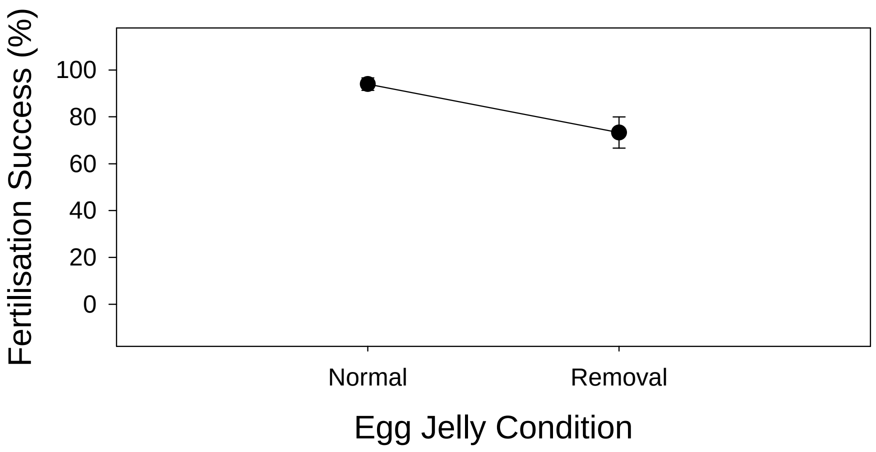

Fig. 3.3.14 *Odontaster validus*. The effect of egg jelly coat removal on fertilisation success. This plot represents the fertilisation success between a single male and female. Data are presented as overall means ± SD of three replicate egg counts. Data are based on normal fertilisation and unfertilised egg counts, although the unfertilised egg counts are not plotted. No abnormal fertilisation was observed. Temperature was maintained at 0.5˚C ± 0.5˚C.

Fecundity *Odontaster validus* (Fig. 3.3.15)

A range in female fecundity between 1.18E+05 and 6.83E+05 eggs in 1L (mean = 4.48E+05 eggs, SD = 2.07E+05, n = 8) was recorded. The variability in egg number between females was size related, in terms of both wet weight and radial length (R) (Table. 3.3.4).

Table. 3.3.4 *Odontaster validus*. Correlations between female fecundity and female wet weight (±0.01g) and size (R and r ±0.01mm) using Pearsons Product Moment Correlation. All variables were normally distributed.

Variables	Test Statistic	P-value
Fecundity vs. Wet Weight	0.743	0.035
Fecundity vs. Radial Length (R)	0.830	0.011
Fecundity vs. Body Radius (r)	0.673	0.067

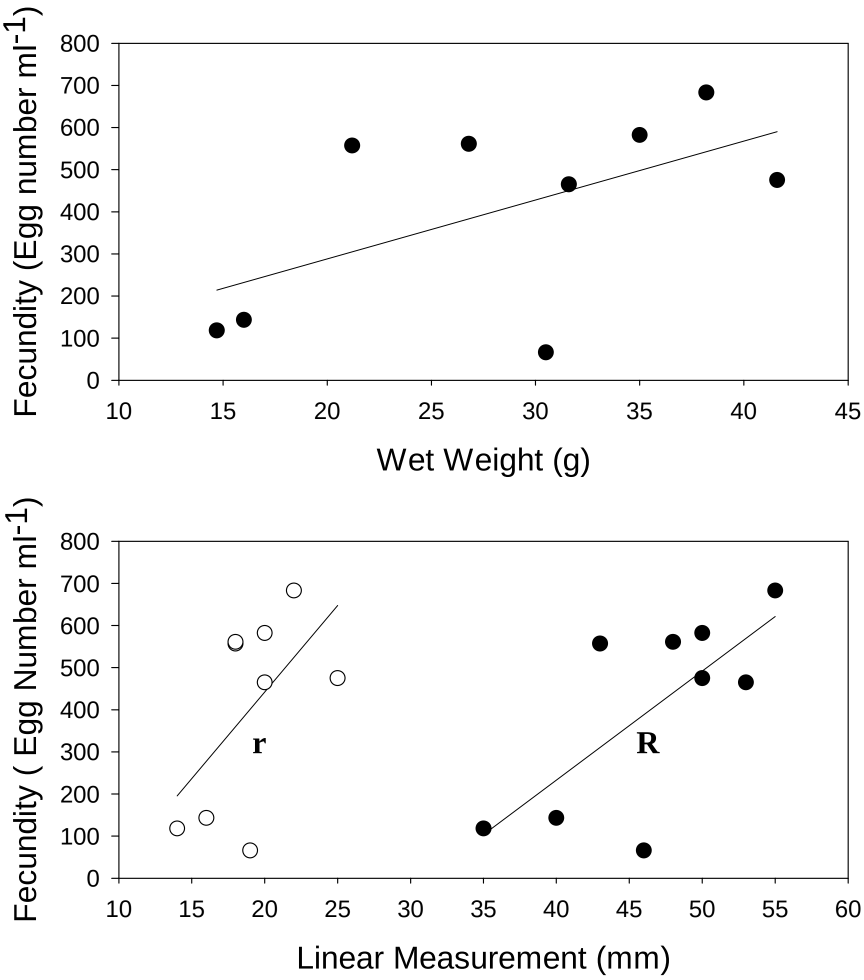

Fig. 3.3.15 *Odontaster validus*. The relationship between female fecundity and animal size (Weight, R and r). Data are presented as egg counts per female (mean = 4.48E+05 eggs, SD = 2.07E+05, n = 8).

Gamete Release *Marthasterias glacialis*

18 males were observed over an hour to quantify gamete release (Table. 3.3.5). The number of sperm released varied between males; 1.04E+09-5.15E+10 sperm. There was no significant correlation between individual size, in terms of wet weight or body size (R and r), and the number of sperm released (Table. 3.3.6). 15 females were observed over the same time period and the number of eggs quantified to determine female gamete release (Table. 3.3.5).

Table. 3.3.5 *Marthasterias glacialis*. Male (mean = 1.80E+10 sperm, SD = 1.86E+10, n = 18) and female (mean = 1.59E+06 eggs, SD = 1.40E+06, n = 15) gamete release (into 1L seawater) over an hour. Data are presented as overall means. Male and female wet weight (+0.01g) and size (R and r ±0.01mm) are also presented.

Date	Sex	Weight (g)	R (mm)	r (mm)	Gametes (ml)	Gamete in 1 L
24/04/2003	Male	61.18	85.00	10.00	4.85E+07	4.85E+10
25/04/2003	Male	46.85	80.00	15.00	4.21E+06	4.21E+09
26/04/2003	Male	52.74	85.00	15.00	8.24E+06	8.24E+09
27/04/2003	Male	47.23	80.00	10.00	4.59E+07	4.59E+10
19/05/2003	Male	63.59	90.00	13.00	2.70E+07	2.70E+10
20/05/2003	Male	64.85	85.00	10.00	5.15E+07	5.15E+10
20/05/2003	Male	72.00	100.00	15.00	1.01E+07	1.01E+10
23/05/2003	Male	86.49	102.00	15.00	3.86E+06	3.86E+09
01/04/2004	Male	84.0	75.00	10.00	2.71E+07	2.71E+10
01/04/2004	Male	52.0	83.00	13.00	6.45E+06	6.45E+09
06/04/2004	Male	49.0	65.00	10.00	6.22E+06	6.22E+09
09/04/2004	Male	74.0	84.00	10.00	5.41E+06	5.41E+09
12/04/2004	Male	41.0	70.00	9.00	4.67E+06	4.67E+09
15/04/2004	Male	122.0	107.00	12.00	1.04E+06	1.04E+09
19/04/2004	Male	56.0	79.00	9.00	6.42E+06	6.42E+09
21/04/2004	Male	51.0	89.00	10.00	5.10E+07	5.10E+10
21/04/2004	Male	69.0	91.00	10.00	1.27E+07	1.27E+10
27/04/2004	Male	61.0	75.00	10.00	3.38E+06	3.38E+09
22/04/2003	Female	60.35	110.00	14.00	852	8.52E+05
19/05/2003	Female	63.29	90.00	10.00	63	6.30E+04
20/05/2003	Female	72.08	95.00	15.00	1120	1.12E+06
20/05/2003	Female	72.02	85.00	15.00	5370	5.37E+06
20/05/2003	Female	54.60	80.00	10.00	3200	3.20E+06
23/05/2003	Female	62.45	100.00	10.00	2480	2.48E+06
06/04/2004	Female	60.00	77.00	10.00	56	5.60E+04
09/04/2004	Female	62.00	85.00	10.00	1603	1.60E+06
15/04/2004	Female	51.00	80.00	8.00	971	9.71E+05
15/04/2004	Female	62.00	85.00	10.00	2173	2.17E+06
19/04/2004	Female	70.00	87.00	10.00	705	7.05E+05
14/05/2004	Female	87.00	89.00	10.00	2553	2.55E+06
14/05/2004	Female	88.00	98.00	11.00	504	5.04E+05
15/05/2004	Female	70.00	87.00	11.00	915	9.15E+05
15/05/2004	Female	315.00	200.00	25.00	1348	1.35E+06

The maximum number of eggs released by a single female was 5.37E+06 eggs and a minimum of 5.60E+04 eggs was recorded in a different female. There was no clear correlation between female size and the quantity of gametes released (Table. 3.3.6).

Table. 3.3.6 *Marthasterias glacialis*. Correlations between the number of sperm and male wet weight (+0.01g) and size (R and r +0.01mm) using Pearsons Product Moment Correlation. Male wet weight and egg number were the only variables to be normally distributed (Appendix Table. 8). Therefore, all data were ranked prior to analysis and tested non-parametrically. Parametric testing on the non-ranked data produced the same statistical conclusions.

	Ranked Data		**Non-Ranked Data**	
Variables	**Test Statistic**	**P-value**	**Test Statistic**	**P-value**
Sperm vs. Wet Weight	-0.073	0.773	-0.195	0.439
Sperm vs. Radial Length (R)	0.104	0.683	-0.018	0.994
Sperm vs. Body Radius (r)	-0.189	0.453	-0.342	0.165
Egg vs. Wet Weight	0.055	0.844	-0.038	0.894
Egg vs. Radial Length (R)	-0.106	0.707	-0.089	0.753
Egg vs. Body Radius (r)	0.060	0.831	0.116	0.682

Gamete Release *Odontaster validus*

Odontaster validus could not be induced to spawn as easily as its temperate counterpart. No spawning or a very poor spawning response was often observed after the 1-methyadenine injection had been administered. Additional injections failed to improve the success rate. Therefore, the quantities described below should be interpreted with some caution. Eight males were used to quantify gamete release over the period of an hour (Table. 3.3.7). The number of sperm released varied between males; 1.73E+09-3.49E+10 sperm.

Table. 3.3.7 *Odontaster validus.* Male (mean = 1.41E+10, SD = 1.61E+10, n = 8) and female (mean = 8.40E+4 eggs, SD = 8.78E+4, n = 9) gamete release over an hour. Data are presented as overall means. Male and female wet weight (+0.01g) and size (R and r +0.01mm) are also presented.

Date	Sex	Weight (g)	R (mm)	r (mm)	Gametes (ml)	Gametes in 1L
22/07/2003	Male	10.20	33.00	15.00	1.73E+06	1.73E+09
22/07/2003	Male	19.60	45.00	20.00	1.12E+07	1.12E+10
22/07/2003	Male	11.90	38.00	15.00	1.20E+07	1.20E+10
21/07/2003	Male	8.40	34.00	13.00	2.48E+06	2.48E+09
21/07/2003	Male	19.50	42.00	16.00	5.56E+06	5.56E+09
31/07/2004	Male	38.25	52.00	22.20	3.49E+07	3.49E+10
31/07/2004	Male	24.15	41.60	19.20	2.23E+07	2.23E+10
05/08/2004	Male	21.62	42.00	17.70	2.29E+07	2.29E+10
21/07/2003	Female	7.30	35.00	14.00	113	1.13E+05
21/07/2003	Female	13.60	38.00	16.00	57	5.70E+04
21/07/2003	Female	23.20	43.00	20.00	270	2.70E+05
22/07/2003	Female	24.10	47.00	19.00	177	1.77E+05
02/07/2004	Female	19.10	41.10	15.00	16	1.60E+04
02/07/2004	Female	11.70	30.00	11.00	35	3.53E+04
31/07/2004	Female	16.29	39.90	15.90	5	4.67E+03
31/07/2004	Female	18.23	43.90	19.00	47	4.65E+04
10/08/2004	Female	17.12	41.00	17.00	37	3.70E+04

These data were comparable with the sperm concentrations described for *Marthasterias glacialis*. However, the number of sperm released in the polar starfish was significantly correlated with individual size, in terms of wet weight, radial length and body radius (Table. 3.3.8). Nine females were monitored over an hour and the numbers of eggs spawned were quantified to determine female gamete release (Table. 3.3.7). The number of eggs released varied between extremes of 4.67E+03 and 2.70E+05 eggs. There was no correlation between the egg numbers released and female size, in terms of wet weight or body size (R and r) (Table. 3.3.8).

Table. 3.3.8 *Odontaster validus.* The correlation between number of sperm and male wet weight (±0.01g) and size (R and r ±0.01mm) using Pearsons Product Moment Correlation. All male variables were normally distributed. All female variables were normally distributed except egg number. Therefore, the female wet weight, size and egg number were ranked prior to analysis and tested non-parametrically. Parametric testing on the non-ranked data produced the same statistical conclusions.

	Ranked Data		**Non-Ranked Data**	
Variables	**Test Statistic**	**P-value**	**Test Statistic**	**P-value**
Sperm vs. Wet Weight	0.883	0.004		
Sperm vs. Radial Length (R)	0.850	0.008		
Sperm vs. Body Radius (r)	0.817	0.013		
Egg vs. Wet Weight	0.094	0.809	0.200	0.606
Egg vs. Radial Length (R)	0.152	0.697	0.217	0.576
Egg vs. Body Radius (r)	0.053	0.893	0.067	0.864

Competence to Spawn

On 14 dates between January 2004 and August 2004, samples of 10-20 individual starfish were injected with 1-methyladenine to assess their competence to spawn. There was a very weak spawning response in the sampled population during the late austral summer (January-March). The radial length (‘R’) of individuals injected ranged between extreme values of 17.20 to 55mm (mean = 36.62mm, SD = 6.64, n = 165), equivalent to a body radius (‘r’) of 11.0 to 26.10mm (mean = 16.54mm, SD = 3.40, n = 165). Starfish injected covered a range in wet weight 3.90 and 52.30g (mean = 19.13g, SD = 9.41, n = 165). No starfish could be induced to spawn during January. However, a small percentage of males released sperm into the surrounding seawater in February and March after being injected (3% and 7% respectively). The competence to spawn increased during the austral winter. 55% of individuals spawned during May and 85% spawned during June. The majority of these individuals were female (82% and 65% respectively). A significant decline in competence occurred between July and August, when only a single female could be induced. Many individuals displayed a classic spawning posture after being injected (65%). However, spawning did not occur in all of the individuals injected, even during the peak months.

There was a significant difference between the size of the competent and non-responsive individuals, with the exception of radial length (Table. 3.3.9).

Table. 3.3.9 *Odontaster validus*. A Mann Whitney statistical test was used to compare the size (R and r ±0.01mm) and wet weight (±0.01g) of competent and non-responsive starfish. The majority of the size variables were not normally distributed, except radial length in the competent and non-responsive individuals (Appendix Table. 9). Therefore, the data were tested non-parametrically.

Variables	Test Statistic	P-value
Weight	W = 3068.5	0.039
Radial Length (R)	W = 2546.5	0.914
Body Radius (r)	W = 3101.0	0.028

In both males and females, there was no significant difference between the individuals competent to spawn and the mean size of all starfish injected (Table. 3.3.10-3.311).

Table. 3.3.10 *Odontaster validus*. A Mann Whitney statistical test was used to compare the size (R and r ±0.01mm) and wet weight (±0.01g) of competent males and females with the total number of starfish injected. The size and weight variables of the competent males and females and the total number of starfish injected were normally and not normally distributed (Appendix Table. 10).

Variables	Test Statistic	P-value
Injected Weight vs Competent Males	W = 14246.5	0.079
Injected R vs Competent Males	T = -1.20	0.230
Injected r vs Competent Males	W = 14297.5	0.154
Injected Weight vs Competent Females	W = 15205.5	0.341
Injected R vs Competent Females	T = -0.62	0.537
Injected r vs Competent Females	W = 15122.0	0.189

Table. 3.3.11 *Odontaster validus*. Test for equal variance in radial length (R ±0.01mm) between competent males and injected starfish and competent females and injected starfish.

Test	Test Statistic	P-value
Competent Males and Injected Starfish		
F-test	0.83	0.592
Levene's test	0.15	0.699
Competent Females and Injected Starfish		
F-test	0.85	0.690
Levene's test	0.40	0.530

Sperm Swimming Speed (Fig. 3.3.16)

The wet weight (± 0.01g) and size (± 0.01mm) of 9 *Laternula elliptica* and 2 *Parborlasia corrugatus* were measured and the individuals strip spawned to investigate sperm swimming speed (Table. 3.3.12).

Table. 3.3.12 *Laternula elliptica* and *Parborlasia corrugatus*. Wet weight (±0.01g) and size (±0.01mm) of bivalves (n = 9) and nemerteans (n = 2) strip spawned to investigate sperm swimming speed.

Sample	Wet Weight (g)	Length (mm)	Width (mm)	Height (mm)
17/8/04 Laternula	84.39	59.1	96.5	42.1
21/8/04 Laternula	70.41	55.30	80.10	44.00
21/8/04 Laternula	71.90	55.50	74.80	43.60
22/8/04 Laternula	165.58	84.10	119.60	58.80
22/8/04 Laternula	70.85	59.00	78.30	41.70
26/8/04 Laternula	122.97	70.90	95.00	48.30
26/8/04 Laternula	78.87	55.20	78.30	42.00
27/8/04 Laternula	67.72	57.00	84.40	37.10
27/8/04 Laternula	73.58	61.00	84.30	39.00
19/8/04 Parborlasia	51.12	220.00	33.30	n/a
20/8/04 Parborlasia	53.84	200.00	40.00	n/a

The number of successful experimental trials for both species was limited, owing to experimental constraints. These data represent a crude measure of sperm swimming speed and the effect of both temperature and viscosity. Swimming velocity increased in both *Laternula elliptica* and *Parborlasia corrugatus* with increasing temperature. Speeds were highest at 6ºC, where sperm reached 73µm s^{-1} and 42µm s^{-1} in *L. elliptica* and *P. corrugatus* respectively. Swimming speed increased linearly with temperature in *L. elliptica*, however speeds in *P. corrugatus* reached a plateau at ~30µm s^{-1} between 2-4ºC, after an initial increase. Velocities increased again to

~48µm s^{-1} at 6˚C. Changes in sperm swimming speed with seawater viscosity were monitored in *P. corrugatus* and viscosity had a marked effect on sperm velocity. Swimming speeds decreased with increasing seawater viscosity, with a maximum speed recorded in normal seawater conditions (~22µm s^{-1}). Sperm swimming speed decreased by 53% in *P. corrugatus,* when the temperature of seawater was reduced from 0ºC (mean = 21.50µm s^{-1}) to –1.9ºC (mean = 10.00µm s^{-1}). When seawater temperatures were maintained at 0.5ºC and only viscosity was adjusted to reflect a drop in temperature from 0ºC to –1.9ºC, sperm swimming speed declined by 25% from 25.10µm s^{-1} to 18.85µm s^{-1}. Thus, about 47% of the decline in speed was attributable to changes in seawater viscosity and the remaining 53% to other effects of temperature.

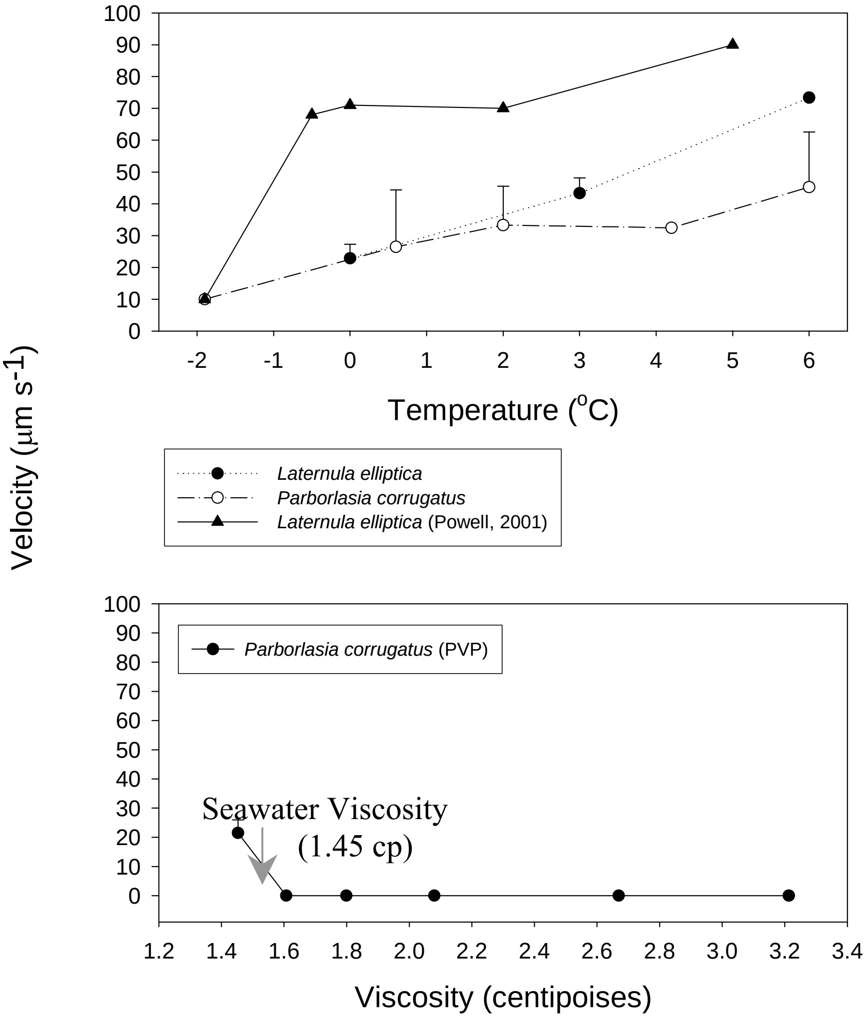

Fig. 3.3.16 *Laternula elliptica* and *Parborlasia corrugatus*. The effect of temperature and viscosity on sperm swimming speed. A single individual was used for each trial and the data are presented as overall means in swimming speed ± SD of three replicate sperm. The experimental temperature was maintained at 0.5˚C ± 0.5˚C when seawater viscosity was varied.

Experimental Constraints

- The spawning response of a number of *Odontaster validus* was poor, especially during the 2004 experimental trials. The long period of transit and artificial aquarium surroundings may have had a detrimental effect on the spawning competence of these individuals. Handling stress may have also altered the normal allocation of energy resources and metabolism in these starfish, possibly diverting reserves to requirements for body maintenance away from reproduction. This behaviour resulted in a number of trials being abandoned and owing to the limited period over which spawning is viable naturally, limited the number of trials conducted. However, on most occasions each trial was completed in triplicate.
- Unfortunately only a single trial was completed to assess temperature effects and the effect of contact time between gametes on fertilisation success in the temperate starfish. This was a result of logistics problems with essential equipment. The thermocirculator and thermogradient block system required for the temperature trial and the Nitex strainers required to assess the effect of contact time, were used in the Antarctic field season during November 2003-March 2004. These items were in transit from Antarctica until late May 2004, at which point laboratory spawning could no longer be induced.
- The counting of sperm using a haemocytometer proved very labour intensive and complicated. Therefore, later experimental trials were quantified using a Coulter Multisizer II. This procedure did save on time and similar, consistent counts were obtained with both methods (Table. 3.2.2).
- The over and underestimation of fertilisation success can occur under laboratory conditions. Fertilisation success was quantified by scoring the presence of a fertilisation membrane around each oocyte. Fertilisation was recorded as ‘normal’ if a fertilisation envelope was scored, unless obvious oocyte abnormalities or polyspermy were evident. However, the presence of a fertilisation membrane does not always result in normal larval development, which can progress abnormally even if early signs are positive (Styan, 1998). This may partly explain the scoring of abnormal development in some experimental trials, but its absence from others even though the same variable is under test. Therefore, many studies score the occurrence of cleaved eggs

instead (Oliver and Babcock, 1992; Desrosiers *et al.* 1996; Keesing and Babcock, 1996). However, the time and resource limitations precluded prolonged observations, and a simpler and earlier means of measuring fertilisation success was used. The artificial induction of spawning can also sometimes cause the release of unripe, unfertilisable eggs (Styan and Butler, 2000), which inevitably reduces the potential number of successful sperm-egg interactions recorded, compared to what would normally be observed in natural populations. However, by choosing to undertake laboratory trials during the natural and published spawning seasons of both starfish, and checking the appearance of the gametes prior to trials, I hoped to minimise this potential problem. These starfish also naturally release 1-methyladenine prior to spawning where it acts as a maturation inducing substance (MIS).

- The assessment of sperm swimming and the effects of temperature and viscosity were difficult to measure and very labour intensive. An automated analysis of the rate of swimming would have been preferable to measuring sperm activity by hand, frame by frame, e.g. a sperm tracker. However, financial constraints precluded this option. It was also difficult to attain an optimum sperm concentration. If the solutions were too concentrated a single sperm could not be isolated, however if the solution was too dilute the sperm cells were very difficult to distinguish. Tracking individual sperm was also made further complicated as most exhibited a helical path of motion and rarely swam along a direct course. Additionally, a number of *Parborlasia corrugatus* were lost after transit preventing more trials.
- Sperm swimming was quantified from a small volume of sperm solution placed on a microscope slide. Several theoretical treatments have suggested that the drag forces associated with motion on the surface of a microscope slide, and exerted on sperm, significantly affect sperm swimming speed and have caused velocity to be exaggerated in the past (Gee and Zimmer-Faust, 1997). Although important, the main objective of this study was not to quantify absolute velocity, but was to observe variations in behavioural patterns and sperm swimming speed under changing external conditions. Additionally, sperm motility can be highly variable even in the absence of physical forces, especially when the influence of sperm age (Levitan, 2000) and paternity are considered (Gee and Zimmer-Faust, 1997).

3.4 Discussion

Sperm Dilution

Sperm limitation was extremely important in the fertilisation success of both the temperate and polar starfish (see Fig. 3.3.1 and Fig. 3.3.2). High numbers of eggs were successfully fertilised over a similar range in both starfish (10^6-10^5 sperm ml^{-1}). Similarly high numbers of eggs were successfully fertilised by sperm concentrations as low as $10^3 ml^{-1}$, but only in *M. glacialis*. In general, *Odontaster validus* required an order of magnitude more sperm than the temperate *Marthasterias glacialis* to achieve optimum fertilisation success. Powell *et al.,* (2001) observed a similar disparity between the concentration of sperm required to optimally fertilise the eggs of the Antarctic limpet and bivalve, *Nacella concinna* and *Laternula elliptica* respectively, compared to comparable temperate molluscs. These patterns may simply reflect the inherent differences between the two species gametes. Alternatively, it may be the harsh external conditions endured by the sperm, which dictates this characteristic. Success levels dropped off dramatically in *M. glacialis* below concentrations of $10^2 ml^{-1}$. However, this was not observed in *O. validus*. Moderate-low fertilisation success (20-40%) was observed in this species even at the lowest sperm concentrations tested. This may be the result of either one or a number of differences in the gametes of the starfish, such as longevity or chemotaxis.

Both starfish achieved high fertilisation levels in the laboratory (85-100%). A number of field studies observing echinoderms spawning have recorded high levels of spawning success (*Acanthaster planci*: 83% Babcock and Mundy, 1992; *Cucumaria miniata*: 86-99% Sewell and Levitan, 1992; *C. frondosa*: 60-85% Hamel and Mercier, 1995). However, comparisons between induced and natural spawning success tend to be complicated by the unpredictable nature of echinoderm spawning episodes (Minchin, 1987; Babcock *et al.*, 1992).

Polyspermy was common at high sperm concentrations in both starfish ($\geq 10^6 ml^{-1}$). Benzie *et al.*, (1994) observed that the larvae developed from the fertilisation of *A. planci* at a laboratory sperm concentration of 10^5 sperm ml^{-1} were often malformed, and they attributed these abnormalities to polyspermy. Invertebrates are able to counter potential polyspermy of their eggs by employing blocks (Styan, 1998). These blocks are extremely effective in avoiding the consequences of high sperm concentrations and are common to many taxa (McClary, 1992; Styan and Butler, 2000). However they may differ in the process by which they act, being either fast-acting electrical blocks or employing a slower cortical reaction. Species-specific differences in polyspermy blocks and their presence or absence in an egg may partly explain the difference between the sperm concentrations observed to cause polyspermy in the starfish of the present study. Styan and Butler (2000) also observed polyspermy in two species of scallop, *Chlamys bifrons* and *C. asperrima* at sperm concentrations >10^4 sperm ml^{-1}. A more rapid decline in success was recorded for *C. bifrons* suggesting this species is more sensitive to polyspermy and less effective at minimising abnormalities at high sperm concentrations. This may suggest that the polyspermy blocks in *O. validus* are either more numerous, or maybe more efficient, than those associated with the eggs of *M. glacialis*, withstanding higher concentrations of sperm before succumbing to abnormal effects.

Many studies have observed sperm limitation of reproductive and fertilisation success of free spawning invertebrates (Pennington, 1985; Denny and Shibata, 1989; Levitan, 1995). In the present study spawning success was greatly limited below sperm concentrations of 10^2 sperm ml^{-1} and 10^4 sperm ml^{-1} in *M. glacialis* and *O. validus* respectively (see Fig. 3.3.1 and Fig. 3.3.2). A gradient in fertilisation success with decreasing sperm concentration has also been recorded in the crown-of-thorns starfish (Benzie and Dixon, 1994). This is a common trait in many fertilisation studies, including those that have described the spawning success of echinoderms (*Strongylcentrotus droebachiensis:* ~ 0% at <10^4 sperm ml^{-1} Pennington, 1985; *S. franciscanus*: 18% at 4.7×10^3 sperm ml^{-1} Levitan *et al.*, 1991) and molluscs (*Cerastoderma edule*: ~0% at 10^3 sperm ml^{-1} André and Lindegarth, 1995; *Chlamys bifrons* and *C. asperrima*: 18% and 35% at 10^2 sperm ml^{-1} Styan and Butler, 2000; *Haliotis tuberculata*: 20% at 10^3 sperm ml^{-1} Baker and Tyler, 2001). Concentrations between 10^4-10^5 sperm ml^{-1} also produced a low percentage of fertilised eggs in an Antarctic bivalve and limpet (Powell *et al.*, 2001).

Temperature

There was a striking difference between *M. glacialis* and *O. validus* in fertilisation success with temperature (see Fig. 3.3.3 and Fig. 3.3.4). Moderate-high fertilisation success was achieved by *M. glacialis* within a broad temperature window (8-19ºC). The optimum number of eggs were fertilised at 12.5ºC. However, spawning success was limited in *O. vali*dus to temperatures below 6ºC and optimum fertilisation levels were restricted over a much narrower temperature range (-1 to +4ºC). Development was abnormal in the Antarctic starfish above these temperatures. Stanwell-Smith and Peck (1998) also showed increasing numbers of non-viable eggs and embryos above 2ºC in *O. meridionalis* from Signy Island, Antarctica. However for *O. validus*, the number of non-viable eggs and embryos did not increase with temperature (-2 to +3ºC), averaging approximately 12.5% throughout Stanwell-Smith and Peck's study. The broader range of temperatures tested in the present study (-2 to +14ºC) may explain the higher frequency of abnormal fertilisation observed at increasing temperatures in this starfish compared to the previous investigation by Stanwell-Smith and Peck (1998). Differences in egg quality between the two studies and the possibility for local adaptations in fertilisation and embryonic development may also be partly accountable for the differences observed.

Hagström and Hagström (1959) reported that elevated temperatures above normal result in reduced fertilisation with associated increases in polyspermy and abnormal cleavages. They observed that a 10-12 degree rise in temperature above normal would commonly result in pathological larvae. In this study fertilisation was reduced to zero at a temperature of 25ºC in *M. glacialis*, some 10ºC above normal. This was also the case in the sea urchin *Parechinus angulosus* (Greenwood and Bennett, 1981). However, temperature was much more restrictive in *O. validus* with zero fertilisation recorded above only a 6ºC rise in ambient temperature.

Nichols and Barker (1984) observed spawning during May-June in the Plymouth Sound population of *M. glacialis*. Seawater temperatures during May were ~12ºC and rose to ~16ºC by July/August. Fertilisation trials were undertaken in the laboratory during a similar time of year in the present study, where high fertilisation success was recorded between 10-14ºC, which corresponds well with observed

spawning in the natural population. A similar rise in seawater temperature was observed by Minchin (1987) in Mulroy Bay, Ireland and was identified as the main spawning cue for *M. glacialis*. Fertilisation was viable over a broad temperature range, in keeping with the considerable fluctuation in temperature recorded both seasonally and annually in Plymouth Sound. Temperatures rarely rose above 18ºC or fell below 5ºC during Nichols and Barker's (1984) study in accordance with with the current study, where fertilisation was adversely affected above 19ºC and below 7ºC.
O. validus spawns during the austral winter (Pearse, 1965, 1969; Stanwell-Smith and Peck, 1998). The current study sampled individuals from South Cove, adjacent to Rothera Research Station on the West Antarctic Peninsula, where winter temperatures rarely rise above +1ºC and fluctuate within a narrow range (Grange *et al.,* 2004). Therefore, the range of temperatures over which an optimum number of eggs was fertilised in the current study is comparable to the timing and occurrence of these temperatures in relation to spawning in natural populations of this starfish. No eggs were normally fertilised at and above 6ºC, after which development proceeded abnormally. A narrow window of opportunity for successful fertilisation with rising temperatures has been recorded before in the Antarctic in an echinoid (*Sterechinus neumayeri*) at Signy Island, Antarctica (Stanwell-Smith and Peck, 1998) and in the infaunal bivalve *Laternula elliptica* at Rothera Research Station (Powell *et al.,* 2001). The number of non-viable eggs and embryos increased for *S. neumayeri* above 1.7ºC, although embryonic development rate remained relatively constant above 0.2ºC (Stanwell-Smith and Peck, 1998). Therefore, a window of optimal temperature between 0.2Cº and 1.7ºC was proposed for this population of the Antarctic urchin. 80% fertilisation success in *L. elliptica* was recorded between –1.9 and +0.2ºC, where success declined rapidly between 0.7 and 5.0ºC. However, all fertilisation was abnormal at this maximum temperature. Consequently, several Antarctic invertebrates experience extremely poor fertilisation success in excess of 5-6ºC.

Benthic invertebrates, especially echinoderms, are extremely sensitive to water temperatures above normal ambient (Farmanfarmaian and Giese, 1963) and the thermal tolerance of cells is correlated well with the thermophily of the species concerned (Andronikov, 1975). Antarctic invertebrates are highly stenothermal (Arnaud, 1977; Peck and Conway, 2000) and possess functional limits within a very narrow range of thermal tolerance (Peck, 2002; Peck *et al.,* 2004). The upper lethal limit recorded for *O. validus* and *L. elliptica*, at which the functional scope to under take fundamental biological activity is diminished, is 5-6ºC (Peck, 2002), which corresponds well with the inability of these invertebrates to fertilise eggs normally at similar temperatures. Most Antarctic invertebrates operate within 20-50% of the temperature window observed for temperate species (Peck and Conway, 2000), which may explain the much broader capacity of *M. glacialis* to fertilise eggs successfully over a wider temperature range. This has important implications for how marine invertebrates are geographically distributed (Andronikov, 1975). Stanwell-Smith and Peck, (1998) concluded that the relationships between development rate and temperature and embryonic mortality and temperature may be important factors delineating the ability of species to colonise different habitats. Therefore, the relationship between fertilisation success and temperature may also affect distributions of Antarctic echinoderms.

Salinity

A decline in fertilisation success with decreasing salinity was observed in both the temperate and Antarctic starfish (see Fig. 3.3.5 and Fig. 3.3.6). Both inhabit typical marine sites where salinity is stable. Therefore, these data are in keeping with the conditions experienced in natural populations of both starfish and suggest both species are stenohaline in reproduction. Most echinoderms have been traditionally considered as stenohaline (Binyon, 1972), There are however exceptions where some species inhabit areas of seasonal or tidal fluctuating salinities (*Luidia clathrata* Hintz and Lawrence, 1994). However, in general fertilisation and development is optimal at the salinity at which parents are maintained (e.g. *Psammechinus miliaris* Gezelius, 1962; *Strongylcentrotus purpuratus* Dinnel *et al.*, 1987).

Zero and abnormal fertilisation success was observed in both *M. glacialis* and *O. validus* below a salinity of 28. A number of studies have recorded detrimental effects of low salinities on fertilisation success (Greenwood and Bennett, 1981; Griffin *et al*., 1998; Litvik and Trippel, 1998), including very low and zero fertilisation being recorded in an Antarctic limpet and bivalve below a salinity of 27 (Powell, 2001). Low salinities can affect both the viability of sperm and ova by arresting sperm motility (Griffin *et al.*, 1998; Litvak and Trippel, 1998) and being injurious to the ova (Greenwood and Bennett, 1981).

Gamete Age

Similarities were observed between the longevity of gametes broadcast by the temperate and Antarctic starfish. The longevity of *M. glacialis* gametes was ~6 hours (see Fig. 3.3.7). Optimum fertilisation (90-100%) was recorded in *M. glacialis* after interactions between dry sperm and eggs and between gametes aged over a 2-hour period. All development proceeded abnormally from interactions between sperm and eggs aged in excess of 6 hours. Optimum fertilisation was restricted in *O. validus* to interactions between freshly spawned gametes and sperm and eggs aged <1hour (see Fig. 3.3.8). Normal fertilisation was still possible, albeit at a very low level, with gametes aged between 6 and 24 hours. However, abnormal development accounted for most fertilisation above 6 hours. The occurrence of long-lived sperm in an Antarctic invertebrate has been reported before, with the sperm of *Laternula elliptica* and *Nacella concinna* able to fertilise eggs after 90+ and 70 hours of ageing respectively (Powell *et al.*, 2001). These longevities are well in excess of the 24hour age recorded in the current study for *O. validus*, although experimental protocols, in terms of the equipment used, were very similar. However, Powell (2000) only investigated sperm longevity, using freshly spawned eggs for each experimental trail. Egg longevity is not static and varies between individuals and studies owing to exogenous and endogenous factors (Meidel and Yund, 2001), e.g. bacterial contaminants can cause lysis of eggs under laboratory conditions (Epel *et al.*, 1998). This was not accounted for in the current study, and may have contributed to the results observed. Alternatively, sperm viability may have been greater than that of the eggs used, although several studies have suggested the opposite based on the ‘respiratory dilution effect’ (Chia and Bickell, 1983). As both aged sperm and eggs were used in the current study it is difficult to say which of the gamete characteristics limited the maximum age at which gametes were viable most, and the results recorded were not necessarily caused by a shared characteristic of the eggs and sperm. However, owing to time constraints I chose to test the characteristic most sensitive in a shared system, i.e. the coincident ageing of eggs and sperm that would naturally occur in the water column especially after synchronous spawning. With more time I would have tested for the effects of both aged sperm and freshly spawned eggs, and aged eggs and freshly spawned sperm on fertilisation success.

The greater longevity observed in the sperm of *L. elliptica* may be attributed to this species' ecology. Individuals remain buried in the sediment for prolonged periods of time acquiring nutrients through filter feeding (Powell, 2000), and therefore accrue benefits from locating to sites of relatively high flow. However, moderate-high flow rates tend to dilute sperm broadcast into the water column and limit fertilisation success downstream of spawning males (Pennington, 1985). Therefore, longer-lived sperm would confer an advantage encouraging fertilisation success to accrue over time and distance. However, *L. elliptica* also occurs at high densities (Ahn, 1993; Ahn, 1994; Ahn *et al.*, 1996; Ahn *et al.*, 2003) and can on occasions spawn *en masse* vast quantities of eggs and sperm, sometimes producing carpets of gametes (Powell, 2001). During these episodes the presence of a rapidly spawned mass may be sufficient to delay the progress of sperm reaching viable eggs, where greater sperm longevity would be beneficial. Mass spawning is common in several broadcast spawners and on some of these occasions when sperm dilution is less important, sperm competition may be more of a concern than sperm limitation, favouring the production of fast sperm at the cost of longevity, thereby capable of rapidly routing out a pool of ever decreasing virgin eggs (Levitan, 1993). Alternatively, increased longevity may simply be a result of slower swimming sperm, however additional experimental trails would be required if this variable was to be measured accurately. Therefore, even if the increased sperm longevity of Antarctic spermatozoa is only a result of low water temperature (Davenport, 1995), the benefits are not reduced. Long-lived sperm can increase the success of synchronous mass spawning events by permitting sufficient time for sperm densities to reach the high levels required for fertilisation success. The current study has already reported a requirement for 1-2 orders of magnitude more sperm to achieve optimal fertilisation in the Antarctic and may be partly accountable for long-lived gametes.

A broad range of sperm longevities has been reported amongst a variety of free spawning invertebrates, including sea urchins (*Strongylcentrotus droebachiensis*: 0.3 hour Pennington, 1985; *S. franciscanus*: 2.5 hours Levitan *et al.,* 1991), seastars (*Asterias rubens*: 24 hours Williams, 1999; *Acanthaster planci*: 7 hours Benzie and

Dixon, 1994), polychaetes (*Nereis virens*: ~24 hours and *Arenicola marina*: ~85 hours Williams, 1999) gastropods (*Haliotis ascina*: 2+ hours Encena *et al.*, 1998; *H. tuberculata* Baker and Tyler, 2001) bivalves (*Mytilus edulis*: 5+ hours Levy and Couturier 1996; *Cerastoderma edule*: 4-8 hours (André and Lindegarth, 1995) bryozoans (*Celleporella hyaline*: 1.2 hours Manriquez *et al.,* 2001), ascidians (*Ascidia mentula*: 48 hours Havenhand, 1991) and coral (*Platygyra sinensis*: ~5 hours Oliver and Babcock, 1992).

Previous studies have also described the detrimental influence of increasing gamete age on fertilisation success in echinoderms and polychaetes (*Lytechinus variegatus* Levitan, 2000; *S. droebachiensis* Meidel and Yund, 2001; *Asterias rubens*, *Arenicola marina* and *Nereis virens* Williams and Bentley, 2002), molluscs (*Mytilus edulis* Levy and Couturier, 1996) bryozoans (*Celleporella hyalina* Manriquez *et al.,* 2001) and ascidians (*Botryllus schlosseri* Johnson and Yund, 2004).

It is generally reported that sperm become senescent more rapidly than eggs in free spawning invertebrates (Williams and Bentley, 2002) and that the longevity of a sperm suspension relies mainly on the concentration of sperm. The latter has been attributed to oxygen consumption and as concentrated sperm respire at a lower rate than dilute sperm- the respiratory dilution effect (Chia and Bickell, 1983)- sperm longevity is reduced at low concentrations. Evidence of the respiratory dilution effect has been demonstrated before, for the seastar *Acanthaster planci* (Benzie and Dixon, 1994), the sea urchin *S. franciscanus* (Levitan *et al.*, 1991) and the ormer *Haliotis tuberculata* (Baker and Tyler, 2001). Oocyte longevity has been correlated with the reproductive mode of a species and longer-lived oocytes are acknowledged to confer an advantage by being fertilisable by sperm a number of hours or days after release (Williams and Bentley, 2002).

The ageing of gametes within the gonad of an animal is also a component of fertilisation success, although the ageing of gametes post-spawning is more commonly studied. The age at which sperm and eggs are broadcast into the water column will affect the competency of those gametes to be fertilised. Inevitably, immature sperm and eggs will fail to fertilise, or to be fertilised successfully, and as a result a lower number of successful fertilisations will result than otherwise would occur from interactions between mature, ripe gametes. Therefore, an animal’s age and reproductive maturity are also important factors in fertilisation success (Levitan, 1995).

However, the influence of gamete age on fertilisation success is contentious, especially as many consider the influence of gamete limitation to be far more important, diluting gametes below fertilisable concentrations well before the viable life of gametes expires (Levitan *et al.,* 1991). This is especially relevant to the success of free spawning invertebrates, and fundamental in the fertilisation traits of individuals experiencing a high flow regime. However, many invertebrates exhibit adaptive spawning patterns to limit the influence of sperm dilution and as a consequence can secure high spawning success, such as synchronous spawning, broadcasting gametes in viscous fluids (echinoderms and polychaetes Thomas, 1994ab; *S. droebachiensis* Meidel and Yund, 2001) or depositing sperm onto the substratum, which remain quiescent until activated by female spawning (*Lepasterias polaris* Hamel and Mercier, 1995). Increases in viscosity have been shown to affect the passage of particles through the water column (Podolsky and Emlet, 1993; Podolsky, 1994), and arresting effects on sperm swimming speed have been inferred from these studies as seawater viscosities increase. A reduction in sperm swimming speed with increasing seawater viscosity was observed in the current study. Therefore, the broadcasting of sperm in a viscous fluid would for a time arrest the activity of the sperm released, conserve energy and minimise the early onset of the ‘respiratory dilution effect’. Synchronous spawning has been demonstrated before in the Antarctic limpet *Nacella concinna* (Picken and Allan, 1983), which forms discrete spawning stacks of individuals, and in the Antarctic brittle star *Ophionotus victoriae* (Grange *et al.,* 2004). In these instances gamete age becomes more important and the possession of longer lived sperm an advantage. This is also true of some invertebrates that internally fertilise their eggs (Bishop, 1998) and maximise success by efficiently disseminating, capturing and storing relatively long-lived sperm (e.g. the bryozoans *Celleporella hyalina* Manriquez *et al.,* 2001).

Contact Time

Contact time did affect the fertilisation success recorded in the temperate and polar starfish. However, successful fertilisation was recorded much sooner in *O. validus* (after 5 seconds) compared to the zero fertilisation levels observed in *M. glacialis,* until 5 minutes contact time between eggs and sperm had elapsed (see Fig. 3.3.9 and Fig. 3.3.10). High fertilisation success (70%) was recorded in *O.validus* during the first minute of contact between gametes. A maximum number of eggs were fertilised after approximately 5 minutes contact time. At sperm-egg contact times > 5minutes, all treatments showed similar fertilisation success (80-100%). Levitan (2000) also observed a significant effect of contact time on fertilisation success in the urchin *Lytechinus variegatus*, particularly within the small time intervals most likely to be important in the field (less than 1 minute). Rothschild and Swann (1951) also came to a similar conclusion for *Psammechinus miliaris*. Fertilisation in *M. glacialis* was notably low, never exceeding ~40% and was absent at contact times <5minutes. The absence of fertilisation in *M. glacialis* before 5 minutes contact time between gametes may reflect the quality of the gametes used in the trial, although the pattern of fertilisation success in this seastar was comparable to that of *O.validus* and other asteroids (e.g. *Asterias rubens* Williams, 1999) that require several minutes to achieve optimal levels of fertilisation. Why *O. validus* should need such a short time for successful fertilisation compared to *M. glacialis* is not overly clear. However, the rate and steps by which fertilisation proceeds in different invertebrates are varied. A similar plan or organisation of the structures involved in fertilisation are recognised in most gametes studied, however large variability is apparent in the way in which gametes recognise each other and interact in different groups and species, e.g. chemotaxis (Rosati, 1995). Therefore, one explanation may be that the eggs of *O. validus* exude greater amounts of, or more effective, sperm attractant than those of *M. glacialis* lending to higher overall success rates during shorter contact times under optimum conditions. Differences in other egg traits, such as the density of sperm receptors on the egg membrane may also influence the rates and efficiency of fertilisation between the starfish. If receptors on the egg surface of *M glacialis* are at a lower density than those on the eggs of *O. validus*, then a larger proportion of the sperm that arrive at the egg surface of *M. glacialis* may not be able to bind to the receptors as quickly. *M. glacialis* also appeared to be more susceptible to the effects of polyspermy at lower sperm concentrations compared to *O. validus*. An increased sensitivity to polyspermy can result in selection for increased resistance to sperm at the level of egg receptor proteins, with the outcome that a lower proportion of attempted sperm-egg receptor fusions are successful, and this may explain the slower rates of successful fertilisation observed in *M. glacialis*. These species-specific differences may partly explain the differences observed between *M. glacialis* and *O. validus*, and have also been discussed by Styan *et al*., (2005) to explain the differences in fertilisation success between *Patiriella calcar* and *P. regularis*. Unfortunately, only a single trial was undertaken to assess the effects of contact time on fertilisation success in *M. glacialis* and therefore, further trials would be required before any firm conclusions could be made.

These data indicate that in the case of *M. glacialis*, eggs need to remain in contact with a sperm suspension for several minutes in order to achieve high numbers of fertilised eggs. Similar results have been demonstrated for the sea urchins *Echinus esculentus* (Williams, 1999) and *Strongylcentrotus purpuratus* (Levitan *et al.,* 1991) and for the polychaete *Nereis virens* (Williams, 1999). However, some species do not exhibit any notable effect of contact time on fertilisation success owing to rapid attachment of a sperm to an egg, which was observed by Babcock and Keesing (1999) in *Haliotis laevigata.* Successful fertilisation was also very rapid, under optimum conditions, in *O. validus* and may possibly describe an adaptation to overcome the problems experienced when broadcasting gametes into a low temperature environment.

Viscosity

Emlet and Strathmann, (1985) commented that most zooplankters and planktonic larvae of benthic marine invertebrates live in a world dominated by viscosity. Our data indicate that the viscosity of the external medium has an important effect on fertilisation success in both the temperate and Antarctic starfish and the bivalve *Laternula elliptica*. However, the range of viscosities tested was broad, some of which were in excess of what would be experienced naturally. Seawater temperatures were maintained at 0.5ºC (1.27cp; *O. validus* and *L. elliptica*) and 15ºC (1.45cp; *M. glacialis*) and viscosities were increased using additions of PVP, methylcellulose and glycerol (only used PVP for *M. glacialis* and *O. validus*). Fertilisation success decreased with increasing viscosity (see Fig. 3.3.11, Fig. 3.3.12 and Fig. 3.3.13). Podolsky (1994) demonstrated a decrease in larval feeding rate with temperature and he attributed over half of the observed effect to viscosity, concluding this effect to be responsible for the slower feeding rates observed in polar filter feeders. Emlet and

Strathmann, (1985) also looked at the effects of viscosity on feeding in small zooplankton and suggested that when viscosity is a dominant factor, there is a thick layer of water that moves with an animal and deflects food-laden pockets of water from its path, inhibiting the capacity of zooplankton to feed. Given the similarities between suspension feeding and other processes involving contact between small particles, temperature-induced viscosity change is likely to impact a range of biological processes including fertilisation kinetics (Podolsky, 1994). A similar response was demonstrated in the swimming speed of larvae in the sand dollar *Dendraster excentricus* (Podolosky and Emlet, 1993). A 10-degree drop in temperature reduced larval swimming speed by 40% and water movement by 35%. 40% of the decrease in swimming speed and 55% of the decrease in water movement was attributed to viscosity effects alone. Viscosity also has an important effect on larval fish swimming (Fuiman and Batty, 1997) and Brokaw (2001) has previously described the propagation of bending waves along a sea urchin sperm tail needed to push the spermatozoan through a viscous environment. At a high viscosity the sperm tail generates bending waves with shorter wavelengths and high bend angles. Normal seawater conditions produced the highest fertilisation success in the current study. However, high fertilisation was still possible in *M. glacialis* and *L. elliptica* outside the normal range of seawater viscosities. This may have been caused by the retention of packets of concentrated sperm in the more viscous solutions, easily capable of fertilising collections of passing eggs.

Egg Jelly

Removal of the jelly coat reduced the number of successful sperm-egg interactions in *O. validus* (see Fig. 3.3.14). Many studies have discussed and reviewed the relative importance and influence of accessory egg structures on invertebrate fertilisation success. Studies have demonstrated both the influence of these structures on effective egg size (Levitan, 1993; Marshall *et al.*, 2000; Podolosky, 2002) and the importance of chemoattractants often associated with the egg (Miller, 1982, 1985; Bolton and Havenhand, 1996; Jantzen and Havenhand, 2001).

Podolsky (2002) demonstrated the important physical role of egg accessory structures on fertilisation success, increasing the size and buoyancy of the egg making it a preferred target for sperm. The egg jelly increased the target egg size of the sand dollar *Dendraster excentricus* six-fold. Podolsky (2002) also recorded a decline in fertilisation success in the same experiment when the egg jelly was removed, and attributed this effect to changes in the rate of sperm-egg collisions. This led some to reason that under sperm dilution, larger eggs would be fertilised with greater probability and would therefore be selected for (Levitan, 1993, 1998). Therefore, a trade off between egg size, sperm velocity and longevity and gamete concentration is likely. This has been demonstrated in three species of sea urchin *Strongylcentrotus purpuratus*, *S. franciscanus* and *S. droebachiensis* (Levitan, 1993) and in a free-spawning ascidian *Pyura stolonifera* (Marshall *et al.*, 2000). However, Podolsky and Strathmann (1996) suggested interspecific differences in egg fertilizability and sperm-half life to be more important than the probability of egg fertilization in the evolution of free-spawning invertebrate egg sizes. Styan *et al.*, (2005) recorded no significant difference in fertilisation success between small and large starfish eggs in the genus *Patiriella* and therefore also concluded an uncoupling of egg size and the likelihood of fertilisation. Consequently, fertilisation may not be a major constraint on the evolution of egg size.

Compounds contained in or released from accessory egg structures have also been shown to influence fertilisation success by affecting sperm morphology, respiration, motility, longevity (Bolton and Havenhand, 1996), chemotaxis (Miller, 1982, 1985), species specificity and acrosome interaction (Hoshi, 1991). The rapid diffusion of sperm chemoattractants from an egg can also contribute to higher fertilisation success by increasing the effective target egg size. This has been demonstrated for the solitary ascidian *Ciona intestinalis* (Jantzen and Havenhand, 2001). Sperm chemotaxis has been exhibited in echinoderms before (Ward *et al.*, 1985; Miller, 1985), as well as in other taxa including the Mollusca, Urochordata and Cnidaria (Bolton and Havenhand, 1996), and acts to counter the damaging effects of sperm dilution. The reduction in fertilisation success observed in *O. validus* in the present study, after the removal of the jelly coat, may have resulted from detrimental effects on both the physical and chemical attributes of the starfish eggs. A recent study by Styan *et al.*, (2005) suggested the lesser importance of egg size on fertilisation success in two species of starfish, concluding that there must be a difference between the species in the likelihood that when a sperm routes out a conspecific egg it can successfully attach and fertilise. The current study also suggests the greater importance of species-specific gamete traits, other than egg size, in fertilisation success as both *O. validus* and *M. glacialis* broadcast eggs of a similar size into the water column, but produce different levels of fertilisation success under optimum conditions. The removal of compounds associated with or released from the jelly coat, when this accessory structure is removed from the eggs of *O. validus*, probably plays a more critical role in the levels of fertilisation success observed.

Competence to spawn

O.validus produced the largest induced spawning response during May (55%) and June (85%). Stanwell-Smith and Peck (1998) also found that spawning could be successfully induced in a Signy Island population of the same species during the winter months (May-July). The reproductive cycle of this polar starfish has been described previously by Pearse (1965, 1969) and confirms the loss of large oocytes and active sperm from the gonad during the austral winter. Feeding larval stages of *O. validus* have been sampled in the waters round the Antarctic as early as May, and most abundantly in July, confirming a spawning during May-June in natural populations (Shreeve and Peck, 1993; Stanwell-Smith and Peck, 1998; Stanwell-Smith *et al.*, 1999).

The males and females induced to spawn in the present study were larger than the mean size of the starfish injected. This has been demonstrated before in *O.validus* by Stanwell-Smith and Clarke (1998), who also reported a higher frequency of females (81%) spawning compared to males (19%). Females accounted for 65% and 82% of the induced individuals in May and June respectively. Both studies confirmed that not all starfish injected could be induced to spawn. Stanwell-Smith and Clarke (1998) attributed this disparity to the prolonged gametogenic cycle exhibited in *O. validus*. An 18-24 month oogenic cycle with distinct overlapping cohorts has been described for a number of free-spawning invertebrates in the Antarctic, including shallow-water echinoderms (Pearse, 1965; Grange *et al.*, 2004). This confirms the simultaneous existence of two oocyte generations in the gonad, where spawning is annual and a proportion of oocytes are broadcast each year. Larval data also confirm that at least some individuals are able to spawn annually. The higher frequency of females being induced to spawn is somewhat counterintuitive to what is normally observed. Most species are reported to show male-first spawning in echinoderms (Soong *et al.*, 2005). However, incidences of male-first spawning are normally in response to the high selective pressure of sperm competition, where males spawn well in advance of females and are thus easier to observe. The preference for female-first spawning in *O. validus* may indicate a higher selective pressure of sperm limitation, which is supported by the observation that an order of magnitude more sperm is required to fertilise eggs under optimum conditions, compared to the amount of sperm required to produce similar results in a temperate equivalent. In these instances males closely follow the release of eggs with sperm, inadvertently reducing the gap in spawning time between the sexes, making female-first release more difficult to observe and less likely to be documented regularly. Therefore, the females collected from natural populations of *O. validus* during the spawning period in 2004 may have been more readily induced to spawn than the males sampled, relying on the appearance of eggs in the water column to synchronise a reproductive response.

65% of the individuals observed to spawn in the current study exhibited interesting pre-spawning behaviour. Each starfish contorted, arched and raised itself above the floor of the plastic aquaria. This ‘spawning posture’ is commonly observed in the field during the spawning episodes of natural echinoderm populations and can be expressed by spawning and non-spawning individuals (Hendler and Meyer, 1982). Many studies have interpreted this behaviour as an adaptive behaviour to ensure the release of gametes into the water column away from the seabed, minimising gamete wastage and maximising the chance of long-range dispersal by raising the gonopores into the field of flow (Hendler and Meyer, 1982; Babcock *et al.*, 1994). A similar benefit has been described from mounting high coral heads to spawn, which has been observed in *Acanthaster planci* (Benzie *et al.*, 1994). However, such behaviour can have an adverse effect of elevating individuals into higher flow regimes, where potential dilution effects may act to counter any benefits gained from dispersal and act as a stronger selective pressure. Consequently, this behaviour is commonly coincident with adult aggregative behaviour and high fertilisation levels have been demonstrated for echinoderms under similar circumstances that act to counter dilution effects (Pennington, 1985; Levitan *et al.,* 1992; Hamel and Mercier, 1995; Levitan, 1995).

Sperm swimming speeds

Sperm speeds are notoriously difficult to measure (Styan and Butler, 2000) and complications experienced in the current study made it difficult to attain an accurate measurement of sperm swimming speed. However, temperature and seawater viscosity had an important effect on sperm swimming velocity (see Fig. 3.3.16). The effects of both temperature and seawater viscosity on motility have been investigated before in the swimming capacity of larvae in the sand dollar *Dendraster excentricus* (Podolsky and Emlet, 1993). The physical influence of viscosity accounted for a large proportion of the observed temperature effect on larval swimming and could explain 40% of the recorded decline in swimming speed with a 10-degree drop in temperature. The current study suggests sperm velocity is severely impaired by increases in seawater viscosity and increased by rising temperatures. The effect of temperature on the swimming velocity of Antarctic sperm has been demonstrated for *Laternula elliptica* (Powell, 2000). Increasing temperatures caused an increase in velocity and although the actual speeds recorded differ from the current study the same pattern was observed. It is likely that the actual speeds varied between these studies because of differences in paternity (Gee and Zimmer-Faust, 1997). However, the similar patterns observed in sperm swimming speed with increasing temperature in both studies were most probably temperature driven. A similar response to temperature is described in the present study for *Parborlasia corrugatus,* including a plateau in sperm swimming speed between 2-4˚C, reminiscent of the similar velocities recorded between –0.5ºC and +2ºC in *L. elliptica* by Powell (2000). Approximately, 47% of the decline in speed was attributable to changes in seawater viscosity alone and 53% to other effects of temperature.

Sperm swimming velocity is important to fertilisation success as it determines the number of sperm-egg collisions (Farley, 2002; Kupriyanova and Havenhand, 2002). Rises in temperature elevate sperm swimming speed (Mita *et al.,* 1984; Powell, 2000), and can impair fertilisation success in free-spawning invertebrates, especially in Antarctic species (Powell *et al.*, 2001). This has been attributed to the trade off between sperm velocity and longevity (Levitan, 2000). A decline in longevity with increasing activity has been demonstrated for sperm of the urchin *Lytechinus variegatus* (Levitan, 2001) and has been attributed to the 'respiratory dilution effect' (Chia and Bickell, 1983). Increases in viscosity also affect the physical attributes of sperm (Brokaw, 2001) and arrest sperm motility by exerting excessive drag on swimming cells (Fuiman and Batty, 1997). Therefore, the physical attributes of seawater exert important influences on sperm motility, including salinity effects (Powell *et al.*, 2001). Seawater salinities below 26 impaired swimming in the sperm of the Antarctic bivalve *L. elliptica*.

Conclusions

Many factors contribute to fertilisation success in free-spawning marine invertebrates. As a consequence fertilisation success can vary between 0-100% (Levitan, 1995). Our study suggests that Antarctic invertebrates have evolved unique spawning patterns to maximise fertilisation success. Antarctic free-spawning invertebrates encounter a very arduous and unforgiving environment, where consistently low temperatures, high seawater viscosities and at times appreciable flow conditions undermine successful sperm-egg interactions.

This study suggests that there is a similar range in sperm concentration over which high numbers of eggs can be fertilised in both a temperate and polar starfish. However, equally high numbers of eggs are only successfully fertilised at lower concentrations in the temperate starfish. Therefore, Antarctic invertebrates require 1-2 orders of magnitude more sperm to ensure optimal fertilisation success. These sperm tend to be long-lived and are capable of fertilising eggs 24+ hours after release. Synchronous spawning, aggregations and specific pre-spawning behaviour help to counter the deleterious effects of sperm limitation.

Antarctic eggs and sperm are also highly stenothermal, with extremely narrow ranges of thermal and salinity tolerance. Therefore, even small modifications in temperature and salinity can dramatically reduce the number of eggs fertilised. This has important implications for the geographical distribution of Antarctic invertebrates, but more intuitively confirms the susceptibility of these invertebrates to environmental modification, making them one of the most temperature sensitive fauna on the Earth. Such stenothermy is of particular relevance if the 1-2ºC rise in global temperature, predicted over the next century, is realised.

4.1 Introduction

The quality and quantity of food arriving at the seabed in the Antarctic has several effects on the composition and condition of tissues in marine invertebrates. It has a direct effect on the amount and composition of material channelled directly into the digestive tissues and diverted to storage, and it can have an indirect influence on the timing of reproduction and how energy is allocated to the gonad. This is important in the understanding of Antarctic reproductive cycles, many of which are underpinned by the intensely seasonal signature in primary production, and especially relevant when the potential impact of environmental change, in terms of seasonality is considered.

The biochemical constitution of an organism is basic to the understanding of its physiology (Giese, 1966a). The relative contribution of different bodily constituents (protein, lipid, carbohydrate, nucleic acids and mineral ash) influences the amount of energy provisioned between maintenance, growth and reproduction, and provides information on the nutritional economy of an organism. However, the organic composition of Antarctic invertebrates has received relatively limited attention, except for the study of pelagic zooplankton.

Pearse and Giese (1966a) were the first to describe the organic profile of a selection of benthic animals collected from an extreme southerly latitude at McMurdo Sound. This investigation questioned the generally-accepted paradigm that polar animals invest in unusually high lipid stores to counter starvation during the long Antarctic winter (MacGinite, 1955; Littepage, 1965). Pearse and Giese (1966a) and some more recent studies have found results contradictory to this theory, especially amongst benthic invertebrates, although it is still considered typical of the planktonic zooplankton in the Antarctic (Clarke and Peck, 1991).

Pearse and Giese (1966a) commented on the bodily constitution of 7 benthic invertebrates and arrived at a number of tentative conclusions, which formed the basis for future investigations into the biochemical composition of Antarctic taxa. Protein accounted for the largest percentage dry mass of the tissues studied and explained approximately 50% of the tissue compositions. These invertebrates also exhibited moderate to low lipid levels and a minimal contribution from carbohydrate, comparable to the values typical in temperate taxa. In most instances all species conformed to this composition. However, a large tissue lipid content was demonstrated for the isopod *Glyptonotus antarcticus,* which was illustrative of the original paradigm describing most previously-studied Antarctic crustaceans. This study thus indicated that the organic constitution of polar invertebrates is not dissimilar from the tissue composition of comparable temperate marine forms, and that a latitudinal gradient in lipid composition does not apply to all polar marine animals.

Many studies have commented upon the biochemical profile of tropical, subtropical, temperate and sub-polar echinoderm species (Giese, 1966a; Lawrence and Guille, 1982; Magniez, 1983; Walker *et al.,* 1987; McClintock *et al.,* 1990; Bishop and Watts, 1992; David and MacDonald, 2002). Pearse and Giese (1966a) were some of the first to turn their attentions poleward and reported on the biochemical composition of 7 benthic invertebrates, including three Antarctic asteroids, *Acodondaster hodgsoni, Diplasterias brucei* and *Perknaster fuscus antarcticus.* Studies into each of the echinoderm classes have followed (McClintock and Pearse, 1987; McClintock, 1989; Stanwell-Smith and Clarke, 1998; Brockington *et al.,* 2001), including the study of deep-water species (Galley, 2004).

The biochemical composition of echinoderms varies between taxa, between sexes and through temporal variations in tissue composition. The role of different bodily constituents also varies between organs, which can be influenced by sex and/or season.

The study of whole body echinoderms has been widespread and has highlighted the predominance of ash in the skeletal tissues, e.g. in the body wall of asteroids and holothurians (~30 to 80%), the disc (~49 to 72%) and arms (~57 to 73%) of ophiuroids, the test (~80%) and spines (~89%) of echinoids and the crinoid arms (~64%), calyx (~79%) and cirri (~83%) (McClintock and Pearse, 1987). The use of calcium carbonate, or silica in some holothurians, in body wall support is thought to explain the elevated ash levels in echinoderm tissues (Giese, 1966a). Some internal tissues are also known to contain calcium carbonate deposits, e.g. the gut and gonad wall of some ophiuroids (Tyler, 1980). High ash levels have also been reported in Antarctic gelatinous zooplankton (Clarke *et al.*, 1992), diluting the presence of other bodily constituents. However, some echinoderms, particularly some species of asteroid, do exhibit elevated levels of protein, mainly with a structural role, in these tissues. The body wall of the Antarctic asteroid *Perknaster fuscus antarcticus* is comparatively rich in protein because the body wall is devoid of the normal calcareous plates typical of echinoderms, mainly being composed of connective tissue (Pearse and Giese, 1966a). Most echinoderms exhibit moderate-low levels of lipid in skeletal tissues, including those species Antarctic in origin (Giese, 1966b; Pearse and Giese, 1966ab; McClintock and Pearse, 1987). These lipids tend to have either a structural role or are stored in reserve. The lipid levels of whole bodies of echinoderms have been compiled previously and describe a generally low lipid content in echinoids and holothurians (1 to 2%), ophiuroids (1 to 2.6%), crinoids (1.5 to 5.3%) and asteroids (2.0 to 12.6%) (Giese, 1966b).

Some tissues contain large reserves of protein and lipid. Protein tends to constitute a large fraction of individual tissues, sometimes accounting for nearly half of the biochemical profile (Giese, 1966a; Giese and Pearse, 1966a; McClintock and Pearse, 1987). They form the basic architecture of cells, therefore where cells contribute to a major part of a tissue, levels of protein are expected to be considerable. Proteins may also be used as an overwintering energy store in some benthic taxa such as brachiopods (Peck *et al.*, 1987). Lipids are often accumulated and sequentially stored to provide a reserve for when energetic demand is high or when nutrients are scarce. The actual lipid content exhibited by invertebrates commonly varies with a species' trophic ecology and lifestyle (Clarke and Peck, 1991). Herbivory is normally associated with low lipid reserves in the Antarctic benthos. Levels are generally higher in carnivorous invertebrates, although benthic accumulations of lipid are diminished in comparison to the inhabitants of the pelagic.

Elevated levels of lipid and protein have been observed in the digestive and nutrient storage organs of echinoderms (Giese, 1966a; McClintock and Pearse, 1987) and may reflect the fact that the gut tends to be highly metabolic tissue. A secondary role of short-term nutrient storage has also been described for the gut tissues of ophiuroids, echinoids and holothurians (Lawrence *et al.*, 1965; McClintock and

Pearse, 1987; Klinger *et al.,* 1988; Bishop and Watts, 1992). However, any capacity for long-term storage is limited.

The role of the pyloric caeca in asteroids is much more diverse (mobilisation of nutrients, provision of nutrients during gametogenesis). One of the most important of its functions is as a long-term storage organ. The role of this body component in nutrient storage is reflected in the high levels of both protein and lipid found in the pyloric caeca of Antarctic asteroids, e.g. in *Odontaster validus* (Pearse, 1965; McClintock *et al*., 1988). McClintock (1989) actually observed a higher proportion of energy in the pyloric caeca of *O. validus* compared to the body wall or the gonads, and argued that this large capacity to store nutrients and energy in the pyloric caeca may have contributed to the success of the starfish in the Antarctic. The role of the pyloric caeca in providing nutrients to the gonad during gametogenic development has also been identified as important, both in terms of the seasonal changes in the biochemical constitution of asteroids and also in the provisioning of energy between somatic and reproductive demands.

Levels of protein and lipid tend to be highest in the reproductive tissues of invertebrates, especially during pre-spawning periods when individuals are gravid. Elevated lipid levels are common in the reproductive tissues of echinoderms, especially in the female ovary (Giese, 1966a). Most studies have described a clear dichotomy between the biochemical compositions of echinoderm ovaries and testes. This has been observed in the Antarctic (McClintock and Pearse, 1987), where 88% of the species studied exhibited higher ovarian lipid compared to the testes. Lipids serve an important role in maturing gonadal tissues and constitute a large amount of reproductive material. Eggs of marine invertebrates often accumulate large volumes of lipid and lipoprotein, which results in some echinoderms having a high overall tissue lipid content, especially those that exhibit lecithotrophy and broodcare e.g. *Porania sp* (lipid = 53.5%) and *Acondaster hodgsoni* (lipid = 62%) (McClintock and Pearse, 1987). McClintock and Pearse (1987) found large lipid reserves in the gonads of a number of Antarctic asteroid species and attributed this to the preponderance for lecithotrophy at high latitudes. A lower lipid content was largely limited to those species favouring investment in planktotrophic larvae, e.g. *Odontaster validus* (~13% lipid) and *O. meridionalis* (23% lipid). Brockington *et al.,* (2001) also recorded a low lipid level in the gonad of the common Antarctic urchin *Sterechinus neumayeri*, a broadcast spawner. However, these trends are probably widespread and more dependent on reproductive pattern than existing at a polar latitude.

In contrast to the female gonad, the testes exhibit higher levels of protein. McClintock and Pearse (1987) recorded high levels of insoluble protein in the testes of many Antarctic echinoderms and concluded that these reflected the presence of nucleic acids associated with sperm. The high levels of insoluble protein recorded also reflect the analytical techniques available at the time of the study, when abilities to dissolve protein were limited. Consequently, the 'insoluble' fraction of nitrogen-containing biochemical constituents probably included both refractory protein and nucleic acids. Comparatively lower levels of lipid are typical in the testes and reflect the inherently larger contribution of lipid to the development of oocytes compared to sperm.

Carbohydrate is often interpreted to represent the allocation of tissue space to food storage (chiefly glycogen) and the nutritional well being of an animal (Giese, 1966a). This constituent often varies with the nutritional condition of the animal and the provision of reserves between different localities. Glycogen is preferentially stored by some invertebrates (e.g. in molluscs) and lacking in others (e.g. echinoderms), and these distinctions are also maintained in Antarctica. Most Antarctic echinoderms possess carbohydrate levels at the lower end of the invertebrate range, which are generally comparable to, or slightly less than, the levels observed in temperate equivalents. McClintock and Pearse (1987) observed low carbohydrate levels in 24 species of Antarctic echinoderm. Pearse and Giese (1966a) did find two exceptions to this general rule, reporting larger than average carbohydrate levels in *Perknaster fuscus antarcticus* and *O. validus*. Pearse and Giese (1966b) also observed elevated carbohydrate values in the gonad of the Antarctic echinoid *S. neumayeri*.

Therefore, several studies have substantiated that there is no latitudinal gradient in organic composition in echinoderms, and that any differences are driven by variations in bodily components.

The intense seasonality experienced in the Antarctic is likely to underpin the seasonal cycles in the biochemical constitution of many benthic invertebrates, especially in terms of stored nutrient and reproductive reserves. The prolonged and arduous winter typical of the Antarctic has led many to postulate that endemic invertebrates would benefit from the accumulation of large ration reserves (namely lipid) in summer to ensure survival during the depauperate austral winter. The lack of any appreciable lipid storage in many benthic invertebrates has encouraged the study of winter survival, especially in filter feeders that appear to flourish irrespective of the harsh winter conditions (Peck *et al.,* 1986; Peck *et al.*, 1987). These invertebrates survive by virtue of their reduced metabolic rates and tend to favour the utilisation of protein over lipid, during late winter when nutrients are scare (Ahn *et al.,* 2000, 2003; Fraser *et al*., 2004).

The tissue composition of invertebrates may also vary with time depending on the tempos and stage of the reproductive cycle (Giese, 1959; 1966a; Boolootian, 1966). Periods of reproductive investment and maturity have exhibited large accumulations of stored lipid and protein in both ovarian and testicular constituents. Comparatively lower levels are common after recent spawning and during gonad recovery. These cycles are common in the Antarctic, where many invertebrates possess discrete sexual cycles in reproduction, e.g. *Odontaster validus* (Pearse, 1965). However, not all individuals display variation in organic levels of the gonad during the reproductive cycle, e.g. *S. neumayeri* (Pearse and Giese, 1966b).

Therefore to summarise, we know that echinoderms have substantially high ash contents by virtue of their skeletal elements, and in some instances internal tissues, being commonly composed of calcareous deposits. Previous studies have also alluded to typically low carbohydrate levels in these invertebrates, whereas the organic fraction of most marine invertebrates is typically dominated by protein. Therefore, the carbon:nitrogen ratio in most tissues is determined by the ratio of lipid:protein, which is a trend generally maintained in Antarctic echinoderms. Current consensus also suggests the importance of protein and lipid in the ovary and protein and nucleic acids in the testis, where energy for metabolism is typically provided by protein.

The main objective of this study was to determine the organic constitution of two Antarctic echinoderms, the starfish *Odontaster validus* and the brittlestar *Ophionotus victoriae*, with particular regard to their reproductive, digestive and nutrient storage organs, and to evaluate the compliance of both species to the previously described biochemical profile 'typical' of most taxa. Both species are extremely abundant in shallow water sites of the marine Antarctic and exhibit seasonal reproductive cycles (Pearse, 1965; Grange *et al.,* 2004). However, these echinoderms differ in their reproductive timing and trophic position and therefore should provide some information on how different species partition their energy between competing processes during an annual cycle, given the same background of environmental conditions (Fig. 4.1.1). *Odontaster validus* spawns during May-August, favouring the release of planktotrophic larvae during the austral winter. These discrete spawning cycles may well influence the cycling of constituents in the starfish gonad. However, I expect the provisioning of nutrients from the pyloric caeca to the gonad during gametogenesis to underpin the main patterns in the elemental and proximate composition of this tissue. The adult starfish are also characterised as scavengers and opportunistic feeders, suggesting that they are somewhat de-coupled from the intense seasonality typical of the Antarctic and that any patterns in tissue composition will also reflect this disassociation. *Ophionotus victoriae* is also characterised by broadcast spawning and the release of feeding larvae into the water column. However, the brittlestar spawns during the austral spring some 3-4 months after the starfish. This ophiuroid is a detritivore consuming mostly decayed and remineralised material from the seabed. The seasonal tempos of this echinoderm have been attributed in part, to the seasonal sedimentation events common in the high Antarctic (Grange *et al.,* 2004), and consequently I would expect patterns in the elemental and proximate composition of both the reproductive and digestive tissues to reflect this seasonality.

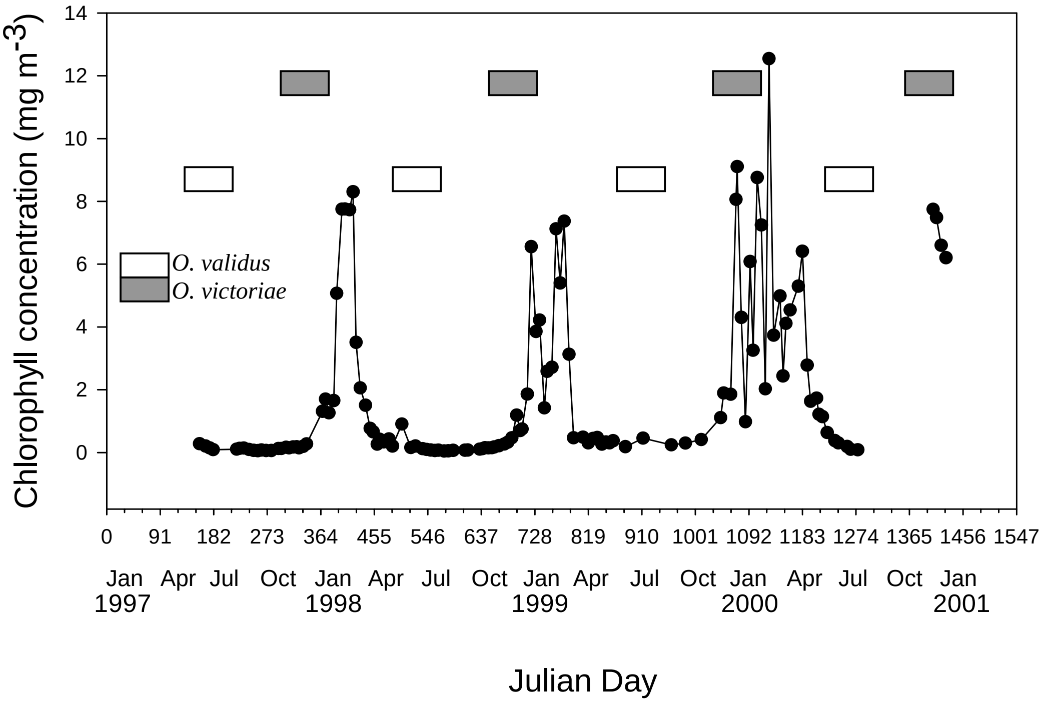

Fig. 4.1.1 *Ophionotus victoriae* and *Odontaster validus.* The timing of the spawning period in two Antarctic echinoderms and the summer phytoplankton bloom.

The main aim of this study was to determine the elemental and proximate composition of the ophiuroid bodily tissues, the ophiuroid gut, the asteroid pyloric caeca and the gonad of both echinoderms. Steps were also taken to compare and contrast the sexual variation in tissue composition in each species and to describe any seasonal variation in tissue composition in the two echinoderms. A final comment was made on the biochemical profile of both echinoderms in relation to each species ecology and reproductive patterns.

4.2 Materials and Methods

Sampling

Samples were collected for elemental (CHN) analysis from shallow water marine sites adjacent to Rothera Research Station on Adelaide Island, to the west of the Antarctic Peninsula (67˚34 S 68˚08 W). Monthly sampling was undertaken during the 2003 and 2004 field seasons and 10-15 individuals were collected each month. The brittle star *Ophionotus victoriae* and the starfish *Odontaster validus* were SCUBA diver collected from depths of 15-20m (Table. 4.2.1).

Table. 4.2.1 Individuals were collected over monthly intervals from shallow water sites adjacent to Rothera Research Station. 10-15 starfish and brittlestars were sampled over a 4 and 10 month period respectively.

Samples	Sampling Site	Number of Individuals
Odontaster validus		
16/12/2003	South Cove	10
19/12/2003	South Cove	10
09/01/2004	South Cove	10
05/02/2004	South Cove	15
19/03/2004	South Cove	15
Ophionotus victoriae		
13/02/2003	Hanger Cove	10
10/03/2003	Hanger Cove	10
09/04/2003	Hanger Cove	10
05/05/2003	Hanger Cove	10
17/06/2003	Hanger Cove	9
10/07/2003	Hanger Cove	10
13/12/2003	Hanger Cove	13
07/01/2004	Hanger Cove	12
11/02/2004	Hanger Cove	15
09/03/2004	Hanger Cove	15

Sampling was not possible during August 2003-end of November 2003, because diving operations were suspended. These measures followed the death of the BAS Marine Biologist Kirsty Brown.

Sample Preparation

Individual *Odontaster validus* were dissected soon after collection. Individuals were maintained at ambient seawater temperature in a thermally controlled aquarium to reduce any stress and the effects of handling prior to dissection. Individuals were weighed wet (±0.01g) and measured using vernier callipers to an accuracy of 0.01mm ('R' and 'r'), and the gonad and pyloric caeca tissue removed for further analysis. A scalpel was used to cut away and peel back the aboral body surface, and the gonad and pyloric caeca were removed using tweezers. It was important to remove the tissues when fresh and at the time of collection, to ensure that tissue degradation and changes to the elemental composition of the tissue were minimised. Tissues were stored and labelled separately, held at –80ºC, and transported back to the UK for elemental analysis.

Initial attempts were made to dissect individual *Ophionotus victoriae* immediately after collection when fresh. However, within a short period of time tissues lost coherence making dissection very difficult. Difficulties were also experienced separating the gonads intact from the skeletal elements of each ophiuroid. Therefore, after collection individuals were placed in a –80ºC freezer for 2-5 minutes before dissection. During this time individuals were bagged separately to ensure that any loss of arms was contained to a single individual. Ophiuroids were removed from the freezer separately and were measured using vernier callipers (±0.01mm disc diameter) and weighed frozen. The whole body volume of each brittle star was measured following Peck (1993). A weighing vessel was fashioned out of a plastic lid and suspended from a clamp stand using two pieces of wire of equal length. The wire was threaded through four drill holes in the plastic lid and twisted together at the top to permit the vessel to hang from the stand. A beaker of seawater was placed on an electronic balance and the weighing vessel positioned over the beaker and lowered until the plastic lid was submerged approximately 5cm below the seawater surface. Particular attention was paid to ensure that the vessel was suspended freely without touching any side of the beaker. The electronic balance was then tared. An individual brittle star could then be lowered carefully into the weighing vessel using tweezers. The specimen was dropped into the vessel from just above the surface of the seawater to ensure water was not lost from the beaker from splash back and to prevent the tweezers from breaking the water surface. The weight observed on the electronic scale was recorded and the density of seawater used to calculate the whole body volume of each brittle star:

$$\text{Weight of sample (g)} \times \text{density of seawater (1.025)} = \text{Volume of sample (cm}^3\text{)}$$

N.B. The weight of each sample was equivalent to the volume of water displaced. Small gas bubbles were commonly produced when a sample was placed into the weighing vessel, causing the weight recorded on the electronic balance to fluctuate slightly (0.01-0.05g). However, this error was very small (0.25-1%).

The aboral surface of the disc was cut away and removed so as the internal tissues were easily accessed. As the tissues began to thaw there was a short time window within which the tissues remained generally intact and fairly easy to separate from the skeletal elements of the ophiuroid. A quantity of both gonad and digestive tissue was removed using a warmed scalpel to cut through any ice, and the tissue removed with tweezers. The tissues were individually weighed to ensure samples were large enough for elemental analysis ($\geq$ 2mg). The sex of individuals was also recorded and determined by the appearance of the gonad (Chapter 2.2). Individual tissues and dissected whole ophiuroids were bagged separately and labelled to identify the sampling site and date of collection. Tissue samples were also labelled to identify the adult of origin. Individual tissue samples were stored in a –80ºC freezer and transported back to the UK.

Grinding, Drying and Ashing

The dissected whole ophiuroids were dried in the Rothera Research Station's Bonner Laboratory. Initially individuals were dried separately in ceramic crucibles of a known weight in a thermally controlled oven at 70ºC (Heraeus Kelvitron) for 24 hours. The samples were then removed and placed in a dessicator containing silica dessicant, designed to keep the atmospheric humidity low and to prevent the tissues from re-absorbing water, and individually weighed. Samples were returned to the oven for a further 12 hours after which the weighing procedure was repeated. A final dry weight was recorded after a full 48 hours. There was no measurable weight loss from the samples after the initial 24-hour oven period. Therefore, subsequent samples were dried for 24hours at 70ºC.

The dissected whole ophiuroids were then ground in a fume cupboard using a mortar and pestle. Samples were ground until the material was homogeneous. The calcareous skeleton of the ophiuroid was quite robust and required approximately 20-30 minutes grinding. Once ground, the dried samples were suitable for ashing. A trial was undertaken to establish the most appropriate ashing time on a subsample of the dried material. Work at the British Antarctic Survey involving the ashing of a variety of benthic marine invertebrate tissues at a range of temperatures, and analysing the carbon content in the ash, has suggested 550-650ºC as the optimum temperature for ashing echinoderms (A Clarke, pers. comm.). This pragmatic approach seeks the temperature, which offers the best compromise to maximise oxidation of organic carbon and minimise losses of inorganic carbonate from a sample. 1 mg samples were ashed in pre-weighed (~2g), pre-ashed aluminium crucibles in a thermally controlled furnace (Carbolite) at 600ºC for 24 hours. To ensure that excess material was not lost from the samples the temperature in the furnace was programmed to ramp up over a period of an hour. A temperature of 600ºC was maintained for the following 12 hours, after which the furnace was programmed to cool progressively to ambient temperature during the final 11 hours. The ashed samples were removed from the furnace, placed in a dessicator and individually weighed. The ashing process was then repeated for a further 24 hours. The ashed material maintained a constant weight after the first 24 hours in the furnace and all of the remaining samples were ashed using the same protocol. Both the dry and ashed whole ophiuroid samples were stored at –80ºC in separately labelled vials and transported frozen back to the UK.

All of the reproductive and digestive tissue samples were dried and prepared at the British Antarctic Survey, Cambridge. The gonad samples of *Odontaster validus* were also sexed at this stage. Small pieces of frozen tissue were sampled and allowed to thaw slightly. This material was used to produce gonad smear preparations from each individual sampled, and viewed under a compound microscope where eggs or pools of sperm were visible.

Several methods were used to prepare the individual tissue samples for analysis. Initially a subsample was removed from each of the frozen tissues using a scalpel and tweezers. The size of the subsample taken was equivalent to a ~1mg sample of dried material. Samples were removed from the –80ºC freezer and dissected on a bench immediately adjacent, to minimise the time that material was exposed to ambient room temperature. Subsamples were placed in labelled, pre-weighed ceramic crucibles and stored in a cool box over ice. The crucibles were then transferred to a thermally controlled oven (Heraeus Kelvitron) and dried for 24 hours at 70ºC. This process proved unsatisfactory, owing to the oily nature of both the digestive and gonad tissues. Drying caused the tissues to liquefy and produce a resultant thin film of protein sitting in a sticky pool of oxidised fat, which remained cemented to the base of the crucible. This was a common occurrence when the tissue was lipid rich. This material was impossible to grind and could not be adequately removed from the crucible without leaving a significant degree of residue on the ceramic surface.

A second method proved more successful, and allowed the direct transfer of dried samples from the oven to the CHN elemental autoanalyser (Carlo Erba EA1108). A series of light-weight tin capsules (7x7mm), specifically designed to retain samples during elemental analysis, were pre-weighed and arranged in a grid of labelled wells in a plastic tray, suitable to withstand oven temperatures. Duplicate subsamples of both frozen reproductive and digestive tissue were placed in the tin capsules and stored in a cool box over ice in the plastic tray. The tray was then transferred to a thermally controlled oven and dried over a 24 hour period at 70ºC. The samples were then placed in a dessicator to cool and were transported to the CHN elemental autoanalyser (Carlo Erba EA1108) using an airtight box containing surplus silica dessicant. The tin capsules were re-weighed and the tissue dry weight recorded as the difference between this value and the original weight of the tin capsule. Care was taken to seal each capsule before re-weighing to ensure against sample loss. This method was adopted for both the starfish and brittle star gonad and the pyloric caeca and gut tissues.

A proportion of the frozen gut, pyloric caeca and gonad tissues were subsampled for ashing. 15 and 30 samples were selected for the starfish and brittle star respectively.

Tissues were selected using random numbers generated by Minitab (version 13, Pennsylvania State University), subsampled frozen and placed into pre-ashed metallic crucibles of a known weight in a cool box over ice. The samples were then transferred to a thermally controlled oven and dried for 24 hours at 70ºC. Once dry the samples were transferred to a dessicator and weighed within the crucibles to obtain a dry weight. Each sample was then ashed for 24 hours at 600ºC and weighed to provide an ashed weight. The overall mean weight of the ashed samples was calculated for each tissue (pyloric caeca, gut and gonad).

The CHN Elemental Autoanalyser (EA1108)

All samples were processed through a CHN Elemental Autoanalyser (Carlo Erba EA1108), set up for the measurement of carbon, nitrogen and hydrogen only. Elemental composition data were generated after each sample was processed, using the software Eager 800 on a compatible workstation PC. The machine was calibrated prior to running any samples and again after every 200 samples had been run (Fig 4.2.1), when the combustion column had to be disconnected and the excess ash and combustion products removed.

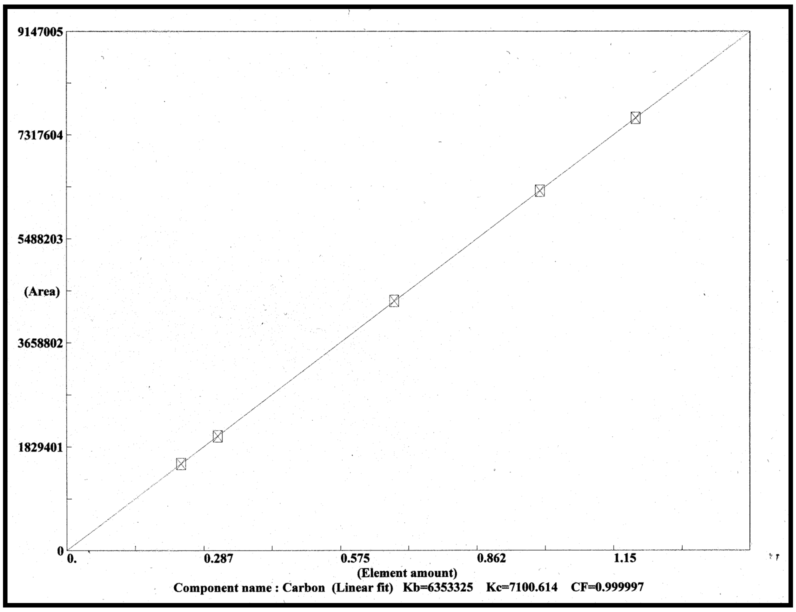

Fig. 4.2.1 Example trace of a standard carbon calibration curve.

This process required an airblank, a sample blank (empty tin capsule Fig. 4.2.2) and 4x1mg standard samples to be processed (Fig. 4.2.3). A final 1mg standard sample was run to ascertain whether the calibration procedure had been a success and to ensure that the autoanalyser was operating at its optimum. The standard used was Acetanilide with a defined N:C:H ratio.

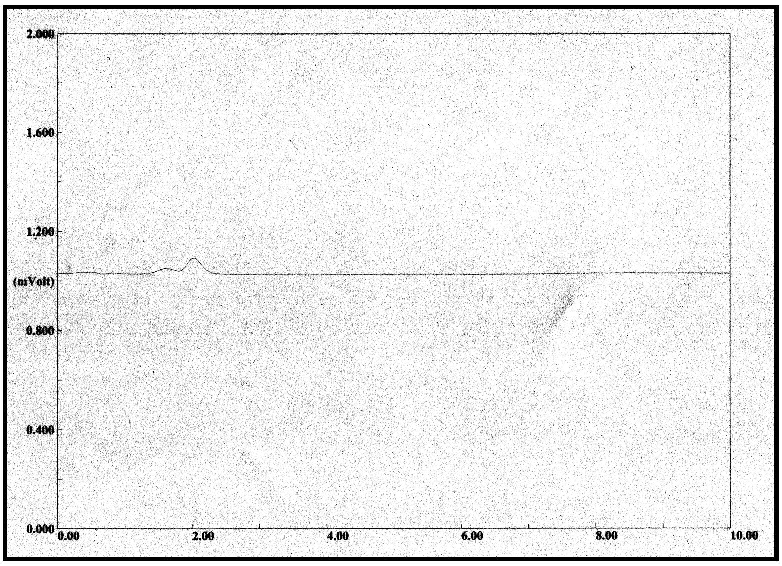

Fig. 4.2.2 Example chromatogram trace of a sample blank (empty tin capsule).

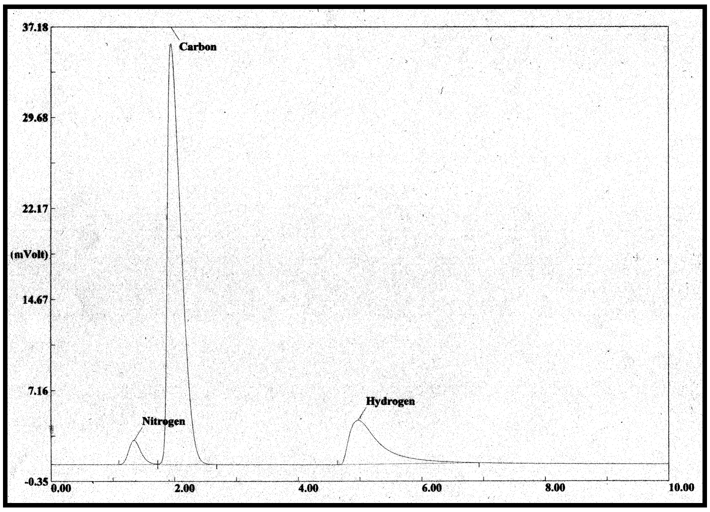

Fig. 4.2.3 An example chromatogram trace of a standard sample run.

The ratio of the standard was accepted within a 2% error margin (Table. 4.2.2). A single Acetanilide standard was run consistently after every 12 samples to monitor the accuracy of the autoanalyser throughout prolonged sample runs.

Table. 4.2.2 The biochemical composition of the standard Acetanilide and the error boundaries accepted for each constituent after calibration.

Element	**Ratio**	**2% error margin**
Nitrogen	10.36	10.17-10.55
Carbon	71.09	69.68-72.50
Hydrogen	6.71	6.58-6.84

It was very important to obtain an accurate weight of both standard and sample. Slightly varied weighing procedures were necessary for the different sample tissues, based on their preparation and drying. The whole ophiuroid samples were already ground and dried, and a subsample ashed prior to arrival at the British Antarctic Survey, Cambridge. These samples (dry and ash) were stored and transported frozen and therefore had to be re-dried in a thermally controlled oven for 24 hours at 70ºC before being processed. This ensured any excess moisture retained during stowage was driven off. Samples were then stored inside a dessicator containing surplus silica dessicant. Individual tin capsules were tared on an electronic microbalance (±0.01mg) and handled using forceps. Particular care was taken not to touch the sample or capsule. A spatula was used to deposit a small amount (~1mg) of dry sample into each pre-weighed capsule (6x4mm). Each capsule was then sealed using forceps, folded into a small ball and placed back onto the tared balance to be weighed. A series of 50 samples could be placed into the carousel of the autoanalyser at one time; each sample required approximately 10 minutes to process. Samples were processed in duplicate and a crucible of silica dessicant was placed into the carousel during each run to keep the pre-loaded samples dry.

The oily nature of the gonad and digestive tissues necessitated a different approach, which has been described above. Samples were dried in pre-weighed tin capsules and the dry weight attained from re-weighing the capsules after 24 hours oven time and calculating the difference. These capsules were sealed using forceps and care was taken when folding the tin foil, as the material was brittle after drying and the capsules easily torn where the tissue samples had liquefied and dried. The samples were then transferred to the carousel and analysed as above. During the initial subsampling of the frozen tissues the amount of material removed into the capsules had to be estimated (~1mg of dry material). On occasion the dry sample was >1mg and therefore an airblank was run after every 12th sample in addition to a standard, to ascertain whether any excess material had swamped the consecutive sample runs.

Data Analysis

The raw data generated from the Eager 800 computer software presented a N:C:H ratio based on the amount of each element as a percentage dry weight of each sample processed. Samples were processed in duplicate and each sample pair was assigned a number based on the numerical order in which they were collected. These preliminary data were checked by plotting each sample, and by comparing the difference between each replicate and the range of monthly samples for each element (%carbon and %nitrogen) (Fig. 4.2.4).

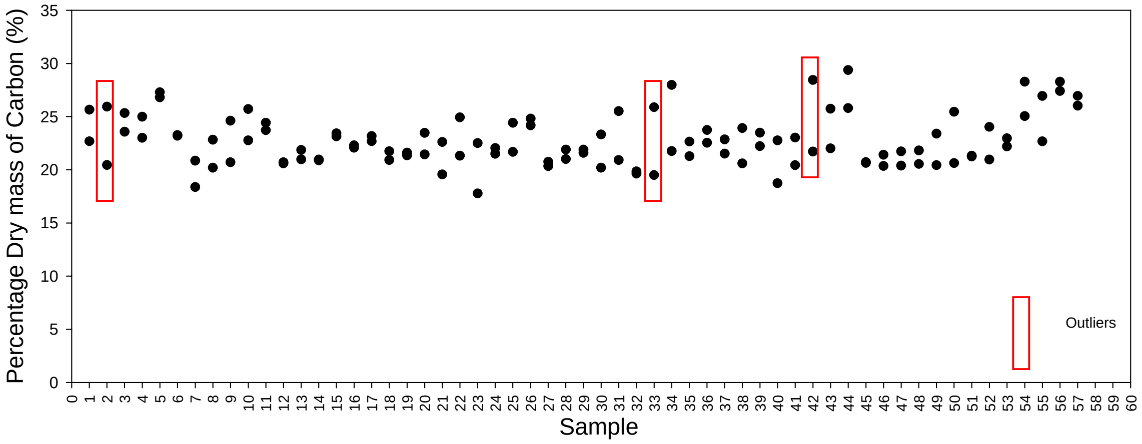

Fig. 4.2.4 An example of a raw data plot to identify sample outliers. Plot shows the %dry mass of carbon in bodily tissue samples of *Ophionotus victoriae*. Samples were ran in duplicate and the range in %carbon between each replicate plotted to identify any outliers. A triplicate sample was ran where large differences were noted between replicate samples.

The difference between duplicate samples was sorted according to magnitude and plotted in rank order to identify any obvious outliers. The range in %carbon and %nitrogen values between sample replicates was also plotted as frequency histograms (Fig. 4.2.5). Any obvious outliers were identified and a triplicate sample processed to ensure the repeatability and reliability of the data.

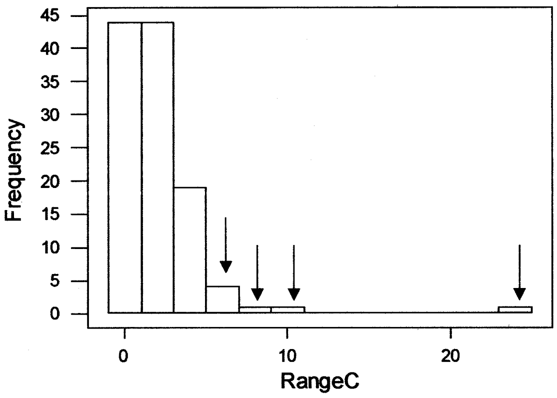

Fig. 4.3.5 Histogram to show the range in %dry mass of carbon between duplicate samples of bodily tissues in *Ophionotus victoriae*. Sample outliers are identified by → and indicate the lower frequency of samples, where the difference in %dry mass of carbon between replicates was large. These samples were processed in triplicate.

CHN Algorithm

An algorithm based on the stoichiometric arguments in Gnaiger and Bitterlich (1984) and modified by Andrew Clarke, was used to calculate a proximate composition from the elemental composition (C, H, N and ash, all as a percentage of dry mass) of each sample through a macro executable in Minitab 13.0.

Before the macro could be executed, it was necessary to set values for three key variables: percentage residual water, percentage of non-protein nitrogen and the mass fraction of nitrogen in non-protein nitrogen (NPN: nucleic acid, chitin):

1. Oven-dried tissues always contain a small amount of tightly-bound residual water, which must be allowed for in the calculations. A default value was inserted into the macro when the percentage residual water was unknown. Gnaiger and Bitterlich (1984) determined this value to be 6.
2. The percentage of total nitrogen that is non-protein nitrogen had to be entered next. In the absence of firm data Gnaiger and Bitterlich (1984) determined this value to be 5, which is considered typical of marine invertebrate tissue.

3. The final value to be entered was the mass fraction of nitrogen in the non-protein nitrogen, which normally lies within a range of 0.01 to 1.0. A default value of 0.15, being the median value for normal amino acids (range 0.08-0.32) was used.

These data were included in the Minitab macro, which organised data into several columns including sample reference number, dry mass (% wet mass), ash content (% dry mass), carbon (% dry mass), nitrogen (% dry mass), hydrogen (% dry mass), phosphorous (% dry mass) and inorganic carbon (% mineral ash). The dry mass (% wet mass) and phosphorous (% dry mass) column were not integral to the algorithm and were left blank. The carbon, nitrogen and hydrogen (% dry mass) data were obtained from the raw data generated for each sample. The autoanalyser could not distinguish between carbon in calcium carbonate or organic carbon. Therefore, to estimate the proximate (lipid, carbohydrate and protein) composition of a tissue from the CHN data, values of ash content, and the carbon content of the ash were also required. The ash content (% dry mass) of samples was obtained from the furnace data and included the individual ash data for all of the dissected whole ophiuroid samples and the average ash data for individual gonad and digestive tissues. The inorganic carbon (% mineral ash) data were obtained from the %carbon values generated by the Eager 800 software after processing ash samples. Real data were only available for the dissected whole ophiuroid samples. The ashed gonad and digestive tissue were not processed through the CHN elemental autoanalyser (Carlo Erba EA1108). Therefore, in these instances a default value of 0.01 was entered. This default value was also used on occasions when a negative %carbon or zero value was detected by the CHN machine, and it reflects the level of carbon in ash in typical marine invertebrate tissue (A Clarke, pers. comm.), although this default is not suitable for those tissues high in ash, e.g. the carbonate skeletons of echinoderms. Direct measurements of these tissues were made in the current study.

The algorithm was executable as follows:

1. Calculate absolute dry mass by subtracting the residual water content from oven-dried mass.
2. Estimate organic carbon by subtraction of inorganic carbon from measurement of the carbon content in the ash.

3. Estimate the fraction of total nitrogen explained by protein.
4. Estimate mass of protein from mass fraction of nitrogen in protein (conversion factor = 0.173).
5. Estimate C and H in this protein and subtract from total organic C and H.
6. Partition remaining organic carbon between lipid (fraction of carbon = 0.776) and carbohydrate (fraction of carbon = 0.444).
7. Sum components (protein, lipid, carbohydrate and non-protein nitrogen) to check that organic components explain between 95 and 105% organic matter.

The routine returns error messages when variables exceed boundary conditions defined within the macro. Negative values of both lipid and carbohydrate can be returned if the fraction of carbon explained by protein has been over or underestimated respectively. The mass fraction of carbon in the non-protein organic matter should lie within the range of 0.776 to 0.444. A sample within the middle of this range is comprised of a mixture of carbohydrate and lipid, whereas a sample at either extreme is pure lipid or pure carbohydrate respectively. A value >0.776 has been overestimated for lipid and will produce negative carbohydrate values. The most plausible explanation for this is the overestimation of non-protein nitrogen (NPN) or that the residual water value is too high. Alternatively, a severe underestimation of the residual water content can cause an overestimation of carbohydrate and negative lipid values. If this occurred the samples were re-run using optimised values of non-protein nitrogen (NPN) or residual water content where necessary, until the algorithm returned values within the boundary limits of the macro. Owing to the variables that have to be set or estimated, the algorithm cannot provide a definitive composition. It can, however, provide a valuable guide to proximate composition.

Statistical manipulation

The elemental and proximate composition data were analysed statistically. Only carbon, nitrogen, protein and lipid were analysed and plotted. However, the average and standard deviation of each tissue component was calculated. Hydrogen and carbohydrate values were not processed further, owing to the large variability inherent in hydrogen data caused by the inconsistent water content of tissues, and the limited percentage dry mass attributable to carbohydrate in most invertebrate tissues (A Clarke, pers. comm.). Although carbon and nitrogen are often considered indicative of lipid and protein respectively, these relationships are not direct, and neither are these data calculated simply using carbon and nitrogen in the algorithm. Therefore, all four data sets were analysed and plotted, and any patterns identified.

The data were separated based on species and tissue type, and box plots of each element and tissue component were plotted over time. These data were statistically analysed for normality using the Anderson-Darling Normality test (Appendix Table. 11). One-Way ANOVA was used to test for the effects of sex and month sampled on each data set, when the data were normally distributed. Data that exhibited significant differences with month sampled were analysed using Tukey's Test for pairwise multiple comparisons. Data that were considered non-normal were also processed using a non-parametric statistical test to identify any significant differences with sex and month sampled. The Kruskal-Wallis test was employed in these cases. Comparisons between tissues and between the two echinoderm species using the non-parametric Mann Whitney test and 2-sample T-test were undertaken, depending on the normality of the data. All data were separated by month sampled and sex, and were plotted by Julian Day (1st January 2003 = Day 1), where any obvious patterns with time could be identified. All statistical tests were under taken using Minitab 13.0 and all data were plotted using Sigma Plot 8.0 (SPSS Software).

4.3 Results

Compatibility of the data and the algorithm

The elemental tissue components of each of the two echinoderms analysed varied beyond the ranges acceptable for the macro used. Subsequently, data had to be separated and the default values optimised individually, so as the majority of values returned fell within the boundary limits of the macro (Table. 4.3.1 and 4.3.2). This mainly involved substituting the default residual water content with values until the macro returned acceptable results. Thus, the different species and tissues types were processed individually, and each elemental data set was separated by month sampled. Further separation according to sex was sometimes necessary where the data continued to deviate outside the normal boundary limits of the macro. However, some data were more easily accommodated by the algorithm and on occasion returned realistic results using the defaults recommended by Gnaiger and Bitterlich (1984).

The elemental data for pyloric caeca tissue from *Odontaster validus* fitted the algorithm reasonably well, returning realistic values for monthly sample sets when the default boundaries were optimised. However, the remaining data had to be separated initially by month, and then by sex in order to fit the macro. The tissues (whole body, gut and gonad) of *Ophionotus victoriae* produced elemental data less consistent with the limits of the macro, necessitating all data to be separated first by month and then by sex. In some instances, despite optimising the default inputs suggested by Gnaiger and Bitterlich (1984), not all values fell within the recommended boundary limits and some reported negative lipid or carbohydrate values. These data were disregarded and removed from any further analysis (Table. 4.3.1 and 4.3.2).

The elemental data for gonad tissue from *Odontaster validus* consistently returned negative carbohydrate values, even after the data were separated by month and sex and ran individually through the macro. Extremely negative carbohydrate values commonly result when the amount of lipid is overestimated by the algorithm, and usually occur when the fraction of non-protein nitrogen is too high or the residual water value excessive. However, the gonad data consistently failed to conform to the

Table. 4.3.1 *Odontaster validus*. Table of values for residual water and non-protein (NPN) used to estimate proximate composition from elemental analysis based on stoichiometry (Gnaiger and Bitterlich, 1984). The values of residual water and NPN show the values that optimised the results and returned data within the boundary limits of the macro (original default = residual water 6, NPN 5). All tissues had a default value of 0.15 for the mass fraction of N in the NPN. The mean %ash and mean %C in ash are also included. The last column details the number of individuals that were removed from further analysis aster returning consistently negative lipid or carbohydrate values.

Species	**Tissue**	**Data**	**Mean Ash (% dry mass)**	**Mean C in Ash (%)**	**% Residual Water**	**NPN**	**Total Number of Individuals**	**Individuals discarded**
Odontaster validus	Gonad	Male (All)	39.92	0.01	6	5	37	27
		Female (All)	39.92	0.01	6	5	33	33
	Pyloric caeca	16/12/2004	19.73	0.01	3	5	10	0
		Male 19/12/2003	19.73	0.01	1	5	3	0
		Female 19/12/2003	19.73	0.01	0.1	5	7	1
		09/01/2004	19.73	0.01	0.1	5	10	0
		Male 05/02/2004	19.73	0.01	0.1	5	9	4
		Female 05/02/2004	19.73	0.01	0.1	1	6	1
		Male 19/03/2004	19.73	0.01	1	5	6	0
		Female 19/03/2004	19.73	0.01	0.01	0.1	9	0

Table. 4.3.2 *Ophionotus victoriae*. Table of values for residual water and non-protein nitrogen (NPN) used to estimate proximate composition from elemental analysis based on stoichiometry (Gnaiger and Bitterlich, 1984). The values of residual water and NPN show the values that optimised the results and returned data within the boundary limits of the macro (original default = residual water 6, NPN 5). All tissues had a default value of 0.15 for the mass fraction of N in the NPN. The mean %ash and %C in ash are also included. The last column details the number of individuals that were removed from further analysis after returning consistently negative lipid or carbohydrate values.

Species	Tissue	Data	Mean Ash (% dry mass)	Mean C in Ash (%)	% Residual Water	NPN	Total number of Individuals	Individuals Discarded
Ophionotus victoriae	Whole Animal	Male 13/02/2003	45.95	3.81	13	5	7	1
		Female 13/02/2003	46.72	3.62	14	5	3	0
		Male 10/03/2003	49.20	5.42	15	1	7	1
		Female 10/03/2003	52.53	7.07	12	5	3	0
		09/04/2003 (all F)	42.58	2.60	7	17	10	0
		Male 05/05/2003	43.80	2.47	8	5	2	0
		Female 05/05/2003	42.80	2.25	17	5	8	2
		Male 17/06/2003	42.93	0.97	15	5	3	1
		Female 17/06/2003	43.68	2.41	15	5	6	2
		Male 10/07/2003	40.72	0.96	14	5	5	0
		Female 10/07/2003	40.44	0.18	15	5	5	0
		Male 13/12/2003	43.77	1.02	16	5	7	1
		Female 13/12/2003	43.53	0.11	15	5	6	0
		Male 07/01/2004	43.70	0.01	16.5	5	4	0
		Female 07/01/2004	45.05	0.31	15	5	8	0
		Male 11/02/2004	49.02	1.62	12	5	9	2
		Female 11/02/2004	45.40	0.79	15	5	6	0
		Male 09/03/2004	43.79	3.32	12	5	10	0
		Female 09/03/2004	42.66	3.17	12	5	5	0

Table. 4.3.2 contd *Ophionotus victoriae*. Table of values for residual water and non-protein nitrogen (NPN) used to estimate proximate composition from elemental analysis based on stoichiometry (Gnaiger and Bitterlich, 1984). The values of residual water and NPN show the values that optimised the results and returned data within the boundary limits of the macro (original default = residual water 6, NPN 5). All tissues had a default value of 0.15 for the mass fraction of N in the NPN. The mean %ash and %C in ash are also included. The last column details the number of individuals that were removed from further analysis after returning consistently negative lipid or carbohydrate values.

Species	**Tissue**	**Data**	**Mean Ash (% dry mass)**	**Mean C in Ash (%)**	**% Residual Water**	**NPN**	**Total number of Individuals**	**Individuals Discarded**
Ophionotus victoriae	Gut	Male 13/02/2003	19.002	0.01	0.01	1	7	2
		Female 13/02/2003	19.002	0.01	0.1	5	3	0
		Male 10/03/2003	19.002	0.01	0.1	5	7	2
		Female 10/03/2003	19.002	0.01	1	5	3	1
		09/04/2003 (all F)	19.002	0.01	1	1	10	2
		Male 05/05/2003	19.002	0.01	6	5	2	2
		Female 05/05/2003	19.002	0.01	0.1	5	8	1
		Male 17/06/2003	19.002	0.01	1	5	3	1
		Female 17/06/2003	19.002	0.01	1	5	6	1
		Male 10/07/2003	19.002	0.01	1	5	5	1
		Female 10/07/2003	19.002	0.01	1	5	5	0
		Male 13/12/2003	19.002	0.01	1	5	7	1
		Female 13/12/2003	19.002	0.01	5	5	6	0
		Male 07/01/2004	19.002	0.01	1	5	4	1
		Female 07/01/2004	19.002	0.01	0.5	5	8	0
		Male 11/02/2004	19.002	0.01	0.01	1	9	0
		Female 11/02/2004	19.002	0.01	1	5	6	0
		Male 09/03/2004	19.002	0.01	1	5	10	4
		Female 09/03/2004	19.002	0.01	0.01	1	5	2

Table. 4.3.2 contd *Ophionotus victoriae*. Table of values for residual water and non-protein nitrogen (NPN) used to estimate proximate composition from elemental analysis based on stoichiometry (Gnaiger and Bitterlich, 1984). The values of residual water and NPN show the values that optimised the results and returned data within the boundary limits of the macro (original default = residual water 6, NPN 5). All tissues had a default value of 0.15 for the mass fraction of N in the NPN. The mean %ash and %C in ash are also included. The last column details the number of individuals that were removed from further analysis after returning consistently negative lipid or carbohydrate values.

Species	**Tissue**	**Data**	**Mean Ash (% dry mass)**	**Mean C in Ash (%)**	**% Residual Water**	**NPN**	**Total number of Individuals**	**No of Individuals Discarded**
Ophionotus victoriae	Gonad	Male 13/02/2003	16.173	0.01	3	5	7	2
		Female 13/02/2003	16.173	0.01	1	5	3	1
		Male 10/03/2003	16.173	0.01	3	1	7	3
		Female 10/03/2003	16.173	0.01	0.1	5	3	0
		09/04/2003 (all F)	16.173	0.01	0.01	5	10	3
		Male 05/05/2003	16.173	0.01	5	5	2	0
		Female 05/05/2003	16.173	0.01	1	5	8	3
		Male 17/06/2003	16.173	0.01	6	5	3	0
		Female 17/06/2003	16.173	0.01	3	5	6	5
		Male 10/07/2003	16.173	0.01	3	5	5	1
		Female 10/07/2003	16.173	0.01	0.1	5	5	1
		Male 13/12/2003	16.173	0.01	6	5	7	1
		Female 13/12/2003	16.173	0.01	0.1	5	6	1
		Male 07/01/2004	16.173	0.01	5	5	4	0
		Female 07/01/2004	16.173	0.01	1	5	8	1
		Male 11/02/2004	16.173	0.01	5	5	9	2
		Female 11/02/2004	16.173	0.01	0.1	5	6	0
		Male 09/03/2004	16.173	0.01	0.1	5	10	5
		Female 09/03/2004	16.173	0.01	6	5	5	5

boundaries of the algorithm. Therefore, this tissue was plotted and analysed only in terms of its elemental composition (C and N).

The elemental and proximate composition data for the individual tissues from each echinoderm analysed are presented in Table. 4.3.13 - 4.3.16. Also note that all energy values are quoted as negative (the thermodynamic convention that illustrates that the energy created is exothermic).

Temporal and Sexual Differences

Odontaster validus Gonad

10-15 starfish were collected on 5 separate dates during the Antarctic summer field season 2003-2004 (December-March). The average ash content of the gonad tissue was high (39.92%). A slightly larger percentage composition was attributed to carbon (mean = 42.74%), and nitrogen (mean = 10.46%) accounted for a much smaller fraction of the gonad (Table. 4.3.3). This pattern was true of both the testes and ovaries.

The elemental composition of the gonad tissue did not differ significantly over the sampling period (Table. 4.3.4), although carbon and nitrogen composition between the sexes were significantly different. Similar carbon and nitrogen values were observed in the gonad of individuals sampled during December 2003-March 2004 (Fig. 4.3.1). The percentage composition of carbon remained consistently high (~42%). Nitrogen levels were comparatively low (~11%). However, the trends in tissue composition varied between the testes and ovaries (Fig. 4.3.2 and 4.3.3). The percentage composition of carbon and nitrogen in the testes were relatively consistent and similar to the mean composition observed (Table. 4.3.3). However, a decline in both carbon and nitrogen content was observed over the Antarctic summer in the ovaries and generally higher levels of carbon were recorded (Fig. 4.3.3).

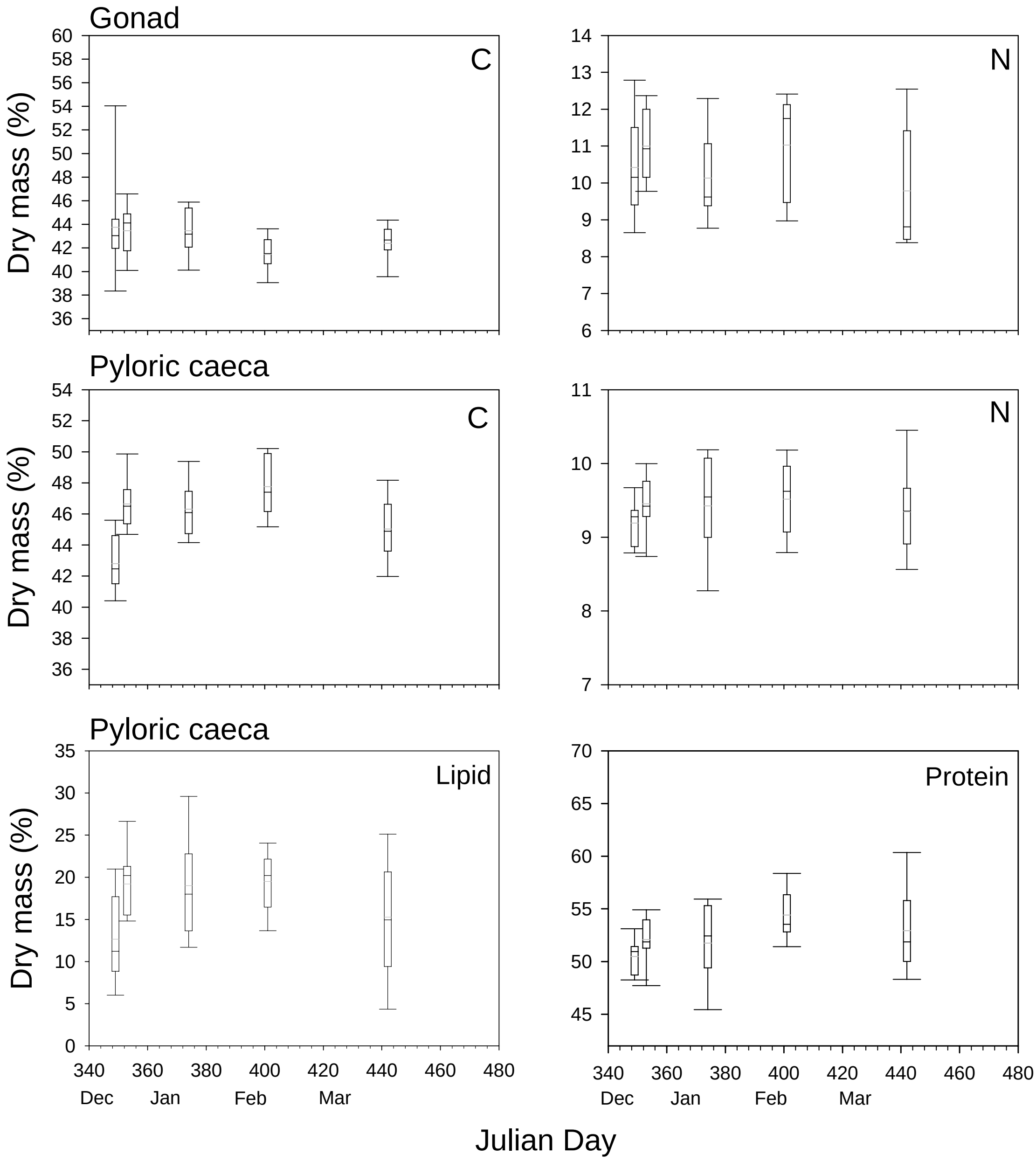

Fig. 4.3.1 *Odontaster validus*. Biochemical and proximate composition of pyloric caeca and gonad tissue collected on a monthly basis between December 2003 and March 2004. Data are plotted as box plots. The boundary of the box closest to zero indicates the 25th percentile, the black and grey line within the box marks the median and mean respectively, and the boundary of the box farthest from zero indicates the 75th percentile. Whiskers (error bars) above and below the box indicate the 90th and 10th percentiles.

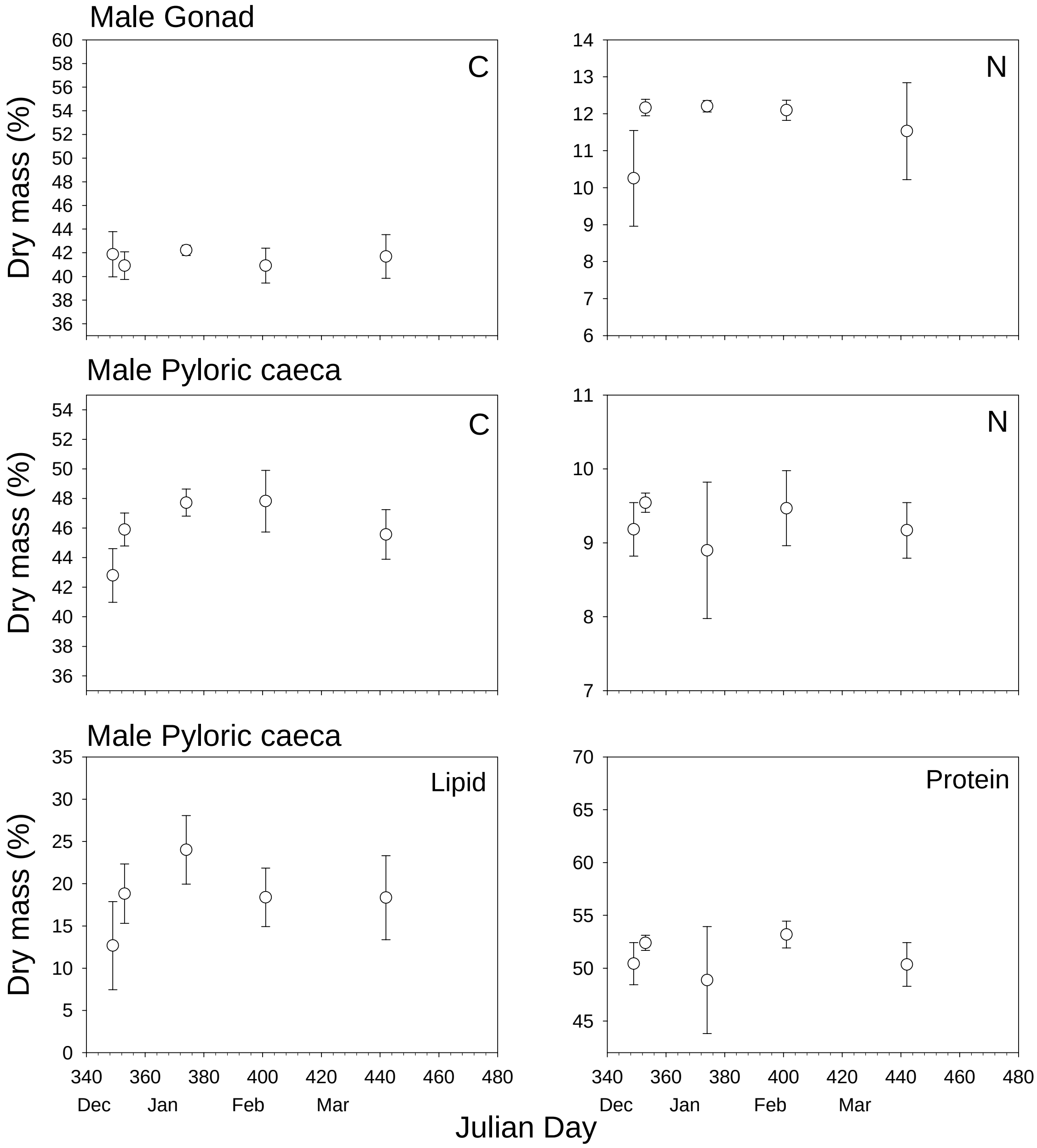

Fig. 4.3.2 *Odontaster validus*. Biochemical and proximate composition of male pyloric caeca and gonad tissue collected on a monthly basis between December 2003 and March 2004. Data are plotted as an overall mean ± SD.

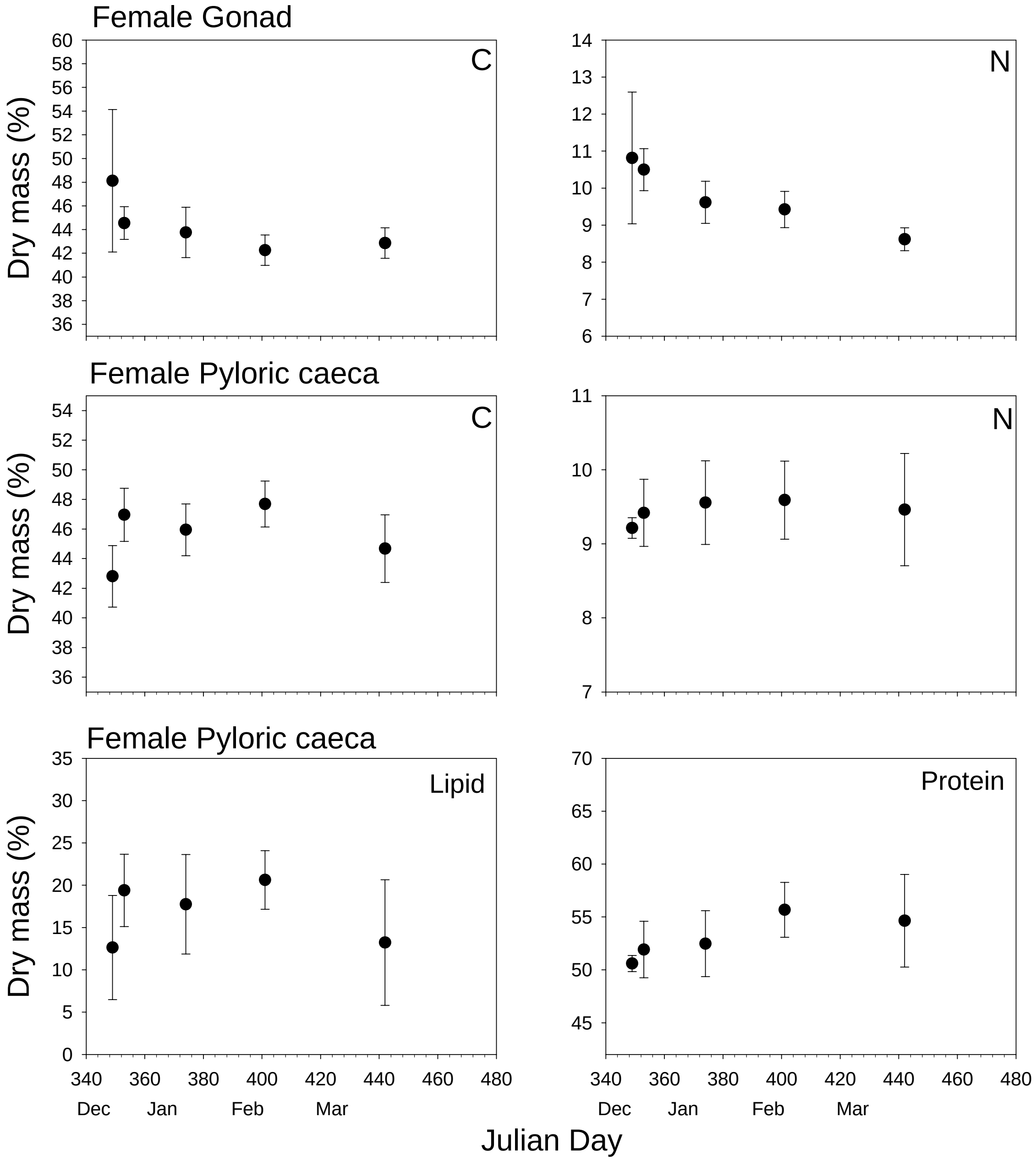

Fig. 4.3.3 *Odontaster validus*. Biochemical and proximate composition of female pyloric caeca and gonad tissue collected on a monthly basis between December 2003 and March 2004. Data are plotted as an overall mean + SD.

Table. 4.3.3 *Odontaster validus*. Descriptive statistics (mean ± SD, range, N) describing the composition of the gonad and pyloric caeca tissues in males and females.

Species	Tissue	Composition (% dry mass)	Average	SD	Min	Max	N
Odontaster validus	Gonad	Carbon	42.74	2.50	38.11	55.03	60
		Male Carbon	41.43	1.58	38.11	43.79	27
		Female Carbon	43.81	2.61	39.91	55.03	33
		Nitrogen	10.46	1.42	8.27	12.85	60
		Male Nitrogen	11.51	1.17	8.61	12.70	27
		Female Nitrogen	9.61	0.99	8.27	12.85	33
	Pyloric caeca	Carbon	45.82	2.44	40.31	50.26	60
		Male Carbon	45.79	2.63	40.31	50.18	27
		Female Carbon	45.85	2.32	41.58	50.26	33
		Nitrogen	9.39	0.52	8.25	10.90	60
		Male Nitrogen	9.29	0.46	8.25	10.17	27
		Female Nitrogen	9.48	0.56	8.52	10.90	33
		Protein	52.39	3.23	45.28	62.93	54
		Male Protein	51.13	2.35	45.28	57.59	23
		Female Protein	53.33	3.50	46.81	62.93	31
		Lipid	16.92	5.86	4.27	29.89	54
		Male Lipid	17.18	5.37	5.73	26.87	23
		Female Lipid	16.72	6.28	4.27	29.89	31
		Carbohydrate	7.80	5.49	0.13	20.36	54
		Male Carbohydrate	7.53	5.45	0.13	20.36	23
		Female Carbohydrate	8.01	5.59	0.16	20.13	31
		Energy	-20.99	1.33	-17.87	-23.60	54
		Male Energy	-20.86	1.34	-17.87	-22.84	23
		Female Energy	-21.08	1.35	-18.56	-23.60	31

Odontaster validus Pyloric Caeca

The pyloric caeca had a lower average ash content compared to the gonad (19.7%), which was calculated from 15 representative tissue samples chosen at random. However, similar carbon (mean = 45.8%) and nitrogen (mean = 9.4%) values were observed. Very similar levels of these constituents were recorded in the digestive tissues of both the males and females (Table. 4.3.3), which were not significantly different between the sexes (Table. 4.3.4).

The elemental composition of the pyloric caeca tissue varied over the sampled months (Table. 4.3.4), although this statistical difference was only significant for carbon. Nitrogen levels did not vary significantly with sampling month and remained

Table. 4.3.4 *Odontaster validus* and *Ophionotus victoriae*. One-Way ANOVA and Kruskal-Wallis test statistics testing for differences between sexes and sampling month in the composition of bodily, digestive and reproductive tissues (normal and non-normal data). AA significant difference was recorded at a significance level P < 0.05.

		ANOVA						**Kruskal-Wallis**					
		Month			**Sex**			**Month**			**Sex**		
Tissue	**Element**	**F**	**P value**	**Significant**	**F**	**P value**	**Significant**	**H**	**P value**	**Significant**	**H**	**P value**	**Significant**
O. validus													
Gonad	C							9.61	0.048	No	17.12	< 0.001	Yes
	N							8.22	0.084	No	23.68	< 0.001	Yes
Pyloric caeca	C	12.34	< 0.001	No	0.01	0.936	No						
	N	0.66	0.624	No	1.88	0.175	No						
	Protein	2.29	0.073	Yes	6.81	0.012	Yes						
	Lipid	3.22	0.020	No	0.08	0.782	No						
O. victoriae													
Whole Animal	C							28.56	0.001	Yes	0.99	0.321	No
	N							24.93	0.003	Yes	2.21	0.137	No
	Protein	3.02	0.003	Yes	4.96	0.028	Yes						
	Lipid							12.59	0.182	No	0.31	0.579	No
Gut	C							30.20	< 0.001	Yes	5.36	0.021	Yes
	N							18.40	0.031	Yes	12.06	0.001	Yes
	Protein	3.27	0.002	Yes	5.62	0.020	Yes						
	Lipid	2.16	0.033	Yes	4.45	0.038	Yes						
	C							17.62	0.071	Yes	63.94	<0.001	Yes
	N							14.29	0.012	No	27.23	< 0.001	Yes
	Protein							17.93	0.040	Yes	40.58	< 0.001	Yes
	Lipid							14.45	0.107	No	30.51	< 0.001	Yes

consistent around ~9.5% (Fig. 4.3.1). Carbon levels increased over the majority of the sampling period, decreasing in the following March. The percentage of carbon recorded in the pyloric caeca on December 16th was significantly lower than levels recorded in tissues collected on the remaining sampling dates (Table. 4.3.5). These patterns were repeated in both the males and females (Fig. 4.3.2 and 4.3.3).

Table. 4.3.5 *Odontaster validus* and *Ophionotus victoriae*. The results of one-way ANOVA and the Tukey Test for pairwise multiple comparisons testing for differences between month sampled in bodily, digestive and reproductive tissues. A significant difference was recorded at a significance level $P < 0.05$. Differences identified by the Tukey Test are described numerically, (1-10 for February 2003-March 2004 in *O. victoriae* and 1-5 for December 16th 2003-March 2004 for *Odontaster validus*) displaying the month tested against the most dissimilar months in brackets. If a general overall difference was recorded the Tukey comparison was recorded as 'none'.

			ANOVA			
			Month			
Species	**Tissue**	**Element**	**F**	**P value**	**Significant**	**Tukey Comparisons**
Odontaster validus	Pyloric caeca	C	12.34	< 0.001	yes	1 (2,3,4,5)
		Lipid	3.05	0.025	yes	none
Ophionotus victoriae	Whole Animal	Protein	3.02	0.003	yes	2 (6)
	Gut	Protein	3.27	0.002	yes	none
		Lipid	2.16	0.033	yes	none

The proximate composition of these tissues was also analysed. Protein accounted for the largest percentage composition of the pyloric caeca (mean = 52.4%). Levels of lipid (mean = 16.9%) and carbohydrate (mean = 7.8%) were smaller (Table. 4.3.3) and accounted for the third and fourth most important constituents respectively. There was a significant difference in the percentage composition of protein between the male and female tissues, although levels of lipid were similar in the pyloric caeca irrespective of sex (Table. 4.3.4).

Despite the sexual difference in percentage composition of protein, no difference was observed over the sampled months. However, a statistical difference in the levels of lipid was evident over the same period (Table. 4.3.4). Protein levels in the pyloric caeca remained high during the sampling period (~50%) and appeared to increase slightly (Fig. 4.3.1). The percentage of protein also increased in the female pyloric caeca over the sampling period (Fig. 4.3.3). A clear pattern in composition was not so apparent in the males, and large error bars associated with the January 2004 samples may be partially accountable for the lack of pattern observed. The percentage composition of lipid varied markedly within each month and differences between months were not significant (Fig. 4.3.1 and Table. 4.3.5). Lipid levels increased during the sampling period, although a decrease in the percentage composition of the pyloric caeca was apparent during March 2004. This trend was clear in the females (Fig. 4.3.3) and males although the percentage lipid content in males declined earlier during February 2004 (Fig. 4.3.2). The allocation of energy to the pyloric caeca was also similar between males and females (2 sample T-test, equal variance (Table. 4.3.6) T = -0.58, P = 0.567, DF = 52).

Table. 4.3.6 *Odontaster validus*. Test for equal variance in energy content of the pyloric caeca between males and females. A P < 0.05 indicates unequal variance.

Test	Test Statistic	P-value
F-test	1.01	0.988
Levene’s test	0.01	0.919

Ophionotus victoriae Whole Animal

The average ash content of the ophiuroids collected was 44.6%. Carbon and nitrogen accounted for a lower average percentage dry mass of the tissue, composing 23.1% and 3.8% of the tissue respectively (Table. 4.3.7). There was no statistical difference in the range of carbon and nitrogen levels observed between the males and females sampled (Table 4.3.4).

Table. 4.3.7 *Ophionotus victoriae*. Descriptive statistics (mean ± SD, range, N) describing the composition of the bodily, gut and gonad tissues in males and females.

Species	Tissue	Composition (% dry mass)	Average	SD	Min	Max	N
	Whole Animal	Ash	44.60	4.13	36.20	59.84	114
		Male Ash	45.30	4.94	36.20	59.84	54
		Female Ash	43.96	3.15	39.20	56.56	60
		Carbon	23.11	2.21	19.53	28.46	114
		Male Carbon	23.31	2.25	19.53	28.22	54
		Female Carbon	22.92	2.17	19.56	28.46	60
		Nitrogen	3.81	0.52	2.90	5.38	114
		Male Nitrogen	3.90	0.58	2.90	5.38	54
		Female Nitrogen	3.73	0.46	2.93	4.66	60
		Protein	20.95	2.68	15.90	29.56	104
		Male Protein	21.57	2.88	15.90	29.56	48
		Female Protein	20.42	2.40	16.10	24.96	56
		Lipid	6.11	4.31	0.03	16.46	104
		Male Lipid	5.75	3.92	0.03	14.13	48
		Female Lipid	6.42	4.63	0.09	16.46	56
		Carbohydrate	13.19	6.62	0.03	24.40	104
		Male Carbohydrate	13.47	6.74	0.03	24.40	48
		Female Carbohydrate	12.96	6.58	1.54	24.16	56
		Energy	-9.92	1.27	-7.08	-13.20	104
		Male Energy	-9.99	1.22	-7.44	-12.69	48
		Female Energy	-9.86	1.32	-7.08	-13.20	56
	Gut	Carbon	46.77	6.66	15.07	57.80	114
		Male Carbon	47.23	7.61	15.07	57.80	54
		Female Carbon	46.36	5.71	18.77	52.32	60
		Nitrogen	7.45	1.25	1.57	9.31	114
		Male Nitrogen	7.17	1.20	1.57	9.07	54
		Female Nitrogen	7.71	1.25	1.82	9.31	60
		Protein	42.68	4.25	31.15	52.65	93
		Male Protein	41.51	3.84	31.98	49.83	40
		Female Protein	43.57	4.37	31.15	52.65	53
		Lipid	25.75	8.21	4.56	41.46	93
		Male Lipid	27.78	8.80	6.12	40.71	40
		Female Lipid	24.22	7.45	4.56	41.46	53
		Carbohydrate	9.41	9.13	0.06	37.93	93
		Male Carbohydrate	9.03	9.19	0.06	37.93	40
		Female Carbohydrate	9.70	9.17	0.48	36.37	53
		Energy	-22.40	1.87	-17.75	-25.50	93
		Male Energy	-22.87	1.90	-17.94	-25.50	40
		Female Energy	-22.06	1.78	-17.75	-25.37	53
	Gonad	Carbon	46.67	6.18	31.97	67.24	114
		Male Carbon	41.80	2.62	31.97	45.99	54
		Female Carbon	51.05	5.09	38.51	67.24	60
		Nitrogen	9.99	2.14	6.55	13.70	114
		Male Nitrogen	11.71	1.17	7.56	13.70	54
		Female Nitrogen	8.44	1.54	6.55	12.68	60
		Protein	56.85	11.14	39.72	72.38	80
		Male Protein	65.48	4.24	55.85	72.38	40
		Female Protein	48.23	8.99	39.72	69.66	40
		Lipid	14.28	13.46	0.43	41.41	80
		Male Lipid	4.66	3.20	0.43	14.61	40
		Female Lipid	23.89	12.94	0.78	41.41	40
		Carbohydrate	7.06	6.02	0.03	36.70	80
		Male Carbohydrate	5.78	4.38	0.03	16.22	40
		Female Carbohydrate	8.34	7.13	0.05	36.70	40
		Energy	-21.06	2.60	-17.42	-26.31	80
		Male Energy	-19.19	0.73	-17.93	-21.31	40
		Female Energy	-22.92	2.46	-17.42	-26.31	40

The elemental composition of the ophiuroids did differ significantly over the sampling period (Table. 4.3.4). This was true for both the percentage dry mass of carbon and nitrogen. Carbon and nitrogen produced similar patterns in the bodily tissues of the ophiuroid (Fig. 4.3.4), decreasing initially between February-March 2003, before increasing over the following months (until July). The percentage composition of both constituents decreased between May-June 2003. A lower percentage carbon and nitrogen were observed in December 2003, and continued to decline until February 2004. These patterns were clearly indicated in the females (Fig. 4.3.6) and in most cases were also exhibited by the males, with the exception of missing data in April 2003 and an anomalous high value being observed in the following May (Fig. 4.3.5). Data were absent during April as all of the individuals sampled were identified as female and owing to the limited amount of individuals returning values consistent within the boundary restrictions of the macro, only 2 males could be reliably plotted for May 2003. Such a small sample size may have skewed the data distribution and resulted in an anonymously high average.

The proximate composition of these tissues was also determined. Protein accounted for the largest fraction of the bodily tissues (mean = 21.0%), with smaller fractions being composed of carbohydrate and lipid (mean = 13.2% and 6.1% respectively). A statistical difference was apparent in the proximate composition of the tissues between the sexes (Table. 4.3.4). However, this was only demonstrated for protein. The average percentage composition of protein was higher in the males (21.6%). The percentage composition of protein also differed significantly over the sampling period. A significant difference in composition was apparent between March and July 2003 (Table. 4.3.5). The patterns exhibited in protein and lipid were similar to the trends observed in nitrogen and carbon (Fig. 4.3.4). The percentage composition of both components increased during March-July 2003 and decreased between May and June 2003. Similar levels were observed in the following December, before a brief period of increase between February and March 2004. Again these patterns were clearly indicated in the female ophiuroids (Fig. 4.3.6). However, an increase in the percentage composition of the male ophiuroids occurred earlier in January 2004 and percentage lipid levels proceeded to decline during the following March, which was not replicated in the female data (Fig. 4.3.5).

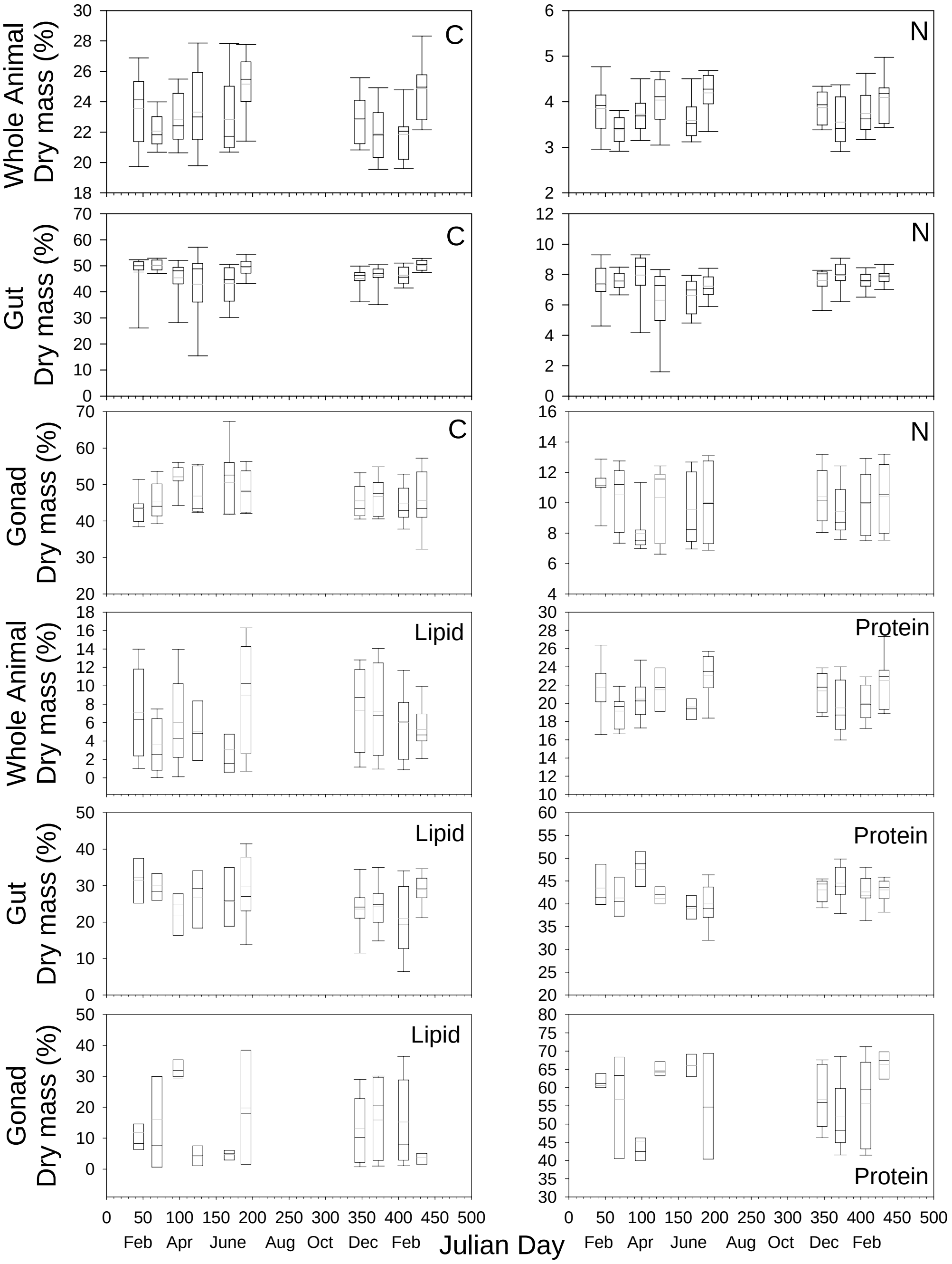

Fig. 4.3.4 *Ophionotus victoriae*. Biochemical and proximate composition of bodily, gut and gonad tissues collected on a monthly basis between February 2003 and March 2004. Data are plotted as box plots. The boundary of the box closest to zero indicates the 25th percentile, the black and grey line within the box marks the median and mean respectively, and the boundary of the box farthest from zero indicates the 75th percentile. Whiskers (error bars) above and below the box indicate the 90th and 10th percentiles.

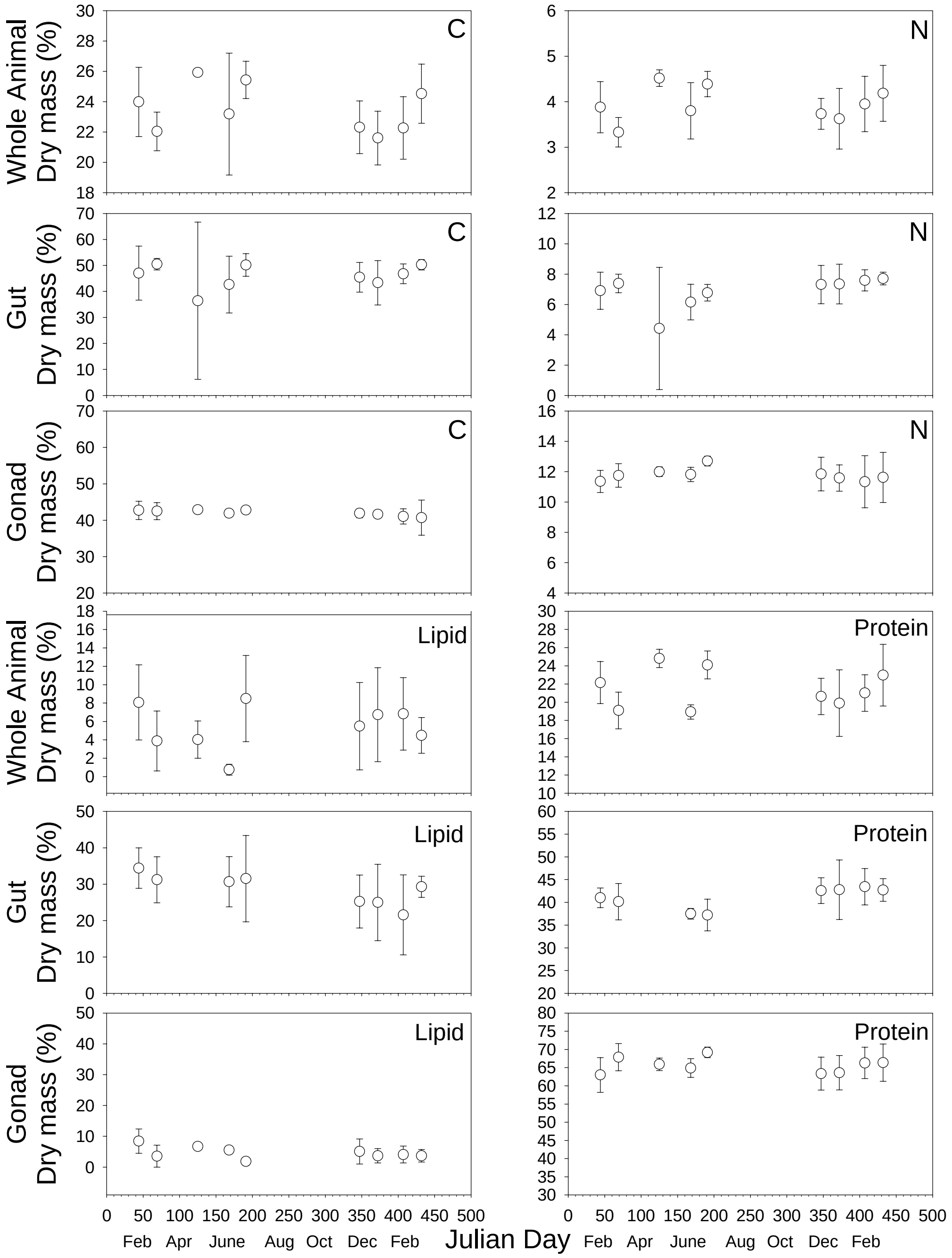

Fig. 4.3.5 *Ophionotus victoriae*. Biochemical and proximate composition of male bodily, gut and gonad tissues collected on a monthly basis between February 2003 and March 2004. Data are plotted as an overall mean ± SD.

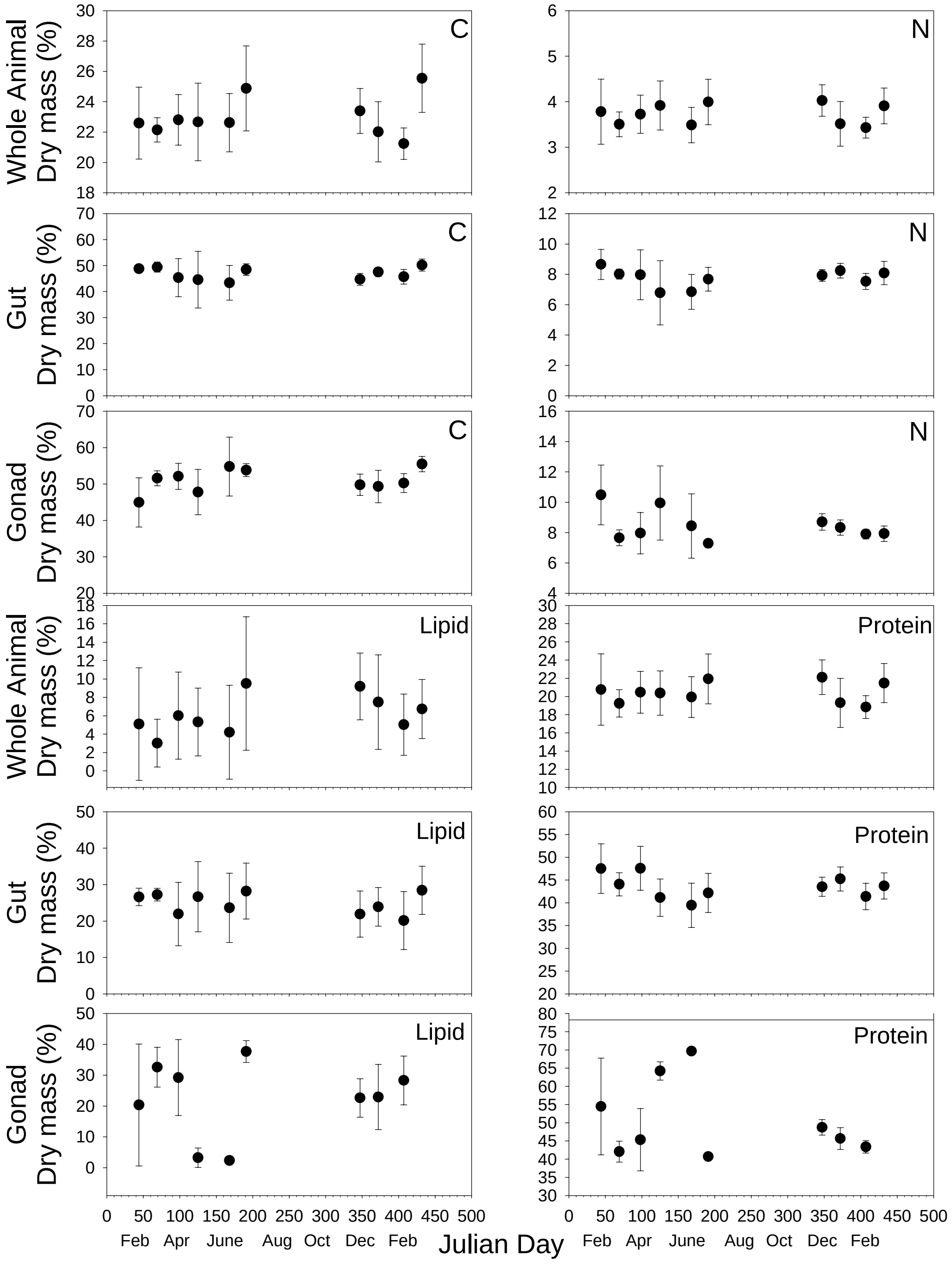

Fig. 4.3.6 *Ophionotus victoriae*. Biochemical and proximate composition of female bodily, gut and gonad tissues collected on a monthly basis between February 2003 and March 2004. Data are plotted as an overall mean ± SD.

The amount of energy allocated to bodily tissues of the ophiuroid was low compared to the other tissues and similar between males and females (Mann Whitney, W = 3059.0, P = 0.440).

Ophionotus victoriae Gut

Lower ash levels were exhibited in the ophiuroid digestive tissues. An average ash composition of 30 random tissues was determined (19%). The remainder of the gut was mainly composed of carbon (mean = 46.8%), with a smaller fraction being attributable to nitrogen (mean = 7.5%) (Table. 4.3.7). The elemental composition of the male and female gut tissue was significantly different (Table. 4.3.4).

A statistical difference in both the percentage dry mass of carbon and nitrogen was apparent over the sampling period (Table. 4.3.4) and similar patterns were observed in both components. The percentage composition of both constituents declined between February and May 2003. A small increase in levels followed during June and July. Similar levels were observed in the following December, and remained fairly consistent during the following months, although a slight increase in the percentage composition of both carbon and nitrogen in the gut could be discerned over the same time period. These patterns were apparent in the both the male and female gut tissues (Fig. 4.3.5 and 4.3.6). However, the missing data and large error associated with the male data in April and May 2003 obscured the trend.

Protein accounted for the largest fraction of material in the ophiuroid gut (mean = 42.7%). Lipid and carbohydrate accounted for the remaining dry mass (mean = 25.8% and 9.4% respectively). There was a significant difference in the proximate composition of the male and female gut tissues (Table. 4.3.4). This was true for both the percentage dry mass of protein and lipid.

A significant difference in the levels of protein and lipid was also evident over the sampling period (Table. 4.3.4), although there was no significant difference between individual sampling months (Table. 4.3.5). Patterns in the percentage composition of lipid and protein were similar. Percentages declined between February and March/April 2003, and April/May and July 2003. The percentage composition increased during April/May. Slightly higher levels of protein were recorded in the gut in the following December, whereas lipid levels were comparatively lower. These values remained relatively consistent in terms of protein, but decreased slightly in lipid during February 2004 before increasing in the following March. These patterns were clearly indicated in both the male and female gut tissues. The allocation of energy to the gut tissues was slightly higher in males (Mann Whitney, W = 2780.0, P = 0.046).

Ophionotus victoriae Gonad

The average ash content was reduced in the gonad (16.2%) compared to the gut. Carbon accounted for the largest fraction of the ophiuroid gonad (mean = 46.7%). The average percentage composition of nitrogen was diminished by comparison (10.0%) (Table 4.3.7). There was a significant difference in the elemental composition of the gonad between males and females (Table. 4.3.4), in terms of both carbon and nitrogen. Generally higher levels of carbon were observed in the ovaries, whereas nitrogen values were generally larger in the testes (Fig. 4.3.5 and 4.3.6).

A statistical difference was not evident in the elemental composition of the gonad over the 10-month sampling period (Table. 4.3.4), despite a sexual difference being identified. The patterns in carbon and nitrogen varied (Fig. 4.3.4). The percentage composition of carbon increased between February-April 2003 and decreased between April and May and between June and July, despite a higher percentage composition in the gonad during June. Comparable carbon levels were exhibited in the following December, with similar values being determined during February and March 2004. A small increase in the percentage composition of carbon was apparent during January 2004. Nitrogen values were more variable and generally decreased during February-April 2003. The percentage composition of nitrogen increased in the gonad during May and July and decreased in June 2003. Comparable levels of nitrogen were observed in the following December. Lower percentage nitrogen was observed during the following month, but increased again during February and March 2004. Similar patterns in both the percentage dry mass of carbon and nitrogen were evident in the ovaries (Fig. 4.3.6). However, the composition of the testes was generally consistent over the sampling period (Fig. 4.3.5).

The proximate composition of the gonad was also determined identifying protein as the main tissue component (mean = 56.9%). Lipid accounted for the third most abundant component of the ophiuroid gonad (mean = 14.3%), and a smaller fraction was contributed by carbohydrate (mean = 7.1%). A clear dichotomy existed between the testes and ovaries composition (Table. 4.3.7). The testes exhibited generally higher fractions of protein (Fig. 4.3.5), whereas lipid levels composed a higher percentage dry mass of the ovaries (Fig. 4.3.6).

A significant difference in the percentage composition of protein was evident over the sampling period (Table. 4.3.4). A large variation in lipid content in the gonad was evident between the individuals sampled on a monthly basis, although no statistical difference was recorded between February 2003 and March 2004. However, a significant difference in composition was apparent between the testes and ovaries (Table. 4.3.4). Patterns in protein and lipid varied in the ophiuroid gonad (Fig. 4.3.4), but were similar to the trends observed in the percentage composition of nitrogen and carbon respectively, and were highly seasonal. The percentage composition of protein declined between February and April 2003, increasing over the following months until July 2003. Similar protein levels were apparent in the following December, before increasing during the remainder of the sampling period. Conversely, the percentage composition of lipid increased during the first three months of sampling. However, lower lipid levels were observed during May-June 2003, although much higher lipid was recorded in July. A similar percentage composition was observed in the following December, which increased during January 2004. A lower percentage composition was apparent during the latter stages of sampling. These patterns were clearly emphasised in the ovaries (Fig 4.3.6).

Patterns exhibited in the testes were much less variable and lacked any obvious seasonality. Protein levels remained consistently high in the testes and increased slightly during February to July 2003 and December 2003 to March 2004. However, the percentage composition of lipid remained consistently low and decreased slightly between February and July 2003. Larger amounts of energy were invested in the gut, compared to the gonad (Mann Whitney, W = 8159.0, P = 0.0005), and females appeared to invest to a greater degree compared to males when allocating reserves to the gonad (Mann Whitney, W = 1004.0, P < 0.0001).

Differences in individual tissue composition

Unfortunately, owing to the problems described earlier, the gonad tissue of *Odontaster validus* could only be analysed in terms of its elemental composition. Therefore, the pyloric caeca and gonad data are only compared in terms of the percentage dry mass of ash, carbon and nitrogen.

The average ash content of the gonad and pyloric caeca tissues was calculated from 15 randomly chosen tissue samples and differed markedly. The average ash level in the gonad was twice as large (mean = 39.9%) as the value recorded in the pyloric caeca (mean = 19.8%). Carbon accounted for the largest fraction of material in both tissues, although the percentage carbon composition of the pyloric caeca was significantly higher (Mann Whitney, W = 2418.0, P < 0.0001). The lowest fraction of material in these tissues was attributed to nitrogen, although a higher percentage composition was recorded in the gonad (Mann Whitney, W = 4363.0, P = 0.0001). However, similar nitrogen levels were observed in the gonad and pyloric caeca of females (2-sample T-test, unequal variance (Table. 4.3.8) T = 0.65, P = 0.517, DF = 50).

Table. 4.3.8 *Odontaster validus*. Test for equal variance in %nitrogen content between female gonad and pyloric caeca tissue. P < 0.05 indicates unequal variance.

Test	Test Statistic	P-value
F-test	3.18	0.002
Levene’s test	5.01	0.029

The ash content of the ophiuroid bodily tissues was determined and compared to an average ash content calculated from 30 randomly chosen gut and gonad tissues. Similar levels of ash were observed in the gut and gonad (mean = 19.0 and 16.2% respectively), and a generally larger fraction of the ophiuroid body was ash (mean = 44.6%). This component accounted for the largest percentage dry mass of the ophiuroid bodily tissue.

The largest percentage dry mass of the gut (mean = 46.8%) and gonad tissue (mean = 46.7%) was composed of carbon, which contributed moderately to the bodily tissues (mean = 23.1%). Similar levels of carbon were observed in the gut and gonad (Mann

Whitney, W = 13848.0, P = 0.111), although differences were apparent in the males and females (Table. 4.3.9). Nitrogen accounted for only a small fraction of the ophiuroid, contributing least to the ophiuroid body. A significant difference in nitrogen was apparent between the gut and gonad tissues (Mann Whitney, W = 9082.0, P < 0.0001), although no difference was observed in the females (Mann Whitney, W = 3429.0, P = 0.293).

Table 4.3.9 *Ophionotus victoriae*. Comparisons between the %carbon content in the gut and gonad tissues in males and females. A Mann-Whitney non parametric analysis was employed and a P < 0.05 indicated a significant difference in tissue biochemical composition.

Variable	Test Statistic	P-value
Female	W = 2684.0	< 0.0001
Male	W = 4023.0	< 0.0001

Protein accounted for the largest fraction of material in the gonad and gut (mean = 56.9% and 42.7% respectively), and contributed moderately to the ophiuroid bodily tissues (mean = 21.0%). A larger percentage composition of the gonad was attributed to protein (Mann Whitney, W = 5585.0, P < 0.0001), although no difference was observed in the females (Mann Whitney, W = 2246.0, P = 0.058).

Moderate levels of lipid contributed to the composition of the gut and gonad (mean = 25.8% and 14.3% respectively), and accounted for the smallest tissue fraction in the ophiuroid body (mean = 6.1%). Larger levels of lipid were recorded in the gut, compared to the gonad (Mann Whitney, W = 9877.0, P < 0.0001), although similar levels were observed between the female gut and ovaries (Mann Whitney, W = 2368.0, P = 0.342).

Carbohydrate accounted for the smallest tissue fraction in the ophiuroid and the percentage composition of the reproductive and digestive tissues was similar (Table. 4.3.10). The amount of energy allocated to the individual tissues varied, although generally higher levels were invested in the gut, compared to the gonad (Mann Whitney, W = 7066.0, P = 00.01). However, a larger allocation of energy was recorded to the ovaries, compared to the female gut (Mann Whitney, W = 2802.0, P = 0.016). A relatively smaller amount of energy was associated with the ophiuroid bodily tissues (mean = -9.92 kJ g^{-1} DM).

Table. 4.3.10 *Ophionotus victoriae*. Comparisons between the %carbohydrate content in the gut and gonad tissues. Comparisons in these tissues in males and females are also recorded. A Mann-Whitney non parametric analysis was employed and a P < 0.05 indicated a significant difference in tissue biochemical composition.

Variable	Test Statistic	P-value
Tissue	W = 8643.0	0.207
Female	W = 2535.0	0.734
Male	W = 1833.0	0.152

Differences between species

The elemental and proximate compositions of individual tissues are compared for the two echinoderms analysed. However, the gonad data can only be compared on an elemental level, owing to the poor conformity of the starfish data to the macro.

The ash content of the digestive gland was very similar between the two echinoderms (~19%). However, the gonad tissues differed quite extensively, where the starfish gonad was composed of more than double the ash content of the brittlestar gonad. The ash content was also important in the ophiuroid bodily tissue accounting for almost 45% of the tissue composition, and although this constituent was important in all tissues, other components dominated the composition of the reproductive and digestive tissues.

The percentage composition of carbon was important in the digestive tissues of both echinoderms, although higher levels were recorded in the ophiuroid gut (Mann Whitney, W = 11249.0, P = 0.0001). A significant difference in the gonad was also apparent in carbon content between the two echinoderm species (Mann Whitney, W = 11212.0, P = 0.0001), with higher levels in the ophiuroid. The bodily tissue was diminished in carbon containing approximately half the carbon content of the aforementioned tissues (~20%). Nitrogen accounted for a much smaller fraction of the tissue composition in both echinoderms. A significant difference in nitrogen content was apparent in the digestive tissues of the two echinoderms (Mann Whitney, W = 8483.0, P< 0.0001), with higher levels being observed in the pyloric caeca of *Odontaster validus*. However, a similar percentage composition of nitrogen was recorded in the gonad of the two echinoderms (Mann Whitney, W = 5777.0, P = 0.096), although a significant difference was apparent between the ovaries of the two species (Mann Whitney, W = 2176.0, P < 0.0001). Levels of nitrogen were lowest in the ophiuroid body tissue (~4%).

Protein was the largest component of tissue in both the pyloric caeca of the starfish and the brittlestar gut tissue (~40-50%). A higher percentage composition of protein was apparent in the pyloric caeca compared to the ophiuroid gut (2 sample T-test, unequal variance (Table. 4.3.11) T = 15.59, P < 0.001, DF = 134). In comparison, the ophiuroid bodily tissue was composed of half the amount of protein (~20%).

Table. 4.3.11 Test for equal variance in %protein content between the gut of *Ophionotus victoriae* and the pyloric caeca of *Odontaster validus*. P < 0.05 indicates unequal variance.

Test	Test Statistic	P-value
F-test	0.58	0.031
Levene's test	4.62	0.033

A more visible contrast was apparent in lipid composition between the two species. The percentage dry mass of lipid was higher in the gut of the brittlestar (2-sample T-test, unequal variance (Table. 4.3.12) T = -7.57, P < 0.001, DF = 138). However, the digestive tissues in both echinoderms contained 2-4 times the tissue composition of lipid compared to the ophiuroid body. The lowest lipid levels were recorded for this bodily tissue (~6%).

Table. 4.3.12 Test for equal variance in %lipid content between the gut of *Ophionotus victoriae* and the pyloric caeca of *Odontaster validus*. P < 0.05 indicates unequal variance

Test	Test Statistic	P-value
F-test	0.51	0.009
Levene's test	4.15	0.043

The lowest percentage dry mass of tissue was attributed to carbohydrate in both the gut and pyloric caeca of the echinoderms analysed (~8-9%). A similar percentage composition of carbohydrate was recorded in the gut and the pyloric caeca (Mann Whitney, W = 3967.0, P = 0.825). The contribution of carbohydrate to the bodily tissues was also slight, although the lowest percentage dry mass of this tissue was attributed to lipid.

A significant difference in the amount of energy allocated to the ophiuroid gut and the asteroid pyloric caeca was apparent (Mann Whitney, $W = 5235.0$, $P < 0.0001$), where a larger allocation in *Ophionotus victoriae* was observed. However, the provisioning of energy to the ophiuroid bodily tissue was much lower (~ 9 kJ g^{-1} DM).

Experimental Constraints

- Sample storage may have affected the biochemical composition of the frozen tissues. Samples were transported and held at the British Antarctic Survey Headquarters, Cambridge at –80ºC. Prolonged periods of storage have been shown to leach nitrogen from stored tissues, which is accelerated in tissues originally rich in nitrogen (R Shreeve, pers. comm.). The loss of nitrogen from samples also appears to be disproportionately high during the first few months of storage. However, each tissue was stored according to the same protocol and for a limited period (~ 6 months), keeping any error consistent and to a minimum.
- A small degree of error is inherent when using the CHN autoanalyser, caused principally by imprecise sample weighing, especially when weighing frozen samples. The detection accuracy of the CHN autoanalyser was also compromised when ashed (whole ophiuroid) samples were processed. Zero and sometimes negative carbon values were returned when the machine was required to operate at the extreme lower end of its detection limits. Therefore, these data were reported and input into the macro as the default (0.01%) as recommended by Gnaiger and Bittlerlich (1984) and any positive values were inputted as normal.
- The algorithm was used with variable success. It has been reported that the algorithm devised by Gnaiger and Bitterlich (1984) works well when the tissues processed vary little in seasonal tissue composition. This may explain the greater degree of conformity observed in the pyloric caeca tissues from *Odontaster validus*. These tissues were collected over a smaller time period (4 months) compared to the 10month sample collections under taken for *Ophionotus victoriae*. Therefore, in comparison the amount of seasonal variation would have been limited in the starfish tissues, especially as

collections were made outside the normal spawning period of this invertebrate, when reproductive activity would have exerted a greater influence on composition. However, as both of these invertebrates have seasonal cycles in reproduction and activity (Pearse, 1965; Grange *et al*. 2004) it is not surprising that the tissue data rarely conformed perfectly to the boundary limits of the macro. Clarke *et al*. (1992) also experienced unresolved difficulties when they attempted to process tissue from gelatinous zooplankton through the Gnaiger and Bitterlich (1984) algorithm, which were attributed to excessive residual water content (~10%) in the tissues. Galley (2004) found similar problems when processing holothurian tissue. However, the algorithm has been shown to give accurate results when compared to direct assays (Brockington *et al*., 2001).

The poor conformity of the starfish gonad tissue is more problematic as this tissue has been successfully processed before (Stanwell-Smith, 1997). However, the ash content determined in the current study (39.92%) is well in excess of any previous estimates (13.90±0.18 Stanwell-Smith, 1997). This will have affected the execution of the macro. Stanwell-Smith (1997) ashed at 480ºC. Subsequent studies have suggested 600ºC as a more appropriate ashing temperature for echinoderm tissues (A Clarke, pers. comm). At 600ºC virtually all of the organic matter in the tissue should have been burnt off and therefore, a lower ash content would be expected in the current study compared to Stanwell-Smith's data (Fig. 4.3.7). However, this was not the case and is difficult to explain. A possible explanation is that the samples used in the current study contained more mineral material than the tissues used in Stanwell-Smith's investigation. At 600ºC it is also possible that excess aluminium, and possibly tissue, may have been burnt off from the weighing crucibles. However, the crucibles were initially ashed empty (pre-ashed) and the furnace temperature ramped to minimise this potential problem. Small sample sizes meant that very small sample weights were recorded for both dry and ashed tissue, and that little difference was recorded in weight between the dry and ashed samples. Consequently, weighing inaccuracies may have affected the final results.

The same method was also used to prepare and ash all of the samples, including the starfish gonad. All of the other gonad and gut tissues used in the current study produced comparable data to previous studies and were amenable to the macro. Therefore, a clear explanation for the high ash content of the starfish gonad is not forthcoming.

Alternatively, the poor conformity of the starfish gonad data to the macro may have been biochemically driven. The macro persistently returned negative carbohydrate values commonly observed when the fraction of non-protein nitrogen is overestimated and the amount of carbon explained by protein underestimated. This may suggest the presence of an unmeasured or underestimated NPN component, possibly a glycoprotein or amino-polysaccharide. This was especially evident in the male gonad tissue and may be attributable to the investment of a large amount of non-protein nitrogen in DNA in sperm, which is not accounted for in the default boundary limits of the macro.

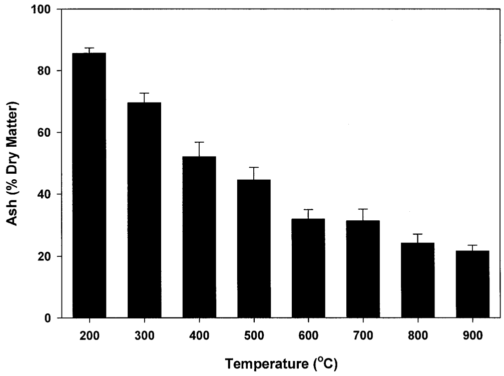

Fig. 4.3.7 *Odontaster validus*. Mineral ash content (percentage dry mass) of echinoderm body tissues versus temperature to illustrate the variation in ash content calculated using different ashing temperatures.

Table. 4.3.13 Biochemical and proximate composition of the gonad and pyloric caeca tissue of *Odontaster validus*. Mean and standard deviation are given for sample sizes > 1. The raw carbon and nitrogen data were used to determine the amounts of protein, lipid, carbohydrate and energy using the Gnaiger and Bitterlich (1984) algorithm. An average &ash content was calculated from 15 random gonad and pyloric caeca samples. Values of C in ash are not included as the default value of 0.01 was used for all samples.

	%DRY MASS													
	PROTEIN		**LIPID**		**CARBOHYDRATE**		**ASH**		**CARBON**		**NITROGEN**		**ENERGY (kJ g^{-1}DM)**	
GONAD	**Mean**	**StDev +**	**Mean**	**StDev +**	**Mean**	**StDev +**	**Mean**	**StDev +**	**Mean**	**StDev +**	**Mean**	**StDev +**	**Mean**	**StDev +**
Male														
Dec 16th							39.92		41.87	1.91	10.25	1.30		
Dec 19th							39.92		40.91	1.17	12.17	0.22		
Jan							39.92		42.21	0.43	12.20	0.16		
Feb							39.92		40.91	1.47	12.10	0.27		
Mar							39.92		41.69	1.84	11.53	1.31		
Female														
Dec 16th							39.92		48.12	6.01	10.81	1.78		
Dec 19th							39.92		44.55	1.38	10.50	0.57		
Jan							39.92		43.76	2.12	9.62	0.57		
Feb							39.92		42.26	1.28	9.42	0.49		
Mar							39.92		42.86	1.29	8.62	0.31		
PYLORIC CAECA														
Male														
Dec 16th	50.43	1.99	12.66	5.21	11.12	6.46	19.73		42.80	1.82	9.18	0.36	-19.53	1.21
Dec 19th	52.40	0.72	18.82	3.52	4.87	2.81	19.73		45.90	1.11	9.54	0.13	-21.37	0.73
Jan	48.86	5.07	24.00	4.06	4.34	1.31	19.73		47.72	0.92	8.90	0.92	-21.29	1.14
Feb	53.18	1.27	18.39	3.47	5.38	4.17	19.73		47.81	2.09	9.47	0.51	-22.44	0.57
Mar	50.35	2.07	18.35	4.96	7.52	5.67	19.73		45.57	1.68	9.17	0.38	-21.13	1.12
Female														
Dec 16th	50.60	0.76	12.64	6.16	10.96	6.53	19.73		42.81	2.07	9.21	0.14	-19.54	1.38
Dec 19th	51.92	2.67	19.39	4.26	5.71	3.36	19.73		46.96	1.79	9.42	0.45	-21.62	0.84
Jan	52.48	3.11	17.75	5.87	6.76	3.88	19.73		45.95	1.75	9.56	0.57	-21.29	1.14
Feb	55.67	2.60	20.62	3.46	3.23	2.60	19.73		47.69	1.55	9.59	0.53	-22.13	0.66
Mar	54.64	4.38	7.42	7.42	12.33	6.25	19.73		44.67	2.28	9.46	0.76	-20.45	1.50

Table. 4.3.14 Biochemical and proximate composition of the bodily tissues of *Ophionotus victoriae*. Mean and standard deviation are given for sample sizes > 1. The raw carbon and nitrogen data were used to determine the amounts of protein, lipid, carbohydrate and energy using the Gnaiger and Bitterlich (1994) algorithm. The ash content was calculated for each individual sample and a mean and a standard deviation reported. Values of C in ash were calculated by running each ash sample through the autoanalyser. Any zero or negative values were recorded as the default value 0.01.

	%DRY MASS															
	PROTEIN		**LIPID**		**CARBOHYDRATE**		**C in ASH**		**ASH**		**CARBON**		**NITROGEN**		**ENERGY (kJ g^{-1}DM)**	
WHOLE	**Mean**	**StDev +**	**Mean**	**StDev +**	**Mean**	**StDev +**	**Mean**	**StDev +**	**Mean**	**StDev +**	**Mean**	**StDev +**	**Mean**	**StDev +**	**Mean**	**StDev +**
Male																
Feb 03	22.15	2.32	8.07	4.08	9.87	6.76	3.81	0.34	45.95	1.32	23.99	2.28	3.88	0.56	-10.44	0.98
Mar 03	19.09	2.02	3.88	3.25	14.38	8.24	5.42	2.95	49.20	7.03	22.04	1.27	3.33	0.32	-8.65	1.04
Apr 03											42.21	0.43	12.20	0.16		
May 03	24.81	1.00	4.03	2.04	17.86	3.39	2.47	1.47	43.80	0.28	25.93	0.0006	4.52	0.18	-10.91	0.46
Jun 03	18.93	0.79	0.75	0.59	21.97	1.42	0.97	1.66	42.93	1.27	23.19	4.02	3.80	0.62	-8.87	0.18
Jul 03	24.09	1.53	8.48	4.69	11.25	8.92	0.96	2.12	40.72	4.50	25.43	1.23	4.39	0.28	-11.33	1.02
Dec 03	20.63	2.00	5.49	4.75	14.96	6.71	1.02	2.21	43.77	6.23	22.31	1.74	3.73	0.34	-9.94	1.13
Jan 04	19.89	3.66	6.74	5.11	11.96	9.57	0.01	0.00	43.70	0.96	21.60	1.77	3.62	0.67	-9.72	1.26
Feb 04	21.00	2.00	6.83	3.94	10.55	6.41	1.62	2.65	49.02	4.68	22.26	2.06	3.95	0.61	-9.79	1.21
Mar 04	22.97	3.38	4.48	1.95	15.36	2.94	3.32	0.58	43.79	2.98	24.53	1.96	4.18	0.62	-10.19	1.04
Female																
Feb 03	20.75	3.92	5.09	6.12	12.17	9.66	3.62	0.38	46.72	0.25	22.59	2.37	3.78	0.71	-9.32	1.60
Mar 03	19.23	1.49	3.01	2.60	12.06	2.88	7.07	2.01	52.53	3.50	22.14	0.80	3.50	0.27	-8.10	1.04
Apr 03	20.46	2.30	3.01	4.74	12.71	6.62	2.60	1.23	42.58	1.90	22.80	1.67	3.73	0.42	-9.71	1.21
May 03	20.37	2.43	5.32	3.69	12.94	6.56	2.25	2.12	42.80	1.84	22.66	2.56	3.92	0.54	-9.45	0.80
Jun 03	19.93	2.24	4.20	5.11	18.33	2.79	2.41	3.09	43.68	2.01	22.62	1.92	3.49	0.39	-9.27	0.31
Jul 03	21.93	2.74	9.50	7.26	11.80	9.74	0.18	0.38	40.44	0.67	24.88	2.80	3.99	0.5	-11.29	1.81
Dec 03	22.11	1.90	9.18	3.62	8.84	6.40	0.11	0.22	43.53	2.61	23.39	1.48	4.03	0.35	-10.69	0.87
Jan 04	19.29	2.70	7.48	5.14	12.00	8.08	0.31	0.83	45.05	1.91	22.01	1.98	3.51	0.49	-9.87	1.29
Feb 04	18.83	1.26	5.03	3.33	14.60	5.93	0.79	1.37	45.40	2.33	21.23	1.04	3.43	0.23	-9.24	0.75
Mar 04	21.46	2.16	6.73	3.22	15.84	2.70	3.17	0.26	42.66	2.78	25.54	2.25	3.91	0.39	-10.79	1.25

Table. 4.3.15 Biochemical and proximate composition of the gut of *Ophionotus victoriae*. Mean and standard deviation are given for sample sizes > 1. The raw carbon and nitrogen data were used to determine the amounts of protein, lipid, carbohydrate and energy using the Gnaiger and Bitterlich (1994) algorithm. The average %ash content was calculated from 30 random gut samples. Values of C in ash are not included as the default value of 0.01 was

	%DRY MASS													
	PROTEIN		LIPID		CARBOHYDRATE		ASH		CARBON		NITROGEN		ENERGY (kJ g^{-1}DM)	
GUT	Mean	StDev +	Mean	StDev +	Mean	StDev +	Mean	StDev +	Mean	StDev +	Mean	StDev +	Mean	StDev +
Male														
Feb 03	41.00	2.15	34.40	5.56	5.11	4.40	19.00		47.06	10.41	6.91	1.22	-24.36	1.14
Mar 03	40.15	4.01	31.21	6.32	7.11	7.42	19.00		50.50	2.22	7.39	0.61	-23.59	1.40
Apr 03														
May 03							19.00		36.40	30.20	4.42	4.03		
Jun 03	37.50	1.18	30.66	6.90	9.57	8.15	19.00		42.67	10.91	6.16	1.17	-23.14	1.59
Jul 03	37.19	3.48	31.50	11.84	9.04	15.52	19.00		50.21	4.37	6.77	0.56	-23.31	2.83
Dec 03	42.58	2.83	25.21	7.27	9.63	5.43	19.00		48.48	5.76	7.31	1.26	-22.27	1.47
Jan 04	42.77	6.55	24.97	10.48	4.98	3.81	19.00		43.35	8.53	7.35	1.31	-22.47	1.89
Feb 04	43.42	3.99	21.55	11.00	15.51	13.04	19.00		46.80	3.79	7.59	0.7	-21.69	2.53
Mar 04	42.71	2.49	39.28	2.91	5.41	3.57	19.00		50.28	2.02	7.71	0.42	-23.17	0.63
Female														
Feb 03	47.51	5.44	26.60	2.42	3.91	3.37	19.00		48.79	0.35	8.65	0.99	-23.05	0.19
Mar 03	44.06	2.55	27.22	1.75	6.04	0.95	19.00		49.41	1.94	8.01	0.33	-22.81	0.22
Apr 03	47.26	4.83	21.90	8.69	9.98	12.36	19.00		45.38	7.31	7.97	1.65	-21.86	2.12
May 03	41.12	4.10	26.66	9.62	10.62	12.12	19.00		44.58	10.88	6.78	2.12	-22.66	2.24
Jun 03	39.44	4.87	23.60	9.51	12.56	12.70	19.00		43.40	6.66	6.84	1.15	-21.37	2.25
Jul 03	42.15	4.30	28.19	7.68	7.10	4.36	19.00		48.46	2.24	7.68	0.78	-22.90	1.46
Dec 03	43.51	2.12	21.89	6.36	7.95	8.34	19.00		44.71	2.26	7.92	0.39	-20.90	1.51
Jan 04	45.23	2.66	23.88	5.31	8.64	4.83	19.00		47.52	1.68	8.24	0.48	-22.24	1.11
Feb 04	41.38	2.90	20.11	7.96	15.99	10.09	19.00		45.72	2.80	7.54	0.53	-21.07	1.87
Mar 04	43.69	2.87	28.41	6.60	8.37	9.48	19.00		50.21	2.30	8.08	0.77	-23.22	1.63

Table. 4.3.16 Biochemical and proximate composition of the gonad of *Ophionotus victoriae*. Mean and standard deviation are given for sample sizes > 1. The raw carbon and nitrogen data were used to determine the amounts of protein, lipid, carbohydrate and energy using the Gnaiger and Bitterlich (1994) algorithm. The average %ash content was calculated from 30 random gut samples. Values of C in ash are not included as the default value of

	%DRY MASS													
	PROTEIN		LIPID		CARBOHYDRATE		ASH		CARBON		NITROGEN		ENERGY (kJ g^{-1}DM)	
GONAD	**Mean**	**StDev ±**	**Mean**	**StDev ±**	**Mean**	**StDev ±**	**Mean**	**StDev ±**	**Mean**	**StDev ±**	**Mean**	**StDev ±**	**Mean**	**StDev ±**
Male														
Feb 03	62.99	4.77	8.45	3.95	5.56	4.26	16.17		42.72	2.52	11.36	0.74	-20.03	0.74
Mar 03	67.86	3.74	3.57	3.57	8.61	7.08	16.17		42.48	2.34	11.75	0.78	-19.27	1.00
Apr 03														
May 03	65.88	1.74	6.73	1.16	2.21	3.01	16.17		42.84	0.53	12.00	0.32	-19.49	0.37
Jun 03	64.87	2.58	5.52	0.72	3.50	2.04	16.17		41.90	0.05	11.81	0.47	-18.99	0.03
Jul 03	69.17	1.45	1.86	1.33	5.60	1.54	16.17		42.77	0.93	12.70	0.32	-18.98	0.27
Dec 03	63.66	4.52	5.10	4.05	5.56	3.06	16.17		41.89	1.19	11.84	1.11	-18.80	0.69
Jan 04	63.59	4.74	3.68	2.32	7.70	6.62	16.17		41.63	1.05	11.58	0.86	-18.68	0.73
Feb 04	66.25	4.32	4.10	2.75	4.46	4.44	16.17		41.03	2.08	11.34	1.71	-18.94	0.57
Mar 04	66.33	5.11	3.70	2.02	7.22	4.85	16.17		40.68	4.82	11.62	1.65	-19.69	0.63
Female														
Feb 03	54.44	13.26	20.30	19.80	4.74	5.70	16.17		444.9 4	6.77	10.48	1.97	-22.45	3.50
Mar 03	42.04	2.87	32.59	6.46	6.55	5.93	16.17		51.57	2.06	7.66	0.52	-24.51	1.36
Apr 03	45.33	8.56	29.22	12.29	6.52	4.50	16.17		52.12	3.57	7.96	1.37	-24.00	2.19
May 03	64.23	2.50	3.25	3.16	11.46	3.91	16.17		47.79	6.24	9.95	2.45	-19.32	0.70
Jun 03	69.66		2.36		5.92	12.70	16.17		54.79	8.06	8.43	2.13	-18.83	0.00
Jul 03	40.68	0.82	37.66	3.54	2.92	2.84	16.17		53.81	1.79	7.29	0.29	-25.54	0.73
Dec 03	48.72	2.13	22.63	6.23	9.42	5.31	16.17		49.79	2.93	8.70	0.55	-22.75	1.30
Jan 04	45.64	2.98	22.94	10.55	11.48	12.62	16.17		49.31	4.47	8.33	0.51	-22.46	2.44
Feb 04	43.36	1.72	28.29	7.88	9.45	7.64	16.17		50.25	5.58	7.90	0.31	-23.65	1.71
Mar 04							16.17		55.49	2.10	7.93	0.51		

4.4. Discussion

The elemental and proximate composition of the echinoderms analysed varied both seasonally and between sexes. Differences between the species and the fundamental compositions of the individual tissues were also observed.

The two Antarctic echinoderm species studied had similar biochemical compositions to those described previously in benthic Antarctic invertebrates (Pearse and Giese, 1966ab; McClintock and Pearse, 1987; McClintock *et al*., 1988; McClintock, 1989; Stanwell-Smith, 1997). These compositions are not dissimilar to many temperate marine forms (Giese, 1966a). However, the percentage composition of ash was elevated in the digestive and reproductive tissues of the echinoderms when compared to other studies (Stanwell-Smith, 1997). High levels of ash have been described in the tissues of Antarctic echinoderms (Galley, 2004) and have been attributed to an investment in structural components in the echinoderm gonad and gut. The employment of different ashing temperatures can also affect the percentage composition of ash determined from the same tissues (A Clarke, pers. comm.). Carbohydrate levels were also slightly elevated in some instances (~13% ophiuroid body) compared to previous studies (<6%) (McClintock and Pearse, 1987; McClintock *et al*., 1988; McClintock, 1989). However, similar data were described by Galley (2004) in a number of deep sea Antarctic echinoderms and were also reported in the tissues of the Antarctic urchin, *Sterechinus neumayeri* (Pearse and Giese, 1966b; Brockington *et al*., 2001). These patterns have been previously associated with diet. Giese (1966a) commented on the possible accumulation of carbohydrate when echinoderms utilise nutrient-rich food sources whilst, Brockington *et al*., (2001) suggested the involvement of diet to explain the higher carbohydrate levels observed in the Antarctic urchin, *Sterechinus neumayeri*.

Ophiuroid Body Tissues

The ophiuroid bodily tissues (including the disc) were mainly composed of ash (~45%). This is comparable to the values previously reported for Antarctic echinoderms (McClintock and Pearse, 1987) and has been attributed to the high mineral content of the calcareous skeletal structures characteristic of the taxon. The ophiuroid disc is composed of many calcareous plates and ossicles. Another interesting feature, originally reported by Mortensen (1933), is the calcareous deposits found in the soft tissue, especially in the gut and gonad wall, of some ophiuroids (Tyler, 1980), which may have contributed to the large ash content observed. The organic composition of the dissected whole ophiuroids used in the current study was lower in comparison to the ash portion, and was mainly dominated by protein. Giese (1966a) attributed the protein content of these tissues to structural components and the heavy musculature of the disc and arms. The contributions of lipid and carbohydrate to the bodily tissues of the ophiuroid were limited in comparison, which is a common observation in the tissues of both Antarctic and temperate echinoderms (Giese, 1966ab; Pearse and Giese, 1966ab; McClintock and Pearse, 1987; McClintock *et al*., 1988; McClintock, 1989; Stanwell-Smith, 1997). Higher levels of organic material have been observed in the body wall of asteroids and holothurians suggesting an additional role as nutrient storage tissue (McClintock and Pearse, 1987).

Ophiuroid gut

The gut of *Ophionotus victoriae* was also relatively high in protein (~43%). Lipid levels were also higher in the digestive tissues of *O. victoriae* (~26%) compared to *Odontaster validus*. McClintock and Pearse (1987) observed comparable levels of these constituents in the same species of brittlestar, although the levels of protein observed were slightly higher in the current study, and suggested the gut to have a role in providing material and energy for metabolic function and possibly gametogenesis. Carbohydrate levels were again elevated in the current study and most probably reflect dietary differences in the ration available. McClintock and Pearse (1987) only collected individuals during October and November between 1984 and 1985, which may partially explain the differences between their study and the current data, which collected animals over a 10-month period. The considerable contribution from lipid to the composition of the gut tissue does suggest a role of nutrient storage (Bishop and Watts, 1992). This may be of particular importance to *Ophionotus victoriae* and other species of ophiuroid when reproductively mature, when the space occupied by the gonads in the ophiuroid disc is large and impairs the normal acquisition of nutrients through feeding (Thorson, 1953).

Asteroid pyloric caeca

The organic composition of the pyloric caeca in *Odontaster validus* was dominated by high levels of protein (~52%) and moderate contributions from lipid (~17%). The amount of ash recorded was similar to the levels of ash observed in the ophiuroid digestive tissue and was relatively low compared to the contents of the other tissues in the study. The mean level of lipid (~17%) in the pyloric caeca was similar to the values (~22%) reported for *O. validus* by McClintock and Pearse (1987). However, slightly higher levels of protein and carbohydrate were observed in the current study. Similar investments in lipid and protein in the pyloric caeca have also been reported by Stanwell-Smith (1997), who also observed higher carbohydrate levels in the pyloric caeca compared to McClintock and Pearse (1987). These differences probably reflect variations in food availability between the sampling sites and the times of each study, especially as *Odontaster validus* reproduces seasonally. The role of the pyloric caeca in asteroids as a nutrient storage organ is evident in the high levels of both protein and lipid observed in this bodily component. The pyloric caeca was generally high in energy and similar between sexes, which was primarily a result of the consistently high levels of protein and lipid invested.

Echinoderm gonads

The mean biochemical and proximate composition of the gonad tissues indicated differences between the sexes of both echinoderm species. Carbon levels were higher in the females of both the asteroid and ophiuroid and nitrogen levels were higher in the testes. A similar pattern was observed in lipid and protein respectively in the gonad tissue of the brittlestar. This reflects the requirement of an inherently greater energetic contribution of lipid to the development of an oocyte and the production of a juvenile when compared to sperm (McClintock and Pearse, 1987). High lipid levels are common in a number of Antarctic echinoderms, and have been associated with the preponderance of lecithotrophy at polar latitudes (McClintock and Pearse, 1987). In contrast high levels of protein are common in the testes of both temperate (Giese, 1966a) and Antarctic echinoderms (Pearse and Giese, 1966a; McClintock and Pearse, 1987; McClintock 1989). Large contributions of non-protein nitrogen reflect the high levels of nucleic acids and DNA commonly associated with sperm. The energetic cost of producing eggs in female *O. victoriae* was significantly larger than the cost of producing sperm in males, which is a common assumption for many marine invertebrates (Giese, 1966a). However, as spermatozoa contain relatively more proteinacious substances than ova, and protein synthesis is an expensive metabolic process in terms of sperm production, the general validity of the assumption that ‘sperm is cheap’ is sometimes doubtful, e.g. in the Icelandic scallop *Chlamys islandica* (Vahl and Sundet, 1985).

The mean biochemical composition of the gonad tissues indicated differences between the two species of echinoderm. Ash levels in the asteroid gonad were much higher than the levels observed in the ophiuroid. This suggests that the asteroid gonad is composed to a greater degree by structural components than the ophiuroid tissue. Feder and Larsson (1968) described the wide distribution of calcareous deposits in the soft tissues of some asteroids. However, a similar occurrence has been described in both abyssal and bathyal ophiuroids, and therefore may not explain the disparity in levels of ash between the two echinoderm species. Previous studies have recorded much lower levels of ash in *Odontaster validus* (Stanwell-Smith, 1977) and suggest that the very high ash content of the gonad in the current study is anomalous. Levels of carbon were also elevated in the ophiuroid gonad, which was especially evident when the mean percentage composition of the ovaries was compared. However, the range in nitrogen levels was similar between the two echinoderm species.

Differences between the two echinoderm species are most probably attributable to subtle differences in their reproductive patterns. Both of these Antarctic echinoderms have been reported to broadcast small planktotrophic eggs into the water column (Pearse, 1965; Grange *et al*., 2004). However, Grange *et al*., (2004) remarked on a flexible egg strategy demonstrated for the brittle star (Chapter 2), producing inter-annually variable numbers of small and large eggs coincident with the quantity and quality of the seasonal arrival of material at the sea-bed. The production of larger eggs (>200μm) by the brittle star and the practice of a more facultative planktotrophy may explain the generally larger contribution of carbon to the ophiuroid ovaries. The absence of any lipid data for the asteroid gonad prevents any further comparison in the current study. However, previous studies have reported generally low-moderate levels of lipid (~9-13%) in the gonads of *Odontaster validus* (McClintock and

Pearse, 1987; McClintock, 1989; Stanwell-Smith, 1997). Elevated lipid levels have been observed in the ovaries of *Ophionotus victoriae* both in previous studies (~27% McClintock and Pearse, 1987) and in the current study (24%). This may reflect a greater maternal investment by the brittlestar and similar contributions from lipid (~23%) have been previously reported in the ovaries of another Antarctic echinoderm with planktotrophic development (*Odontaster meridionalis* McClintock and Pearse, 1987).

Despite differences in the levels of carbon and lipid observed in the gonads of the asteroid and ophiuroid energy contents were similar between the two echinoderms (McClintock and Pearse, 1987; Stanwell-Smith, 1997; current study). This most probably reflects a trade-off between fecundity and egg size.

Seasonal Variation in Tissue Composition

Seasonal variation in the tissue composition of *Odontaster validus* was small. A significant difference in the biochemical constitution of tissue was only observed in the asteroid pyloric caeca and could only be proven for carbon and consequently lipid. The levels of other bodily constituents were generally consistent over the sampling period. The absence of any obvious seasonality in the composition of the asteroid gonad probably reflects the limited period over which the study was undertaken (~4 months December 2003-March 2004). Many studies have also commented upon the close relationship between the asteroid gonad and pyloric caeca as a nutrient storage organ (*Asterias rubens, Oreaster reticulatus, Stichaster australis* and *Luidia clathrata*; McClintock, 1989). The transfer of nutrients from the pyloric caeca to the gonad may explain the lack of any systematic trend in the biochemical composition of the asteroid reproductive tissue. The composition of gonad tissue observed by Stanwell-Smith (1997) in the same species also remained broadly similar throughout the adult reproductive cycle.

Generally consistent levels of protein were observed in the pyloric caeca tissues. A seasonal cycle in lipid was more apparent and both the male and female storage tissues exhibited a period of investment during late 2003 when the percentage lipid composition increased. This period coincides with the Antarctic phytoplankton bloom and although this asteroid is a scavenger and somewhat decoupled from the availability of primary production in the water column, the elevated presence of dead and digested material and potential prey items is likely to enhance the capacity of the asteroid to sequester and store nutrients. A decrease in the percentage lipid composition in both the males and females occurred in February to March 2004 and may be related to the mobilisation of lipid energy reserves to the gonad for gametogenesis.

Seasonal variation was evident in the biochemical composition of *Ophionotus victoriae*. The Antarctic experiences a distinct seasonal and inter-annual cycle in primary production. The intense seasonality characteristic of the Antarctic is recognised as pivotal, not only for the filter feeding community but also for the benthic individuals reliant on the recycled and remineralised material which arrives at the sea-bed during the late austral summer. Such variation in the quality and quantity of food supply may have several effects on the biochemistry of body components. It may have a direct effect on the amount and composition of material channelled directly into the digestive tissues and diverted to storage such as in the gut lining, or it may have an indirect influence on the timing of reproduction and how energy is allocated to the gonad. This ophiuroid is a detritivore consuming decayed and remineralised material from the seabed and the seasonal tempos of this echinoderm has been attributed in part, to the seasonal sedimentation events common in the high Antarctic (Grange *et al.,* 2004).

A clear cycle in the composition of the ophiuroid was difficult to decipher owing to the absence of data between July-December 2003, especially as this period encompasses the months when individuals are gravid and likely to spawn (Grange *et al*., 2004). However, the most significant seasonal variation in tissue composition was observed in the ovaries. Variation in the testes was small, where the lipid content remained consistently low during the sampling period and protein remained consistently high. A clear dichotomy between the male and female gonad has been reported before in most temperate (Giese, 1966a) and Antarctic echinoderms (Pearse and Giese, 1966a; McClintock and Pearse, 1987; McClintock *et al*., 1988; McClintock, 1989) and has been attributed to the inherently higher accumulations of lipid required to produce oocytes, and the large fraction of insoluble protein concentrated in nucleic acids and the DNA associated with sperm. A higher percentage lipid composition was apparent in the ovaries during February-April 2003 and may reflect the transfer of material to the gonad, which has sedimented to the benthos after the break-up of the austral summer phytoplankton bloom. A similar percentage composition was also apparent at the same time in the following year. A much lower percentage lipid composition was recorded during the following May-June 2003, which is somewhat counterintuitive as this time period precedes the perceived spawning event by approximately 4 months and is commonly when reproductive reserves are being invested in the gonad (Chapter 2.3). The increase in lipid composition in the following July further suggested that reserves were being built and that the diminished values reported in the preceding May-June were most probably anomalous, either because of the small sample size in some of the sampled months or because of the problems experienced when processing this material. The lower lipid content recorded in the ovaries in the following December may have been due to spawning and the removal of lipid rich oocytes from the ovary, already identified from the reproductive index and female fecundity data as occurring in November/December each year (Chapter 2.3).

The amount of protein in the ovaries also appeared to vary quite considerably over the sampling period. However, if the high percentage composition recorded in May-June 2003 are disregarded the percentage composition of protein in the ovaries remained relatively high and consistent over the sampling period.

The biochemical composition of the ophiuroid gut and the bodily tissues varied to a lesser degree but seasonal differences were still apparent in both the male and female tissues. The percentage lipid composition was generally higher in the male gut tissue compared to the percentage composition observed in the females. The relatively large contribution of lipid to the composition of the ophiuroid digestive tissues suggests the gut may reserve a nutrient storage role, accumulating nutrients for both general maintenance and reproduction. The percentage composition of lipid remained relatively high between February-July 2003, suggesting the short-term storage of this component in the gut. A decrease in lipid content was observed between July and December 2003 and may reflect the transfer of nutrients to the gonad for gametogenesis and/or a prolonged period when reserves are slowly utilised and the food supply is very low during the prolonged austral winter. Protein levels generally declined during the initial part of the study (February-July 2003). Protein is commonly high in most gut tissues and the pattern observed probably reflects the use of this material as a major substrate to fuel metabolic and reproductive demands as seen in other Antarctic marine invertebrates (Peck *et al*., 1987). The percentage composition of protein was elevated in the following December and persisted in the gut until March 2004, and may be indicative of a higher food supply in the austral summer. The seasonal changes in the biochemical composition of the ophiuroid gut suggest this invertebrate responds rapidly to changes in the availability of food and the quality of the ration.

Despite the small contribution of organic components to the ophiuroid bodily and skeletal tissues the percentage composition of protein and lipid did exhibit some degree of seasonality in bodily composition during the sampling period. A large variation in the percentage composition of lipid was apparent between the individual ophiuroids sampled and obscured any obvious temporal trends in the bodily tissues. The percentage composition of protein did significantly differ over the same sampling period and may reflect the complicated balance between the acquisition of nutrients from the environment and the distribution of bodily constituents between maintenance, somatic and reproductive demands.

Seasonal changes in the biochemical composition of tissues caused either by a direct response to external food resources or by an indirect influence of discrete gametogenic cycles could be seen in both of the echinoderm species. However, this was not true of the asteroid gonad. This tissue showed no significant variation in biochemical composition over the sampling period. It may be that in providing stored nutrients to the gonad, the pyloric caeca act as a make-shift buffer, smoothing any evidence of seasonal cycling in the asteroid gonad. The greatest variation in biochemical composition was evident in the female gonad of the ophiuroid *Ophionotus victoriae*. This species has a highly plastic reproductive pattern, whereby the proportion of oocyte sizes and the annual female fecundity produced is variable depending on the quantity and quality of the seasonal flux event arriving at the benthos (Grange *et al*., 2004). Some degree of seasonality was also apparent in the pyloric caeca and gut tissue of the asteroid and ophiuroid reflecting the role of these tissues in nutrient storage and the inherent response of each to both changing food levels and reproductive demands.

5.1 Synopsis

The most pressing challenge for environmental scientists at present is to investigate the potential impact of global climate change and to explain the recent phenomenon of global warming. Most importantly scientists are being asked how such changes will affect our and other species' capacity to inhabit the earth. Scientists have suggested a rise of sea temperature of at least 1 to 2 degrees is likely over the next 100 years (Hadley Centre Model 3, Mitchell *et al.*, 1998) and in 2001 the Intergovernmental Panel on Climate Change (IPCC) reported a global mean warming of 0.6±0.2ºC during the 20th Century, attributing much of this elevation to the anthropogenic increases in greenhouse gases. However, regional patterns of warming are much more complex and temperatures have not risen uniformly. Regions that are considered to have undergone a very rapid warming are all at high latitude, and have become focal areas for climate change research, including the Antarctic Peninsula and the Bellingshausen Sea.

Certain areas of Antarctica have been increasing in temperature as fast as any other region on earth, where the average temperature trend for all Antarctic stations for 1959 to 1996 was +1.2ºC per century, which is well above the global mean (Vaughan *et al.*, 2001). Reliable records from the Antarctic Peninsula confirm that this region has been warming since the 1950s and that this trend has been exceptional and considerably more pronounced than the mean Antarctic trend. The climate of the Western Antarctic Peninsula is thought to be the most rapidly changing in the Southern Hemisphere, with a rise in atmospheric temperature of nearly 3ºC since 1951 (Meredith and King, 2005). For the first time, the adjacent ocean has also shown profound coincident changes in temperature, with summer temperatures rising more than 1ºC (Meredith and King, 2005). There have also been associated cryospheric impacts. Three out of the four ice core records from the Antarctic Peninsula have yielded data to support warming over the last 50 years, and in some cases have provided convincing evidence for a close coupling between increasing greenhouse gas levels (CO_2 and CH_4) and Antarctic air temperature records (Petit *et al.*, 1999). Current studies also suggest a significant environmental impact, e.g. the receding of glaciers and ice shelves (Vaughan and Doake, 1996), the shrinking of seasonal snow cover (Fox and Cooper, 1998), and dramatic changes in freshwater lake and pool ecosystems (Quayle *et al*., 2002, 2003). This warming is believed to have caused the disintegration of both the Wordie Ice Shelf (Doake and Vaughan, 1991) and the northern extent of the Larsen Ice Shelf (Vaughan and Doake, 1996), and current concern over the stability of the West Antarctic ice sheet is also growing (Zwally, 1991). However, the proximate mechanism or mechanisms that have acted as the impetus for this exceptional warming are not yet evident.

Interest in this region's climate has also grown, as Antarctica and areas of the Southern Ocean are major components of the global climate system through the connective circumpolar pathway between the major ocean basins and the formation of deep and intermediate water (Drewry, 1993). Consequently, Antarctica plays a key role in global change as interactions between the atmosphere, ice, oceans and biota affect the entire earth system through a number of feedback mechanisms. It is therefore even more important that the responses of Antarctica, its inhabitants and the surrounding oceans to radiative forcing are understood.

Climate change has long been regarded as an important factor in species life history, the origins of species and evolution (Clarke, 1993). Therefore, temperature or temperature-associated factors probably play an important role in determining the limits of performance and success for many species. Consequently, early detection of change in the normal biological functions of animals has proven invaluable in the study of environmental change and the ecological implications of climate change are now being comprehensively studied in Antarctica.

Changes in seawater temperature directly affect many aspects of the marine Antarctic environment and ultimately aspects of species survival. Some factors that will alter with temperature are water viscosity (important in larval and sperm swimming and in the passage of particles through the water column), gas solubility (especially when the dynamics of oxygen and carbon dioxide dissolution in seawater are considered) and carbonate dissolution (important in cold water where a higher energy expenditure is required to sequester calcium carbonate from seawater). Temperature also influences animal physiology especially when the processes of metabolism, protein synthesis, enzyme activity and reaction rate are considered, and very few physiological processes tend to be compensated for temperature completely (Clarke, 2003ab, 2004; Clarke and Fraser, 2004). The rate of temperature change is also very important, where long-term climate change may allow the adaptation of physiology not possible when sudden changes in temperature are experienced. This is even more important in high polar seas where many organisms have evolved to become closely coupled with the thermally-stable environment and function within a very narrow range of thermal tolerance (Peck, 2002). Most Antarctic invertebrates exhibit temperature windows only 20-50% as large as those observed for temperate species, with a strict upper lethal limit between 5ºC and 9ºC for most species. Pörtner *et al.*, (1999) described the most extreme example of this stenothermy in the Weddell Sea bivalve *Limopsis marionenis*, which has an upper lethal limit between 2-4˚C depending on the length of temperature exposure. Such stenothermy sets the functional limits at which Antarctic animals can complete fundamental biological functions, e.g. feeding, growth, reproduction and larval physiology and development (Peck and Prothero-Thomas, 2002; Peck *et al.*, 2004) and illustrates well the sensitivity of these animals to environmental modifications and their suitability as early indicators for even the smallest changes in climate.

However, recent evidence suggests that extinction in the sea is unlikely as a direct thermal effect of temperature change, and is instead more likely from ecological factors (Clarke, 1993). These extinctions normally describe an event where the causal agent of extinction is associated with a change in temperature, e.g. a change in the degree of seasonality, although it is often difficult to distinguish between the co-varying effect of temperature and seasonality.

The Antarctic provides a unique natural laboratory for scientific study, where the affects of temperature and seasonal food availability can be examined individually. The nearshore environment is characterised by consistently low temperatures, which are contrasted by an intensely seasonal signal in food supply. The development of these conditions over millennial timescales has resulted in a fauna whose physiology is highly stenothermal and in some respects is closely associated with the environmental seasonality. Therefore, Antarctic marine animals are some of the most temperature sensitive fauna on earth and are likely to be susceptible to even the smallest environmental modifications. Consequently, the Antarctic benthos is a useful barometer for climate change, especially as these taxa have already undergone a prolonged but dramatic change since the break up of Gondwana (Clarke and Crame, 1992). Owing to the extreme sensitivity and stenothermal nature of many Antarctic marine taxa, responses in these organisms are expected to be much larger and earlier than the responses of the terrestrial ecosystem and in organisms at lower latitudes (Manabe and Stouffer, 1979; Clarke and Harris, 2003; Peck *et al*., 2004). Reproductive success is a vital characteristic in species survival and evaluation of change in reproductive condition with time key to identifying vulnerable taxa. Therefore, the study of invertebrate reproductive ecology in the Antarctic may provide an early insight into the effects of global environmental change, and the long-term patterns in Antarctic reproduction used to predict future trends if warming continues. The studies undertaken within this thesis lend some weight to that argument and illustrate well the complex and diverse reproductive patterns, previously dismissed by many, in the Antarctic.

The current study provides an example of inter-annual variation in the long-term reproductive cycles of four shallow water Antarctic invertebrates. Previous studies of invertebrate reproductive cycles in the Antarctic are limited and this study provides a basis upon which longer-term studies can be based and the causal environmental factors identified. Most investigations to date have been based on a PhD or Masters duration (1 to 2 years). However, although this thesis presents four years of data, the proximate factor or factors most important in driving the inter-annual variation in invertebrate reproduction are difficult to discern. The long-term cycles intrinsic in the Antarctic, e.g. the 18-24 month gametogenic cycle common in many invertebrates, necessitate observations to be made over prolonged time periods and a number of co-varying environmental variables to be tested. These cycles obscure any obvious relationships between biological and environmental patterns from being easily and quickly identified. The difficulty in deciphering between the effects of temperature and seasonality has been exemplified well in the advancement of knowledge in reproductive ecology in the Southern Ocean. The differing degrees of inter-annual variation exhibited by the 4 invertebrates in the current study suggest that, the seasonal food supply and trophic position, and not the low temperatures *per se*, appear to be the major influences on the long-term reproductive patterns observed. This was well demonstrated for the detritivore *Ophionotus victoriae*, where the reproductive tempos and effort was associated with the annual sedimentation event.

This species exhibited a significant degree of inter-annual variation in reproductive effort. This inter-annual variation was expressed less in species decoupled from the seasonal phytoplankton bloom, e.g. in the scavenger *Odontaster validus* and more so in the predatory nemertean *Parborlasia corrugatus*. The limited amount of inter-annual variation demonstrated for the sea cucumber *Heterocucumis steineni* was somewhat counterintuitive, as this species is a filter feeder and very reliant on the intense summer production. However, the difficulties experienced in sample processing and analysis probably obscured any real variability from being identified. The reproductive plasticity of these invertebrates, illustrates their ability to succeed and their role as important and abundant members of the Antarctic benthos.

This study also presents evidence to suggest that Antarctic invertebrates have achieved many reproductive adaptations to succeed in the conditions experienced in the Antarctic environment. These include adaptations to achieve high fertilisation success. Antarctic invertebrates need 1-2 orders of magnitude more sperm to ensure optimal fertilisation than temperate species (Powell *et al*., 2001) and sperm tend to be long-lived and capable of fertilising eggs 24+ hours after release. Synchronous spawning, aggregations and specific pre-spawning behaviour help counter the deleterious effects of sperm limitation. Antarctic eggs and sperm are also highly stenothermal, with extremely narrow ranges of thermal and salinity tolerance (Powell, 2001; Powell *et al*., 2001). Therefore, even small modifications in temperature and salinity can dramatically reduce the number of eggs fertilised. This has important implications for the geographical distribution of Antarctic invertebrates, and more importantly confirms the susceptibility of these invertebrates to environmental modification. Such stenothermy is of particular relevance if the 1 to 2ºC rise in global temperature, predicted over the next century, is realised.

This thesis also suggests that the quality and quantity of food arriving at the seabed in the Antarctic has several effects on the composition and condition of tissues in marine invertebrates. It has a direct effect on the amount and composition of material channelled directly into the digestive tissues and diverted to storage, and it can have an indirect influence on the timing of reproduction and how energy is allocated to the gonad. The allocation of reserves between reproductive and maintenance processes varies temporally, spatially and between the sexes. The echinoderms studied displayed seasonal cycles in biochemical and proximate tissue composition and exhibited tradeoffs between reproductive and metabolic requirements. The occurrence of a reduced growth rate and low metabolism, for any given food intake in the Antarctic means more energy should be available for reproduction and activity (Clarke, 1980). There is a reduced metabolic cost at low temperature, which is correlated with a reduced annual energy requirement, an increased ecological growth efficiency (Clarke, 1987), and a reduced requirement for an overwintering energy store (in contrast to pelagic zooplankton; Clarke and Peck, 1991). The allocation of reserves to organs such as the asteroid pyloric caeca and ophiuroid gut provides nutrients for both metabolic maintenance and gametogenesis. These adaptations are the most efficient way of adapting to a cold seasonally-productive environment (Clarke, 1979) and reflect why these invertebrates are abundant and conspicuous members of the Antarctic benthos. Therefore, any temperature-induced change in seasonality would have important implications for many Antarctic marine animals, directly affecting the allocation of reserves to digestive tissues and storage, and indirectly influencing reproductive processes.

These studies have identified some of the ways by which Antarctic invertebrates have adapted to exploit the unique conditions characteristic of the Antarctic environment, especially in terms of its seasonality, and have contributed to the current consensus which suggests that switches in reproductive mode in the Antarctic have arisen through changes in seasonality in primary production, and not temperature directly. However, these studies have also demonstrated the close coupling between Antarctic invertebrates and the seasonally-productive, stenothermal environment and suggest that the majority of taxa now known in the Antarctic would be adversely affected by any future rise in sea temperature that could elevate current summer temperatures 1 to 2ºC above their annual mean. The combination of very limited functional scopes, with slow rates of adaptation (Peck *et al*., 2004) confirms the vulnerability of many Antarctic marine taxa to any future warming and promotes the use of Antarctic marine animals as early indicators of environmental change, especially if the current climate model predictions are realised, e.g. the HADCM3 model predicts that global sea temperature at 1.5m depth will rise by approximately 2ºC over current temperatures by 2100 (King, 1994; Murphy and Mitchell, 1995; Mitchell *et al*., 1998) and the IPCC have suggested a possible global atmospheric rise of 3ºC if greenhouse gases double in the atmosphere, with a rise of 6ºC occurring in the Antarctic (IPCC, 2001) having grave consequences for the inhabiting fauna. Irrespective of the underlying physiology, it is clear that animals with such a limited capacity to cope will be adversely affected by even modest environmental warming. Consequently, we should be looking to the Antarctic marine benthos for early signs of large-scale population loss and/or reproductive failures, or species extinction in the coming decades.

Future Work

In the past many studies have only investigated the short-term response of organisms to temperature variation, many of which have involved eurythermal subjects so organisms survive the initial change. However, data have only alluded to the short-term phenotypic capacity of animals to respond and survive. The close coupling between environmental variation and long-term reproductive patterns observed in this thesis, confirms the importance of prolonged monitoring projects, possibly over decadal timescales, if the evolutionary change over protracted time scales is to be determined.

This study could be employed as part of a much larger project developed to measure the reproductive success of Antarctic invertebrates over a decadal timescale and the data used to produce a model which would be predictive of future climate change effects (global warming) on population biology.

Such a project would develop and build on the existing BAS long-term monitoring data sets, which have included the collation of environmental parameters (Rothera Time Series Programme: RaTS) and biological samples for invertebrate reproductive analysis in the coastal Antarctic (Rothera research station). It would involve the completion of reproductive and environmental analyses for a 10-year monitoring period (1997-2007), and combine these data with field population size frequency studies, investigations into invertebrate recruitment and *in situ* growth measures in order to develop the data into a powerful predictive model. These analyses would also allow us to go some way towards identifying the factor or factors most important in driving Antarctic reproduction, i.e. is it food availability or temperature that is most important or some other factor? The data presented in this thesis could be used and combined with data gathering of future samples to develop a unique long-term data set, exceptional in terms of Antarctic research history and possibly worldwide.

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Table. 1 The division of histological samples between different workers. Laura Grange (LG) completed histology for all four species and repeated image analysis for all of the samples described. This required new photographs to be prepared and calibrated for each species and 100 oocytes per female to e re-measured. New photographs could not be prepared and analysed for *Heterocucumis steineni* collected between April 1998-December 1999 owing to the original slides being missing. Therefore, the original blocks were re-cut and new slides prepared for analysis by LG. The original histology completed by LG was not repeated and all repeated image analysis was carried out by original methods employed by LG. Several months contained additional individuals not processed by the original workers and this was especially common in *H. steineni* collected between 1998-1999. LG processed these individuals through histology and image analysis.

Species Name	**Worker**	**Histology**	**Blocks re-cut (LG)**	**Images re-photographed (LG)**	**Images re-measured (LG)**
Ophionotus victoriae	Nils Cornelius	Sept 1997 – Aug 1998	N/A	Sept 1997 – Aug 1998	Sept 1997 – Aug 1998
Ophionotus victoriae	Laura Grange	Sept 1998 – Dec 2000	N/A	N/A	N/A
Odontaster validus	Anna Leach	July 1997 – Dec 1998	N/A	July 1997 – Dec 1998	July 1997 – Dec 1999
Odontaster validus	Ben Murphy	Jan 1998 – Dec 1999	N/A	Jan 1998 – Dec 1999	Jan 1998 – Dec 1999
Odontaster validus	Laura Grange	Jan 2000 – Jan 2001	N/A	N/A	N/A
Parborlasia corrugatus	Brioni Caswell	Dec 1998 – Jan 2000	N/A	Dec 1998 – Jan 2000	Dec 1998 – Jan 2000
Parborlasia corrugatus	Laura Grange	July 1997 – Dec 1998 Feb 2000 – Dec 2000	N/A	N/A	N/A
Heterocucumis steineni	Richard Hunt	Apr 1998 – Dec 1999	Apr 1998 – Dec 1999	Apr 1998 – Dec 1999	Apr 1998 – Dec 1999
Heterocucumis steineni	Vicky Clark	Jan 1998 Jan 2000 – Jan 2001	N/A	Jan 1998 Jan 2000 – Jan 2001	Jan 1998 Jan 2000 – Jan 2001
Heterocucumis steineni	Laura Grange	Sept 1997 – Mar 1998 June 1998, Dec 1998 Jan 1999, Feb 1999 Nov 2000	N/A	N/A	N/A

Table. 2 *Ophionotus victoriae, Odontaster validus, Parborlasia corrugatus* and *Heterocucumis steineni*. Anderson-Darling Normality test on reproductive data. Normally distributed data are given by P-values >0.05.

Species	**Variable**	**AD Test Statistic**	**P-value**
Ophionotus victoriae	Disc Diameter (mm)	0.246	0.755
	Disc Volume (mm)	7.859	< 0.005
	Actual Fecundity	0.465	0.240
	Gonad Index (%)	30.934	< 0.005
	Oocyte Feret Diameter (μm)	5.469	< 0.005
	Pre-vitellogenic Oocytes (Proportion)	1.129	< 0.005
	Vitellogenic Oocytes (Proportion)	1.129	< 0.005
	Average Monthly Oocyte Size (μm)	0.521	0.172
	Average Monthly Gonad Index (%)	2.421	< 0.005
	Temperature (deg C)	2.156	< 0.005
	Phaeophytin (mg m^{-3})	4.689	< 0.005
	Chlorophyll (mg m^{-3})	5.315	< 0.005
Odontaster validus	Wet Weight (g)	2.916	< 0.005
	Radial Length (R mm)	0.624	0.104
	Body Radius (r mm)	1.724	< 0.005
	Gonad Index (%)	7.798	< 0.005
	Pyloric caeca Index (%)	4.276	< 0.005
	Oocyte Feret Diameter (μm)	0.598	0.113
	Pre-vitellogenic Oocytes (Proportion)	1.019	0.010
	Vitellogenic Oocytes (Proportion)	1.019	0.010
Parborlasia corrugatus	Retracted Length (mm)	2.823	< 0.005
	Oocyte Feret Diameter (mm)	0.719	0.056
	Pre-vitellogenic Oocytes (Proportion)	0.894	0.020
	Vitellogenic Oocytes (Proportion)	0.894	0.020
Heterocucumis steineni	Wet Weight (g)	7.284	< 0.005
	Length (mm)	5.937	< 0.005
	Gonad Index (%)	0.688	0.072
	Oocyte Feret Diameter (mm)	0.630	0.093
	Pre-vitellogenic Oocytes (Proportion)	4.427	< 0.005
	Vitellogenic Oocytes (Proportion)	4.584	< 0.005

Table. 3 *Ophionotus victoriae*. Test for equal variance in actual fecundity between individual years (1997 to 2001). Years with equal variance were indicated by an F-test and a Levene's test when P > 0.05.

Variable	Test Statistic	P-value
1997-1998		
F-test	0.73	0.671
Levene's Test	0.01	0.922
1997-1999		
F-test	1.10	0.897
Levene's Test	0.11	0.740
1997-2000		
F-test	1.45	0.610
Levene's Test	0.27	0.609
1998-1999		
F-test	1.50	0.580
Levene's Test	0.02	0.892
1998-2000		
F-test	1.98	0.353
Levene's Test	0.06	0.812
1999-2000		
F-test	1.32	0.703
Levene's Test	0.02	0.883

Table. 4 *Odontaster validus*. Test for equal variance in radial length (R ±0.01mm) between individual years (1997 to 2001). Years with equal variance were indicated by an F-test and a Levene's test when P > 0.05.

Variable	Test Statistic	P-value
1997-1998		
F-test	0.67	0.071
Levene's Test	2.35	0.127
1997-1999		
F-test	0.50	0.005
Levene's Test	8.84	0.004
1997-2000		
F-test	0.61	0.027
Levene's Test	6.54	0.011
1998-1999		
F-test	0.74	0.147
Levene's Test	3.11	0.079
1998-2000		
F-test	0.91	0.593
Levene's Test	1.21	0.272
1999-2000		
F-test	1.23	0.312
Levene's Test	0.89	0.346

Table. 5 *Heterocucumis steineni*. Test for equal variance in overall gonad index between individual years (1997-2001). Years with equal variance were indicated by an F-test and a Levene's test when P > 0.05.

Variable	Test-Statistic	P-value
1997-1998		
F-test	0.85	0.530
Levene's Test	1.78	0.184
1997-1999		
F-test	0.64	0.076
Levene's Test	4.83	0.029
1997-2000		
F-test	0.52	0.009
Levene's Test	2.52	0.114
1998-1999		
F-test	0.75	0.109
Levene's Test	2.22	0.137
1998-2000		
F-test	0.61	0.005
Levene's Test	1.05	0.307
1999-2000		
F-test	0.81	0.270
Levene's Test	0.04	0.834

Table. 6 *Odontaster validus*, *Marthasterias glacialis* and *Laternula elliptica*. Anderson-Darling Normality test on fertilisation success variables.

Species	Variable	AD Test Statistic	P-value
M. glacialis	**Viscosity**		
	< 4.0cp	1.042	0.008
	> 4.0cp	1.193	< 0.005
O. validus	**Viscosity**		
	Seawater	0.262	0.375
	PVP-seawater	0.322	0.230
L. elliptica	**Viscosity**		
	Glycerol < 2.0cp	0.220	0.787
	Glycerol > 2.0cp	0.581	0.072
	PVP < 2.0cp	0.480	0.188
	PVP > 2.0cp	0.581	0.072
	Methylcellulose < 4.0cp	0.552	0.120
	Methylcellulose > 4.0cp	0.392	0.253

Table. 7 *Odontaster validus*. Anderson-Darling Normality test on female wet weight (±0.01g), size (R and r ±0.01mm) and fecundity (egg number). Normally distributed data were indicated by P > 0.05.

Variable	AD Test Statistic	P-value
Wet weight	0.228	0.722
R	0.274	0.558
r	0.173	0.892
Fecundity	0.652	0.055

Table. 8 *Marthasterias glacialis*. Anderson-Darling Normality test on male and female wet weight (±0.01g), size (R and r ±0.01mm) and on sperm and egg numbers released during gamete release experiments.

Variable	AD Test Statistic	P-value
Sperm	1.903	< 0.005
Male Wet Weight	0.732	0.046
Male R	0.299	0.548
Male r	1.865	< 0.005
Egg	0.638	0.078
Female Wet Weight	3.589	< 0.005
Female R	2.556	< 0.005
Female r	2.029	< 0.005

Table. 9 *Odontaster validus*. Anderson-Darling Normality test on the wet weight (±0.01mm) and size (R and r ±0.01mm) of both competent and non-responsive starfish.

Variable	AD Test Statistic	P-value
Competent Wet Weight	1.533	< 0.005
Competent R	0.729	0.051
Competent r	1.328	< 0.005
Non-responsive Wet Weight	2.908	< 0.005
Non-responsive R	0.272	0.666
Non-responsive r	1.238	< 0.005

Table. 10 *Odontaster validus*. Anderson-Darling Normality test on the wet weight (±0.01mm) and size (R and r ±0.01mm) of competent males and females, and the total number of starfish injected.

Variable	AD Test-Statistic	P-value
Competent Male Wet Weight	1.295	< 0.005
Competent Male R	0.527	0.158
Competent Male r	0.833	0.026
Competent Female Wet Weight	0.454	0.211
Competent Female R	0.354	0.385
Competent Female r	0.687	0.050
Injected Wet Weight	4.253	< 0.005
Injected R	0.535	0.168
Injected r	2.358	< 0.005

Table. 11 *Odontaster validus* and *Ophionotus victoriae*. The Anderson-Darling Normality test statistics and recorded p-values for the biochemical and proximate composition of bodily, digestive and gonad tissue in two shallow-water Antarctic echinoderms. Normally distributed data are indicated by P > 0.05.

Species	Tissue	Element	Anderson-Darling Statistic	P value	Normal
Odontaster validus	Gonad	C	0.968	0.014	Not
		N	1.755	< 0.005	Not
	Pyloric caeca	C	0.3	0.572	Normal
		N	0.232	0.793	Normal
		Protein	0.494	0.207	Normal
		Lipid	0.344	0.476	Normal
Ophionotus victoriae	Whole Animal	C	1.12	0.006	Not
		N	0.794	0.039	Not
		Protein	0.584	0.126	Normal
		Lipid	1.699	< 0.005	Not
	Gut	C	8.655	< 0.005	Not
		N	4.713	< 0.005	Not
		Protein	0.289	0.609	Normal
		Lipid	0.639	0.093	Normal
	Gonad	C	3.046	< 0.005	Not
		N	4.929	< 0.005	Not
		Protein	3.968	< 0.005	Not
		Lipid	5.346	< 0.005	Not

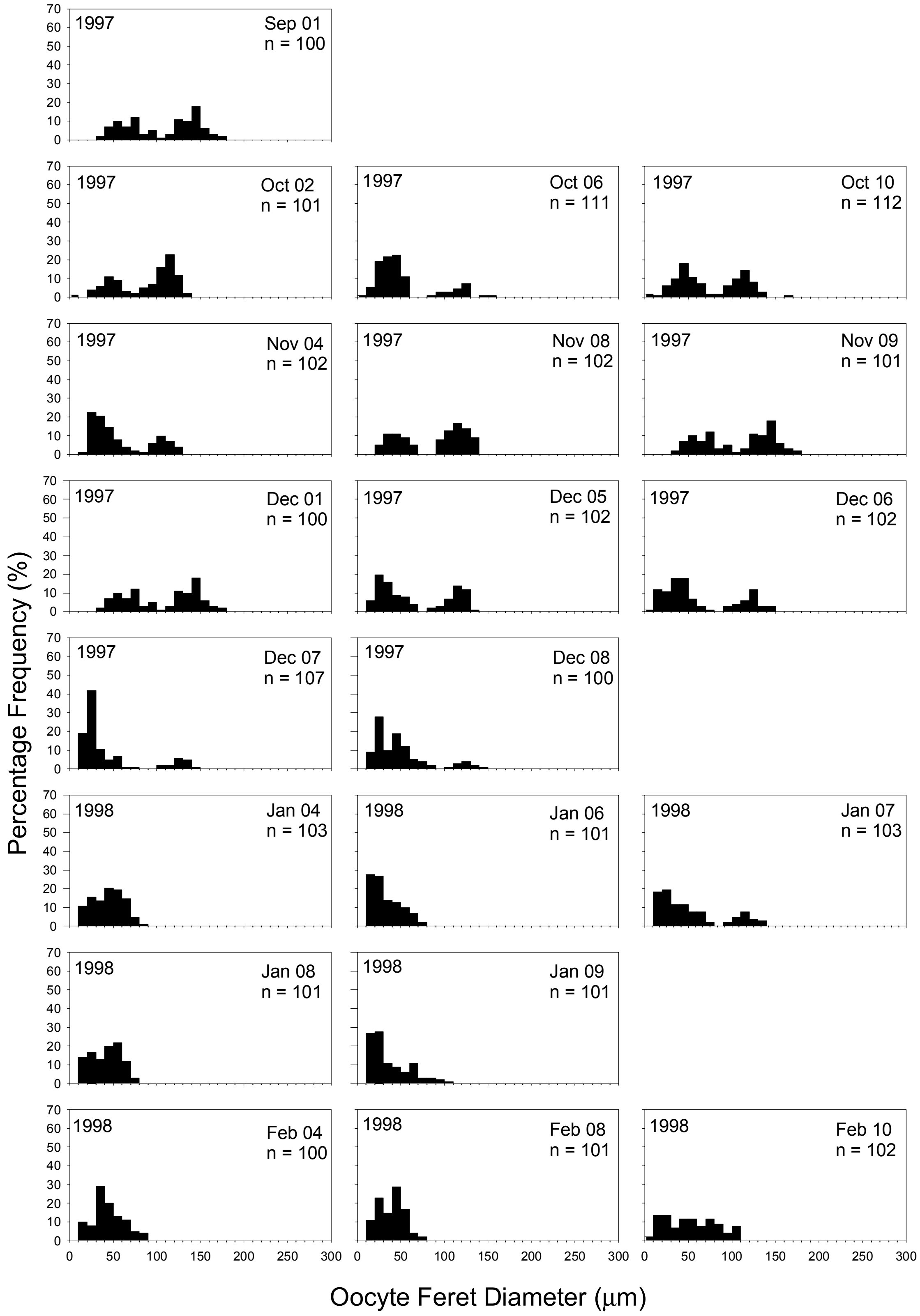


Fig. 1 *Ophionotus victoriae*. Individual oocyte feret diameter histograms, where month sampled and the number individual are indicated (e.g. Sep 01 = Individual number 1 sampled in September), and n = number of eggs counted (1997-2001).

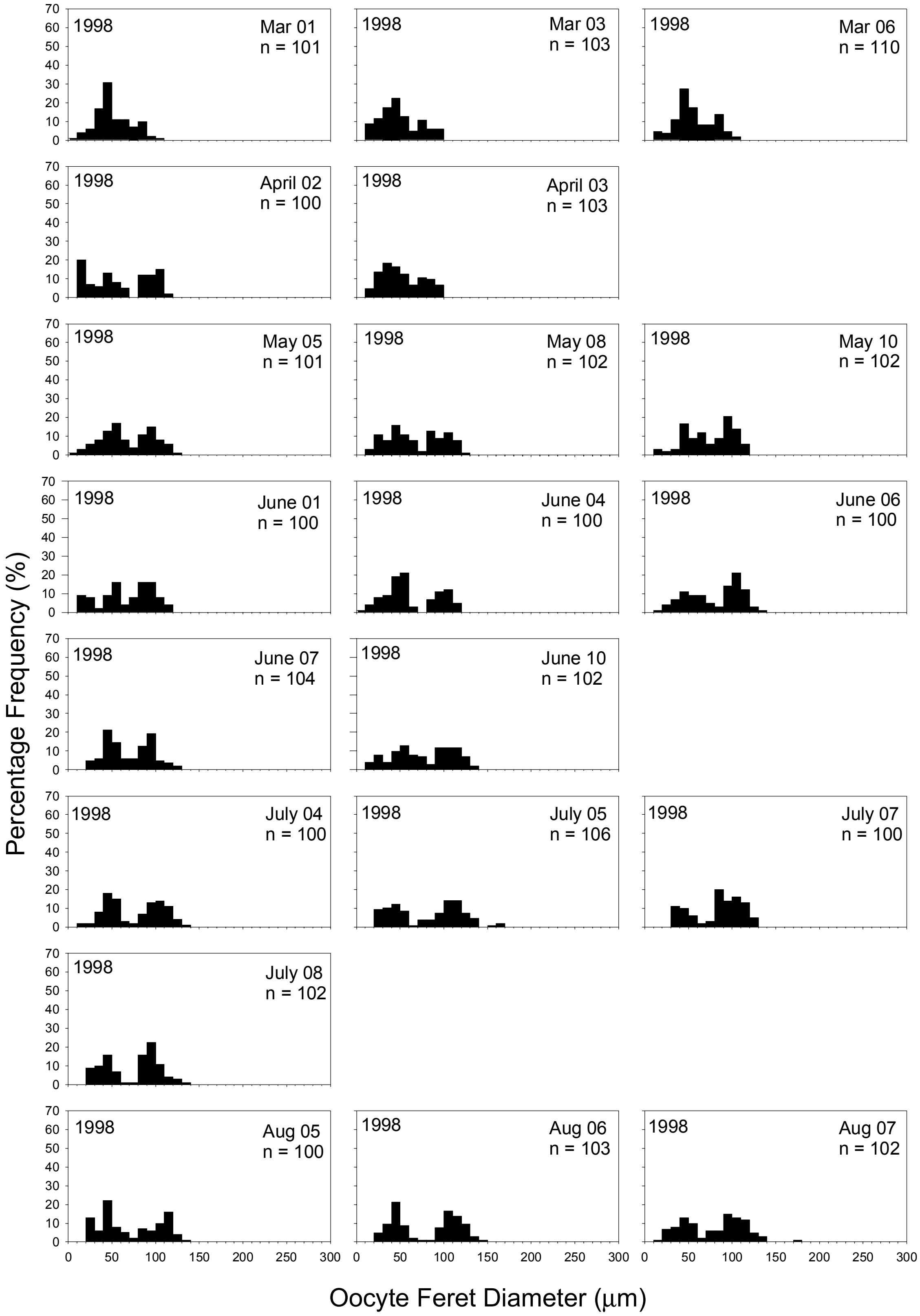


Fig. 1 contd *Ophionotus victoriae*. Individual oocyte feret diameter histograms, where month sampled and the number individual are indicated (e.g. Sep 01 = Individual number 1 sampled in September), and n = number of eggs counted (1997-2001).

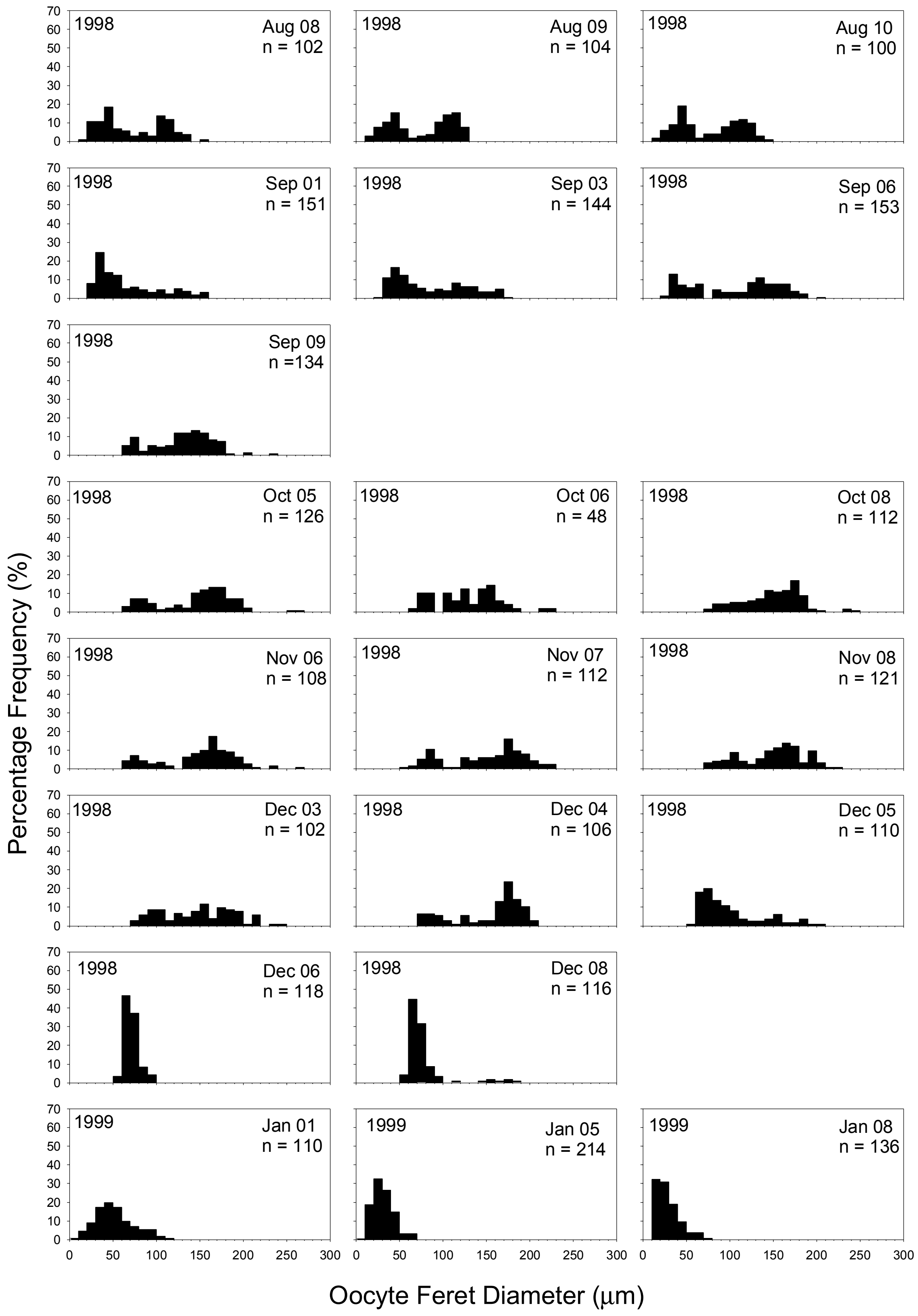


Fig. 1 contd *Ophionotus victoriae*. Individual oocyte feret diameter histograms, where month sampled and the number individual are indicated (e.g. Sep 01 = Individual number 1 sampled in September), and n = number of eggs counted (1997-2001).

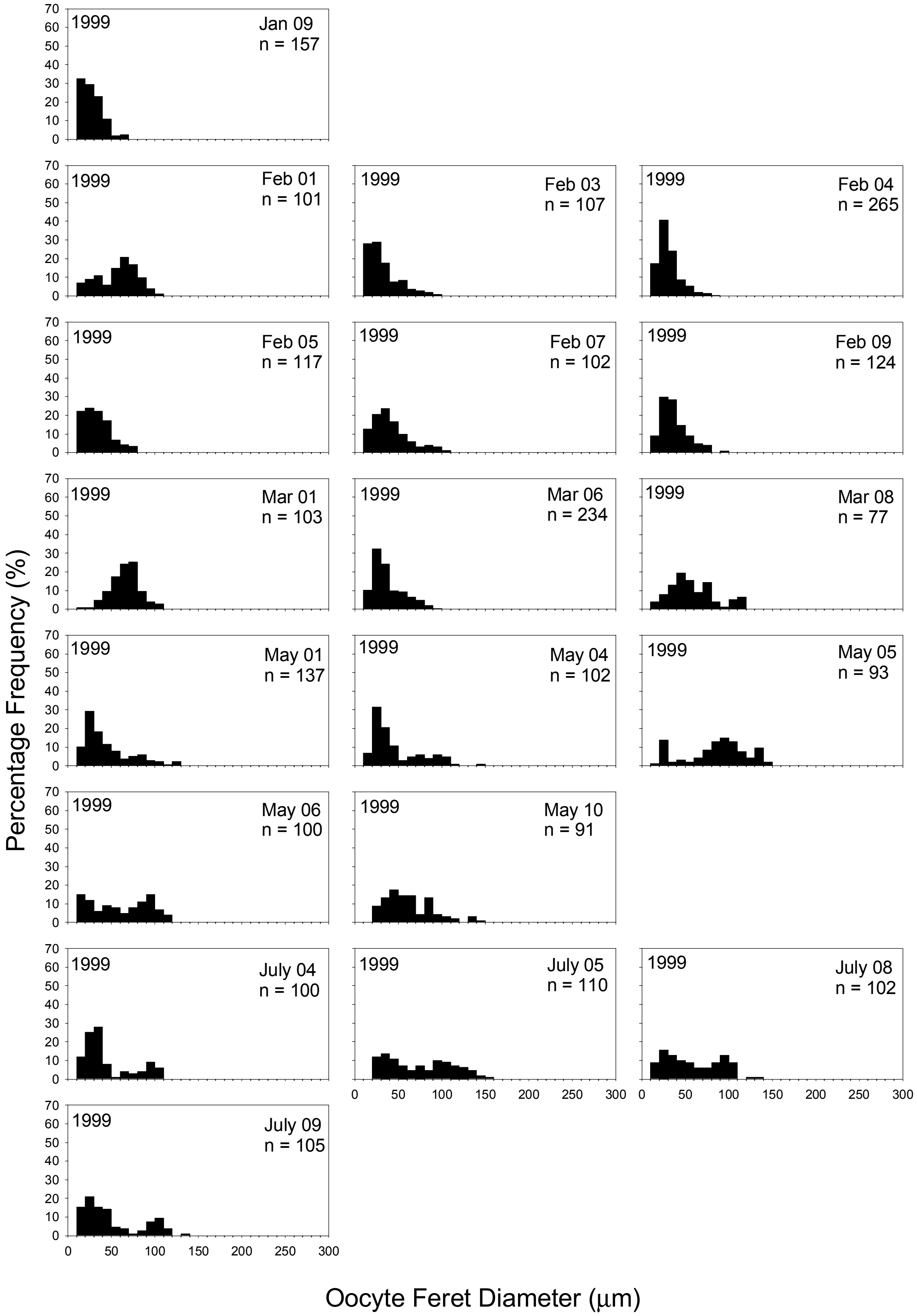


Fig. 1 contd *Ophionotus victoriae*. Individual oocyte feret diameter histograms, where month sampled and the number individual are indicated (e.g. Sep 01 = Individual number 1 sampled in September), and n = number of eggs counted (1997-2001).

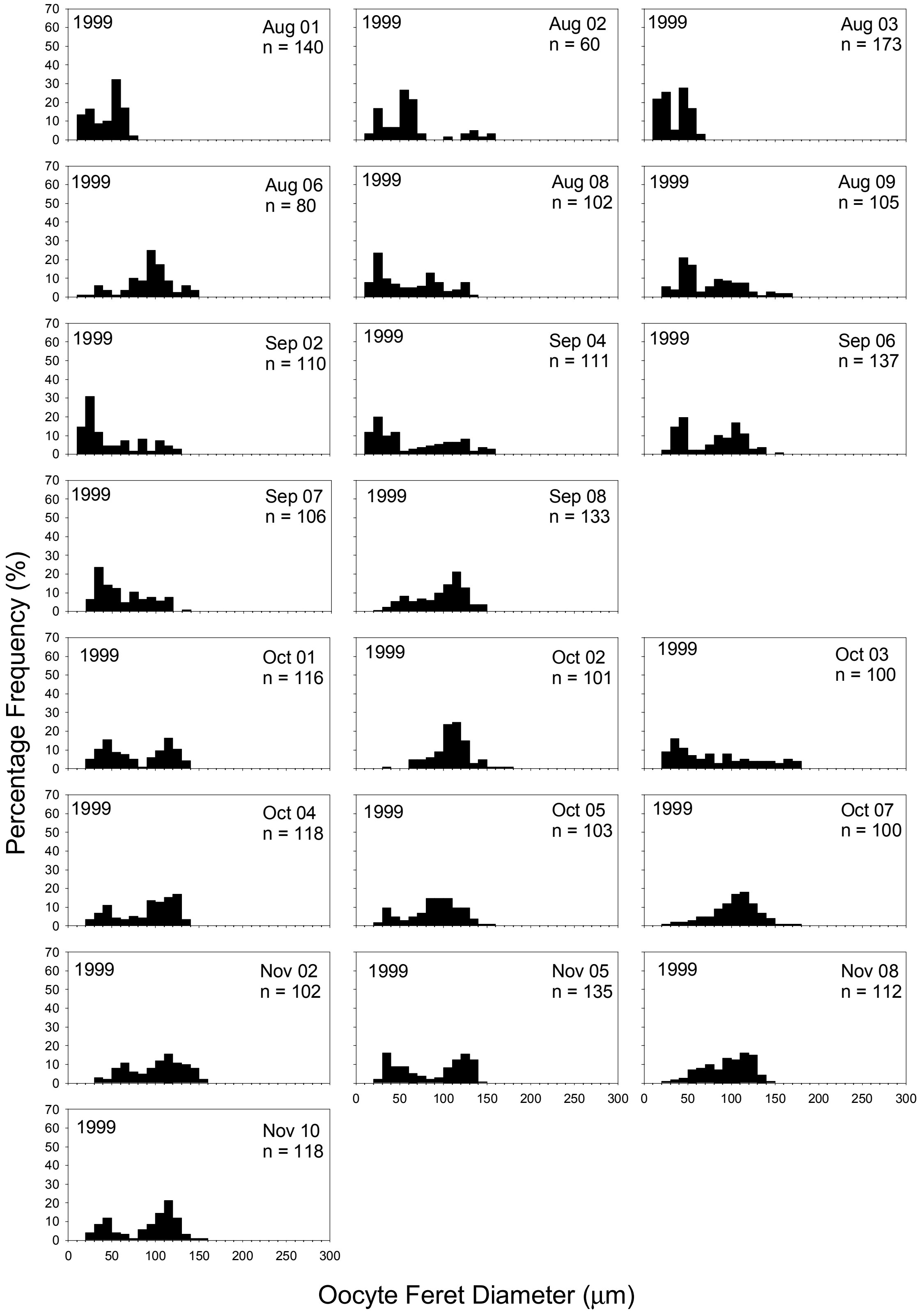


Fig. 1 contd *Ophionotus victoriae*. Individual oocyte feret diameter histograms, where month sampled and the number individual are indicated (e.g. Sep 01 = Individual number 1 sampled in September), and n = number of eggs counted (1997-2001).

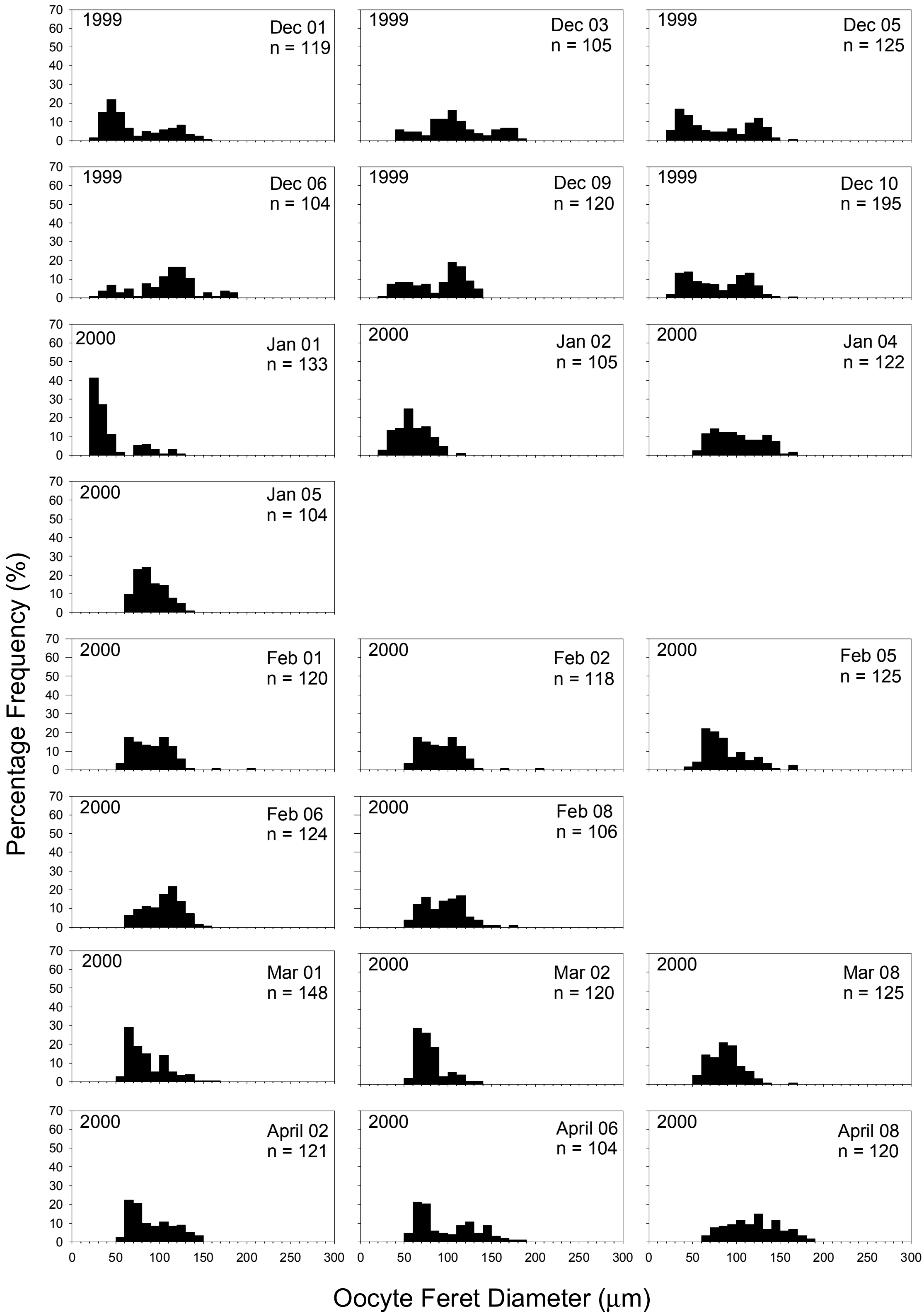


Fig. 1 contd *Ophionotus victoriae*. Individual oocyte feret diameter histograms, where month sampled and the number individual are indicated (e.g. Sep 01 = Individual number 1 sampled in September), and n = number of eggs counted (1997-2001).

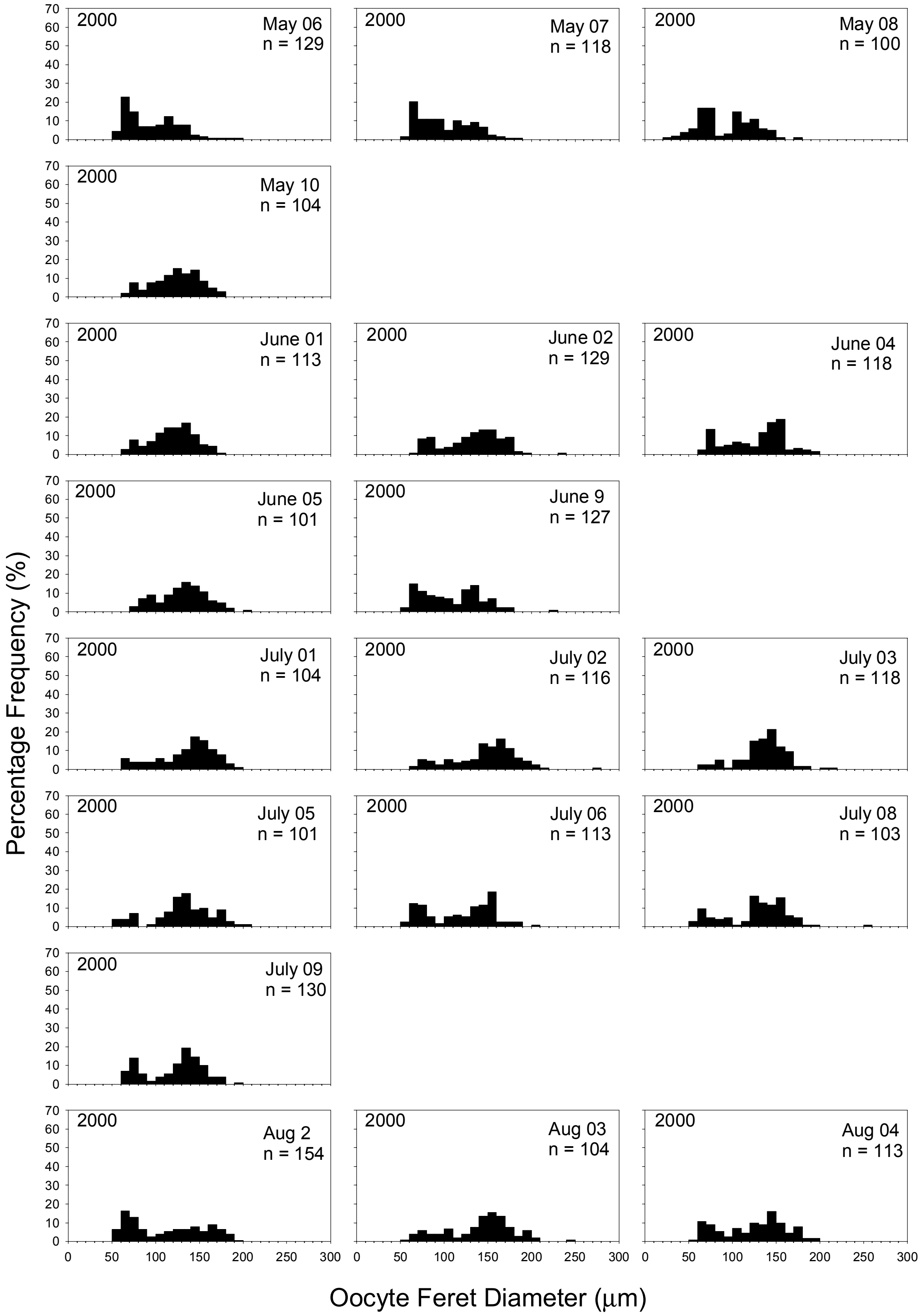


Fig. 1 contd *Ophionotus victoriae*. Individual oocyte feret diameter histograms, where month sampled and the number individual are indicated (e.g. Sep 01 = Individual number 1 sampled in September), and n = number of eggs counted (1997-2001).

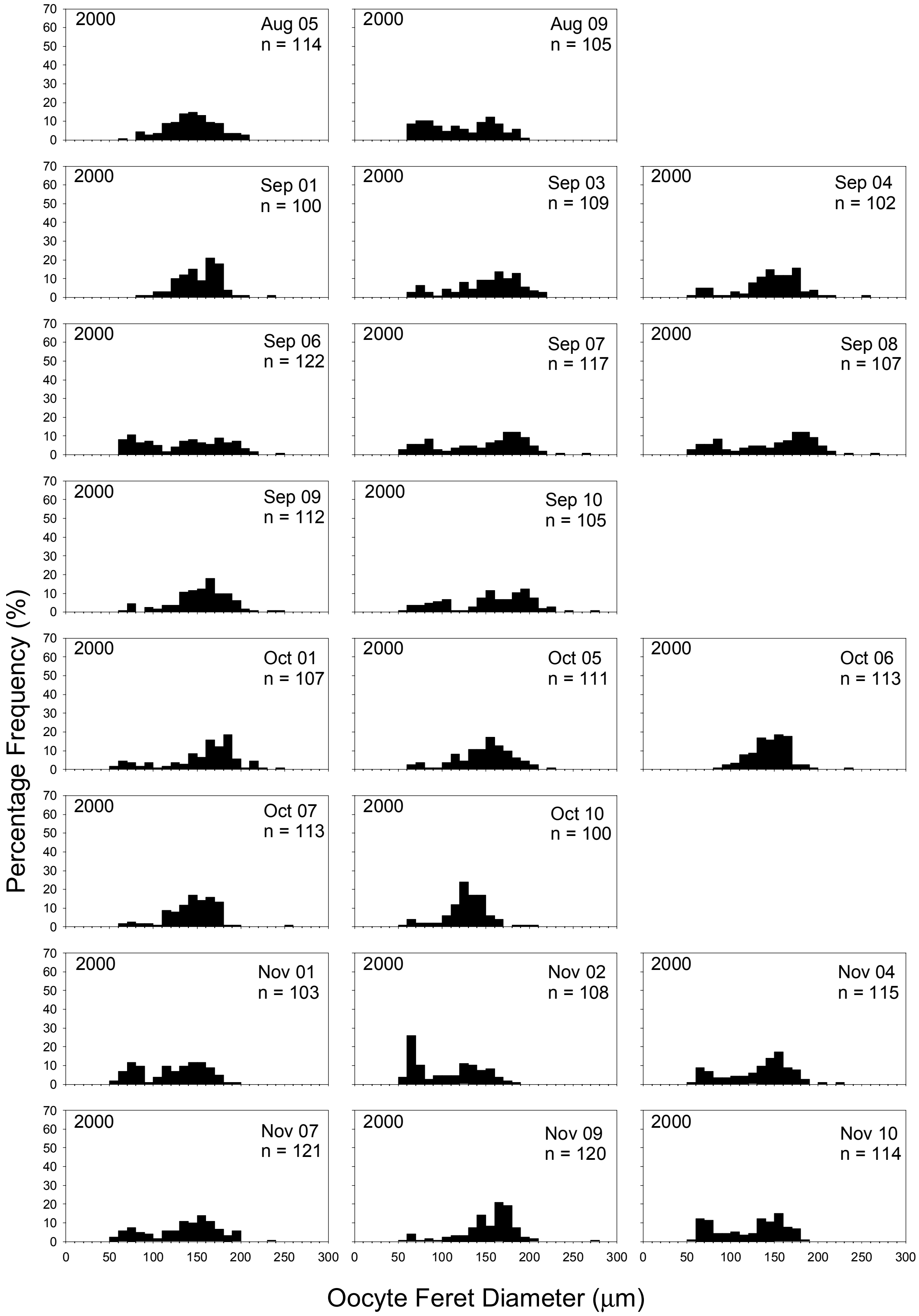


Fig. 1 contd *Ophionotus victoriae*. Individual oocyte feret diameter histograms, where month sampled and the number individual are indicated (e.g. Sep 01 = Individual number 1 sampled in September), and n = number of eggs counted (1997-2001).

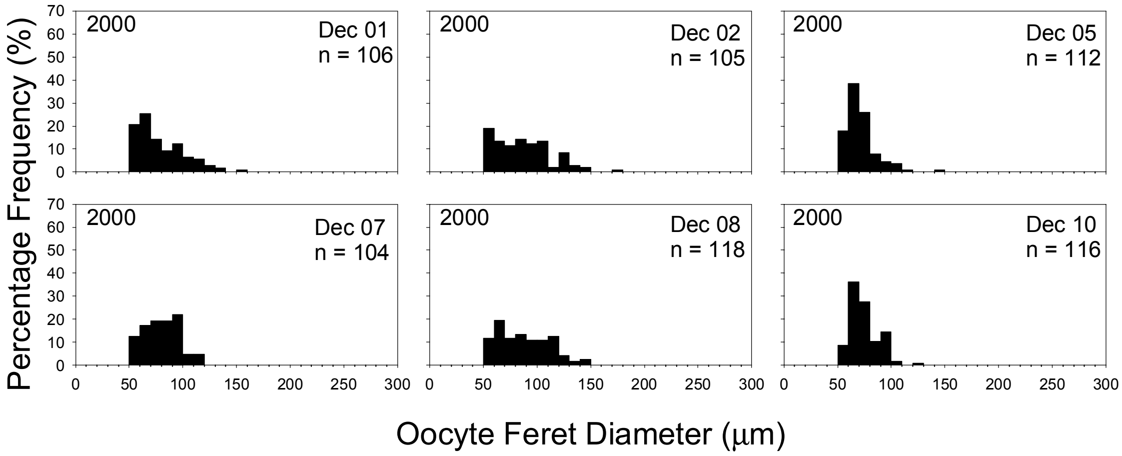


Fig. 1 contd *Ophionotus victoriae*. Individual oocyte feret diameter histograms, where month sampled and the number individual are indicated (e.g. Sep 01 = Individual number 1 sampled in September), and n = number of eggs counted (1997-2001).

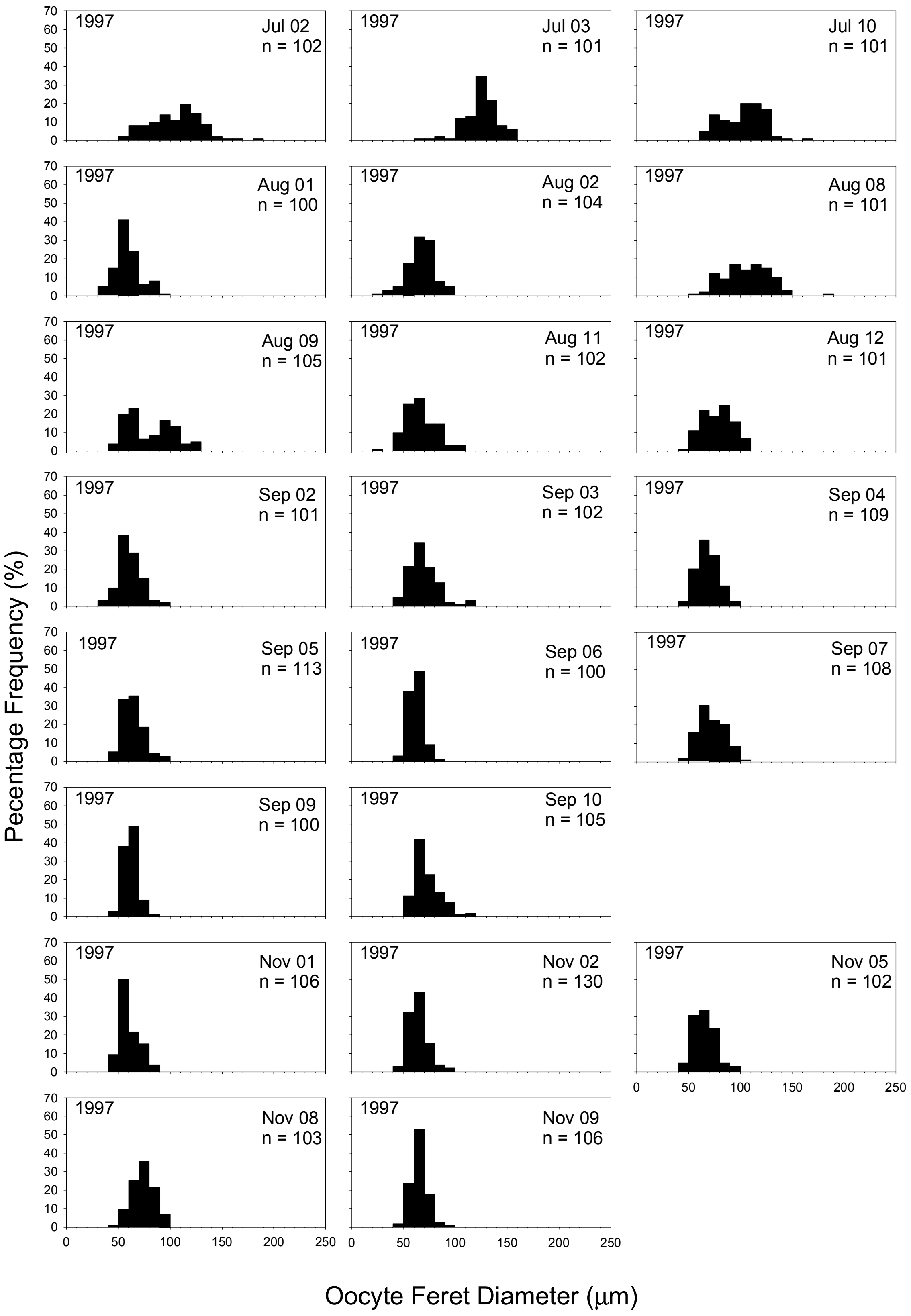


Fig. 2 *Odontaster validus*. Individual oocyte feret diameter histograms, where month sampled and the number individual are indicated (e.g. Sep 01 = Individual number 1 sampled in September), and n = number of eggs counted (1997-2001).

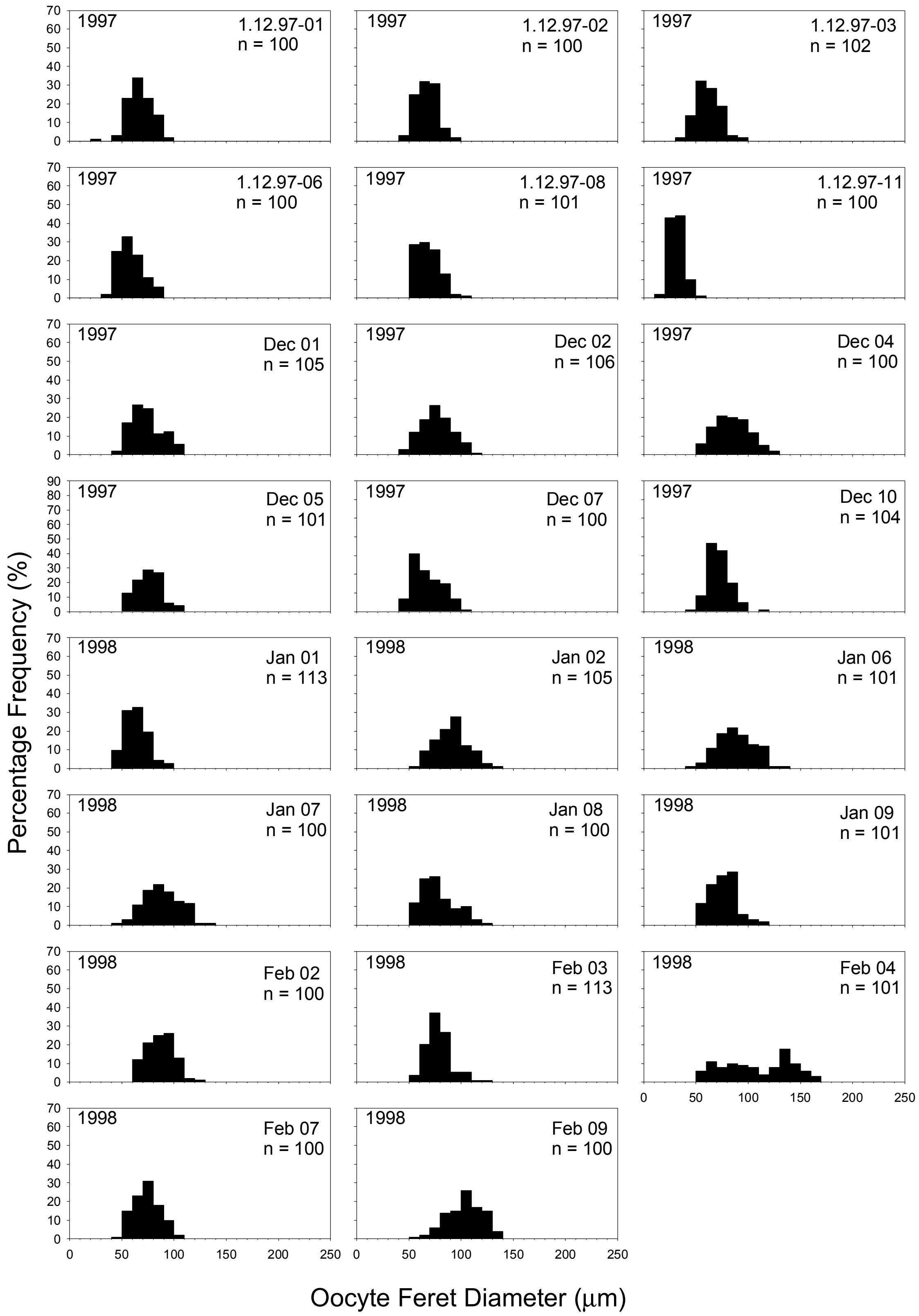


Fig. 2 contd *Odontaster validus*. Individual oocyte feret diameter histograms, where month sampled and the number individual are indicated (e.g. Sep 01 = Individual number 1 sampled in September), and n = number of eggs counted (1997-2001).

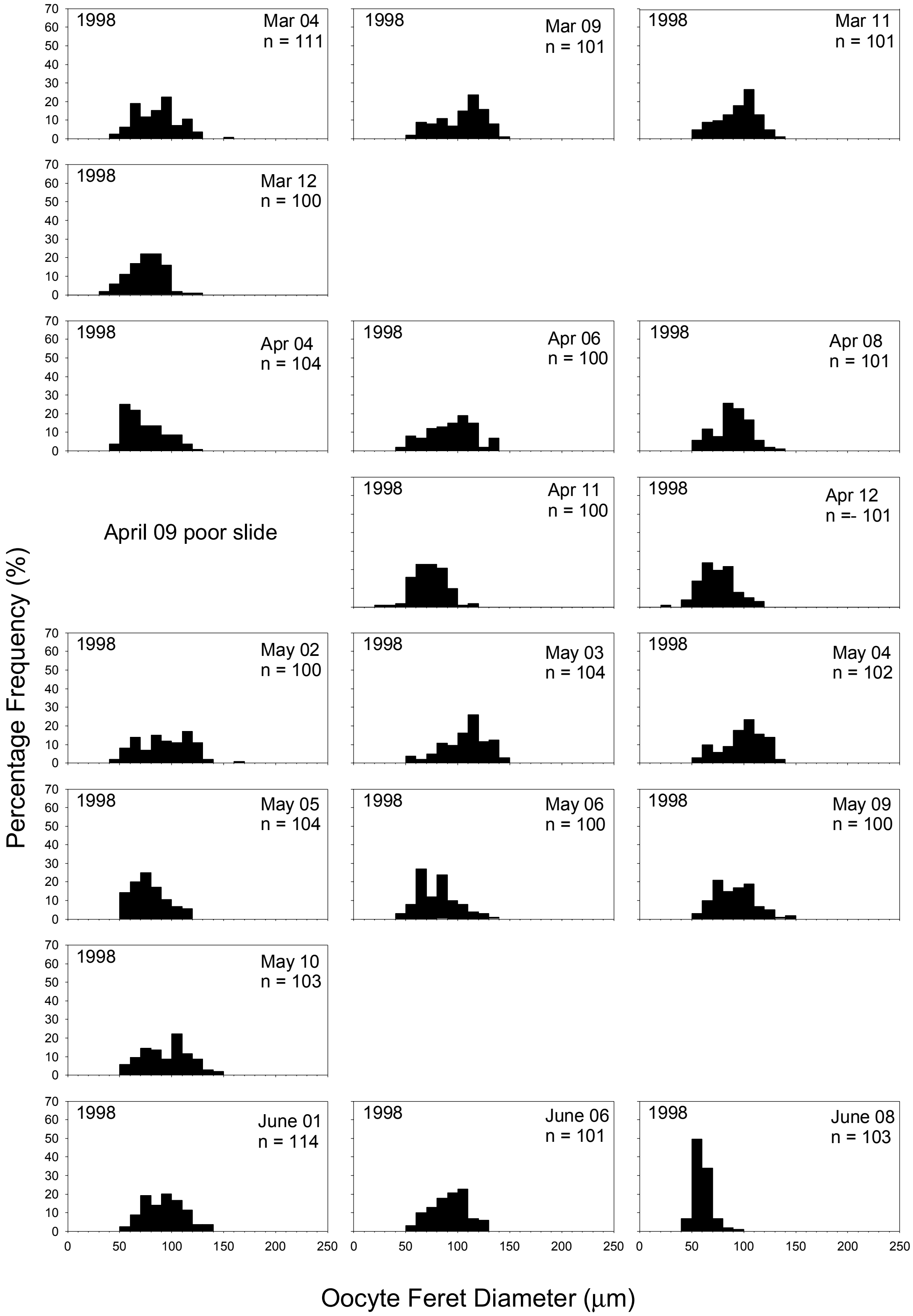


Fig. 2 contd *Odontaster validus*. Individual oocyte feret diameter histograms, where month sampled and the number individual are indicated (e.g. Sep 01 = Individual number 1 sampled in September), and n = number of eggs counted (1997-2001).

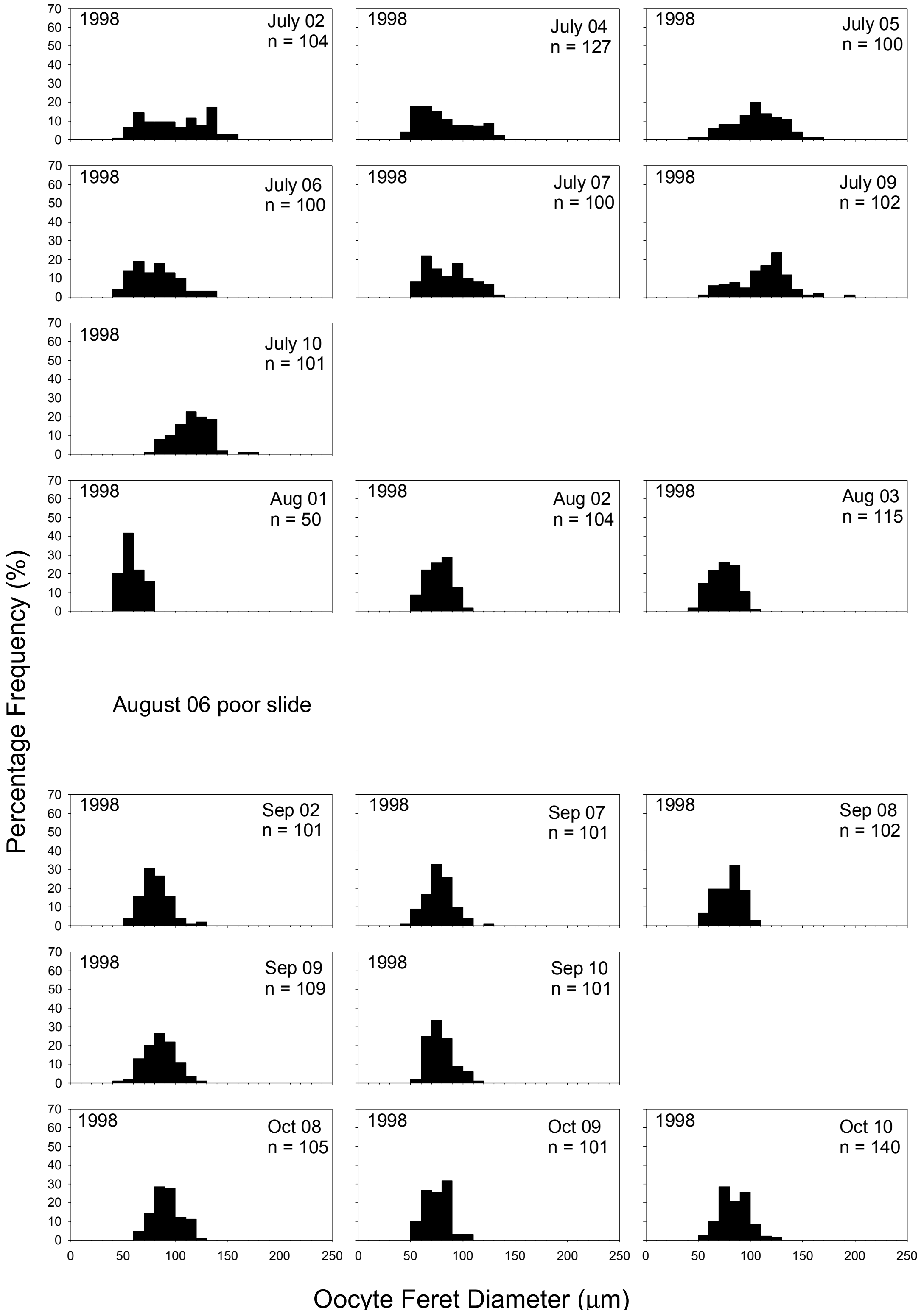


Fig. 2 contd *Odontaster validus*. Individual oocyte feret diameter histograms, where month sampled and the number individual are indicated (e.g. Sep 01 = Individual number 1 sampled in September), and n = number of eggs counted (1997-2001).

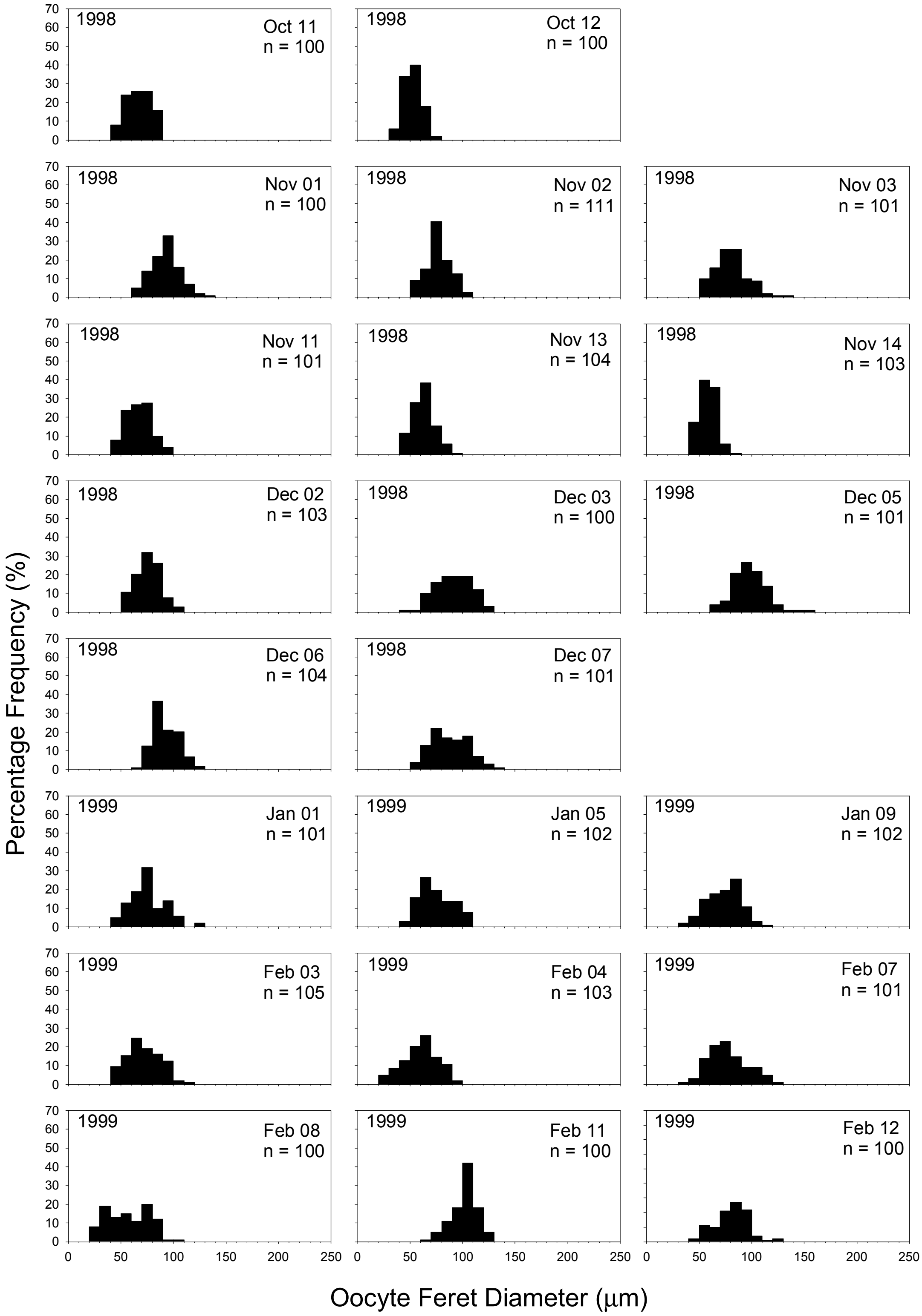


Fig. 2 contd *Odontaster validus*. Individual oocyte feret diameter histograms, where month sampled and the number individual are indicated (e.g. Sep 01 = Individual number 1 sampled in September), and n = number of eggs counted (1997-2001).

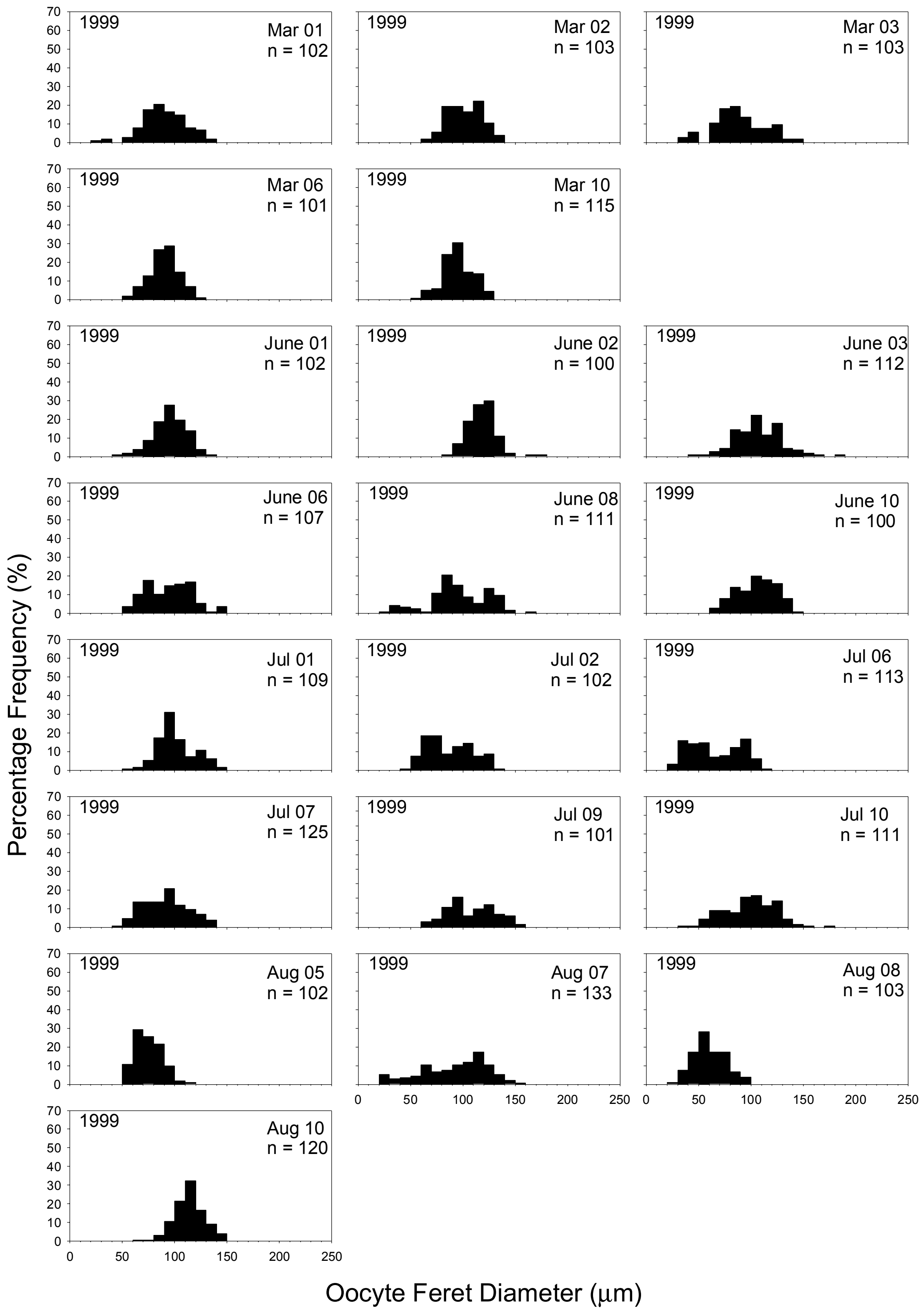


Fig. 2 contd *Odontaster validus*. Individual oocyte feret diameter histograms, where month sampled and the number individual are indicated (e.g. Sep 01 = Individual number 1 sampled in September), and n = number of eggs counted (1997-2001).

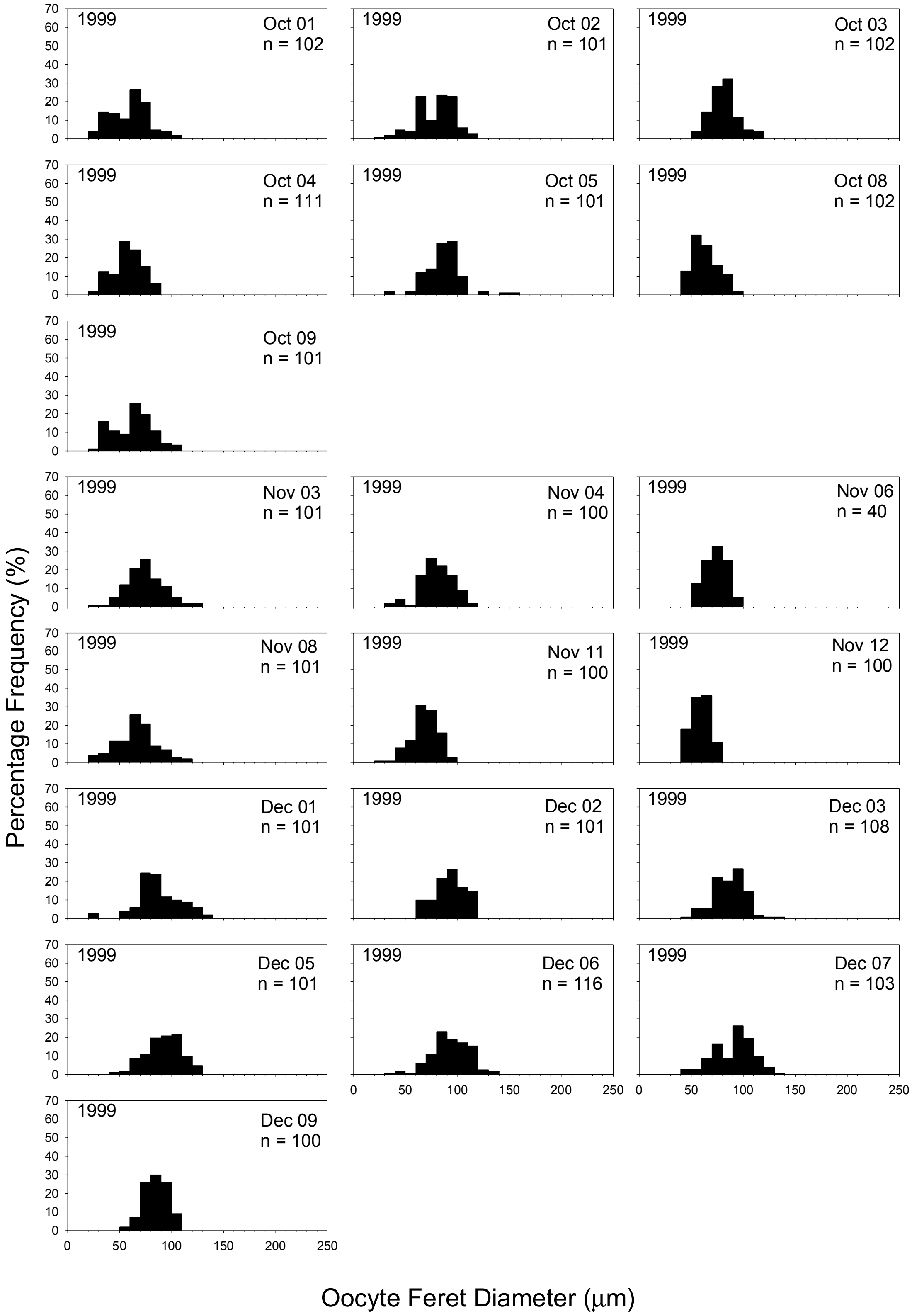


Fig. 2 contd *Odontaster validus*. Individual oocyte feret diameter histograms, where month sampled and the number individual are indicated (e.g. Sep 01 = Individual number 1 sampled in September), and n = number of eggs counted (1997-2001).

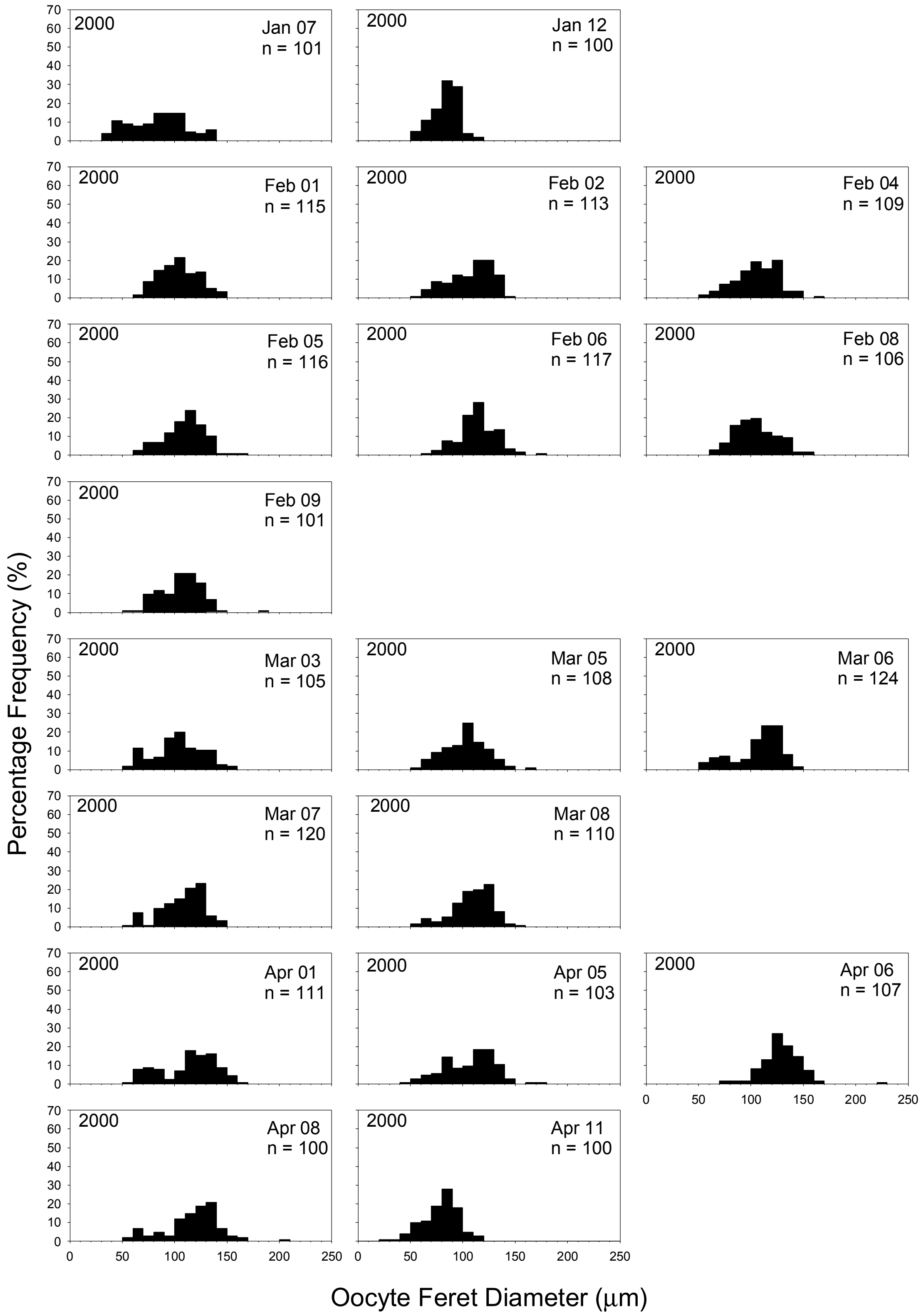
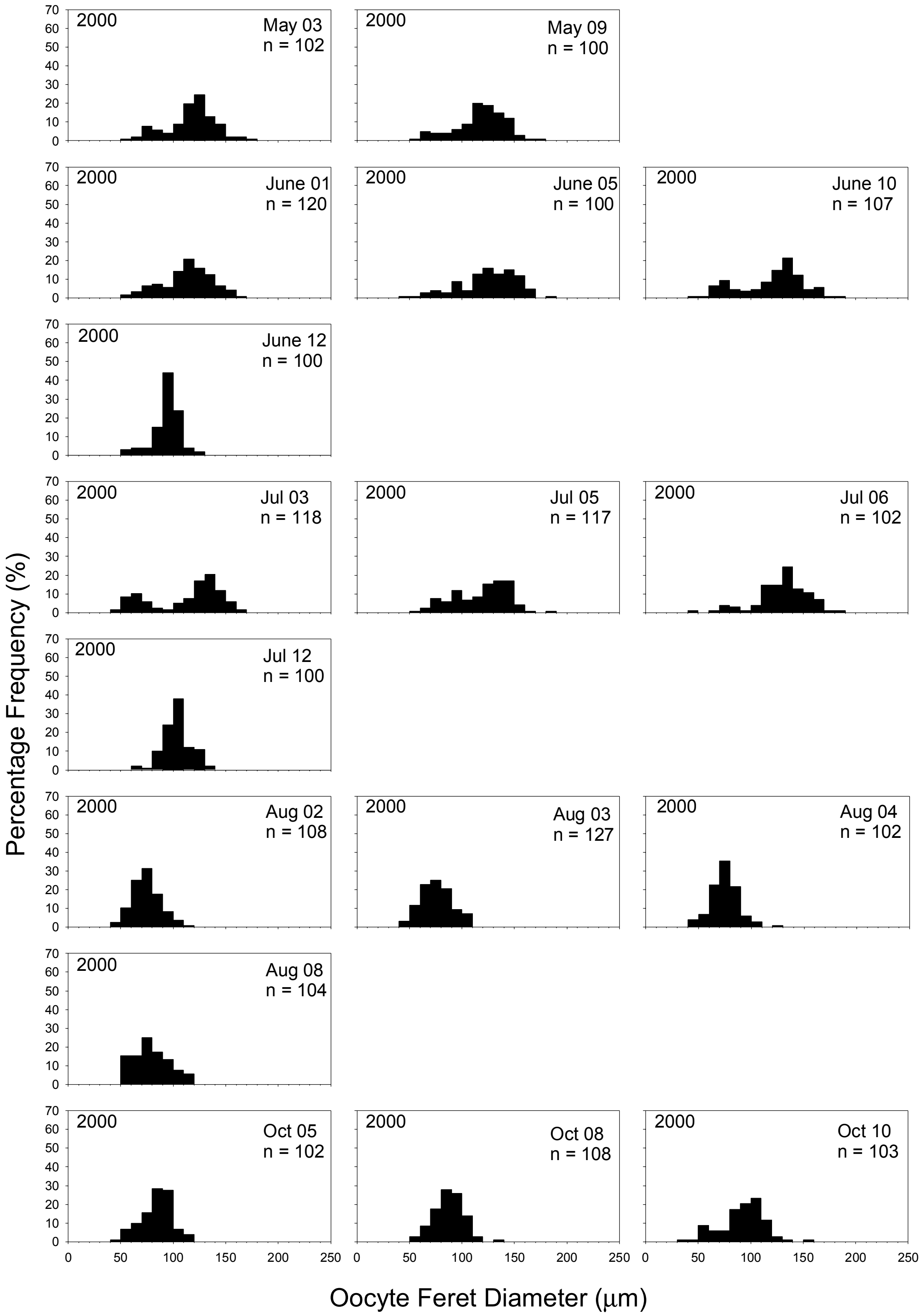


Fig. 2 contd *Odontaster validus*. Individual oocyte feret diameter histograms, where month sampled and the number individual are indicated (e.g. Sep 01 = Individual number 1 sampled in September), and n = number of eggs counted (1997-2001).



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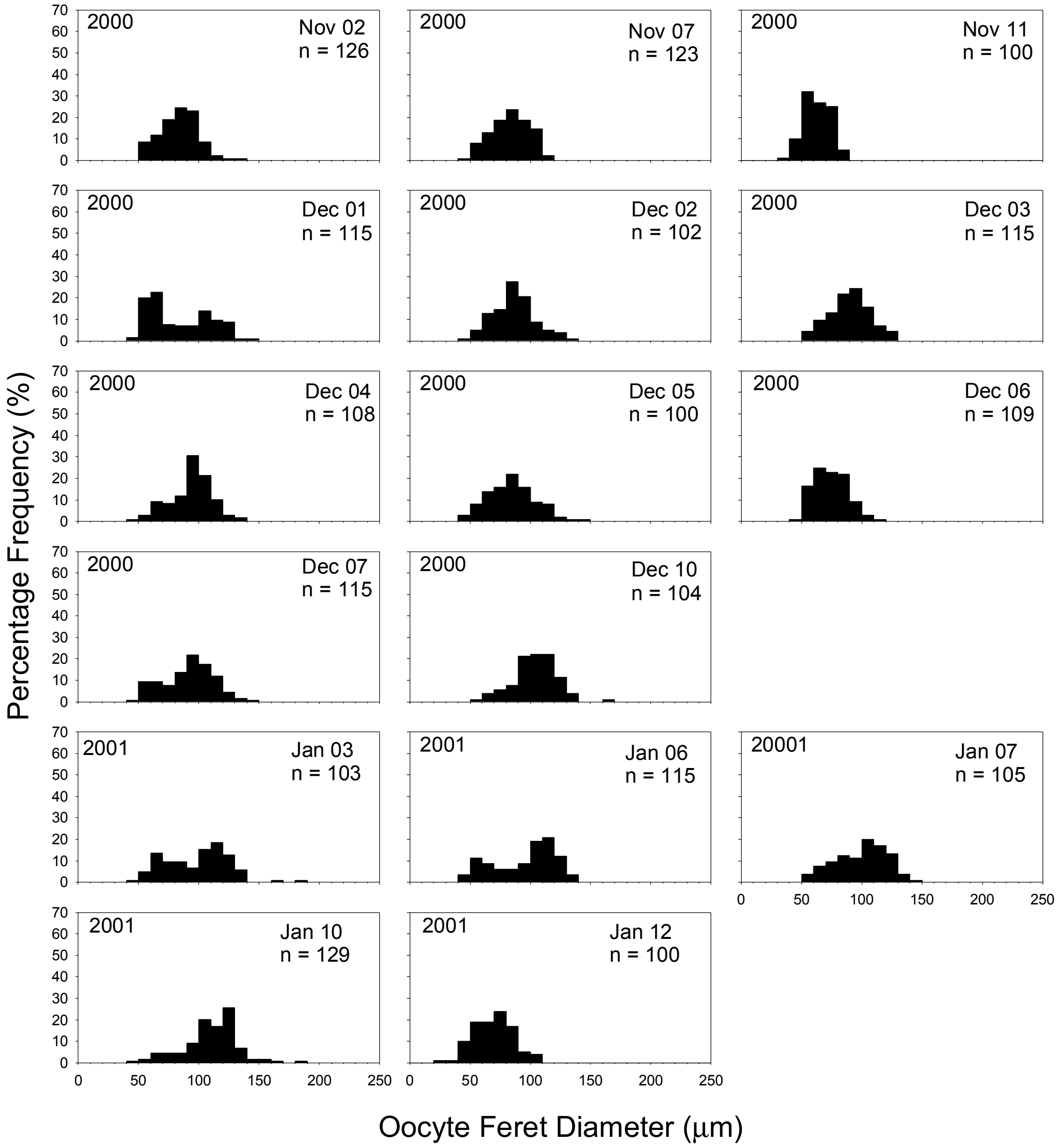

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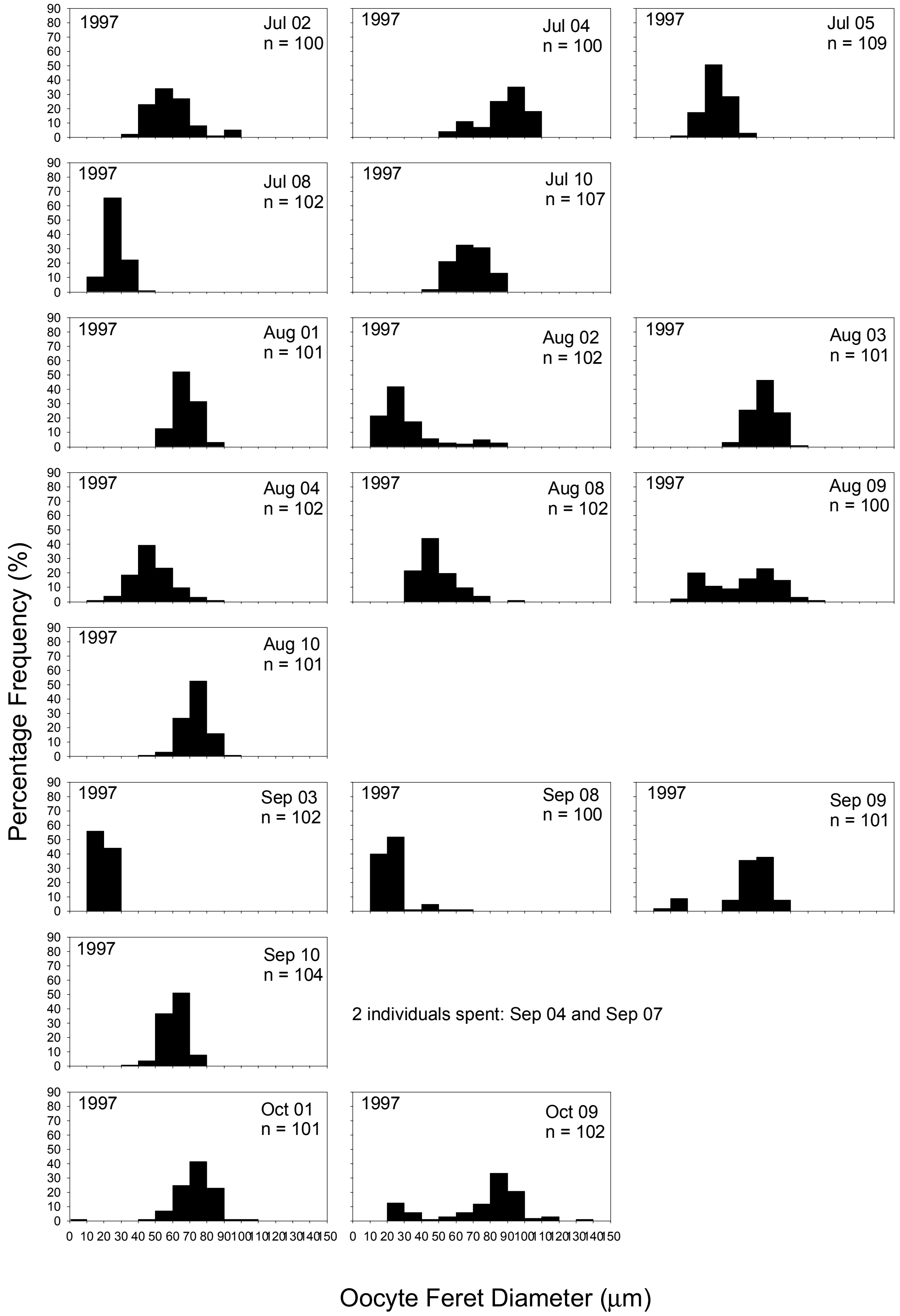


2 individuals spent: Sep 04 and Sep 07

Fig. 3 *Parborlasia corrugatus*. Individual oocyte feret diameter histograms, where month sampled and the number individual are indicated (e.g. Sep 01 = Individual number 1 sampled in September), and n = number of eggs counted (1997-2001).

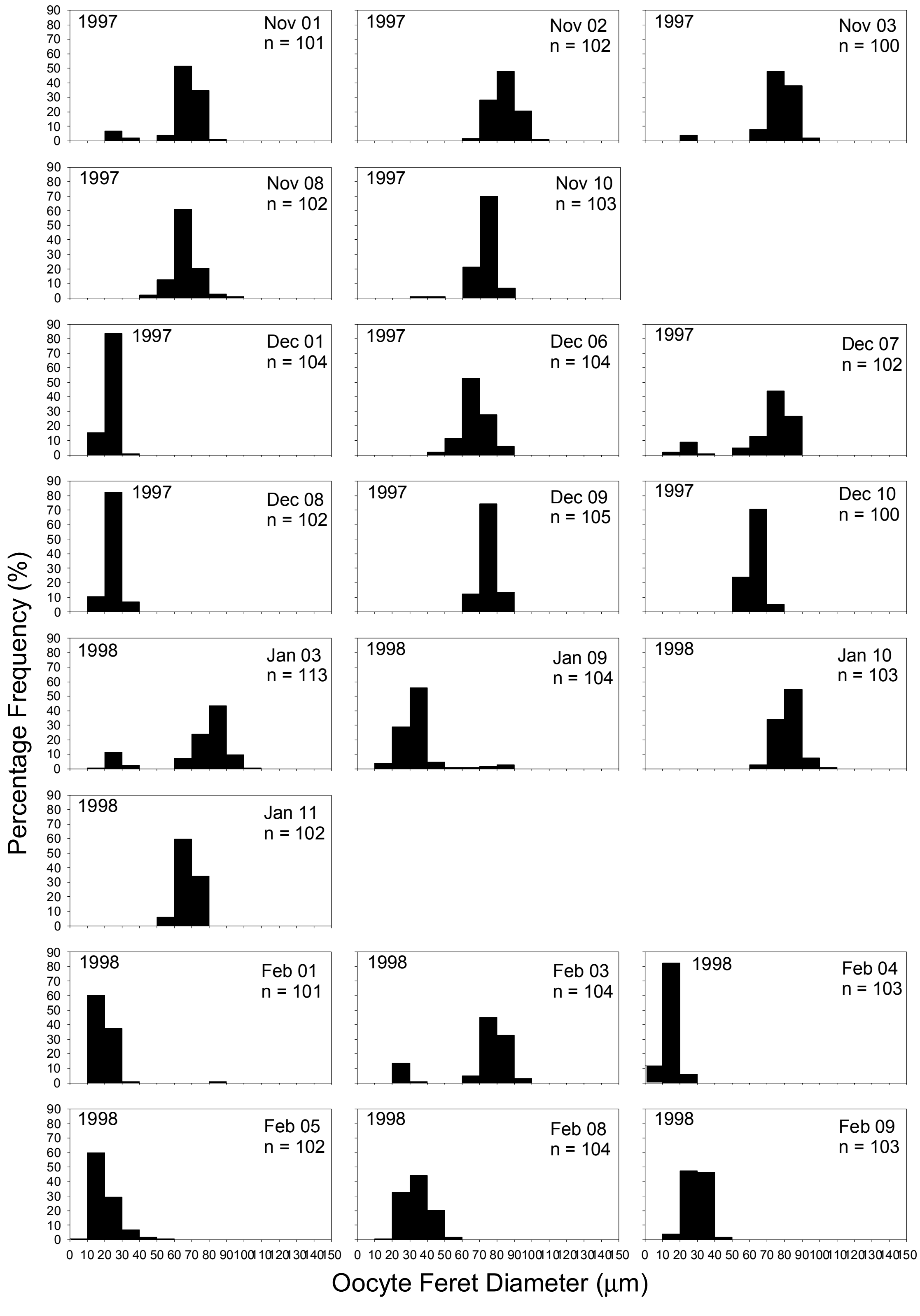


Fig. 3 contd *Parborlasia corrugatus*. Individual oocyte feret diameter histograms, where month sampled and the number individual are indicated (e.g. Sep 01 = Individual number 1 sampled in September), and n = number of eggs counted (1997-2001).

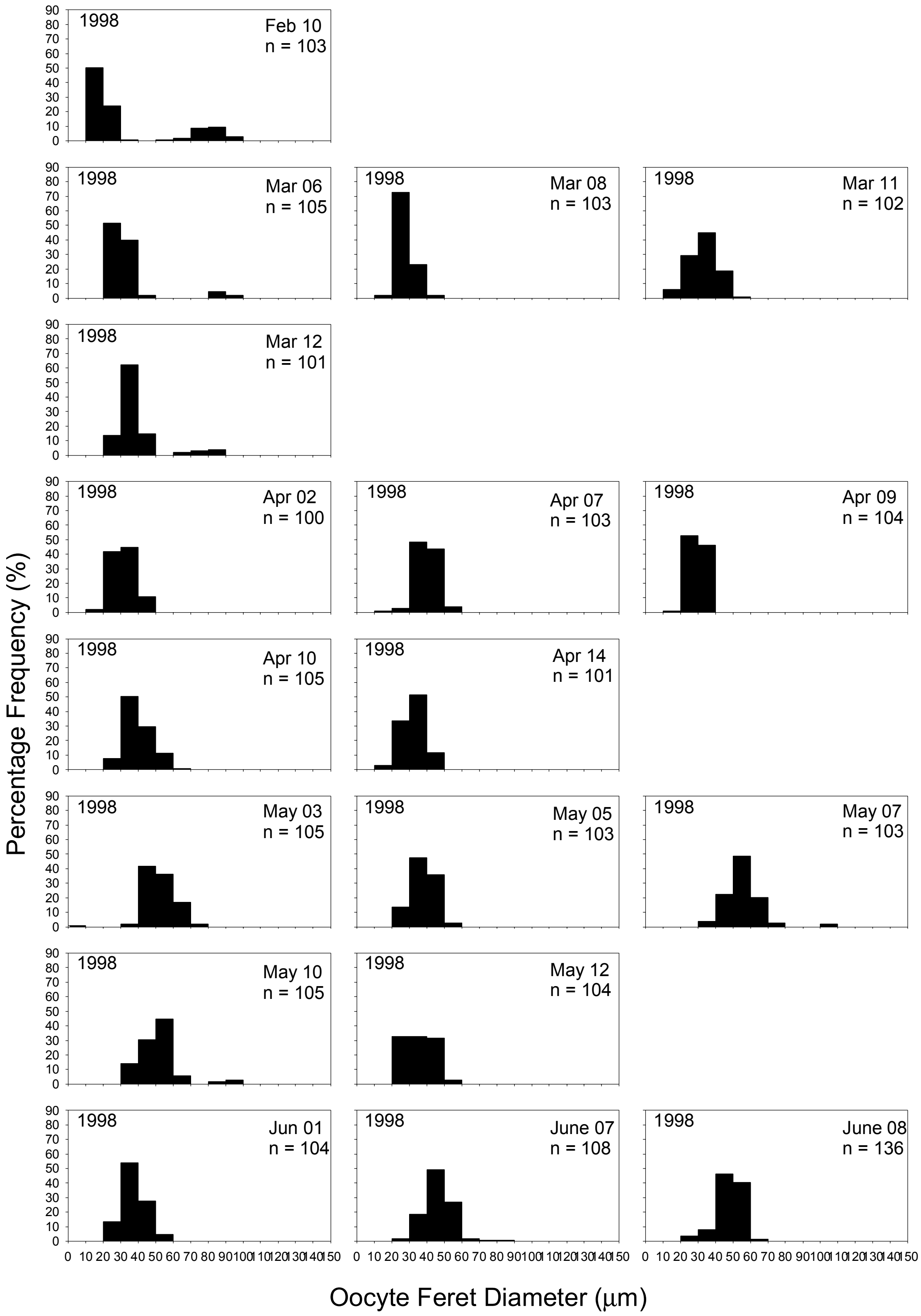


Fig. 3 contd *Parborlasia corrugatus*. Individual oocyte feret diameter histograms, where month sampled and the number individual are indicated (e.g. Sep 01 = Individual number 1 sampled in September), and n = number of eggs counted (1997-2001).

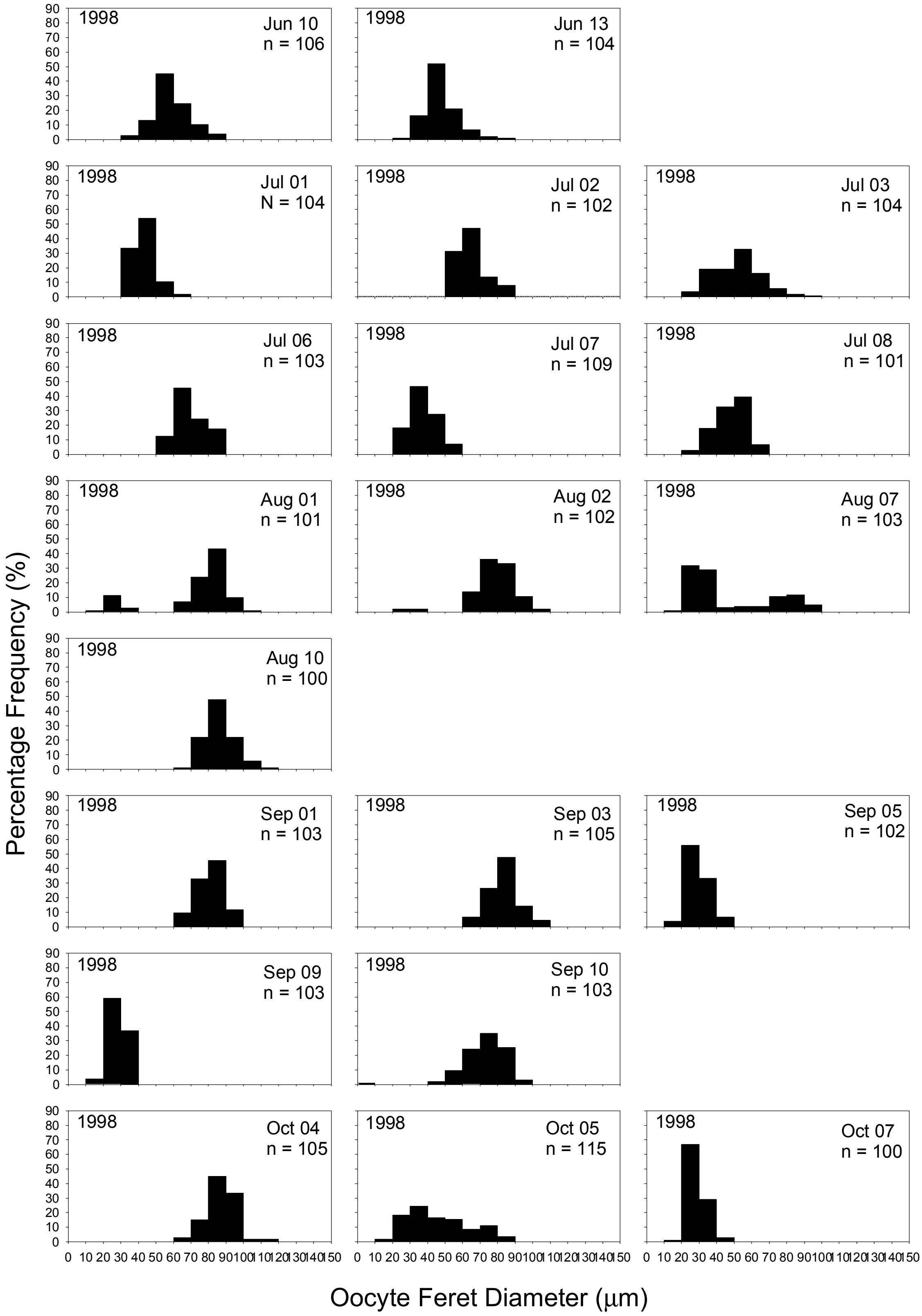


Fig. 3 contd *Parborlasia corrugatus*. Individual oocyte feret diameter histograms, where month sampled and the number individual are indicated (e.g. Sep 01 = Individual number 1 sampled in September), and n = number of eggs counted (1997-2001).

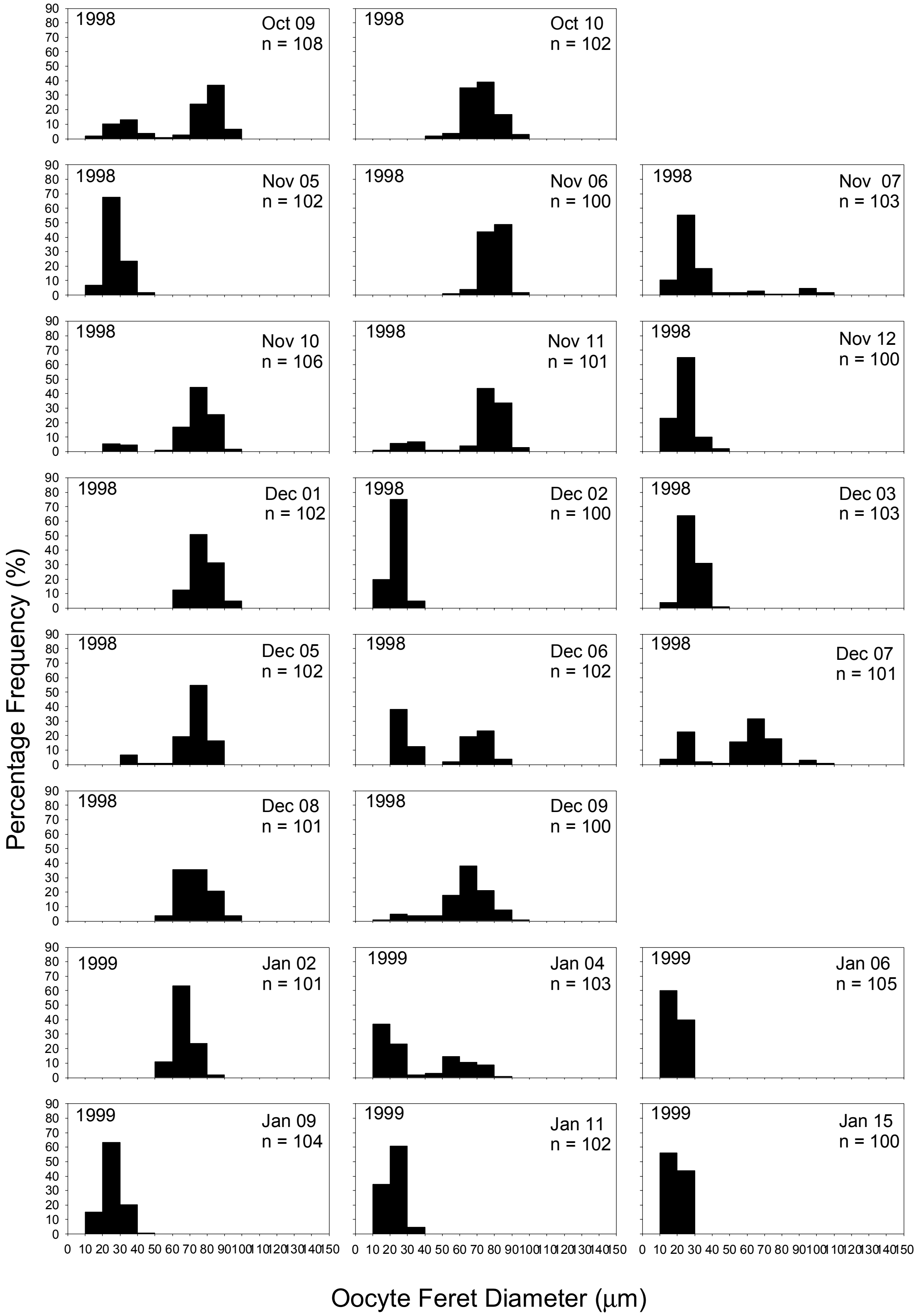


Fig. 3 contd *Parborlasia corrugatus*. Individual oocyte feret diameter histograms, where month sampled and the number individual are indicated (e.g. Sep 01 = Individual number 1 sampled in September), and n = number of eggs counted (1997-2001).

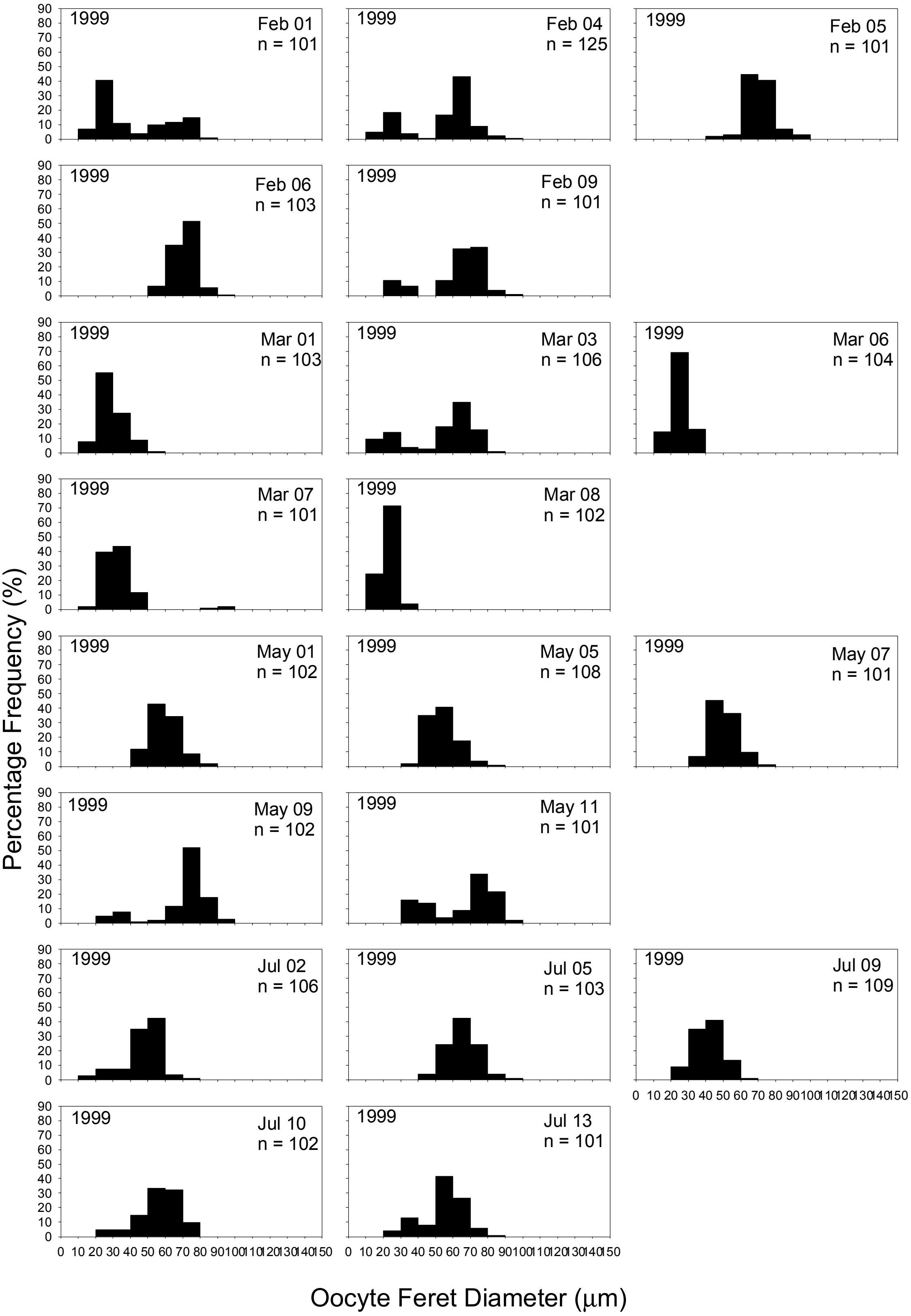


Fig. 3 contd *Parborlasia corrugatus*. Individual oocyte feret diameter histograms, where month sampled and the number individual are indicated (e.g. Sep 01 = Individual number 1 sampled in September), and n = number of eggs counted (1997-2001).

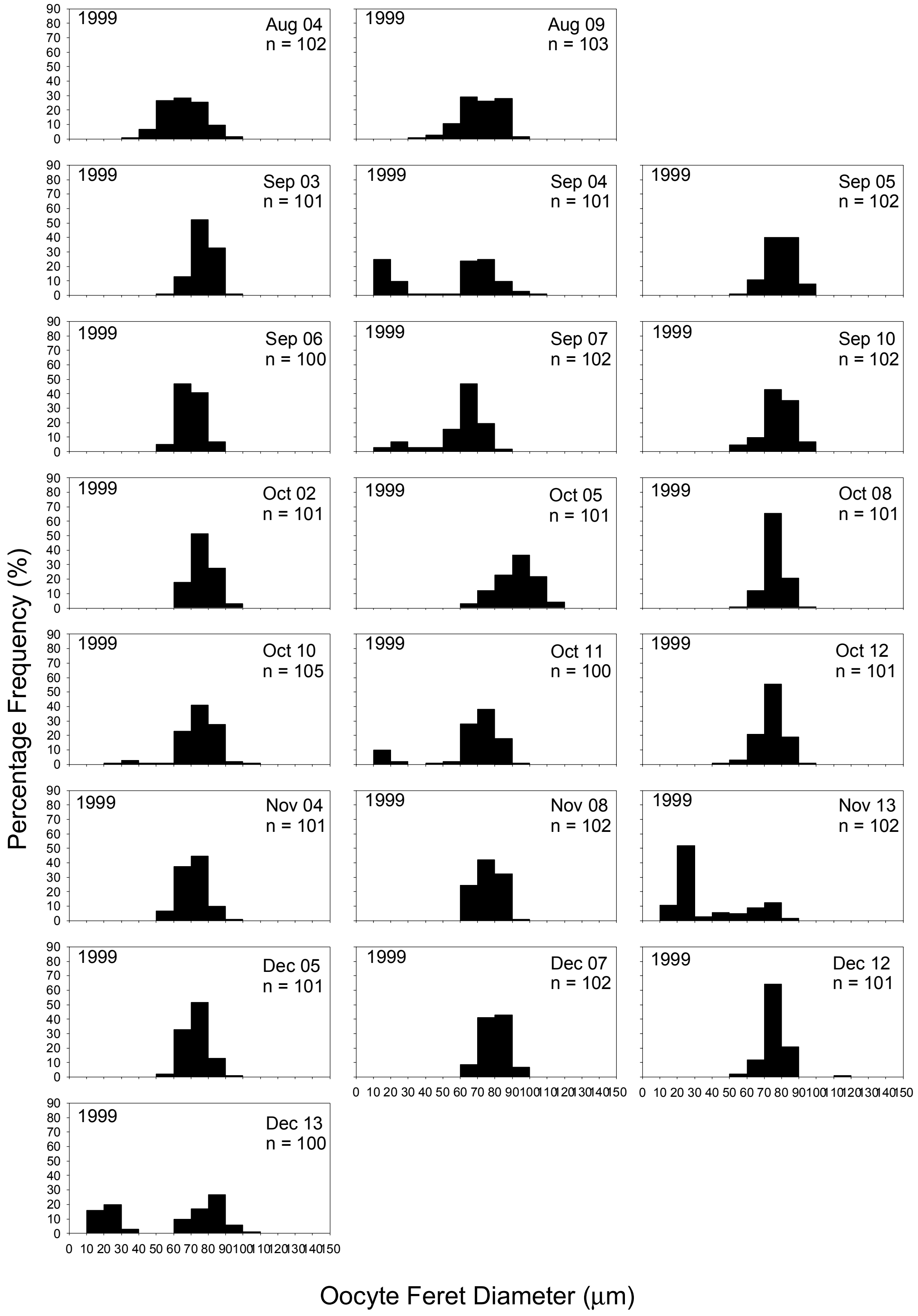


Fig. 3 contd *Parborlasia corrugatus*. Individual oocyte feret diameter histograms, where month sampled and the number individual are indicated (e.g. Sep 01 = Individual number 1 sampled in September), and n = number of eggs counted (1997-2001).

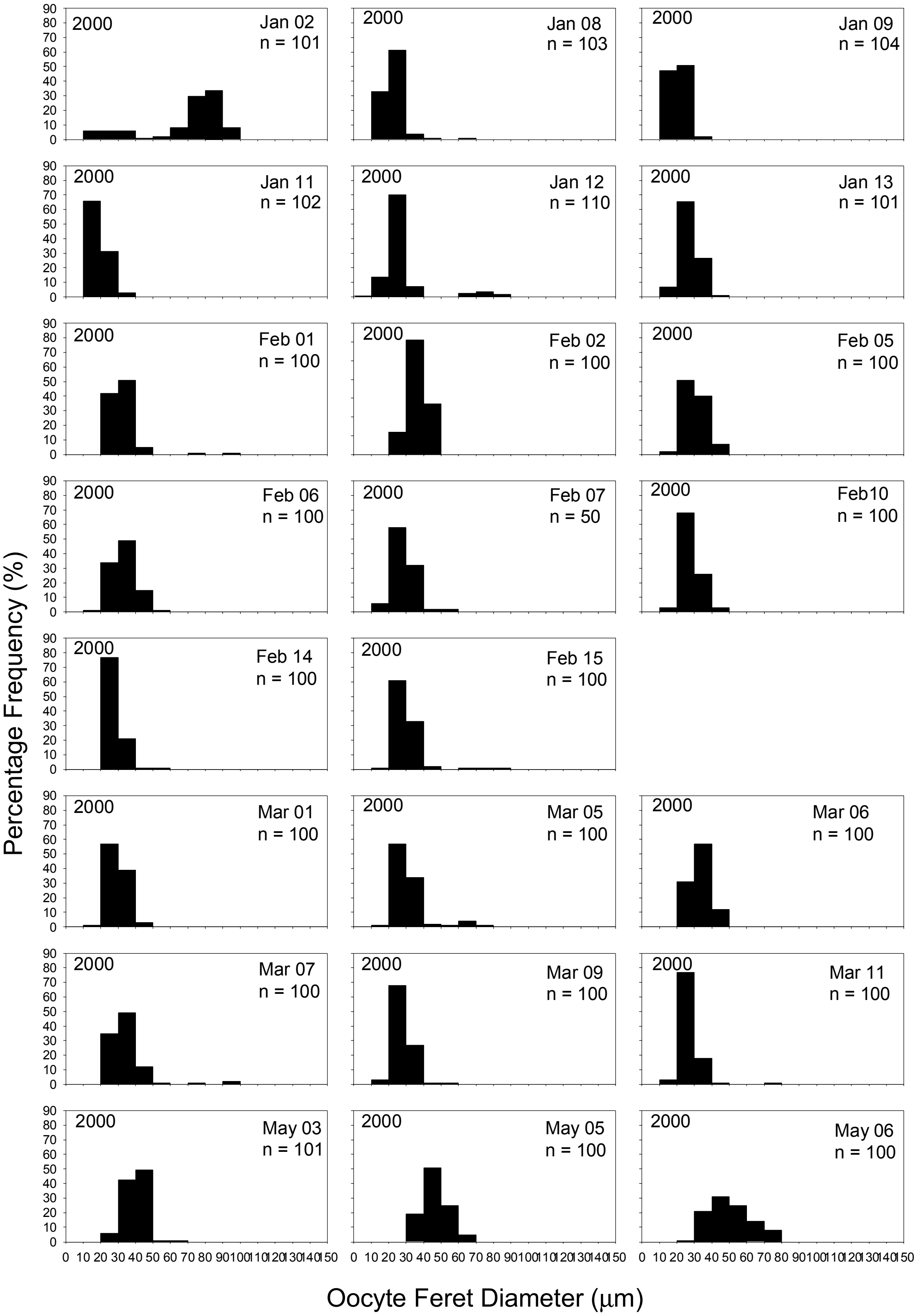


Fig. 3 contd *Parborlasia corrugatus*. Individual oocyte feret diameter histograms, where month sampled and the number individual are indicated (e.g. Sep 01 = Individual number 1 sampled in September), and n = number of eggs counted (1997-2001).

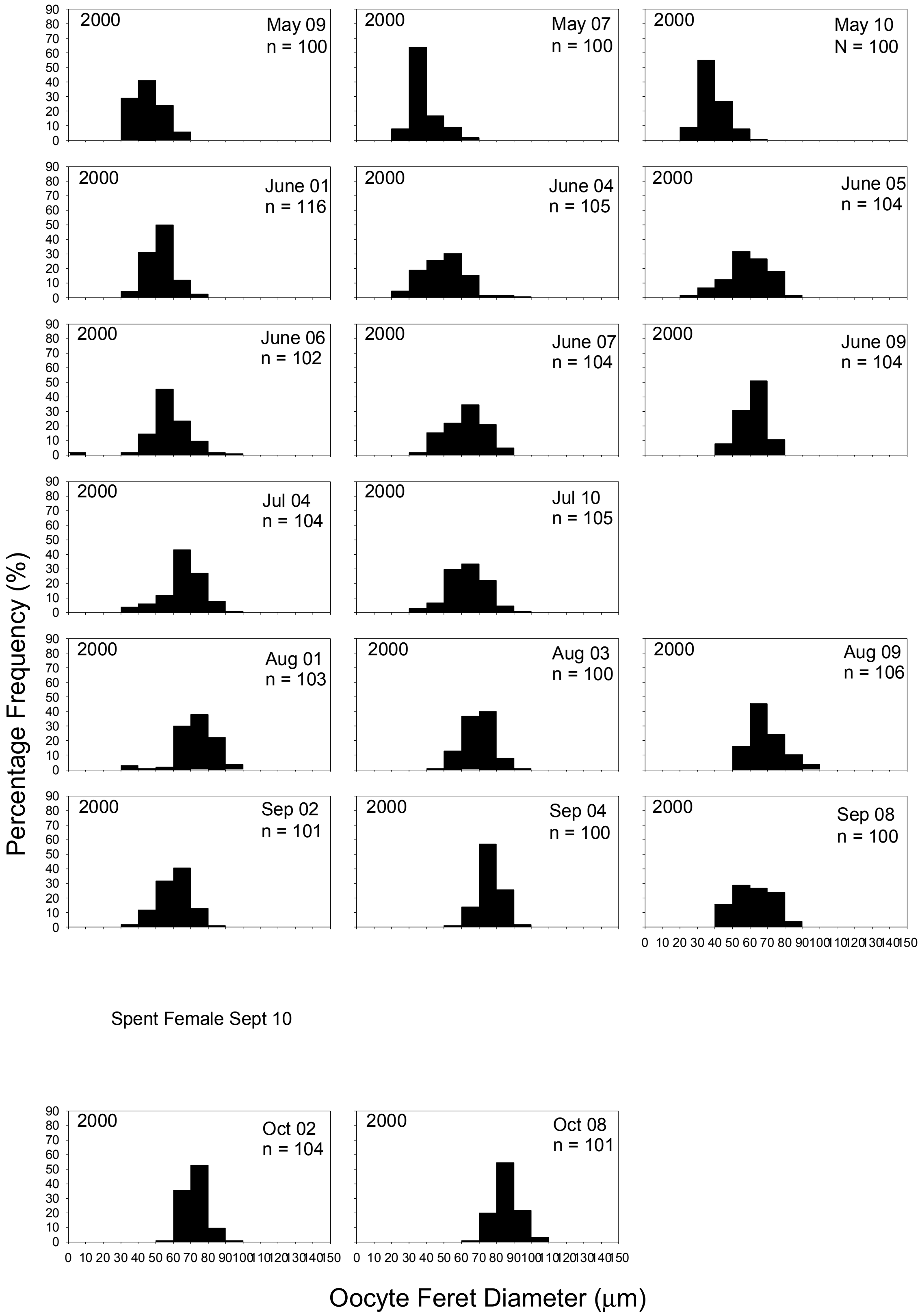


Fig. 3 contd *Parborlasia corrugatus*. Individual oocyte feret diameter histograms, where month sampled and the number individual are indicated (e.g. Sep 01 = Individual number 1 sampled in September), and n = number of eggs counted (1997-2001).

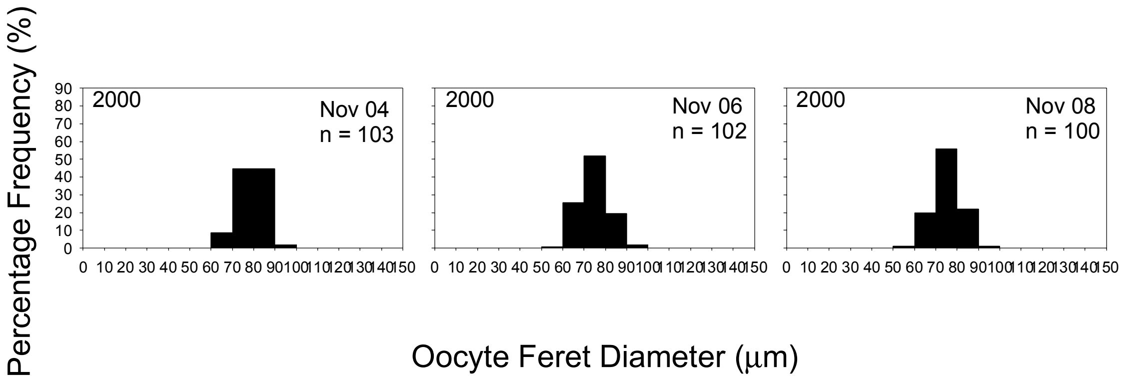


Fig. 3 contd *Parborlasia corrugatus*. Individual oocyte feret diameter histograms, where month sampled and the number individual are indicated (e.g. Sep 01 = Individual number 1 sampled in September), and n = number of eggs counted (1997-2001).

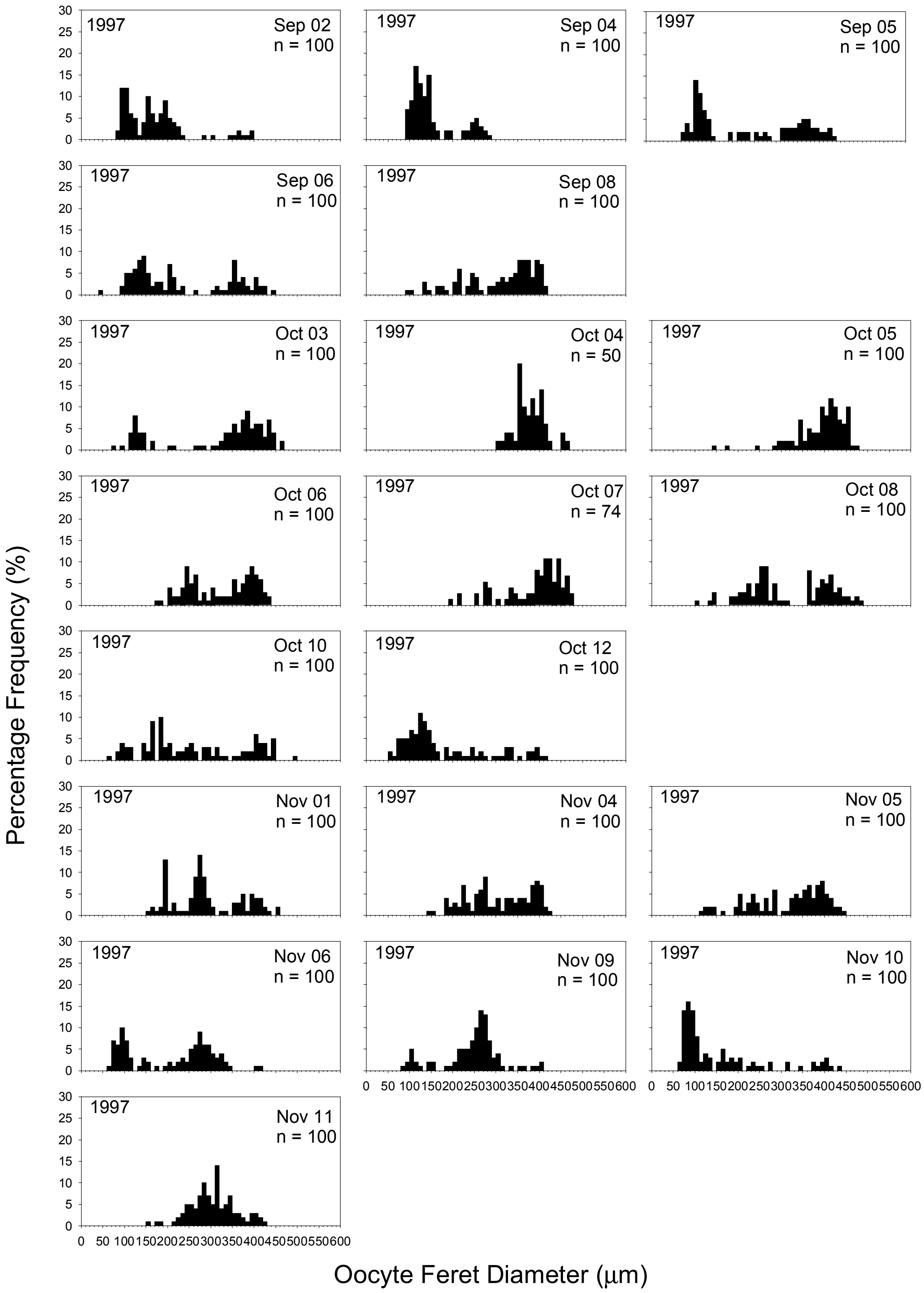


Fig. 4 *Heterocucumis steineni*. Individual oocyte feret diameter histograms, where month sampled and the number individual are indicated (e.g. Sep 01 = Individual number 1 sampled in September), and n = number of eggs counted (1997-2001).

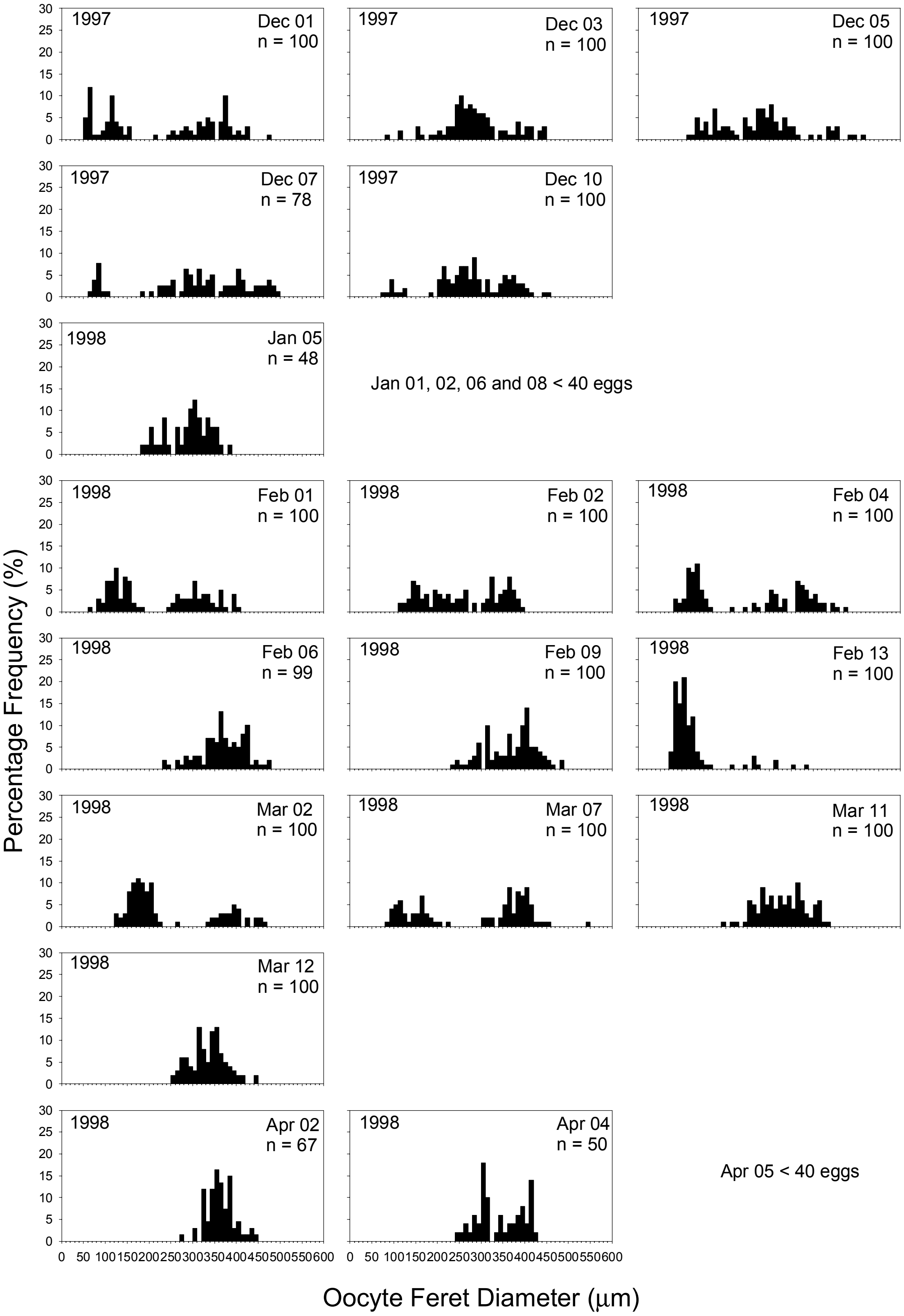
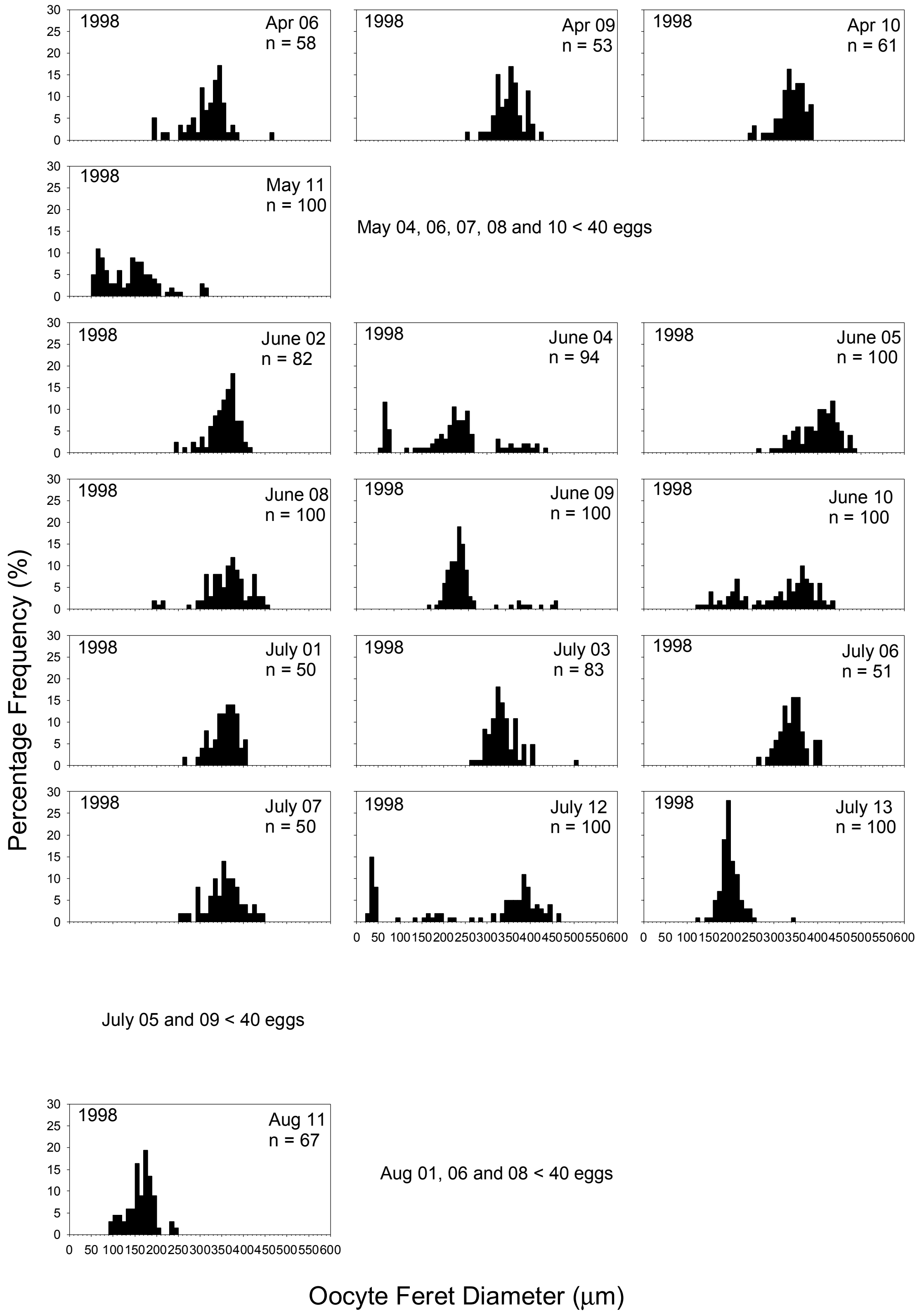


Fig. 4 contd *Heterocucumis steineni*. Individual oocyte feret diameter histograms, where month sampled and the number individual are indicated (e.g. Sep 01 = Individual number 1 sampled in September), and n = number of eggs counted (1997-2001).

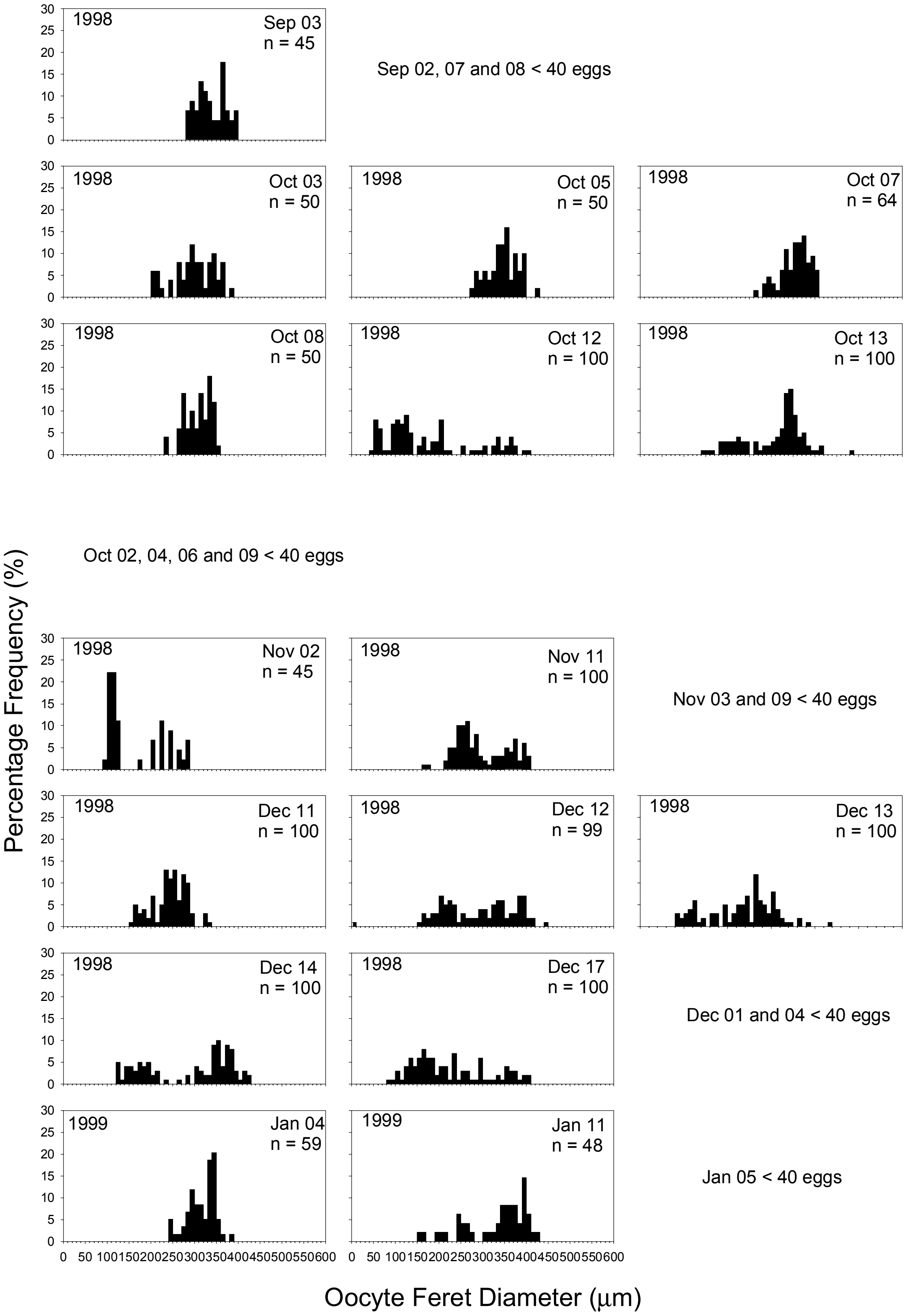


May 04, 06, 07, 08 and 10 < 40 eggs

July 05 and 09 < 40 eggs

Aug 01, 06 and 08 < 40 eggs

Fig. 4 contd *Heterocucumis steineni*. Individual oocyte feret diameter histograms, where month sampled and the number individual are indicated (e.g. Sep 01 = Individual number 1 sampled in September), and n = number of eggs counted (1997-2001)



Sep 02, 07 and 08 < 40 eggs

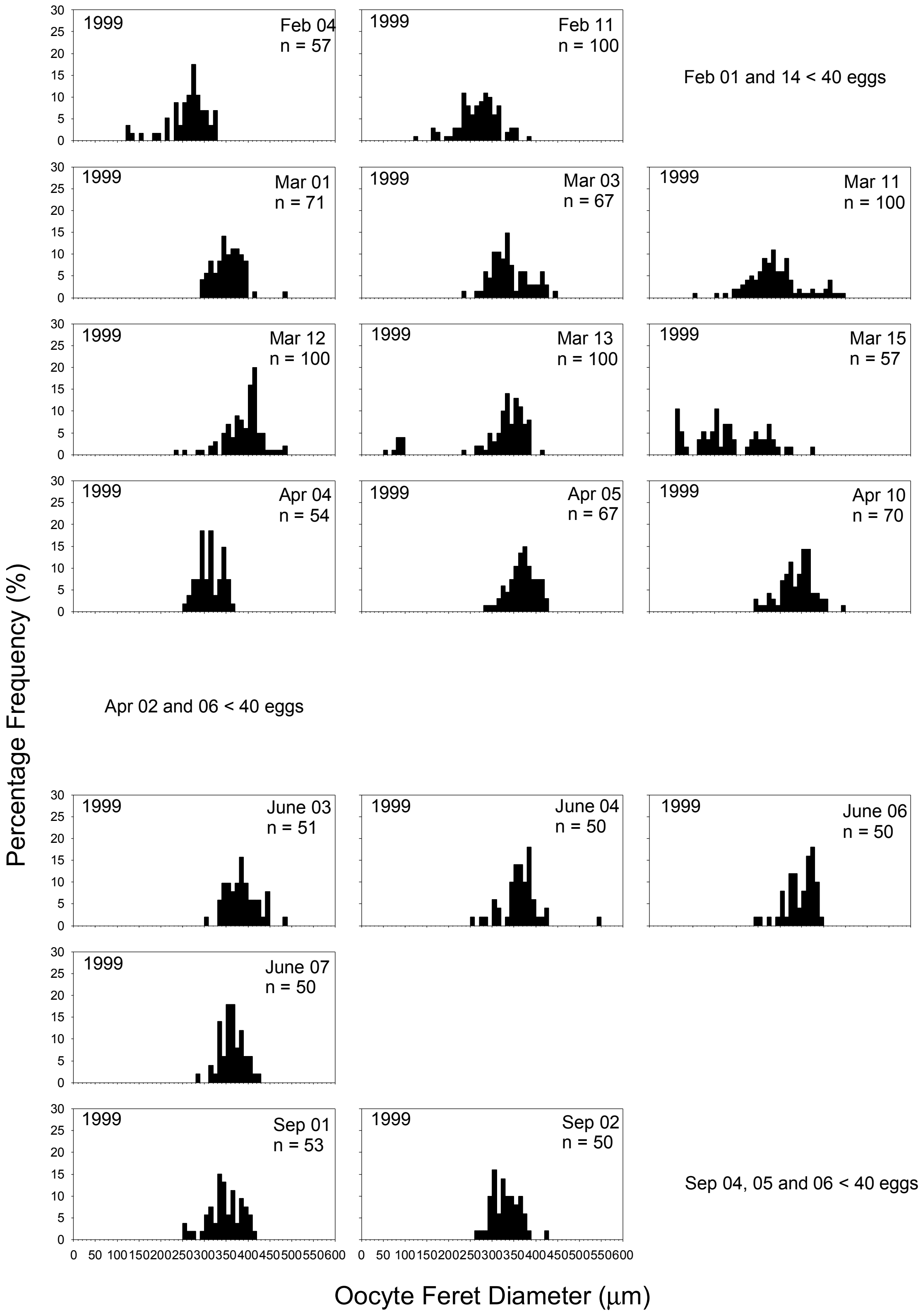
Oct 02, 04, 06 and 09 < 40 eggs

Nov 03 and 09 < 40 eggs

Dec 01 and 04 < 40 eggs

Jan 05 < 40 eggs

Fig. 4 contd *Heterocucumis steineni*. Individual oocyte feret diameter histograms, where month sampled and the number individual are indicated (e.g. Sep 01 = Individual number 1 sampled in September), and n = number of eggs counted (1997-2001).



Feb 01 and 14 < 40 eggs

Apr 02 and 06 < 40 eggs

Sep 04, 05 and 06 < 40 eggs

Fig. 4 contd *Heterocucumis steineni.* Individual oocyte feret diameter histograms, where month sampled and the number individual are indicated (e.g. Sep 01 = Individual number 1 sampled in September), and n = number of eggs counted (1997-2001).

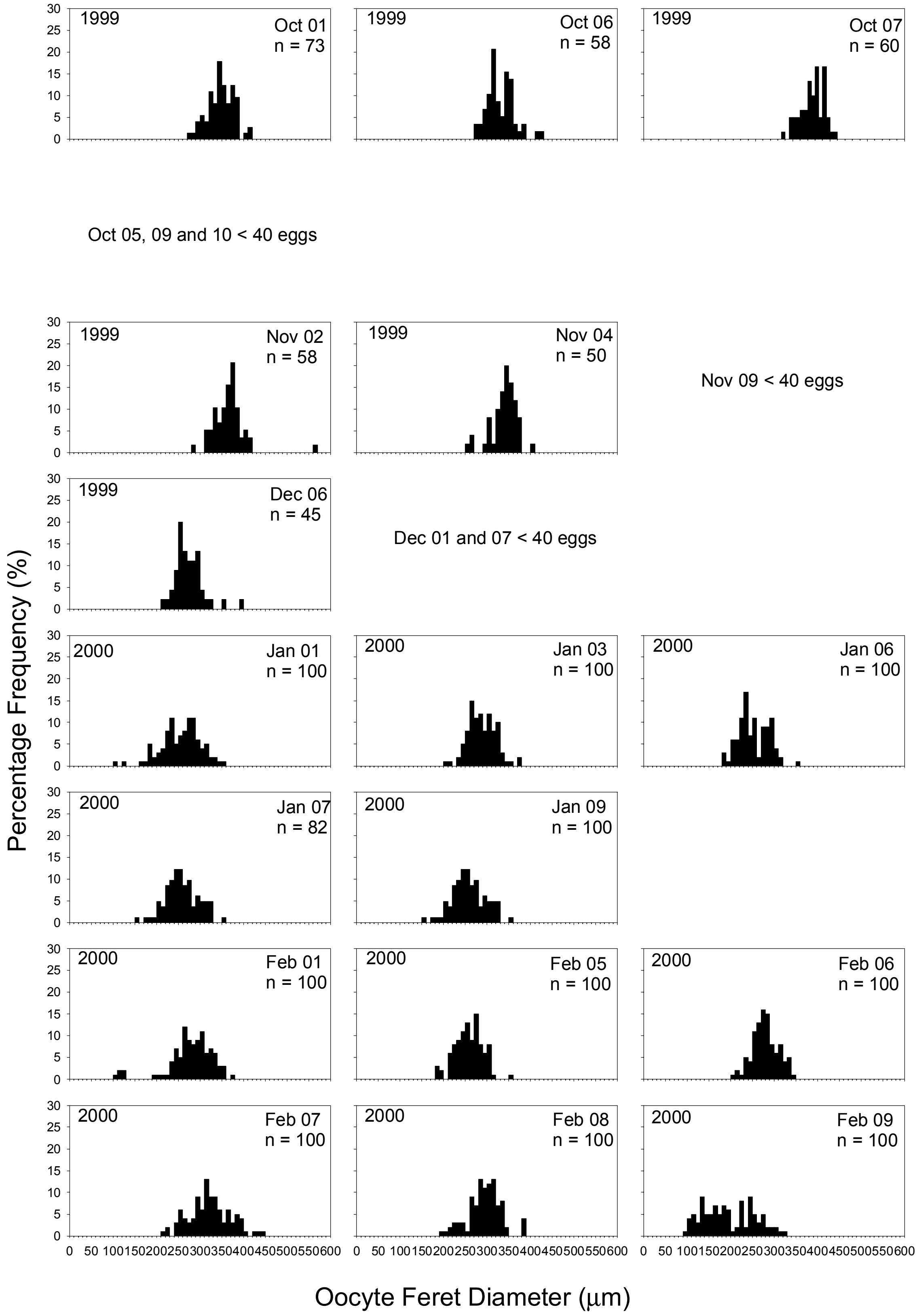


Fig. 4 contd *Heterocucumis steineni*. Individual oocyte feret diameter histograms, where month sampled and the number individual are indicated (e.g. Sep 01 = Individual number 1 sampled in September), and n = number of eggs counted (1997-2001).

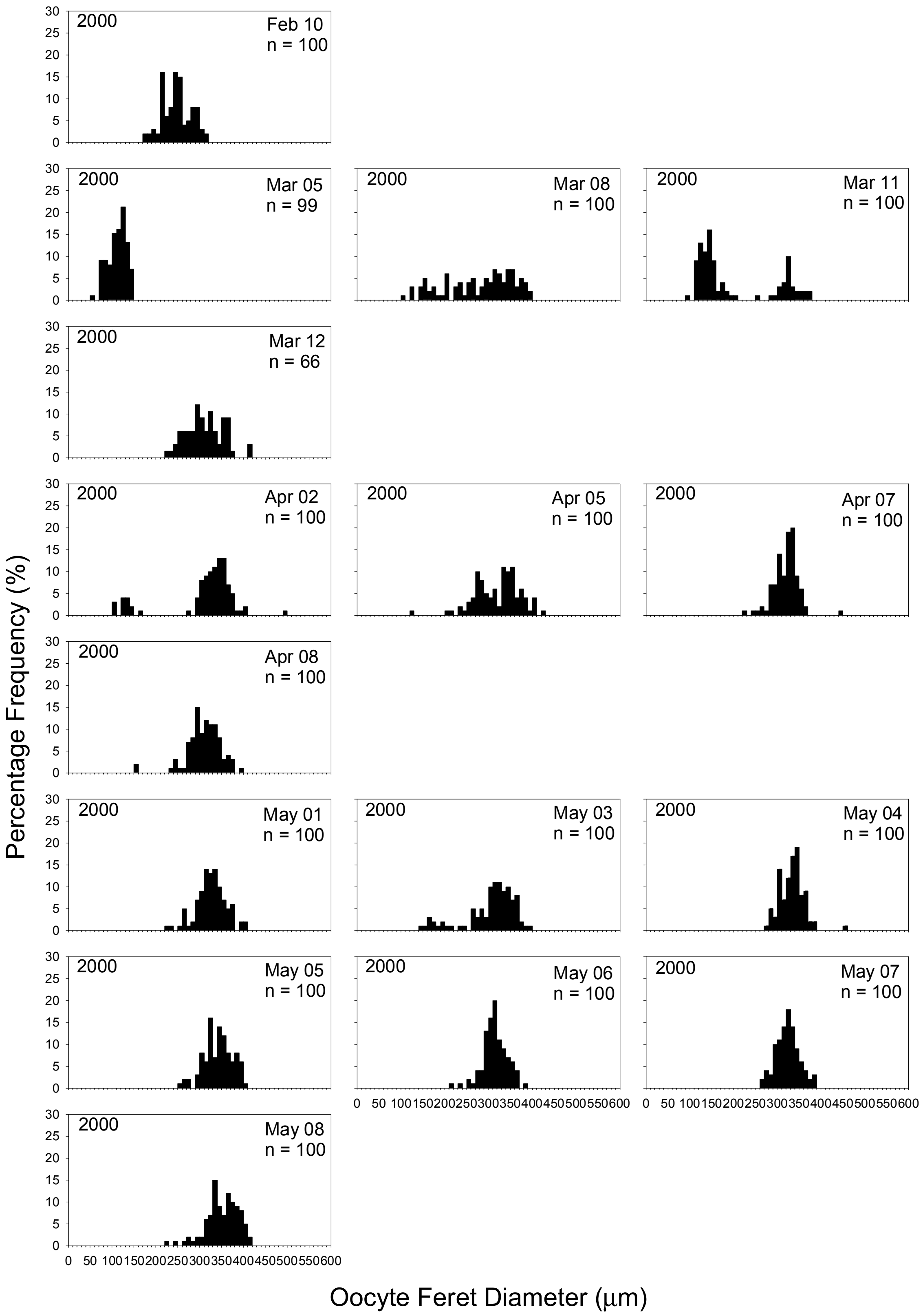


Fig. 4 contd *Heterocucumis steineni*. Individual oocyte feret diameter histograms, where month sampled and the number individual are indicated (e.g. Sep 01 = Individual number 1 sampled in September), and n = number of eggs counted (1997-2001).

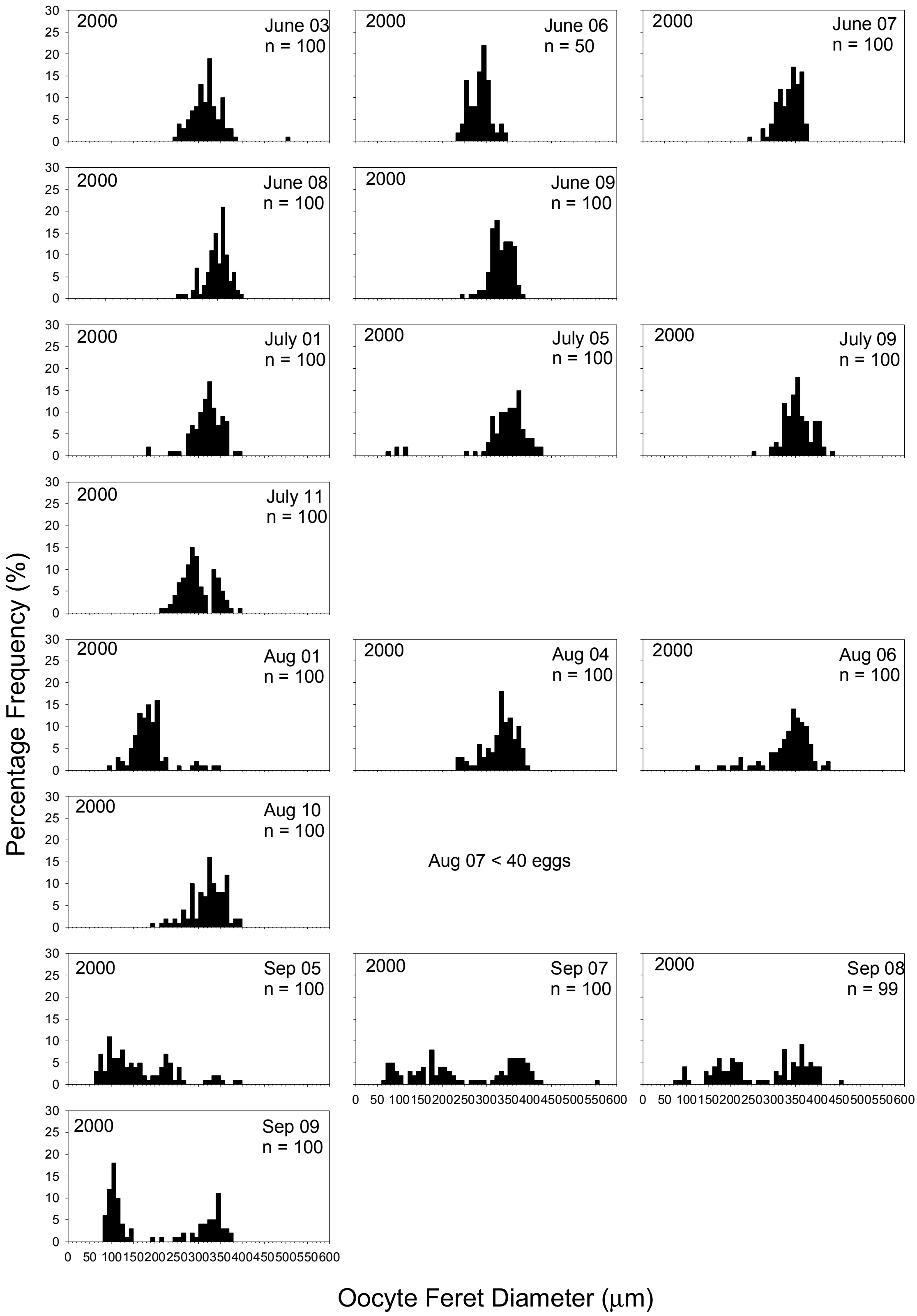


Fig. 4 contd *Heterocucumis steineni.* Individual oocyte feret diameter histograms, where month sampled and the number individual are indicated (e.g. Sep 01 = Individual number 1 sampled in September), and n = number of eggs counted (1997-2001).

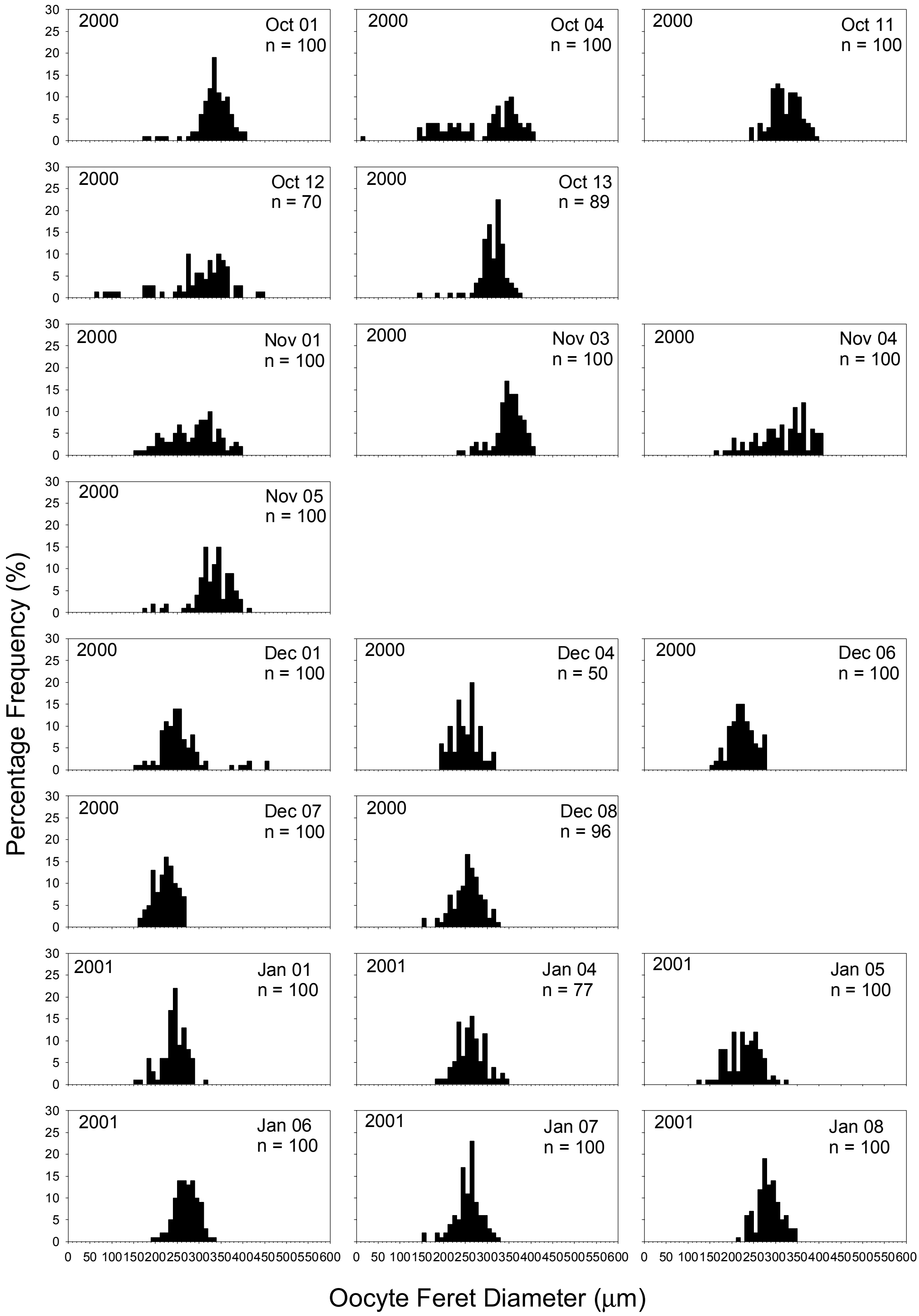


Fig. 4 contd *Heterocucumis steineni*. Individual oocyte feret diameter histograms, where month sampled and the number individual are indicated (e.g. Sep 01 = Individual number 1 sampled in September), and n = number of eggs counted (1997-2001).