UNIVERSITY OF SOUTHAMPTON

Responses Of Benthic Organisms On The Deep Antarctic Continental Shelf To A Highly Seasonal Food Supply

Doctor of Philosophy

By Elizabeth Galley

SCHOOL OF OCEAN AND EARTH SCIENCE

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ABSTRACT

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RESPONSES OF BENTHIC ORGANISMS ON THE DEEP ANTARCTIC CONTINENTAL SHELF TO A HIGHLY SEASONAL FOOD SUPPLY

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Primary production in Antarctic coastal waters and the subsequent flux of biogenic material to the deep continental shelf (600m depth) is intense and highly seasonal. Benthic organisms have been shown to exhibit certain physiological responses to a seasonal food supply. These include reproductive periodicity, feeding rates and nutrient storage. It was hypothesised that organisms living on the Antarctic continental shelf, west of the Antarctic Peninsula, have reproductive and energetic responses coupled to the seasonal pulse of phytodetritus.

Echinoderms formed a conspicuous proportion of the megabenthos on the shelf and from these six species from five different orders were chosen. Specimens were sampled from two locations on the west Antarctic Peninsula shelf during five separate cruises between November 1999 and March 2001. Reproductive strategies were determined by histological analyses of gonad tissue, and elemental (CHN) analyses were used to estimate the nutritional and energetic status of the body tissues.

Highly opportunistic reproductive strategies were observed from two species of holothurian, *Protelpidia murrayi* and *Peniagone* sp., which were closely tied to the phytodetrital pulse. In *Protelpidia murrayi* the initiation of gametogenic cycles were clearly linked to the arrival of phytodetritus, and fecundity in *Peniagone* sp. was also related to the intensity of the pulse. *Sterechinus antarcticus* exhibited a seasonal gametogenic cycle similar to the shallow congener *Sterechinus neumayeri*, although the timing of spawning in *S. antarcticus* was five months later than its shallow water counterpart. The brooding echinoids, *Ctenocidaris perrieri* and *Amphipneustes lorioli*, exhibited a continuous gametogenic pattern; however, *Amphipneustes lorioli* showed a seasonality of recruitment in the brooding pouches. In contrast the asteroid, *Psilaster charcoti*, which shows a seasonal reproductive cycle in shallow-water, showed no such periodicity on the deep Antarctic continental shelf.

Biochemical composition of body components of the six species of echinoderm indicated a significant difference in composition between male and female gonad tissues for all species. Ovaries contained a much higher proportion of lipid compared to the testes. The ovaries of *Protelpidia murrayi* indicated a seasonal variation in composition that correlated to the reproductive cycle; highest values of lipid were observed when the ovaries contained mature vitellogenic oocytes. The variation in composition of the gut lining tissue in several species indicated a role as a nutrient storage organ; the tissue had highest energy values during the period of seasonal phytodetrital flux. The pyloric caeca proved to be a dynamic nutrient storage organ that indicated significant variation both among stations and seasons, and was therefore very sensitive to the quality and quantity of food available.

This thesis is the result of work completed wholly while registered as a postgraduate in the School of Ocean and Earth Science, University of Southampton.

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Chapter 1 – General Introduction

Introduction

The Antarctic Ecosystem

The Antarctic continent is an isolated land mass centred over the South Pole, and is completely surrounded by water. The waters of the Southern Ocean flow unobstructed all the way around the continent driven by winds and as a result of the earth's rotation, form a unique semi-enclosed ecosystem. Mixing with other water masses may therefore only occur at its most northerly boundary, the Antarctic Polar Front. Waters of the Southern Ocean are characterised by extreme seasonal variability in incident solar radiation, near freezing temperatures and by the influence of sea-ice. During the austral summer (December-January) the highest Antarctic latitudes receive 24 hours of sunlight a day, whilst more northerly latitudes such as the Antarctic Peninsula region, may receive up to 21 hours of sunlight a day (Smith *et al.*, 1995). Conversely, during the austral winter (June-August) solar radiation is at a minimum and the Antarctic continent is in permanent darkness, whilst the Peninsula region receives only ~3.2 hours of light per day (Smith *et al.*, 1995).

The Antarctic marine ecosystem is characterised by low temperatures and is one of the most stable thermal environments in the world. Seasonal variations in temperature are very low at locations close to the main Antarctic continent and only have an annual variation of 2.8°C at Rothera research station (Figure 1.1) and 2.2°C further north at Signy Island (Clarke and Leakey, 1996).

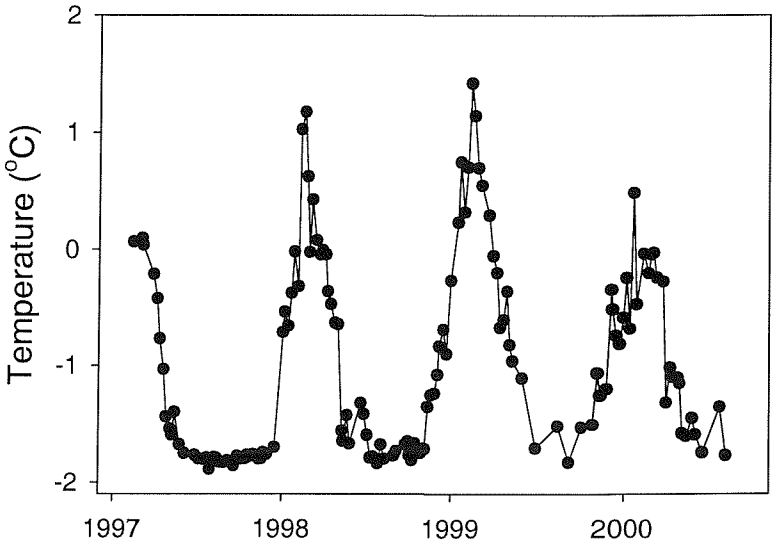

Figure 1.1. Temperature measurements from Rothera Research station (15m depth) from 1997 to 2000, British Antarctic Survey.

Antarctic marine organisms have therefore evolved to live in an environment of thermal stability and partly as a consequence of this many organisms are stenothermal. Such low seasonal variation in temperature is unlikely to drive any seasonal changes in physiological processes (Clarke, 1996). Therefore, any seasonal changes in the biology of an organism may be uncoupled from temperature variability. However, other physiological adaptations may be coupled to temperature. For example, polar marine invertebrates exhibit much lower basal metabolic rates than temperate or tropical species (Clarke, 1991). A reduced metabolic rate may have important consequences for the over-wintering energy requirements of an organism during times when food availability is limited (Clarke and Peck, 1991).

The Antarctic benthic environment is greatly influenced by the presence of ice, including sea-ice, ice-shelves and terrestrial ice caps (Knox, 1994; Gutt, 2001; Clarke, 2003). In shallow waters close to land, ice has a major direct effect on the benthic population by scouring and anchor ice. This has the effect of eradicating the local fauna and as a result there is a distinct zonation of benthic fauna in shallow waters around the Antarctic continent. The effect of ice-shelves and recently calved icebergs also extends in to deeper waters (≤600 m) where in some areas of the continental shelf (the Weddell Sea in particular) impact from tabular icebergs scouring the seabed can be relatively frequent (Gutt, 1996). Ice-berg scour may completely eradicate slow growing organisms (Gutt, 1996), and cause the shallow-water Antarctic benthos never to reach peak maturity. This makes iceberg scour among the five most significant disturbances experienced by any large ecosystem (Gutt, 2001). In addition, icebergs may raft glacial debris to deeper waters (Clarke, 1996), where the drop stones can provide isolated patches of hard substratum in areas otherwise dominated by soft sediments. One of the most prominent effects of ice on the benthic environment is the isostatic depression of the Antarctic continent by the polar ice cap. This continental depression has also resulted in the depression of the continental shelves around Antarctica, these are typically ~600m in depth. In some places the continental shelves may reach 1000 m depth, this may be a result of scouring by ice-shelves as they extended seawards at the previous glacial maxima (Clarke, 2003).

The Antarctic continent is surrounded on all sides by deep oceans; the current regimes and major water masses surrounding the continent exert a considerable impact on the ecology of the marine ecosystem. Two main current systems can be found within the surface waters. Easterly winds close to the continent drive the inner Antarctic Coastal Current anticlockwise around the continent (Knox, 1994). The passage of this current is largely uninterrupted with the exception of the waters to the west of the Peninsula region. The second current system is the Antarctic Circumpolar Current (ACC). This is the dominant of the two currents and is unique in that it is the only current that entirely circles the globe. The lateral extent of this current varies, and its outer limits determine the position of the Polar Front and hence delimit the boundary of the Southern Ocean. The ACC interacts with the coastal current deflecting it northwards around the Peninsula (Hofmann *et al.*, 1996). Evidence suggests that the interaction of these different current systems results in the formation of one or two clockwise gyres on the West Antarctic Peninsula (WAP) shelf (Smith *et al.*, 1995); as a result of these gyres a southward water flow may occur on the inner shelf. Such a circulation pattern would have implications for the larval dispersal and gene flow of free spawning organisms found at different locations on the continental shelf.

Excluding the surface waters, three main water masses dominate the deep Southern ocean. These are the Sub-Antarctic Intermediate Water (SAIW) (~1000m depth); Antarctic Bottom Water (AABW) (near the bottom) and the Circumpolar Deep-Water (CDW) which flows between the other two bodies of water (Knox, 1994) (Figure 1.2). Antarctic surface waters originate close to the Antarctic continent and flow north until they converge with the Sub-Antarctic Surface Water. Here Antarctic surface water sinks, owing to its greater density, and mixes with the SAIW. The second main water mass is formed by the repeated formation of sea-ice in surface waters causing the salinity of the surrounding waters to increase. The subsequent increase in water density causes the waters close to the continent to sink. Sinking water then spreads northwards along the sea floor forming the heaviest of the ocean water masses, the AABW. In areas such as the Weddell Sea, large volumes of Weddell Sea Bottom Water originate from Ice Shelf Water formed under floating ice shelves, which flows down the continental slope forming Weddell Sea Bottom Water (Knox, 1994, Carmack, 1990).

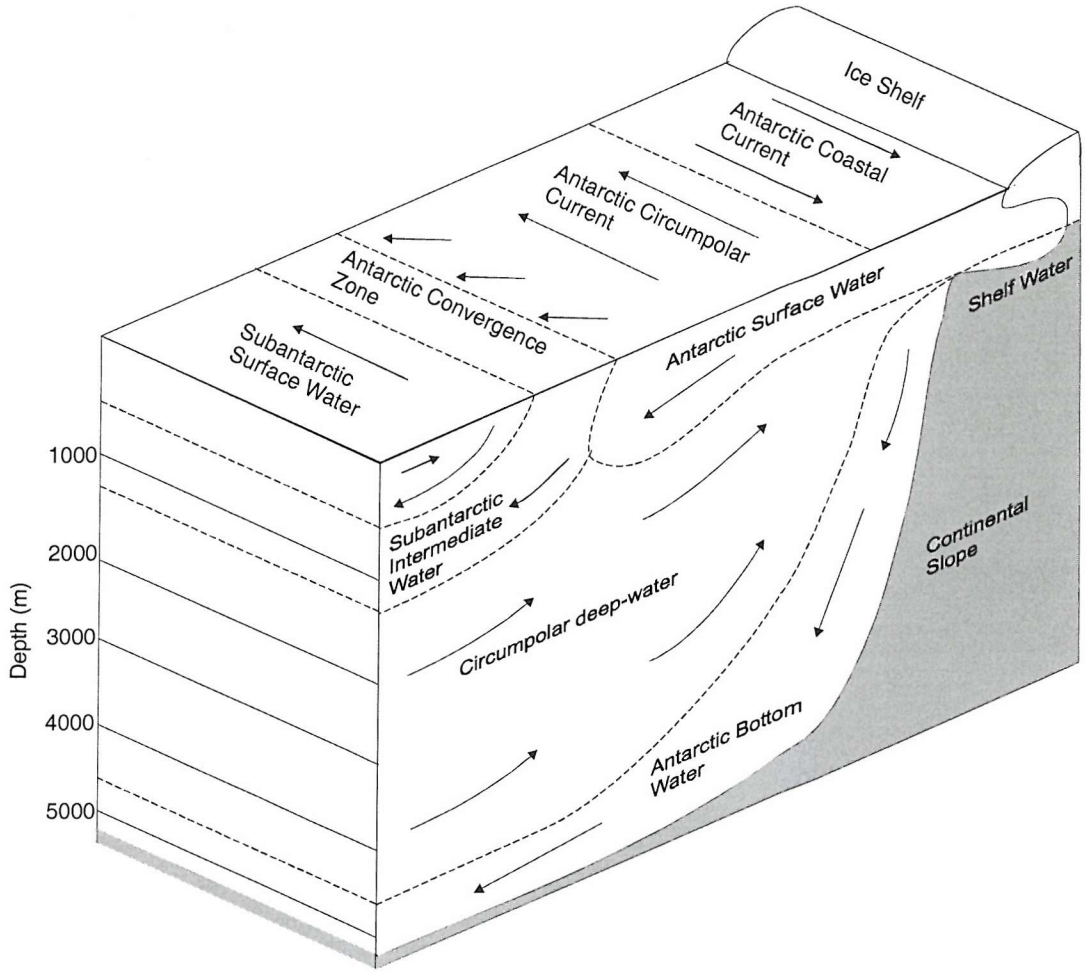

Figure 1.2. Schematic diagram of the meridional and zonal flow and water masses of the Southern Ocean. Redrawn from Knox, 1994.

The dominant water mass in the Southern Ocean is the CDW, which lies below the Antarctic surface waters (Figure 1.2). A southwards and upward flow of CDW near the continent occurs in response to the northward and downward flow of Antarctic surface waters. CDW is the most prominent water mass west of the Antarctic Peninsula. Whilst this water mass is generally found at depths of 1000m, CDW rises in response to the equator-ward Ekman transport of surface waters and may be found at depths of 400-700m on the shelf region (Hofmann *et al.*, 1996). Smith *et al.* (1995) reported that CDW floods the continental shelf throughout the year and characterises the deep-water in the Palmer LTER study area (West of the Antarctic Peninsula) (temperature >0.5°C, salinity 34.75). Values of temperature and salinity recorded during the 'FOODBANCS' programme (temperature ~1.1°C, salinity ~34.6, Smith and DeMaster unpublished data) are in a similar range to those of the Palmer LTER study, indicating the presence of CDW on the continental shelf throughout the

duration of this study. The occurrence of this relatively warm, salty water mass on the Antarctic continental shelf has biological implications for the shelf benthos. In particular, when compared to the surface waters over the WAP continental shelf where temperatures ranged from –1.9 to 0.6°C and salinity ranged from ~33 to 33.9 (Figure 1.3) (Smith and DeMaster unpublished data). This indicates that two separate water masses are responsible for the physical conditions recorded on the shelf and in shallower waters (Figure 1.3). It is therefore entirely possible that species whose bathymetric distributions encompass both of these environments may show specific adaptations depending on their location. Adaptations may include the need to be eurythermal considering that the CDW only floods the shelf environment periodically, and perhaps some deep-sea adaptations such as an increased pressure tolerance given the maximum depths reached on the continental shelf.

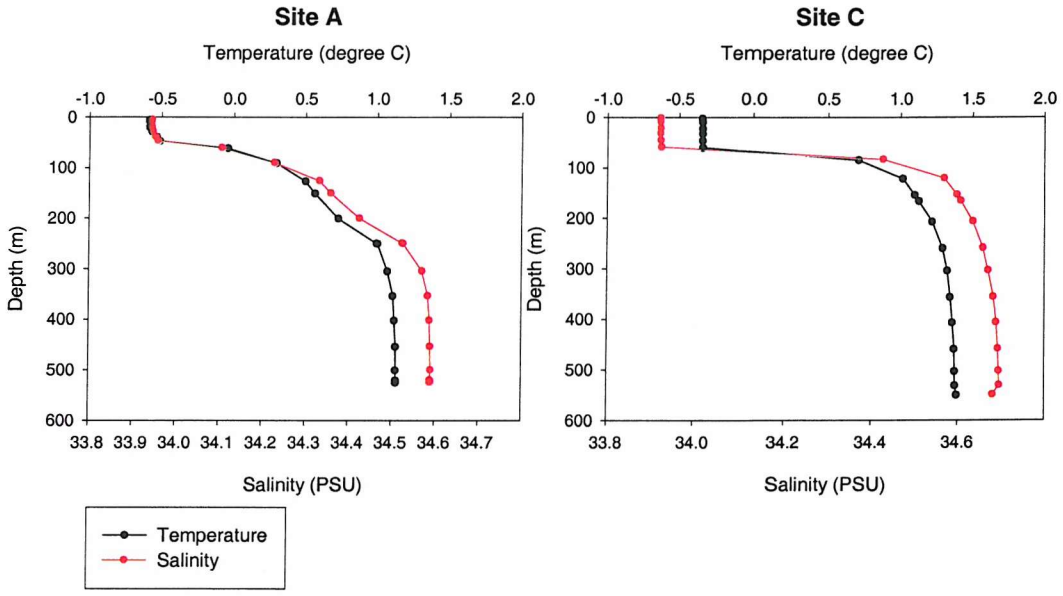

Figure 1.3. Temperature and salinity plot against depth for Sites A and C during June 2000 (Smith and DeMaster unpublished data).

The WAP is one of the most well studied areas of the Antarctic marine ecosystem. The presence of many research stations such as Rothera Station (BAS), Palmer Station (USA), Vernadsky (Ukraine), Captain Arturo Prat (Chile) and the San Martin Base (Argentina) (Figure 1.4) means that research may take place through the year at least within the vicinity of these stations and that longer-term research projects may

be carried out. In addition, the Peninsula environment has also been the major focus of several ship-based research programmes including: Research on Antarctic Coastal Ecosystem Rates (RACER) and the Palmer LTER programme, both of which focussed on water column biology and physical properties in the vicinity of Palmer station west of the Peninsula. In particular the Palmer LTER project provides extensive background data for the area where the FOODBANCS programme is based.

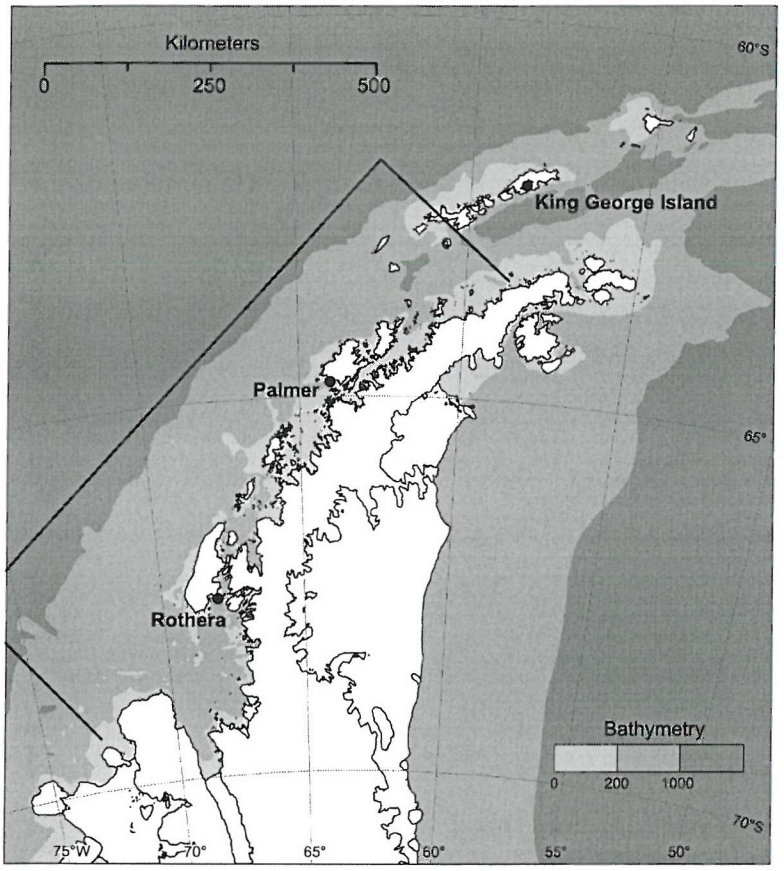

Figure 1.4. Map of the locations of Antarctic research stations supported by various countries. Boxed area indicates the Palmer LTER study area.

The Palmer LTER programme is focussed on ecological processes which link the extent of annual pack ice to the biological dynamics of different trophic levels within the Antarctic pelagic marine community. Research at Palmer Station and in the surrounding near-shore marine environment concentrates on seabirds, the prey of sea birds, primary production and bio-optical and hydrographic characteristics of the water column. Processes such as reproduction and recruitment, and parameters such as food availability that are sensitive to environmental change are monitored. Long

term monitoring of this ecosystem led the Palmer LTER group to devise a conceptual model of annual time lines of key physical and biological components of the water column. Extreme seasonality and the relatively large inter-annual variability (both in magnitude and timing) of physical forcing in the Antarctic marine ecosystem may be compared and contrasted with conditions for biological growth, development and survival of key species from each trophic level providing a conceptual model for the discussion of trophic linkages (Figure 1.5)

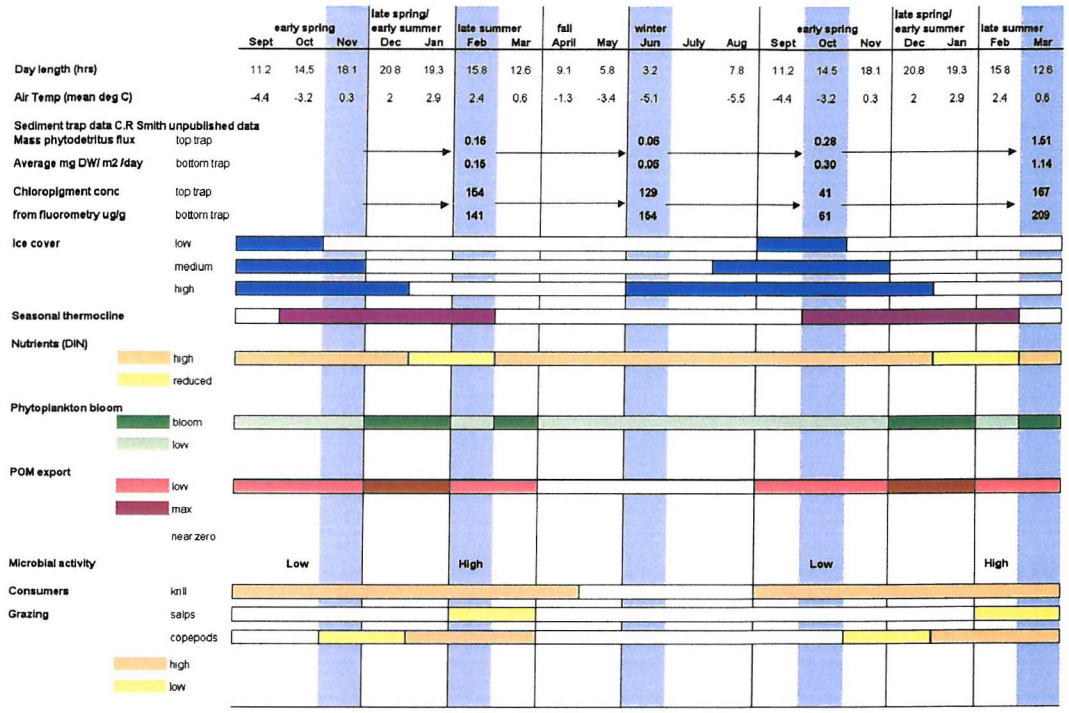

Figure 1.5. Annual time-lines of key physical and biological components in the vicinity of Palmer station (within 100km) adapted from Smith *et al.* (1995). Vertical blue zones indicate timing of FOODBANCS cruises. Sediment trap data from FOODBANCS cruises, Smith and Mincks, unpublished data.

Seasonality in Antarctica

The advance and retreat of annual sea-ice around Antarctica is one of the most dynamic features of the global ocean. During the austral spring months a stable layer of low salinity water is produced at the surface by the melting pack ice. The increase in water column stability and subsequent formation of the thermocline, coupled with an increase in solar radiation produces a stable environment favourable for phytoplankton growth (Figure 1.5). The upwelling CDW is warmer than Antarctic surface waters and therefore carries water with high temperatures, low oxygen and

high nutrients towards the surface (Knox, 1994). The nutrient-rich upwelling waters support the growth and maintenance of large quantities of phytoplankton. Nevertheless, whilst the supply of macronutrients (N, P, Si) to the surface waters exceeds that of the demand made by phytoplankton, the SOIREE (Southern Ocean Iron Release Experiment) programme showed that low levels of dissolved iron in Southern Ocean waters limit phytoplankton growth (Boyd and Law, 2001; Frew *et al.*, 2001). There is however, a marked contrast in productivity between the austral summer and austral winter (Clarke, 1988). Exceptionally high productivity occurs during the perpetually light summer, and very low levels occur during the ice-bound winter months when it is dark almost 24 hours a day (Figure 1.6).

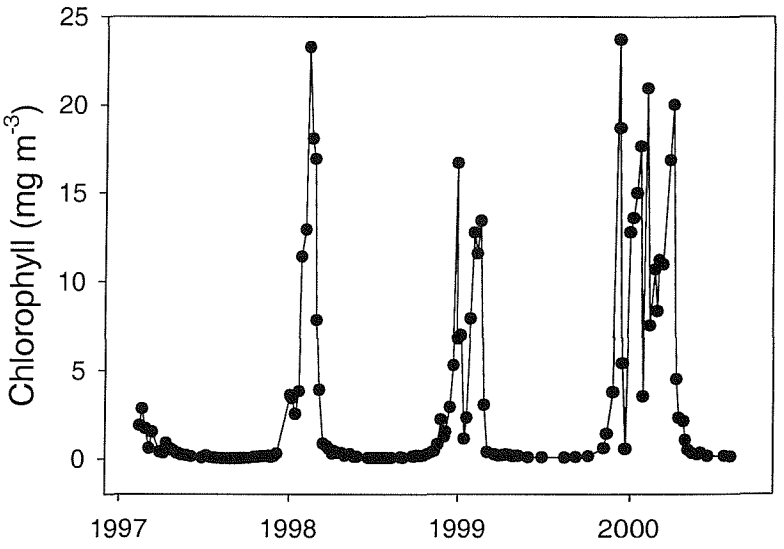

Figure 1.6. Chlorophyll-a measurements from Rothera Research station, British Antarctic Survey.

In addition to the seasonal variation of primary production, there is a distinct latitudinal variation south of the Polar Front. Regions north of the continental shelf break have been found to have low phytoplankton biomass, while some stations in continental shelf waters have a much higher phytoplankton biomass, exceeding 700 mg Chl *a* m^{-2} at some stations (Holm-Hansen and Mitchell, 1991). The greatest phytoplankton blooms were found to occur in the southwestern Weddell Sea, and elevated levels of production have also been recorded west of the Antarctic Peninsula, near Kerguelen and Heard Islands and in the southern Ross Sea (Knox, 1994). Phytoplankton in the Palmer LTER study area starts to accumulate towards the end of November in a year of average ice cover (Smith *et al.*, 1995) (Figure 1.5). These phytoplankton blooms are mostly dominated by diatoms, as are the majority of

phytoplankton blooms in the Southern Ocean. Coccolithophoroids, a common component of temperate water blooms, are absent from Antarctic waters as a result of the cold temperatures.

Bentho-Pelagic Coupling

Large fluxes of bloom material have been observed in sediment trap samples at depths of 150m in the Palmer LTER area (Karl *et al.*, 1996), indicating a large export of seasonal bloom material to the seafloor of the continental shelf. Sediment trap data from the FOODBANCS programme has also provided evidence for intense pulses of particulate organic carbon (POC) flux (Figure 1.5) over the duration of the sampling programme (Nov 1999 to March 2001). Such intense bursts of phytoplankton production and subsequent particle flux would be expected to facilitate the transport of much of the bloom material to the continental shelf sediments. The arrival of particulate organic material to the benthic shelf environment is likely to have a major role in the life histories of deposit and suspension feeding organisms, as well as structuring the benthic community (Grebmeier *et al.*, 1988). Studies carried out in other areas of the world's oceans provide evidence confirming this benthopelagic coupling. In deep-sea environments there has been an increasing interest in the coupling of seasonal variation in the vertical flux of organic material, and the responses of benthic invertebrates to this flux (Billett *et al.*, 1983; Tyler, 1988; Gooday, 1990; Young *et al.*, 1992; Campos-Creasey *et al.*, 1994). It has been demonstrated that some deep-sea benthic invertebrates (mainly deposit feeders) may respond to a seasonal supply of organic material by having a seasonal reproductive periodicity (Tyler, 1986). In all cases of echinoderm species that exhibit a seasonal reproductive cycle the maximum egg size is indicative of planktotrophic development. In the northeast Atlantic the main input of organic material is in late May to August (Lampitt, 1985) which is soon followed by the main period of vitellogenesis (the reproductive process requiring the most energy) in most species from June to November (Tyler *et al.*, 1982; Tyler 1986, 1988). Therefore the presence of bloom material may synchronise reproduction either at the onset of vitellogenesis or by timing larval release to coincide with the POC flux so that feeding larvae may benefit from bloom material (Eckelbarger and Watling, 1995).

Some invertebrates respond to the seasonal flux of phytodetritus by showing a variation in growth rates. The test of the deep-sea regular echinoid *Echinus affinis* has skeletal banding that is thought to result from seasonally varying growth patterns. These growth patterns have been linked to the annually pulsed fallout of phytodetrital food to the deep-sea floor (Gage and Tyler, 1986; Campos-Creasey *et al.*, 1994). Further evidence of seasonal growth was demonstrated by Lampitt (1990) from direct measurements of growth rates of a deep-sea barnacle. Growth was found to be greatly enhanced by the annual deposition of phytodetrital material. In addition to individual growth rates, Gooday (1988) found that some species of deep-sea benthic foraminifera respond to the seasonal flux of phytodetritus in the northeast Atlantic by a subsequent rapid population growth. Similar responses have also been reported from the shelf of the northern Bering and Chukchi Seas, where the quality and quantity of organic carbon deposited to the benthos directly influenced the structure of benthic assemblages (Grebmeier *et al.*, 1988). In these studies a direct comparison of faunal abundance, biomass and sediment oxygen uptake was made between the highly productive (~250 to 300 g C m^{-2} yr^{-1}) Bering Shelf-Anadyr Water (BSAW) and the less productive (~50 g C m^{-2} yr^{-1}) Alaska Coastal Water (ACW). Mean benthic biomass showed a positive correlation with increased surface productivity, the highest benthic biomass values being found under the highly productive BSAW (Grebmeier *et al.*, 1988, Grebmeier and McRoy 1989).

In the shallow water Antarctic environment many studies have been carried out to investigate the physiological responses of benthic invertebrates to a seasonally driven food supply. Seasonal changes in feeding rates have been observed in several species, for example *Sterechinus neumayeri*, which was found to feed most actively during the austral summer months and cease feeding during the winter when sediment chlorophyll concentrations were generally <20mg.m^{-2} (Brockington *et al.*, 2001). Studies of shallow-water suspension feeders found that whilst periods of starvation were much shorter than previously thought, all species showed a marked seasonality of feeding (with one exception) and that feeding stopped for a period of up to 2-3 months during the austral winter (Barnes and Clarke, 1994, 1995). In this instance winter starvation was not synchronized between all species, however changes in feeding activity of most taxa occurred during winter sea-ice and minimal

temperatures, when the water column was undisturbed with chlorophyll levels and particle flux at their lowest.

Investigations into reproductive periodicities of Antarctic benthic invertebrates have drawn particular interest, and in many cases provided evidence of a seasonal spawning period. In some cases seasonality of reproduction is coupled with the onset of pelagic production, as is the case for the filter-feeding bivalve *Lissarca miliaris* (Clarke, 1988); whilst in other species no connection has been made. Ten of the fourteen species of asteroid occurring in McMurdo Sound were found to display discrete seasonal spawning periods (Bosch and Pearse, 1990). However, the correspondence between spawning times and period of mid-summer phytoplankton production is not particularly close, and there is evidence that bacteria are the main source of food for larvae (Rivkin *et al.*, 1986). Several authors have provided evidence of seasonal growth curves in various taxa of benthic invertebrates as summarised by Clarke (1988). In more recent studies, Brey *et al.* (1995) demonstrated that two growth bands were formed each year in *Sterechinus neumayeri.* They are comprised of both translucent and opaque bands, translucent bands being formed during periods of slow growth and opaque bands during periods of rapid growth (Brey *et al.*, 1995), indicating therefore, that *Sterechinus neumayeri* grows slowly during the austral winter and more rapidly during the austral summer.

The FOODBANCS Programme

While the Southern Ocean maintains a low but stable temperature it boasts the world's most intense seasonal production cycle. Karl (1996) summarised sediment trap data for the WAP shelf, which indicates that this extreme seasonality in primary production produces dramatic oscillations of particulate organic carbon export from the euphotic zone. The seasonal phytoplankton bloom near the Antarctic continent has prompted studies to examine the extent of the coupling of benthic production to the production of the overlying water column (summarised in Clarke, 1988; Grebmeier, 1992). Knox (1994) found that the labile organic matter on the sea floor is more persistent and concentrated than the phytoplankton biomass present in the water column. The main phytoplankton bloom on the WAP has been found to occur between December and January when particulate organic matter export from the photic zone is also at its maximum (Smith *et al.*, 1995). Smith and DeMaster

proposed that phytodetritus and/or faecal pellets accumulating on the sea floor might persist for longer periods and provide a more predictable signal relative to the sinking phytodetritus in the water column. Phytodetrital deposits on the continental shelf probably persist longer than those in the water column if only for the simple reason that it cannot sink any further. This extended persistence of organic material may in part explain why some benthic macrofauna do not appear to store lipid in their body tissues (Clarke, 1985; Clarke and Peck 1991). However, a lack of available food in the water column may affect the timing of reproduction, as animals may synchronise their spawning to coincide with periods of high productivity, possibly in an attempt to ensure that there is sufficient food for developing larvae.

The FOODBANCS (FOOD for Benthos on the ANtarctic Continental Shelf) sampling programme was designed to study a wide variety of biological and chemical aspects of the benthic environment on the Antarctic continental shelf in the vicinity of the Palmer LTER study area. The main objective was to evaluate the accumulation, fate and impact on the benthic community of the phytodetrital flux following the spring/summer phytoplankton bloom. In order to achieve this objective samples were collected at regular intervals, using a seasonal series of five cruises over two successive years. The timing of this programme was designed to sample pre-bloom (November 1999), the end of the particulate organic carbon (POC) pulse (March 2000), the end of the ice-free austral summer period (June 2000), the end of the winter ice period, pre-spring bloom (October 2000), and the end of the second POC pulse (February 2001) (Figure 1.5). Thus the scheme covered one full seasonal cycle, and repeated the post-bloom period in a second successive year. These short but intensive sampling periods took place along a transect of three sites A, B and C, (Figure 1.7) crossing the Antarctic shelf close to Palmer Station on Anvers Island. The sampling programme was designed to encompass a wide range of protocols intended to investigate all aspects of the benthic ecosystem. The overall sampling protocol for the FOODBANCS project will provide a comprehensive set of background data for this study. Of particular relevance to this study are sediment trap data and sediment cores taken for chlorophyll-a analyses.

Study Area

The three primary sites were established on an east west transect crossing the continental shelf south of Anvers Island. The transect crosses the shelf from the Peninsula coast towards the shelf break, crossing near the Palmer LTER mooring site (Figure 1.7). Site A was situated close to coastline and was subject to ice-rafted debris such as large drop-stones that were recovered in several of the benthic trawls at this site. Depths at site A ranged from 410 to 650m, measured *in situ*. However, these depths do not correspond to those on the bathymetric map (Figure 1.7), which was constructed using GEBCO data (perhaps as a results of incomplete data in the GEBCO archive). Site C was located on the outer continental shelf at depths of 550 to 580m. The quantity of phytodetrital flux to the seabed differed considerably between sites A and C. Site A was characterised by large quantities of detrital material forming a carpet across the seabed during the phytoplankton bloom (Smith and DeMaster unpublished data). Whilst bloom material was observed at site C it was in much smaller quantities than at site A. Site B was located more centrally on the shelf, between sites A and C, with depths ranging from 550 to 700m. In terms of sediment and phytodetrital flux site B was very similar to site C.

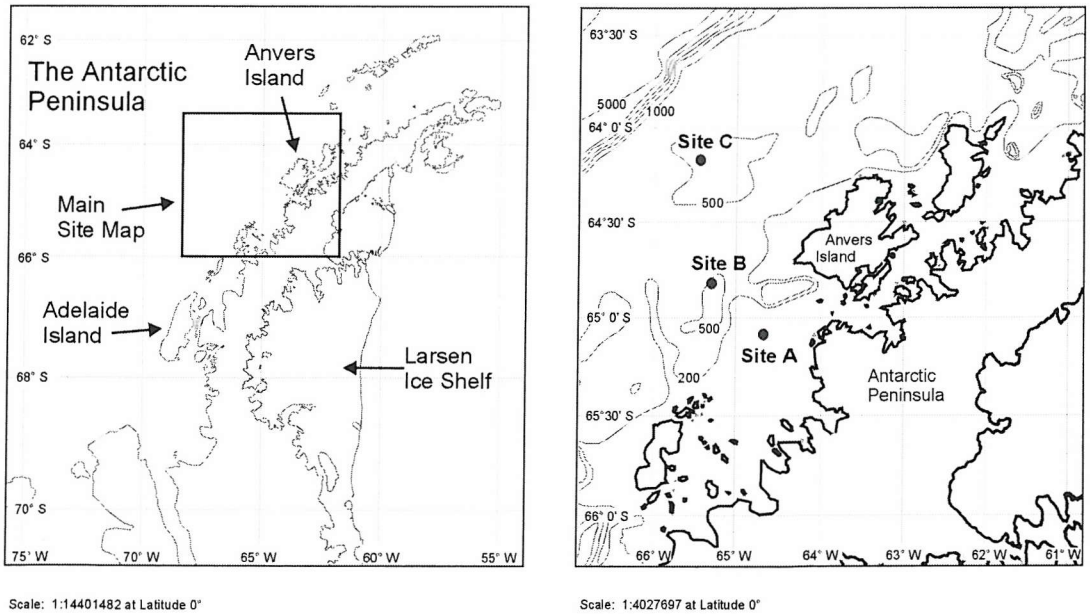

Figure 1.7. Location of sampling sites used in the FOODBANCS study. Map compiled from GEBCO data.

Project Aims

The primary aim of this study was to investigate the reproductive biology of six species of echinoderms (*Protelpidia murrayi, Peniagone* sp., *Psilaster charcoti, Sterechinus antarcticus, Ctenocidaris perreri* and *Amphipneustes lorioli*) found at sites A and C. Site B was excluded from reproductive studies owing to the time and fiscal constraints of a PhD. Site B was excluded because of its general similarity in physical properties to site C. Any difference in reproductive effort or strategy were deemed more likely to occur at sites with the greatest difference in phytodetrital input, sites A and C. The reproductive biology was analysed with regards to the seasonally driven downward flux of phytodetritus to the continental shelf. Specifically to test the following hypotheses:

1. The gametogenetic cycles of benthic invertebrates on the Antarctic continental shelf are controlled by the arrival of a seasonal pulse of phytodetritus.
2. Gamete release is temporally related to this pulse.
3. Fecundity and reproductive effort are related to the intensity of this pulse.
4. A significant proportion of energy input from phytodetritus is re-exported as sexual products.

Four of the six species of benthic megafauna targeted in the study occur at all three primary sites in the Palmer LTER area, whilst two species occur at only one site (*Sterechinus antarcticus*, occurred at site C, and *Amphipneustes lorioli* occurred at site A only).

In addition to investigating the reproductive response to the pulse of phytodetritus, elemental analysis (CHN) was used to provide an indication of the nutritional status of body tissues. CHN profiles were converted to estimated concentrations of protein, lipid, carbohydrate and energy using the stoichiometric relationship described by Gnaiger and Bitterlitch (1982). Analyses of the nutritional status of the body tissues would provide evidence of the physiological state of the organisms. It is hypothesised that a prolonged presence of labile POC within shelf sediments could in part explain why benthic fauna do not lay down lipid stores during the summer months. The need for lipid storage is dictated by a balance between availability of food and the minimum metabolic rate. Where the metabolic rate is low, as it typical for Antarctic benthos, reserves of energy may not be necessary for survival during the winter

months (Clarke and Peck, 1991). This method will investigate the presence or utilisation of any lipid stores in body tissues, and changes in the biochemical composition of gonad tissues that may occur during synchronised spawning of gametes.

Chapter 2 – Species Introduction and Observations

Introduction

The FOODBANCS programme 1999-2001 was the first major temporal benthic sampling programme on the continental shelf west of the Antarctic Peninsula. During this programme the various species compositions of the three sampling sites on the continental shelf were identified and described. An important part of this study was to identify all species present from the original literature, as no key to Antarctic continental shelf megafauna exists. The species chosen for this particular study comprised six species of echinoderm, from three classes (Echinoidea, Holothuroidea and Asteroidea), all of which required identification to species level before meaningful ecological research could be carried out. During the sample collection, dissection and identification process some additional observations were made for each species and these are also reported in this chapter.

Methods

Alpha taxonomy (the description and naming of species) was used for identifying each species. Echinoids were identified using Mortensen's *Monograph of the Echinoidea* (Mortensen, 1928; 1943; 1950; 1951), Holothurians with the *Galathea Reports* (Hansen, 1975) and the *BANZARE Reports* (Clark, 1961) were used for identifying the Asteroids.

In order to study the structure of the test in the echinoid species it was essential that it be well cleaned. Three individuals from each species of echinoid were dried, then brushed gently with a toothbrush to remove the spines, and strong bleach applied to clean each specimen. It is important that the bleach be applied with care and that the test is immediately rinsed in fresh water once clean, to prevent it from disintegrating. Pedicellaria were picked from intact tests mounted on well slides and treated with bleach to reveal their calcareous structure.

Holothurians were examined intact; features were examined in more than ten individuals to ensure reliability (this is because delicate parts of the body such as the tentacles or papillae may be damaged or lost during sampling). In order to establish identification it was necessary to remove a small area of intact skin, which was treated with bleach to expose the spicules and calcareous deposits. Identification of the asteroid species was carried out under a dissecting microscope (Leica MZ-8) on

specimens of the preserved animal. No pedicellaria were found in the asteroids examined in this study.

Class: Holothuroidea, Order: Elasipoda, Family: Elpidiidae

Protelpidia murrayi Theel, 1879*

Material examined: 20 specimens Site C (64° 11.45 S, 65° 29.80 W).

Additional material: 96 specimens Site A (65° 8.32 S, 64° 44 W), 78 specimens Site C.

Diagnosis

The description is based on 20 well-preserved specimens from site C, ranging in size from 22.5 to 133.9 mm long and the ventral sole 12.5 to 87.3 mm broad.

The body is ovoid and varied in shape from broad and vaulted to slightly elongate (Figure 2.1A). Body width values range between 50 and 90% of the body length values. The skin is pink and smooth in live specimens, the thickness of the skin varying considerably from one specimen to another.

The number of tubefeet varies from 6 to 7 depending on the size of the specimen. Tubefeet are evenly spaced around the entire ventral sole, the anterior pair are reduced in size in most specimens.

The first pair of papillae are situated a short distance behind the mouth. The second pair of papillae are situated anteriorly behind the first pair and up to the middle of the dorsal surface. A rudimentary third pair of papillae are present in all specimens immediately behind the second pair. The first and second pairs of papillae are not fused, but free along the whole length.

Each specimen has 10 tentacles surrounding the mouth, which is terminal in position but downward facing. Tentacles are peltate in structure.

The calcareous deposits in the skin consist of primary deposits (spinuous rods) and c-shaped deposits (Figure 2.1 C and D). C-shaped deposits are densely packed throughout the surface of the skin, and the strongly spinuous rods are scattered throughout the skin below the c-shaped deposits.

* Identification of this species was performed by Andrey Gebruk, P.P. Shirshov Institute of Oceanology.

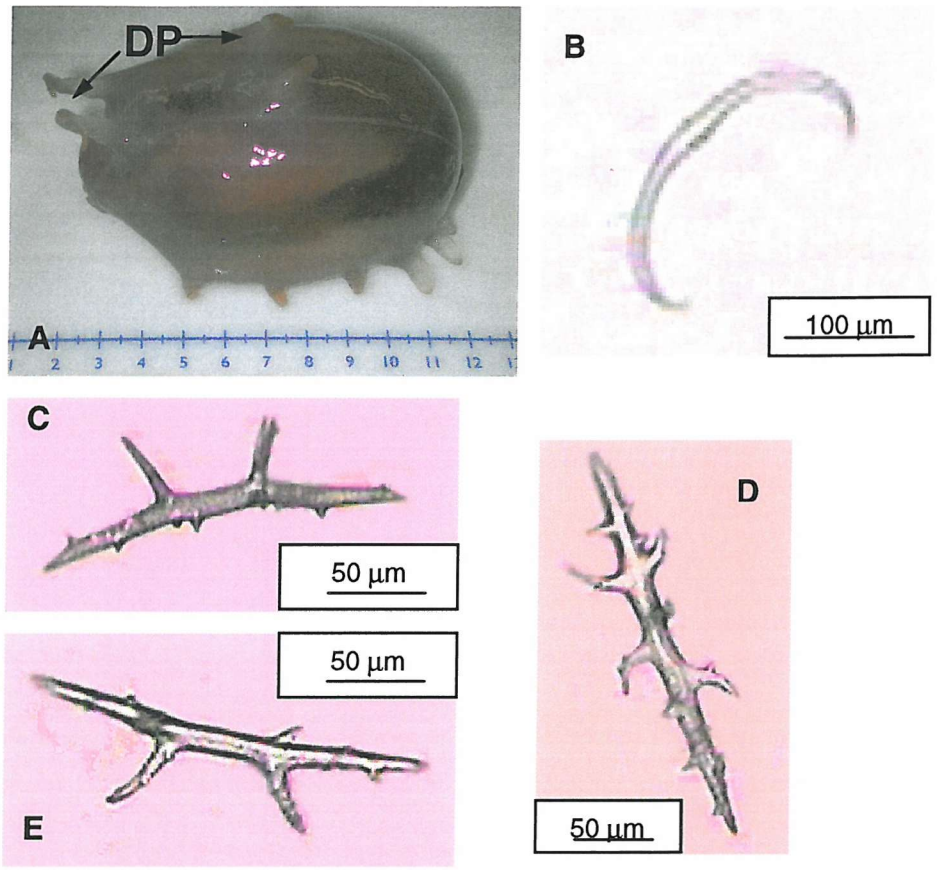

Figure 2.1. Taxonomic characteristics of *Protelpidia murrayi.* (**A**) whole specimen; each number on the blue scale bar = 1cm. DP = dorsal papillae. (**B**) C – shaped spicule (**C-E**) spinuous rods.

Records

Bathymetric range: 590-5300m (Gebruk, 1990).

Distribution: Antarctic. (Gebruk, 1990).

Biology

Feeding: Very little is known of the ecology of *Protelpidia murrayi.* However, as a member of the elpidiid family (which are deposit feeders) and given its morphology and gut contents it would suggest that this species deposit feeds. Many deep-sea photographs suggest that elasipods are primarily rake feeders, grazing on the uppermost sediment surface, leaving no or light traces (Massin, 1982). Elasipods are also known to be able to concentrate the sediment fractions richest in organic matter in their guts (Massin, 1982).

Reproduction: The maximum egg size of 357 µm suggests that *Protelpidia murrayi* produces pelagic lecithotrophic larvae (Chapter 3).

Class: Holothuroidea, Order: Elasipoda, Family: Elpidiidae

Peniagone sp. Theel, 1882

This organism could not be identified any further than the genus level because there have been no previous descriptions of organisms from this study area and from the specimens available the identification could not determine between *Peniagone challengeri* and *Peniagone papillata.*

Material examined: 20 specimens Site C (64° 11.45 S, 65° 29.80 W).

Additional material: 100 specimens Site A (65° 8.32 S, 64° 44 W), 100 specimens Site C.

Diagnosis

The following description is based on 20 well-preserved specimens from site C. Body size ranged from 44.3-125 mm length and 11.3-40 mm width.

The body is elongate and flattened posteriorly. The length of the body is between 2 and 5 times that of the width. The skin is smooth and clear in live specimens, gonad and gut organs can clearly be seen through the body wall (Figure 2.2 A and C).

Specimens have 8-9 pairs of tubefeet, which border the posterior half to two thirds of the ventral sole. The tubefeet decrease in size towards the posterior end of the body, and the posterior 2-3 pairs of tubefeet are positioned at closer intervals (Figure 2.2 A).

The dorsal papillae comprise one pair of large papillae and two smaller pairs (Figure 2.2 B and C). The papillae may be fused at the base, but do not form a velum as they are separate along their length. The two smaller pairs of papillae are situated immediately behind and at the base of the large pair.

Tentacles number 10 in all specimens. Tentacles are well developed with a large stalk and an enlarged disc (Figure 2.2 A, B and C).

The deposits consist of primary crosses and smooth curved rods (Figure 2.2 D, E and F). The primary crosses have a well-developed stem and four low apophyses at the base of each arm.

* Identification of this species was performed by Andrey Gebruk, P.P. Shirshov Institute of Oceanology.

Records

Bathymetric range: 400-600 m (pers. obs. 1999-2001; Hansen, 1975).

Distribution: Antarctic (pers. obs.1999-2001; Hansen, 1975).

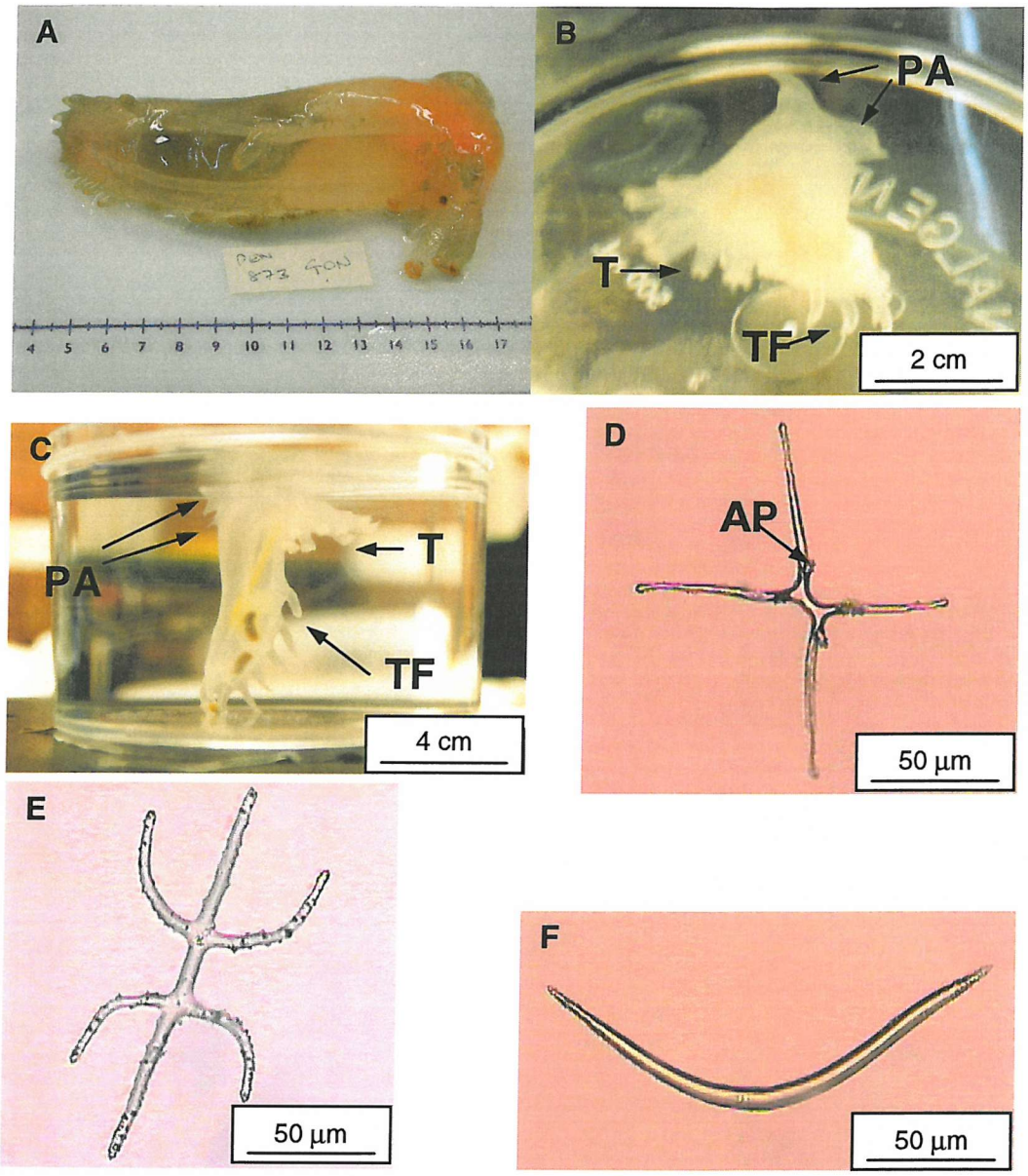

Figure 2.2. Taxonomic characteristics of *Peniagone* sp. (**A**) whole specimen, each number on the blue scale bar = 1cm; (**B**) Live *Peniagone* sp.; (**C**) Live *Peniagone* sp.; (**D**) Primary cross deposit; (**E** and **F**). Deposits. AP = apophyses; PA = papillae; T = tentacles; TF = tube feet.

Biology

Feeding: Deposit feeder (see behaviour below).

Reproduction: A maximum egg size of 569 μm (Chapter 3) suggests lecithotrophic development, as found for the congeneric species *Peniagone azorica* and *Peniagone diaphana* (Tyler *et al.*, 1985).

Behaviour: The FOODBANCS research cruises had a multidisciplinary sampling programme that involved sediment sampling using both boxcorers and megacorers. On one sampling occasion an individual of *Peniagone* sp. was collected in a megacore tube, the animal had been caught intact in the centre of the tube and was still alive. On recovery of the megacorer the holothurian began to swim vertically upwards in the tube using an undulating motion of its oral tentacles and tube feet. Holothurians that have developed a pelagic lifestyle have adapted their body shape in ways to facilitate swimming behaviour (Billett, 1986). *Peniagone diaphana* has a benthopelagic lifestyle (Hansen, 1975; Barnes, 1976; Tyler *et al.*, 1985; Billett, 1986) and is an example of a modified body shape: the body is slightly dorso-ventrally compressed; the tube feet are fused into an anterior brim that acts as a paddle-like postanal fan and the velum is broad and is composed of 4 papillae that only project slightly (Barnes, 1976). The body of *Peniagone* sp. is elongate and only flattened posteriorly. There are 9 pairs of tubefeet that border the posterior half of the ventral sole. The velum is very low with almost free papillae. The body form of *Peniagone* sp. suggests that it spends most of its time on the sea floor moving over the sediment using its small tubefeet. The tubefeet are not fused to form a brim for swimming and they extend around at least half of the ventral sole to support the body in a horizontal position. It is most likely that *Peniagone* sp. depends on the sediment on the sea floor for food. Barnes (1976) found on analysis the gut contents of the benthopelagic holothurian *Peniagone diaphana* to be of benthic origin. It may be possible that *Peniagone* sp. also feeds on suspended particles close to the seabed by raising the anterior part of the body vertically and extending its tentacles into the water column.

Class: Asteroidea, Order: Phanerozonida, Family: Astropectinidae

Psilaster charcoti Koehler, 1906

Material examined: 3 specimens Site A (65° 8.32 lat, 64° 44 long).

Additional material: 42 specimens Site A, 63 specimens Site C (64° 11.45 lat, 65° 29.80 long).

Diagnosis

The body is stellate in shape with the disk and arms merging, the body being flattened from above (Figure 2.3A). The ratio of disk radius (r) to arm length (R) is about 5, thus indicating a relatively small disk. The arms are triangular and the inter-radial arcs between the arms are blunt angled. Pedicellaria are absent.

The abactinal plates are paxilliform (Figure 2.3E). The paxillae are arranged in approximately transverse lines on the arms near to the disc and near to the superomarginal plates. Paxillae are irregularly distributed down the centre of the arms and on the disc. The madreporite is fairly inconspicuous on the central disc (Figure 2.3B). The marginal (supero and infero) plates are prominent (Figure 2.3F) down the sides of the arms, with the superomarginal plates conspicuous from the dorsal view. The supero- and inferomarginal plates are moderately wide and have equal height. Broad-based spines provide a coarse armament on both the supero- and inferomarginal plates, the spines are directed away from the disc and may be of sufficient length to overlap those of succeeding plates.

On the oral side of the arms the actinal plating is moderate in size (Figure 2.3C). There are 4-5 furrow spines per plate that are aligned as a blunt or angled fan. Two rows of tubefeet line the furrow, the tips of the tubefeet narrowing to a blunt point. The oral plates are of moderate size and almost bar-like in shape (Figure 2.3D).

Records

Bathymetric range: 10-3246 m (Clark, 1961).

Distribution: Circumpolar, including Biscoe Islands, Wandel Island, Palmer Archipelago, South Shetlands, Clarence Island, South of South Orkneys, South Sandwich Islands, South Georgia, Bouvet Island, Ross Sea (Fisher, 1940).

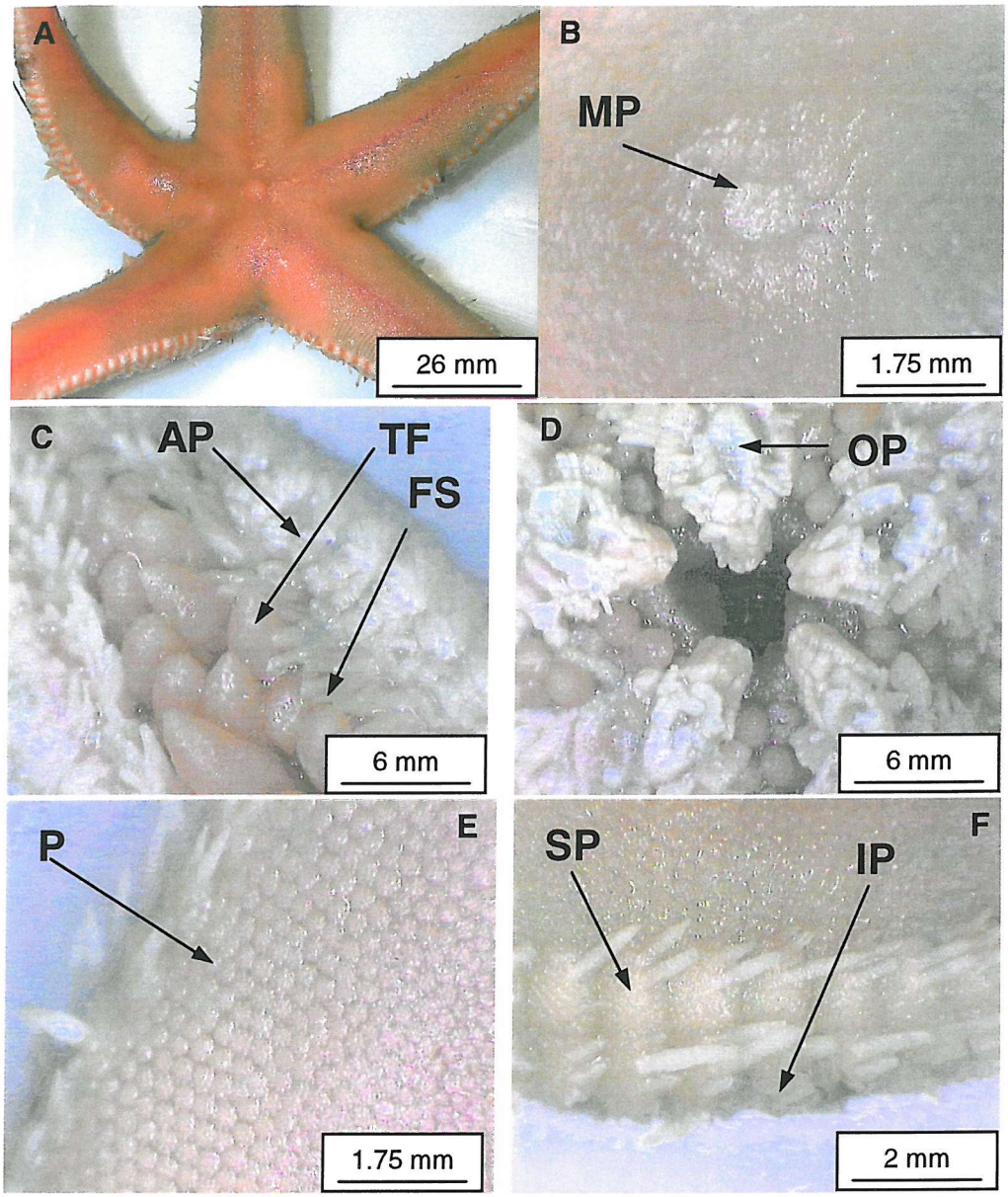

Figure 2.3. External characteristics of *Psilaster charcoti.* (**A**) dorsal view; (**B**) central disc; (**C**) ambulacral area; (**D**) mouth; (**E**) dorsal surface of arm; (**F**) lateral view of arm. AP = actinal plate; FS = furrow spines; IP = inferomarginal plates; MP = madreporite; OP = oral plates; P = paxilliform abactinal plates; SP = superomarginal plates; TF = tubefeet.

Biology

Feeding: Gut contents of *Psilaster charcoti* were examined on dissection of the specimens (Figure 2.4A) and were found to contain either mud or decapod crustaceans. The crustaceans were all identified as *Euphausia superba* and were most prevalent in the gut during October 2000 at Site A. *Psilaster charcoti* is thought to be a predator/scavenger and may feed on the dead *Euphausia superba* that sink through the water column. It is unlikely that they feed on live decapods owing to their lack of pedicellaria with which to catch them. Specimens of *Psilaster charcoti* from shallow

water have been recorded to feed on fish meat from baited traps, sediment and faecal pellets (Dearborn, 1977). It is likely that *Psilaster charcoti* is a facultative scavenger. It is also an opportunistic feeder that exhibits dietary specialisation when a specific food type is present, in this case *Euphausia superba.* As a species of the Astropectinidae, *Psilaster charcoti* is an intra-oral feeder, taking all food into the stomach prior to digestion. In common with other species exhibiting intra-oral feeding, the tubefeet of *Psilaster charcoti* lack suckers and the digestive tract is incomplete, lacking both rectum and anus.

Reproduction: Bosch (1990) reported *Psilaster charcoti*, found in shallow water at New Harbor, McMurdo Sound, to broadcast spawn with resulting non-feeding pelagic lecithotrophic larvae. These data were based on observations of females induced to spawn between September 1984 and December 1985. Natural spawning took place during late October.

Associates: During the dissection of *Psilaster charcoti* many of the adults were found to contain a small (<20 mm long) unidentified organism (Plate 2.4B). The organism was soft bodied and had two tissue types visible through the body wall. All of the organisms were attached to the internal side of the of the abactinal surface, a thin pocket of the host's internal membrane separating it from the coelom. The potential parasites were usually undetectable without dissection and a maximum of 9 organisms were found to inhabit one individual starfish. The identification of this organism has not yet been completed, however there are 4 kinds of parasite known to infest echinoderms: Gastropoda, Myzostomida, Ascothoracida and Copepoda. This organism is lacking several key features from which it would be classified as a crustacean or annelid worm, these include: segmentation, carapace, apparent head or anus, appendages or setae. Clarke (1968) describes a group of parasitic molluscs that are internal parasites and are more degenerate in form and more specialised for reproduction than external parasites. An example is a very degraded parasitic snail, *Parenteroxenos dogieli*, which lives inside the holothurian *Cucumaria japonica* and has no shell and no internal organs except reproductive organs. It seems likely that this particular organism is a degraded parasitic snail that lives in the body cavity of the asteroid and has lost is shell and internal organs except for the reproductive ones.

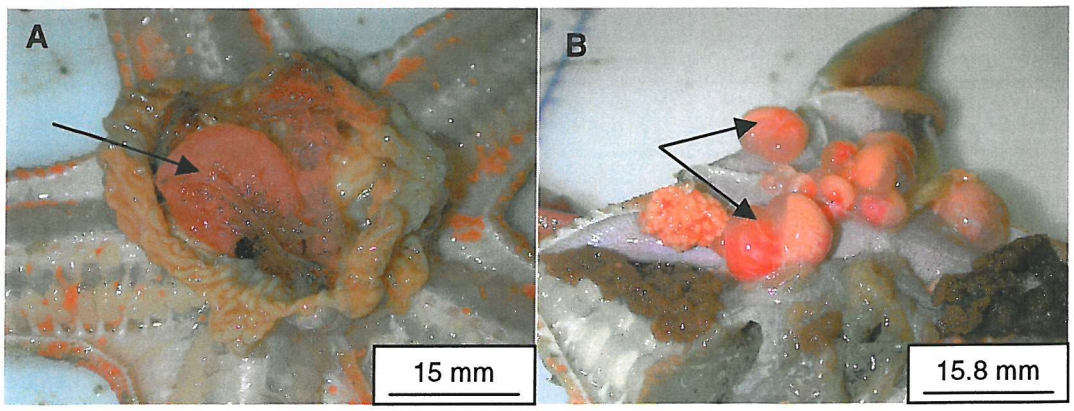

Figure 2.4. Gut contents and parasites in *Psilaster charcoti* (**A**) Gut contents (*Euphausia superba)* of *Psilaster charcoti* as indicated by the arrow, (**B**) Potential parasites in *P. charcoti* as indicated by the arrows.

Class: Echinoidea, Order: Camarodonta, Family: Echinidae

Sterechinus antarcticus Koehler, 1901

Material examined: 3 specimens Site C (64° 11.45 S, 65° 29.80 W).

Additional material: 97 specimens Site C.

Diagnosis

This description was based on 3 specimens from site C. The test is low and hemispherical in shape, the test height ranging from 45 to 70% that of the test diameter. Test diameter ranges in size from 20.5 to 60 mm.

Ambulacral plates have three pore pairs to each plate; the pores are set at a slightly oblique angle on the plate. Primary tubercles are found only on every second or third ambulacral plate (Figure 2.5C).

The apical system in *Sterechinus* exhibits 2 to 5 of the ocular plates are insert (Figure 2.5B). This means that the ocular plate sits between two adjacent genital plates making contact with the periproct. The periproct is large, covered with numerous small plates, among which the small round suranal plate is conspicuous (Figure 2.5B). The peristome is somewhat larger than the apical system as seen in Figures 2.5B and D.

The primary spines are moderate in length (never exceeding the diameter of the test) and slender, tapering to a point. In contrast the secondary spines are thorny and numerous, forming a dense coat over the test (Figure 2.5A).

Specimens exhibit only globiferous pedicellaria, which are adorned by a single poison gland. The blade, distinctly offset from the basal part of the valve, forms a more or

less closed tube, the edges of the blade being connected by varying numbers of cross beams. There are 2-3 lateral teeth on both sides at the end of the blade (Figure 2.5E). Paired rod-like epiphyses bridge across the v-shaped space at the upper end of the five pyramids forming the Aristotle's lantern (Figure 2.5F). In some families a pair of processes issues from the epiphyses of the Aristotle's lantern as a support for the tooth, however this is not exhibited in this specimen.

Records

Bathymetric depth: 100-1080 m (Mortensen, 1943).

Distribution: Circumpolar distribution. In the Atlantic sector of Antarctica, it may be found as far north as South Georgia (Mortensen, 1943).

Biology

Feeding: On dissection the gut was found to contain benthic sediment sometimes consolidated into food pellets. Mud and bottom material have also been reported as forming part of the diet in both *Sterechinus dentifer* and *Sterechinus diadema* (Mortensen, 1943), and it is known that deep-sea species in particular ingest soft substrata. It seems that regular echinoids are opportunistic feeders, their diet varying with food availability, both quantity and type (De Ridder, 1982). In shallow water, *Sterechinus neumayeri* may use the Aristotle's lantern to consume algae, diatoms, plant materials and seal faeces. Whilst in deeper waters, *Sterechinus antarcticus* uses the Aristotle's lantern as a grab to ingest bottom material.

Reproduction: Brey (1991) suggests that the small size of the eggs (0.25 mm) indicates the development of a planktotrophic larva in *Sterechinus antarcticus*. However, as discussed by Emlet (1987) there are reports of species with both planktotrophic or lecithotrophic developments that have overlapping egg sizes with those recorded for *Sterechinus antarcticus*. Thus *Sterechinus antarcticus* could have either planktotrophic or lecithotrophic larval development.

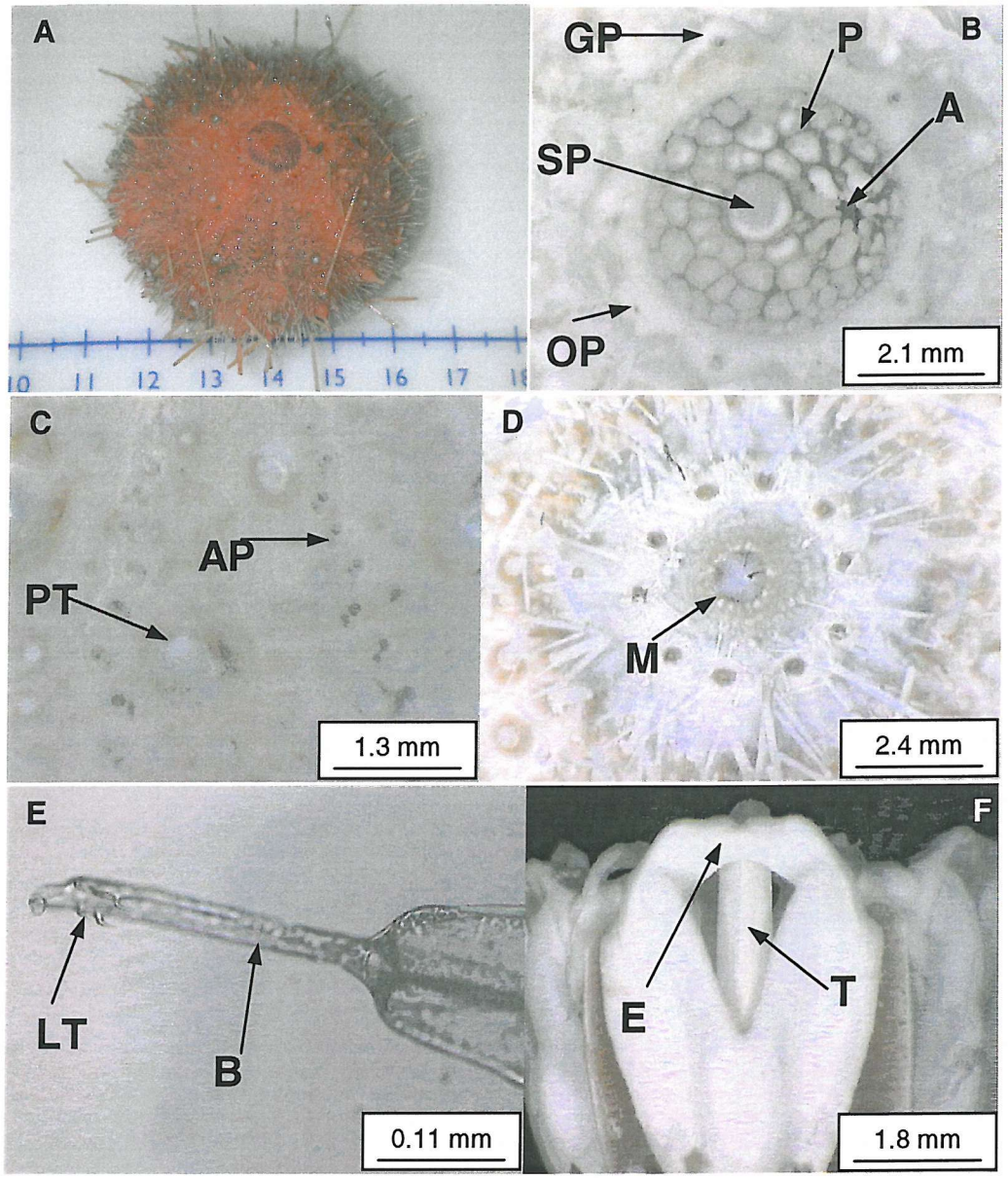

Figure 2.5. Taxonomic characteristics of *Sterechinus antarcticus* (**A**) dorsal view of *Sterechinus antarcticus*, each number on the blue scale bar = 1cm; (**B**) apical system; (**C**) ambulacral plates; (**D**) peristome; (**E**) valve of globiferous pedicellaria; (**F**) pyramids of Aristotle's lantern. A = anus; AP = ambulacral pores; B = blade; E = epiphyses; GP = gonopores and genital plates; LT = lateral teeth; M = mouth; OP = ocular plate; P = periproct; PT = primary tubercles; SP = suranal plate; T = tooth.

Class: Echinoidea, Order: Cidaroidea, Family: Cidaridae

Ctenocidaris perrieri Koehler, 1912

Material examined: 3 specimens Site A (65° 8.32 S, 64° 44 W).

Additional material: 45 specimens Site A, 100 specimens Site C (64° 11.45 S, 65° 29.80 W).

Diagnosis

This description is based on three well-preserved specimens from site A. The test is almost spherical with test diameter ranging from 18.7 to 62.3 mm in all FOODBANCS specimens. Test height is between 65 and 95 % that of the diameter. The test is covered by numerous primary and secondary spines (Figure 2.6A). Single primary spines are found on each interambulacral plate and are ~ 2 x horizontal test diameter in length. In *Ctenocidaris perrieri* a spongy coat of hairs covers the primary spines. Oral primary spines are shorter than those of the main test, the ends of the spines are spear shaped and coarsely serrated (Figure 2.6D). Secondary spines rarely exceed 5-6mm in length and are flattened or club-shaped. Of the secondary spines, those surrounding the primary spines are the most developed. These spines are situated close together to protect the base of the primary spine. The spines of the ambulacrum are shorter (2-3mm) and protect the tubefeet.

The coronal (test) plates do not overlap, but are connected by a rigid, grooved line (Figure 2.6 B and C), they comprise five pairs of ambulacral plates and five pairs of interambulacral plates. Ambulacral plates are narrower and more numerous than the interambulacral plates. The pore pairs of the ambulacra are arranged in a single series, the pores are very close together but have no uniting furrow (Figure 2.6C). There are few interambulacral plates owing to their large size. Each plate carries one primary tubercle, which consists of a cone terminating in a knob (Figure 2.6C). One end of a ligament is attached to the deep depression in the end of the tubercle; the other end of the ligament is attached to the base of the primary spine.

These specimens only exhibit globiferous pedicellaria of the three known types in cidarids. The valve of the globiferous pedicellaria is without a distinct end tooth. The opening of the pedicellaria valve is sub-terminal.

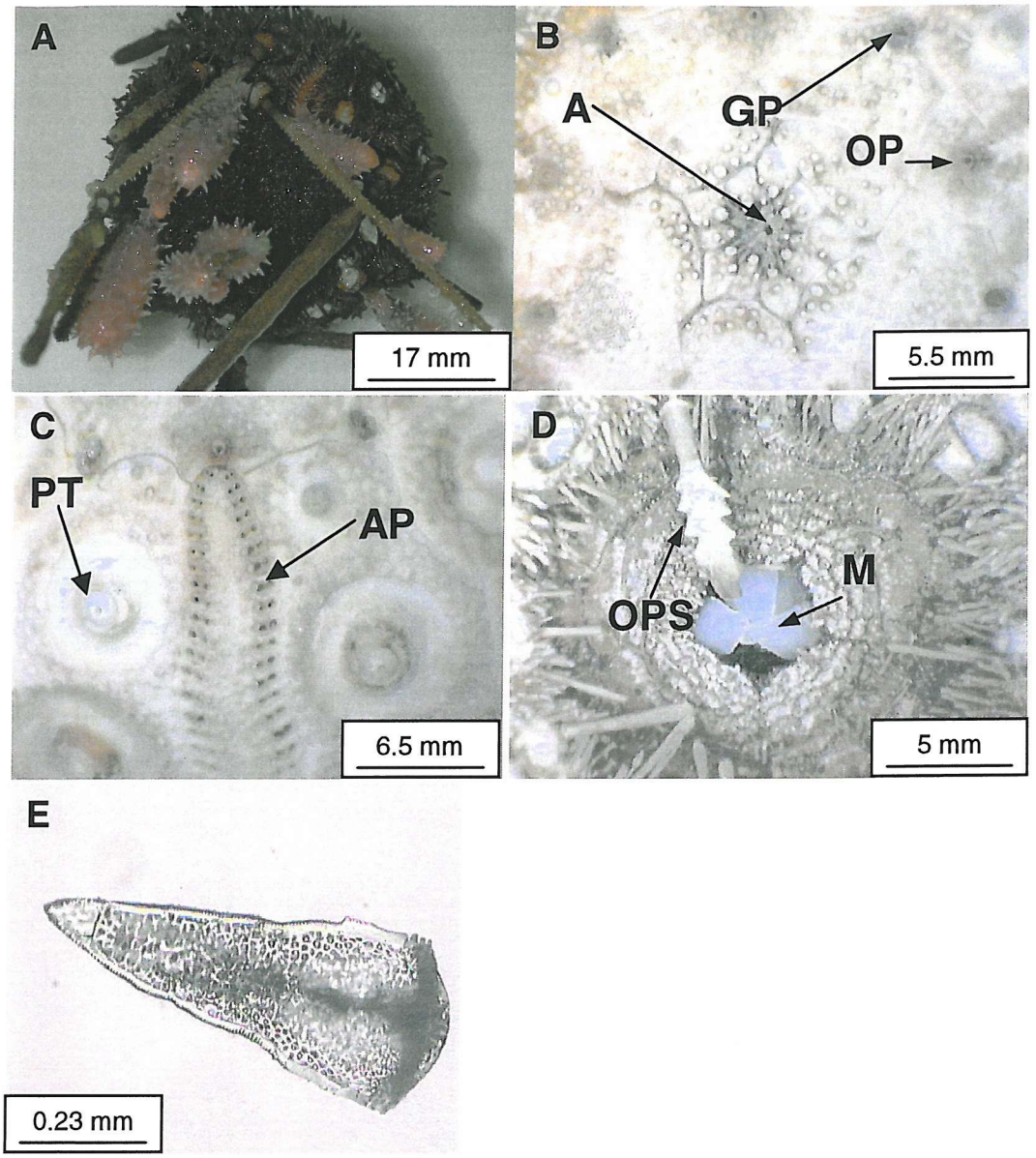

Figure 2.6. Taxonomic characteristics of *Ctenocidaris perrieri* (**A**) Live *Ctenocidaris perrieri* with the holothurian (*Echinopsolus acanthocola*) attached to spines; (**B**) apical system; (**C**) ambulacral plates; (**D**) peristome; (**E**) valve of globiferous pedicellaria. A = anus; AP = ambulacral pores; GP = gonopores; M = mouth; OP = ocular pores; OPS = oral primary spines; PT = primary tubercles.

Records

Bathymetric range: 6-602 m (http://scilib.ucsd.edu/sio/nsf/fguide/echinodermata20.html).

Distribution: Circumpolar, Kerguelen Island, Crozet Island, Heard Island (http://scilib.ucsd.edu/sio/nsf/fguide/echinodermata20.html).

Biology

Feeding: Mortensen (1928) describes the food of cidarids as consisting mainly of hard-shelled organisms, which they crush with their powerful teeth i.e. Bryozoa, serpulids, molluscs and Foraminifera. However, deep-sea forms often have their alimentary canals filled with sediment and detritus, as is the case for the species found in this study that were collected from 600m depth.

Reproduction: *Ctenocidaris perrieri* is known to brood its embryos on the peristomal membrane (Pawson, 1969; Dell, 1972).

Associates: On collection the primary spines of *Ctenocidaris perrieri* were found to serve as a substratum for other invertebrates. The most common epibionts found on the spines of the cidarid include holothurians, bivalves, tubeworms and sponges (Figure 2.7A-D). These relationships can be considered as commensal, in which one animal profits and the others are not affected (Gutt and Schickan, 1998). The filter feeding holothurian *Echinopsolus acanthocola* (Figure 2.7D) has been found on the spines of *Ctenocidaris perrieri* (Gutt, 1990). Brey *et al.* (1993) found the bivalve *Lissarca notocadensis* on the spines also. It has previously been assumed that the position, within a range of a few centimetres to a few decimetres above the sea floor, where a sharp increase in current has been observed (Cacchione *et al.*, 1978), is advantageous for these epibionts since they feed on drifting living or detrital particles.

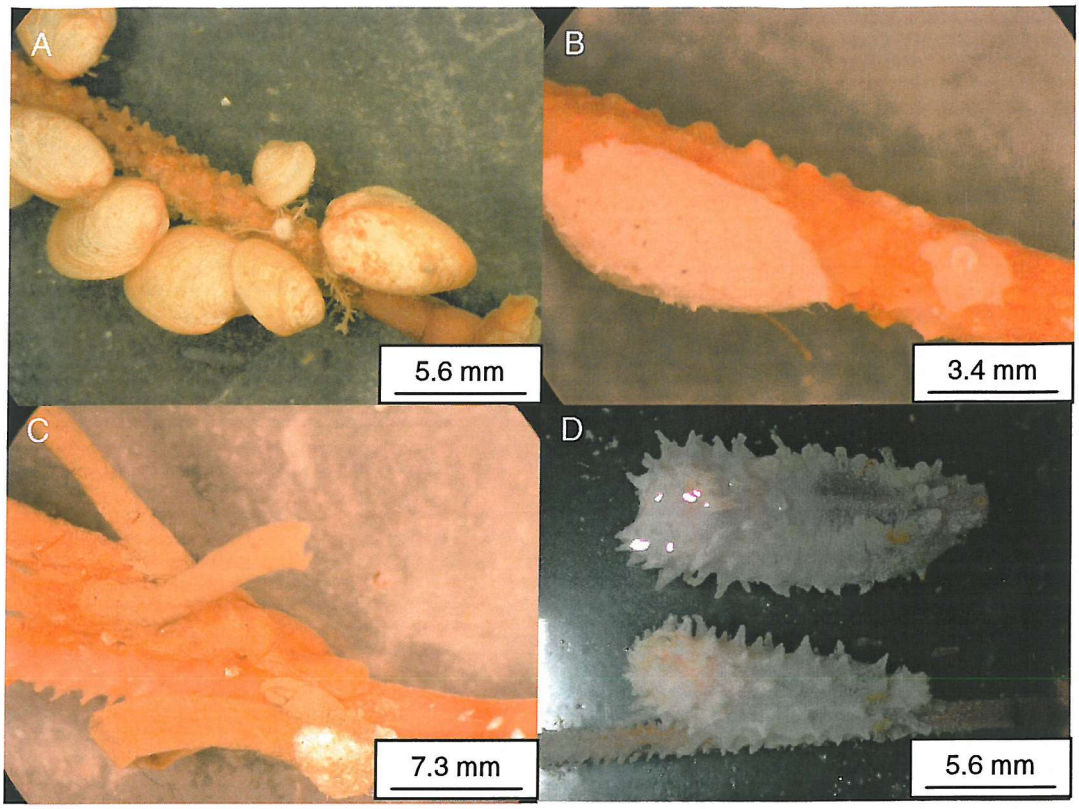

Figure 2.7. Commensal species living on the spines of *Ctenocidaris perrieri*. (**A**) *Lissarca notocadensis*; (**B**) Sponge and barnacle; (**C**) Tube worm; (**D**) *Echinopsolus acanthocola*.

Class: Echinoidea, Order: Spatangoida, Family: Schizasteridae

Amphipneustes lorioli Koehler, 1901

Material examined: 3 specimens Site A (65° 8.32 S, 64° 44 W).

Additional material: 59 specimens Site A.

Diagnosis

The base of the test is oval in outline (Figure 2.8A) and there is a good linear relationship between test length and both width and height. The test length ranges between 47.5 and 79.6 mm, the width ranges between 40.7 and 71.3 mm and the height ranges from 29.5 to 54.9 mm.

Both the Aristotle's lantern and teeth are absent and the test is completely lacking fascioles. When present the fasciole is a band of minute tubercles bearing modified spines.

The ambulacral plates are expanded to form five distinct petals. In females the frontal ambulacrum is quite simple and flush with the test (Figure 2.8 C), whilst the posterior paired ambulacra are deeply sunken to form marsupia in which the brood is reared.

Male urchins also have distinctly petaloid ambulacra but paired ambulacra remain simple and not deeply sunken. In both males and females these petals stop short of the ambitus (widest part of the shell). The pores on the ambulacral plates are arranged in single regular series of vertically placed pairs.

The interambulacrum is of interest where the posterior interambulacrum on the oral side forms a distinct plastron (Figure 2.8D). In this case the labrum adjoins two equally sized plates instead of one plate. The peristome is labiate in shape and positioned to the anterior of the test.

The apical system is slightly sub-central and comprises 5 ocular plates and 5 genital plates (Figure 2.8 B). Ocular plates are the heads of the ambulacra and all support an ocular pore. Genital plates are the heads of the interambulacrum and support a total of only 3 gonopores. One genital plate is modified into the madreporite and it is lacking a gonopore. Of the remaining four plates one is also lacking a gonopore.

The periproct (plates around the anus) (Figure 2.8 F) is situated on the posterior, truncated end of the test, sub-marginal in position it can be seen from the ventral side.

The valves of the globiferous pedicellaria terminate in a series of short teeth (Figure 2.8E).

Records

Bathymetric range: 160-600 m (Mortensen, 1951).

Distribution: Known only from the Antarctic continent region (Mortensen, 1951).

Biology

Feeding: Spatangoida ingest the sediment in which they live (Emlet, 1987). Consequently the gut contains sediment and the small infaunal organisms associated with it. The main feeding mechanism of spatangoids involves the tube feet (phyllodes) on the enlarged oral ambulacral plates. The tube feet stretch out over the substratum, sediment particles adhere to their surface, and then they fold back into the mouth where they deposit the particles (De Ridder, 1982).

Reproduction: *Amphipneustes lorioli* produces large eggs >1000 μm diameter, which are brooded in sunken marsupia on the external surface of the test.

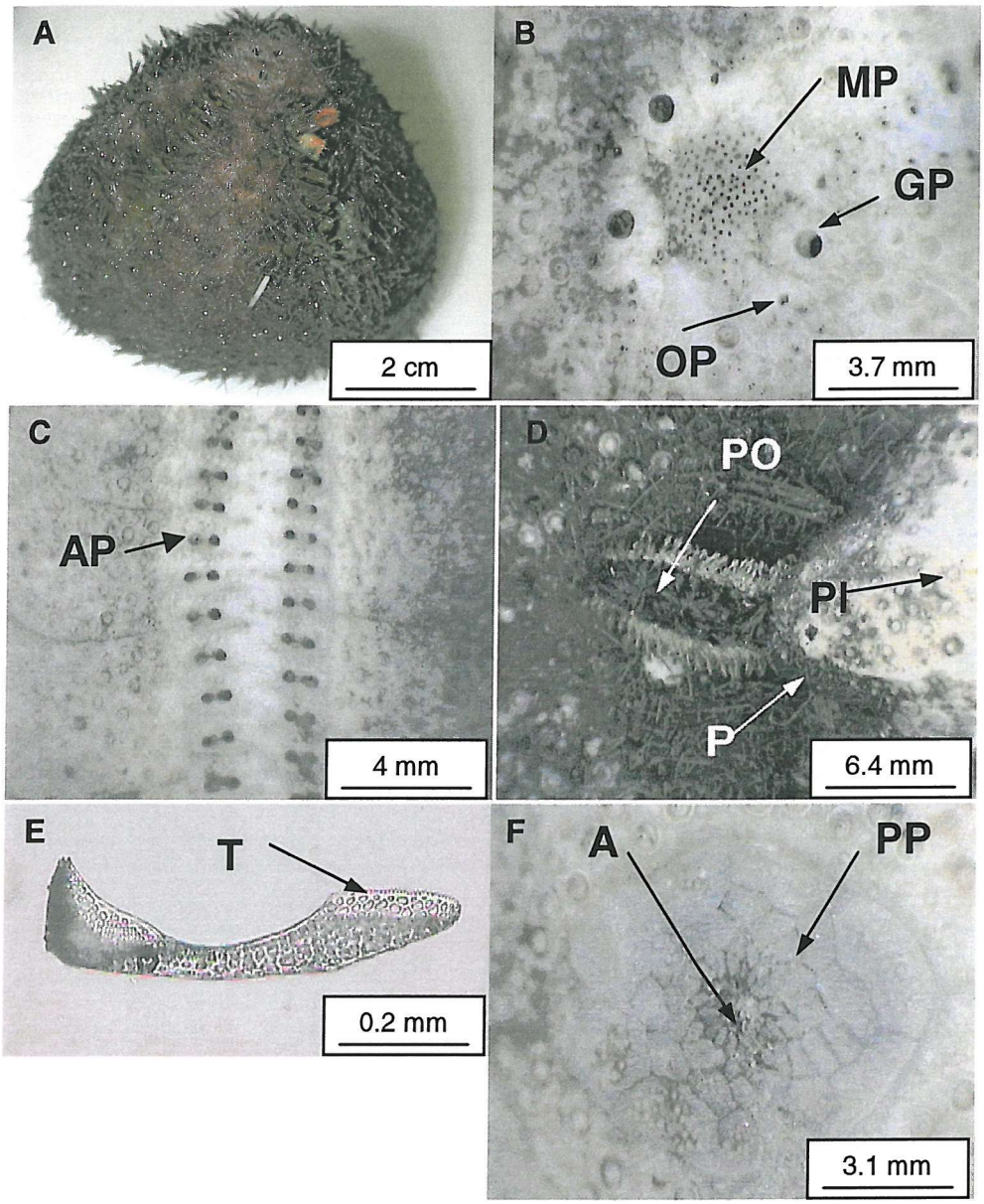

Figure 2.8. Taxonomic characteristics of *Amphipneustes lorioli.* (**A**) *Amphipneustes lorioli*; (**B**) apical system; (**C**) ambulacral plates; (**D**) peristome; (**E**) valve of globiferous pedicellaria; (**F**) anus. A = anus; AP = ambulacral pores; GP = gonopore; MP = madreporite; OP = ocular pore; P = peristome; Pl = plastron; PO = polychaete; PP = periproct plates; T = short teeth.

Associates: During removal and dissection of the gut of the urchin some individuals were found to have a polynoid polychaete living in their gut cavity. Out of 53 individuals examined only 4 were found to contain the polychaete living in its gut. In one urchin, which was subsequently dried, a polychaete was found half in and half out of the anus (Figure 2.8D) suggesting that the polychaete can leave its host without any difficulty. It is possible that some of the polychaetes were sufficiently disturbed during the trawl and recovery that they attempted to leave the host during this period. Records of polychaetes inhabiting the guts of urchins are rare. Emson *et al.* (1993)

found the polychaete *Benthoscolex cubanus* in the guts of a sea urchin from bathyal depths near the Bahamas. The polychaete found by Emson *et al.* (1993) was associated with a spatangoid urchin *Archeopneustes hystrix* and was found to feed by selecting foraminiferans and other fragments of organic material from the gut contents of the sea urchin. Any adverse effects on the urchin resulting from the polychaete living in its gut have not been established. Based on this, Emson *et al.* (1993) describes the nature of the relationship as either parasitic or commensal.

Chapter 3 – Reproductive Biology of Antarctic Benthic Echinoderms

Introduction

Throughout the world's oceans it is generally accepted that the deep-sea starts at the edge of the continental shelf (Gage and Tyler, 1991), at depths in excess of 200m. However, the continental shelves of the Antarctic continent are much deeper than those around the rest of the world's continents. The isostatic depression and scouring by previous extensions of the ice-sheet over the shelf, means that the Antarctic continental shelves may reach depths of 800m (Clarke, 1996). Therefore organisms living on the Antarctic continental shelf are living at depths traditionally regarded as bathyal. Organisms living at these depths will have developed physiological adaptations to elevated pressures, similar to those found in true deep-sea species. However, Clarke (2003), proposed that true deep Antarctic fauna be considered as that living at greater than 2000m depths. Therefore the ecology of benthic organisms living on the Antarctic shelves is a mixture of continental shelf and bathyal influences.

The Antarctic shallow-water environment

The reproductive biology of megafaunal invertebrates, particularly echinoderms, has been well studied in both shallow-water Antarctic and deep-sea environments (see Table 3.1). However there is still very little known about the reproductive biology of the megafauna on the continental shelf around Antarctica, this is because most ecological work in Antarctica has been confined to the deepest depths amenable to SCUBA. Polar latitudes are characterised by extremely low and fairly constant temperatures, contrasting with a strikingly seasonal primary production cycle (Clarke, 1988). As a result of living in this environment polar organisms have a limited thermal tolerance and have developed low resting metabolic rates in comparison to tropical organisms (Clarke and Johnston, 1999). Despite this apparent metabolic advantage, most polar marine organisms grow very slowly and reproduce slowly in comparison to their temperate and tropical counterparts (Clarke, 1983). In addition to cold temperatures the short periods of summer phytoplankton production create a seasonal food limitation in the benthic environment. This food restriction may force Antarctic organisms to reduce metabolic costs by growing slower, reproducing later and exhibiting a lower locomotor activity (Arntz, *et al.*, 1994). Reproductive strategies have previously been linked to food availability in several ways

(Eckelbarger and Watling, 1995). In particular the timing of spawning has been linked to the timing of the spring/summer phytoplankton bloom as it provides a source of food for developing larvae, and may stimulate the process of vitellogenesis by providing an increased food supply to the adult organism. There have been many reproductive studies of individual taxa in the Antarctic shallow-water environment, some which exhibit evidence of coupling to the seasonal supply of food and some that are totally uncoupled. The echinoderms, particularly the asteroids and the echinoids, form a conspicuous part of the benthic megafauna and as such have been studied in some detail.

Previous work

The reproductive biology of shallow-water Antarctic asteroids from McMurdo Sound has been described in detail (Pearse, 1965; Pearse *et al.*, 1985; Pearse *et al.*, 1986; McClintock *et al.*, 1988; Bosch, 1989; Bosch and Pearse, 1990; Pearse *et al.*, 1991). Other studies include asteroids from Anvers Island (Bosch and Slattery, 1999) and Signy Island (Stanwell-Smith and Clarke, 1998). The 15 species covered by these studies exhibited a range of developmental types (Table 3.1). These included pelagic and non-pelagic embryos and larvae, and feeding (planktotrophic) or non-feeding (lecithotrophic) modes of larval nutrition. Three of the 15 species are broadcast spawners with feeding larvae, a further 9 species are broadcast spawners with non-feeding larvae and the remaining 3 species brood their young. Many of these species showed a seasonal reproductive periodicity. The three species possessing a planktotrophic mode of development have seasonal cycles of reproduction; 6 of the 9 species with lecithotrophic development were also seasonal spawners (the periodicity of 2 species is unknown); of the brooders one is seasonal and the remaining 2 species are competent to spawn throughout the year. This variety of reproductive patterns demonstrates the wide diversity of reproductive modes employed by shallow-water asteroids.

The Antarctic echinoids are represented by three families from three separate orders; Cidaridae (order Cidaroidea), Schizasteridae (order Spatangoida), and Echinidae (order Echinoidea). Both the families Cidaridae and the Schizasteridae within the Antarctic consist almost entirely of brooding species (Poulin and Féral, 1996). In contrast to the cidarids and schizasterids, the Antarctic Echinidae are represented by

only five species all within a single genus, *Sterechinus* (Pawson, 1969). *S. neumayeri* has a known planktotrophic larva (Pearse and Giese, 1966), of the remaining four species the mode of development is not known, although Emlet *et al.* (1987) suggested, based on egg size, that either planktotrophic or lecithotrophic development remain a possibility.

The prevalence of brood protection within the Antarctic cidarids is well documented (Pawson, 1969). However, to date very little work has been carried out on the reproductive biology of this group. Many cidarids brood their young on the peristome using their primary spines to hold the juveniles in place. Of the 10 species of cidarid urchin described by Lockhart *et al.* (1994) from Prydz Bay, Antarctica, individuals of 4 species were brooding juveniles. A maximum brood size of 535 juveniles was recorded for on one specimen of *Ctenocidaris* sp.; 2 specimens of *Ctenocidaris geliberti* were carrying broods of 46 and 50 embryos; 2 specimens of *Notocidaris remigera* had brood sizes of 96 and 140 juveniles; and a single specimen of *Notocidaris gaussensis* carried 11 juveniles. Both embryos and juveniles were carried in the sunken peristomal cavity of the female adult urchins. In each of the four species juveniles from the same brood were all of a similar size and at the same stage of development. However no data exist for their gametogenic cycles, times of spawning or reproductive synchrony.

In most species of the Antarctic spatangoid genera *Abatus, Amphipneustes* and *Tripylus* (Mortensen, 1951) the lateral petaloid ambulacra are deeply sunken as marsupia in which the young are brooded. The reproductive biology of three spatangoid irregular urchins has been studied from Antarctic waters, including *Abatus cordatus* (Magniez, 1980, 1983; Schatt, 1988; Schatt and Féral, 1991), *Abatus shackletoni* and *Abatus nimrodi* (McClintock and Pearse, 1988; Pearse and McClintock, 1990). Of these species, annual gametogenic and brooding cycles were established for *A. cordatus*. The brooding cycle of *A. cordatus* lasted for 8.5 months, from the end of March to the beginning of December. Whilst this cycle was synchronous for populations situated within the Golfe du Morbihan, a the spawning period was displaced by six months at the deeper (~50m) and open-sea intertidal site. This evidence suggested that the timing of these cycles was linked to the availability of trophic resources rather than depth (Schatt and Féral, 1991). In contrast to *A.*

cordatus, Pearse and McClintock (1990) found no evidence of seasonality in gonad cycle or stage of brood juvenile in either *A. shackletoni* or *A. nimrodi*. In both these species a continuous reproductive strategy has been suggested.

Table 3.1. Reproductive periodicity and egg size in Antarctic shallow-water and temperate deep-sea echinoderms. PS= Porcupine Seabight; PAP= Porcupine Abyssal Plain. Length of oogenesis is referred to as (Cycle); modes of development are listed as either AL = abbreviated lecithotrophy; B = brooding; DD = direct development; DL = direct lecithotrophic; L = lecithotrophic; P = planktotrophic. Periodicities are listed as either: A = aseasonal; S = seasonal; P = periodic.

Species	Location	Depth (m)	Fecundity	Egg size	Mode	Perio-dicity	Cycle months	Reference
Antarctic shallow-water								
ASTEROIDEA								
Neosmilaster georgianus	Anvers Island	2-15		2170	B	A		Bosch & Slattery, 1999
Diplasterias brucei	McMurdo	<33	300	2800	B	A		Bosch & Pearse, 1990
Notasterias armata	McMurdo	<33	50	3500	B	S		Bosch & Pearse, 1990
Acodontaster elongatus	McMurdo	<33	$3\text{-}4x10^3$	540	L	S		Bosch & Pearse, 1990
Porania sp.	East Cape Evans	15-33	100-310	550	L	A		Bosch, 1989; Bosch & Pearse, 1990
Acodontaster hodgsoni	McMurdo	<33		550	L	S		Bosch & Pearse, 1990
Acodontaster conspicuus	McMurdo	<33	$3\text{-}4x10^3$	700	L	S		Bosch & Pearse, 1990
Bathybiaster loripes	McMurdo	<33		930	L	S		Bosch & Pearse, 1990
Psilaster charcoti	McMurdo	<33		950	L	S		Bosch & Pearse, 1990
Lophaster gaini	McMurdo	<33	$3\text{-}4x10^3$	1280	L	S		Bosch & 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 & Pearse, 1990
Porania antarctica	New Harbor	15-33	$3\text{-}4x10^4$	550	P	S		Bosch, 1989; Bosch & Pearse, 1990
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
ECHINOIDEA								
Abatus nimrodi	McMurdo	shallow	30	1280	B	A		Pearse & McClintock, 1990
Abatus cordatus	Kerguelen	intertidal		1300	B	S	24	Magniez, 1983
Abatus shackletoni	McMurdo	shallow	30	1970	B	A		Pearse & McClintock, 1990
Sterechinus neumayeri	McMurdo	1-3		125	P	S	18-24	Pearse & Giese, 1966
	Rothera	15-30		120	P	S	18-24	Brockington, 2001
OHIUROIDEA								
Ophiosparte gigas				350	L			Pearse 1994
Ophionotus victoriae	Circumpolar (Rothera)		140000	180	P	S	18-24	Pearse, 1994; Mortensen, 1936
Temperate deep-sea								
ASTEROIDEA								
Hymenaster membranaceus	Rockall Trough	2200		1100	DL	A		Pain *et al.*, 1982a
Hymenaster gennaeus	Rockall Trough	2200		1100	L	A		Pain *et al.*, 1982a
Pontaster tenuispinus	Rockall Trough	523-2000		800	DD	A		Pain *et al.*, 1982b

Species	Location	Depth (m)	Fecundity	Egg size	Mode	Perio-dicity	Cycle months	Reference
ASTEROIDEA continued								
Pectinaster filholi	Rockall Trough	1752-2909		850	DD	A		Pain *et al.*, 1982b
Benthopecten simplex	Rockall Trough	2200		950	DD	A		Pain *et al.*, 1982b
Bathybiaster vexillifer	Rockall Trough	2200		1000	DL	A		Tyler *et al.*, 1982
Psilaster andromeda	Rockall Trough	1050-2170	low?	950	L	A		Tyler & Pain 1982
Plutonaster bifrons	Rockall Trough	1600-2965	10^6	120	P	S	12	Tyler & Pain 1982
Dytaster grandis	Rockall Trough	2200, 2900		120	P	S	12	Tyler & Pain 1982; Tyler *et al.*, 1990
ECHINOIDEA								
Poriocidaris purpurata	Rockall Trough	631-2906		1500	DD	A		Tyler & Gage, 1984b
Cidaris cidaris	Rockall Trough	631-2905		110	P	A?		Tyler & Gage, 1984b
Echinus affinis	Rockall Trough	2200		110	P	S	13-14	Tyler & Gage, 1984a
Echinus alexandri	Rockall Trough	2200		110	P	S	13-14	Tyler & Gage, 1984a; Tyler *et al.*, 1984
Echinus acutus norvegicus	W. Scotland	500-1271		110	P	S	12-14	Gage *et al.*, 1986
Echinus elegans	W. Scotland	500-1271		110	P	S	12-14	Gage *et al.*, 1986
Phormosoma placenta	Rockall Trough	631-2900	low	1100	DL	A		Tyler & Gage, 1984b
Sperosoma grimaldi	Rockall Trough	631-2900	low	1100	DL	A		Tyler & Gage, 1984b
Hygrosoma petersi	Rockall Trough	631-2900	low	1150	DL	A		Tyler & Gage, 1984b
Calveriosoma hystrix	Rockall Trough	631-2900	low	1250	DL	A		Tyler & Gage, 1984b
Araeosoma fenestratum	Rockall Trough	631-2900	low	1250	DL	A		Tyler & Gage, 1984b
Stylocidaris lineata	N. Bahamas	510-640		120	S	S	12	Young *et al.*, 1992
HOLOTHUROIDEA								
Laetmogone violacea	PS	960-1506		400	AL	A		Tyler *et al.*, 1985a
Benthogone rosea	N.E. Atlantic	1387-2120		750	AL	A		Tyler *et al.*, 1985a
Cherbonniera utriculus	Rockall Trough	2878-4050	100	200	L	P		Tyler *et al.*, 1987
Molpadia blakei	Rockall Trough	2878-4050		200	L	P		Tyler *et al.*, 1987
Bathyplotes natans	PS	1080-1694		280		A		Tyler *et al.*, 1994
Peniagone azorica	NE Atlantic	2220-2900		300	AL	A		Tyler *et al.*, 1985b
Peniagone diaphana	NE Atlantic	3700-4700		300	AL	A		Tyler *et al.*, 1985b
Ypsilothuria bitentactulata	Rockall Trough	2175-2907	<50	350	L	A		Tyler & Gage, 1983
Amperima rosea	PS	4800	12,800	200	P/L?	P		Wigham *et al.*, 2003
Benthodytes sordida	PAP	3680-4515	260-4000	>1000	DD	A/P?		Tyler & Billett, 1987
Psychropotes longicauda	PAP	4043-4795	8-250	~2000	DD	A/P?		Tyler & Billett, 1987
Psychropotes depressa	PS	2405-2440	250-5000	>750	DD	A/P?		Tyler & Billett, 1987
Psychropotes semperiana	PAP	5432-5440		3000	DD	A/P?		Tyler & Billett, 1987
Deima validum	PAP	4080-4795		700	DD	A/P?		Tyler & Billett, 1987
Oneirophanta mutabilis	PS	2900-4795		950	DD	A/P?		Tyler & Billett, 1987
OPHIUROIDEA								
Ophiomusium lymani	Rockall Trough	2200	12,000	460	L	A		Gage & Tyler, 1982
Ophiura ljungmani	Rockall Trough	2900		120	P	S	8-10	Tyler *et al.*, 1982

Of the five species of *Sterechinus* the reproductive biology of one species, *Sterechinus neumayeri*, has been described in some detail (Pearse and Geise, 1966; Bosch *et al.*, 1987; Brey *et al.*, 1995; Stanwell-Smith and Peck, 1998; Marsh *et al.*, 1999; Tyler *et al.*, 2000; Brockington, 2001). This is perhaps owing to its wide distribution in shallow waters and ease of collection by SCUBA divers, in comparison

with other congeneric species. The reproductive biology of *S. neumayeri* is relatively consistent throughout the three different sampling locations, McMurdo Sound (Pearse and Geise, 1966), Signy Island (Stanwell-Smith and Peck, 1998) and Rothera Research Station (Brockington, 2001). *S. neumayeri* has a discrete reproductive periodicity; oocyte development taking 18-24 months to completion with populations spawning during the austral spring months. The larvae of *S. neumayeri* are planktotrophic, and benefit from a spring spawning as the presence of feeding larvae coincides with the spring phytoplankton bloom in the water column.

To date very little is known of the reproductive biology of holothurians in the Southern Ocean. Two species of dendrochirote holothurians; *Ekmocucumis steineni* and *Psolus dubiosus* have been investigated on the Weddell Sea Antarctic shelf (Gutt *et al.*, 1992). Seasonal differences were observed in the relative female fecundity and gonad weight for *E. steineni*, inferring a seasonal gametogenic cycle. Conversely, no seasonal pattern was found in *P. dubiosus*, which is a brood protecting species. Although the guts of both species were filled with more organic matter in the autumn than in the spring, the occurrence of mature oocytes in *E. steineni* was not directly linked with food availability.

The deep-sea environment

In terms of reproduction, the echinoderms are the best-studied group of the deep-sea benthic invertebrate taxa (Young, 2003). The reproductive biology of a wide variety of deep-sea echinoderms from the northeast Atlantic has been examined (see Table 3.1) from samples collected by the Scottish Marine Biological Association at their permanent stations in the Rockall Trough. Analysis of the data from these studies revealed that the reproductive biology of deep-sea echinoderms could be categorised into one of the following three groups, based on egg size, fecundity and apparent mode of development (Tyler, 1986).

1. Quasi-continuous

Quasi-continuous reproduction is the predominant reproductive pattern found in deep-sea echinoderms. This strategy involves the production of a relatively small number of large eggs (600 – 4400 μm diameter) at a continuous rate throughout the year. Such

large egg sizes suggest direct development, so that the zygote develops directly into a juvenile before settling to the benthic population. In these species oocyte size-frequency distributions are heavily biased towards the smaller oocyte diameters (<300 μm), indicating that females have a reserve of small previtellogenic oocytes at all times of the year. Some of these small oocytes undergo vitellogenesis by laying down yolk in the cytoplasm to reach maximum size. In some asteroid species there is evidence to suggest that some small previtellogenic oocytes degenerate or take up materials by phagocytosis, acting as nurse cells, to provide energy for maturing oocytes (Tyler *et al.*, 1982).

2. Seasonal spawning

Despite the early paradigm that pelagic development was an unlikely strategy in the deep-sea (Thorson, 1950, Mileikovsky, 1971), and predictions that reproductive efforts should be aperiodic based on the constant thermal conditions of the deep-sea (Orton, 1920), evidence for seasonally reproducing deep-sea invertebrates came to light in the late 1960s and '70s (Schoener, 1968; Rokop, 1977). Following the comprehensive work carried out by Tyler and co-workers during the 1980s and 90s seasonal reproduction and the production of planktotrophic larvae has been demonstrated for several deep-sea species of echinoderm. These include *Ophiura ljungmani* (Tyler *et al.*, 1982), *Ophiocten gracilis* (Sumida *et al.*, 2001), *Plutonaster bifrons* (Tyler and Pain, 1982), *Dytaster grandis* (Tyler and Pain, 1982, Tyler *et al.*, 1990), and *Echinus affinis* (Tyler and Gage, 1984b). Typically, seasonal breeders are characterised by a small maximum egg size (~100-200 μm diameter), high fecundity, synchrony of gametogenesis and a seasonal development cycle. The reproductive condition of individuals revealed that initiation of oogenesis occurred in March-April with the most active vitellogenesis occurring during the summer and spawning taking place during January-April each year (Tyler, 1988). The main input of organic material to the northeastern Atlantic sea floor occurs in late May-August (Lampitt, 1985) and Tyler (1986, 1988) proposed that this material might provide the energy required for vitellogenesis in the summer months.

3. Intermediate egg size

The last of the three patterns of reproduction in deep-sea environments is also the least common. Mature vitellogenic oocytes are of intermediate size (~420 μm) and adult females have an intermediate fecundity (12,000 eggs per individual: Gage and Tyler, 1982). These characteristics are indicative of a lecithotrophic mode of development (Tyler, 1986). Although development may be pelagic no seasonality of oocyte production has been observed although in *Ophiomusium lymani* there is seasonality in recruitment to the adult population (Gage and Tyler, 1982)

This Study

The present investigation presents the results of a study of the gametogenic cycles of six species of echinoderm living at 600 m depth on the west Antarctic Peninsula continental shelf. Specimens were collected on 5 sampling occasions during a sixteen-month study. The six species are from five different orders of Echinodermata and cover a wide range of reproductive strategies. These species include: two elasipod holothurians, *Protelpidia murrayi* and *Peniagone* sp.; one asteroid, *Psilaster charcoti*; and three species of echinoid, *Sterechinus antarcticus*, *Ctenocidaris perrieri* and *Amphipneustes lorioli*. Very little is known of the biology of these species (Chapter 2) and no such studies have yet been carried out on the unusually deep Antarctic continental shelf. It was the aim of this investigation to examine the reproductive biology of these six echinoderms, and specifically to test the following hypotheses:

1. The gametogenic cycles of benthic invertebrates on the Antarctic continental shelf are controlled by the arrival of a seasonal pulse of phytodetritus.
2. Gamete release is related to the timing of this pulse.
3. Fecundity and reproductive effort are related to the intensity of the pulse.

In terms of deep-sea sampling this study benefits from a relatively high frequency of sampling events, taken at regular periods throughout several consecutive seasons.

Materials and methods

Sample collections

Specimens were collected from two locations (site A and C, Figure 1.1) at ~600m depth on the continental shelf west of the Antarctic Peninsula. Samples were obtained during the FOODBANCS cruise programme (Chapter 1) over a period of sixteen-months, on five different cruises to the Antarctic Peninsula; 20th November to 12th December 1999, 9th to 19th March 2000, 9th to 19th June 2000, 24th to 31st October 2000 and 24th February to 8th March 2001. An eighteen-foot otter trawl was used to collect samples at depths ranging from 400 to 600 m. The trawl catch was washed and sorted on deck using a purpose-built sieving table. This was a three-tiered design, each tier consisting of a wooden frame supporting a galvanised steel mesh (three mesh sizes: 3 cm, 1 cm and 4 mm). Approximately 20 individuals (where available) of each species were stored in an aquarium awaiting dissection onboard the research ship. Table 3.2 lists the number of each species collected from each cruise. Animals were dissected and all of the tissues removed. For histological purposes, gonad tissues were fixed in 8% seawater-buffered formalin for 24 hours, and then transferred to tubes of 70% alcohol for storage.

Histological procedures

In the laboratory the gonads were weighed prior to processing, for future gonad index analysis. Reproductive cycles were assessed histologically for 6 female and 4 male specimens (where available) from each cruise and site. The tissue was first immersed in 95% alcohol for 24hrs, and then in absolute alcohol for 2hrs, with two subsequent alcohol changes each after 2hrs to ensure effective dehydration. A wax solvent (histoclear or xylene) was used to replace the alcohol, as alcohol and wax are not miscible. Most solvents have the effect of raising the refractive index of tissue, which makes them appear clear and this stage is consequently known as clearing. Tissues were lightly blotted during transfer from one reagent to the next to remove any excess of the previous reagent. Once in histoclear the tissues were left for at least 8 hours. The tissue was blotted lightly with absorbent paper and then transferred from the clearing agent to molten paraffin wax where it was left to impregnate for around 8 hours. The tissue was then set in a wax block in preparation for sectioning.

SPECIES	Site	Nov –Dec 1999	March 2000	June 2000	October 2000	Feb -Mar 2001
Protelpidia murrayi	A	20	20	16	20	20
	C		18	20	20	20
Peniagone sp.	A	20	20	20	20	20
	C	20	24	20	20	20
Psilaster charcoti	A		10	4	18	10
	C		19	8	16	20
Sterechinus antarcticus	A					
	C	12	22	21	25	20
Ctenocidaris perrieri	A		16	7	12	15
	C	20	20	20	20	20
Amphipneustes lorioli	A	8	21	7	10	15
	C					

Table 3.2. Location of sampling sites of the six species of echinoderm, showing date of cruise and the number of individuals collected from each site.

Prior to cutting, the wax blocks were chilled in iced water to facilitate sectioning. Fine (7 μm) sections of each gonad were cut from the wax blocks using a rotary microtome. These sections were then floated in a water bath (40°C) to remove the creases. A clean slide was half submerged in the water to remove the wax section. The mounted sections were left on a drying rack at 37°C over night to dry, prior to staining. Gonad sections were taken for analysis of oocyte size frequency and for calculation of actual fecundity of each individual. The method for estimating the actual fecundity involved sectioning the ovary at a distance equal to that of the average vitellogenic egg diameter for each species (*Protelpidia murrayi* 200 μm and *Peniagone* sp. 305 μm, *Psilaster charcoti* 370 μm, *Sterechinus antarcticus* 150 μm,). The average vitellogenic oocyte size was used under the assumption that the majority of oocytes would thereby only be counted once. Oocytes larger than the average size would be counted twice and oocytes smaller than average diameter would be missed between sections balancing out the total (Pendlebury, 2002). The echinoid species *Ctenocidaris perrieri* and *Amphipneustes lorioli* had such large eggs (>1000 μm) that if they were sectioned every average diameter not enough oocytes would be sectioned through the nucleus to measure 100 oocytes for oocyte size frequency distributions.

To measure oocyte size frequency it was therefore necessary to use the same sections cut for fecundity as not enough individuals were sampled from each cruise to enable to the use of separate individuals. The gonads of *Ctenocidaris perrieri* and *Amphipneustes lorioli* were sectioned every 200 μm to ensure that enough sections were taken from the gonad to measure 100 oocytes. Each vitellogenic egg was identified and assigned a number that was marked on each section throughout the gonad on which that egg appeared. This ensured that each oocyte was only counted once, and on the section displaying the maximum diameter.

Staining

The slides were transferred through a series of different stains, starting with removal of the wax in Histoclear™ for 2 min. Hydration of the tissue with graded alcohol started in 100% alcohol for 1min and then 70% alcohol for 1 min. The slides were transferred to haematoxylin and left for 5 min. After draining off excess haematoxylin, slides were transferred to a slide washing tray and washed in running water for 15 min until sections were blue and no further run off was observed. Slides were then transferred to 1% eosin for 2-4 min and subsequently rinsed in the slide-washing tray. The tissue was then rehydrated from 70% alcohol for 2 min, to 100% alcohol for 2 min, and then cleared in Histoclear™ for a further 2 min. Excess Histoclear™ was wiped off the slides, one or two drops of DPX were placed on the section, and then the cover slip applied.

In the female gonad the oocytes were divided into two groups, previtellogenic oocytes and vitellogenic oocytes. When stained with Haemalum and Eosin the previtellogenic oocytes were characterised by a large, dark stained nucleus, which occupied most of the smooth basophilic cytoplasm. In contrast, the vitellogenic oocytes were characterised as large cells with a granular cytoplasm and a smaller cytoplasm/nucleus ratio.

Image analysis

Sections of the gonad slides were examined under a dissecting microscope (Leica MZ-8) and a compound microscope (Olympus BH-2). Microscope images of the gonads were transferred to bitmap image files using a JVC video camera (TK 1280E)

and Matrox-Rainbow Runner™ PC-VCR software. In order to analyse patterns of oogenesis, the eggs within the female gonad sections were measured and calibrated using Jandel Scientific SigmaScanPro™ 4 image analysis software.

To ensure that maximum oocyte diameter was recorded and that each egg was only measured once, only eggs that had been sectioned through the nucleus were measured. The feret diameter of each oocyte was chosen for measurement. The measure of feret diameter gives the diameter of a fictitious circular object that has the same area as the object being measured. Where possible a minimum of 100 eggs per female was measured. As a precaution against heterogeneity of development within an ovary oocyte sizes were measured from at least two sections of ovary. The oocyte measurements were grouped into 100 μm, 40 μm or 20 μm size classes depending on the oocyte size range. These size frequencies were then converted into percentage size frequencies for each individual. Histograms were constructed of oocyte percentage size frequency for each species at different sites and cruises. The bars represent the mean percentage frequency for all individual replicates at each site/cruise and error bars of ±StDev represent individual variability.

Gonad indices were calculated for each species, except *Protelpidia murrayi* for which the single gonad was arbitrarily divided into two and the weight of one portion was not considered an accurate measurement of half of the gonad. Variation in test diameter and animal length in adults will have an effect on the gonad mass. It is therefore necessary to correct for differing adult sizes by converting each individual animal size (test diameter or animal length) to that of a standard size chosen for each species (the standard animal size being the mean animal size of all specimens studied). Individual scaling relationships were established for each species, in Minitab12, and observed data for each individual were then converted to that estimated for a standard animal. This scaling correction for size was undertaken using a General Linear Model and $\log_e$-transformed data. This allowed a test for a linear relationship of gonad mass with animal size, and simultaneously test for differences between sex, cruises and sites (Table 3.2). Data for both variables were $\log_e$ transformed to ensure that they met with the assumptions of normal distribution for a regression analysis. Where necessary data were split into sex, cruise or site. A linear

regression provided an estimate of the scaling exponent (the slope). Size correction was then carried out using the following equation:

$$\log_e (Sgm) = [\text{slope} \times [\log_e (Ss) - \log_e (Os)]] + \log_e (Ogm) \quad (1)$$

$$Sgm = \exp (\log_e(Sgm)) \quad (2)$$

Where Sgm = standard gonad mass (g), Ss = standard animal size (mm), Os = observed animal size (mm), Ogm = observed gonad mass (g).

Variable	Standard animal size (mm)	*b*	r^2	SD	*F*	*n*
Peniagone sp.	74.4					
GM Cruise 1		1.9457	0.529	0.4325	20.24	19
GM Cruise 2		1.6563	0.561	0.2409	47.29	38
GM Cruise 3		2.7934	0.515	0.4461	39.22	38
GM Cruise 4		2.2098	0.248	0.6810	10.53	33
GM Cruise 5		3.2088	0.435	0.6269	26.20	35
Psilaster charcoti (Cruises 2-5)	109.5					
GM Site A		7.094	0.506	1.2580	31.81	32
GM Site C		4.3146	0.358	0.7656	31.76	58
Fecundity		6.401	0.501	1.5970	16.06	17
Sterechinus antarcticus (Cruises 1-5)	38					
GM Female		3.9985	0.729	0.4310	86.05	33
GM Male		3.3936	0.629	0.3843	77.99	47
Fecundity		4.760	0.643	1.122	18.00	11
Ctenocidaris perrieri (Cruises 1-5)	46.7					
GM Female		4.5709	0.432	0.6455	50.15	67
GM Male		3.1859	0.425	0.4747	45.04	51
Amphipneustes lorioli (Cruises 2-5)	65.7					
GM		2.6812	0.160	0.9143	8.60	46
Fecundity		2.6603	0.298	0.9372	8.06	20

Table 3.3. Scaling exponents relating animal size to gonad mass (GM). Regression relationships all had P values < 0.05).
The Gonad Index was expressed as corrected gonad mass / corrected animal size and plotted against season to represent the average reproductive state of the population.

In addition the mean sizes of previtellogenic and vitellogenic oocyte size classes were calculated for each individual and the mean of means calculated for each population. These data were plotted for both sites A and C against date for each species. The mean fecundity was calculated for October 2000 (pre-bloom) site A and February/March 2001 (mid-bloom) at sites A and C. It was also necessary to standardise these data to that of a mean animal size as there is a positive relationship between fecundity and animal size; the same procedure was used as above (Table 3.3).

Results

Protelpidia murrayi (Holothurian)

External gonad morphology

Protelpidia murrayi had a single gonad located in the anterior part of the coelom. The gonoduct travelled the length of the gonad, branching into numerous tubules at various intervals (Figure 3.1). The gonad wall of the ovary was transparent revealing the orange developing oocytes inside the rounded end tubules. The testes were white in colour and the tubules had a feathery appearance.

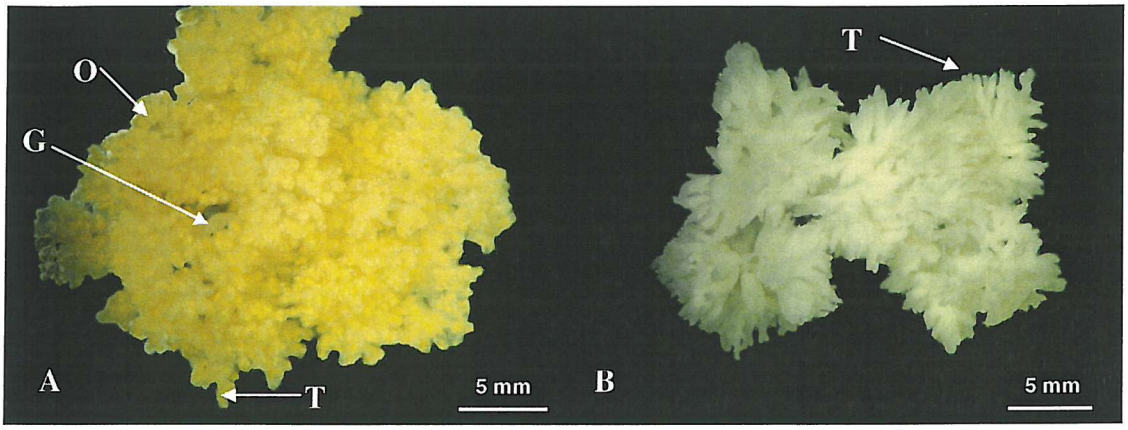

Figure 3.1. External gonad appearance of *Protelpidia murrayi* (**A**) Female, (**B**) Male. G = gonoduct; O = oocyte; T = tubule.

Gametogenesis in *Protelpidia murrayi*

Oocyte development. The structure of the ovary wall in *Protelpidia murrayi* comprised three components; an outer wall covered by coelomic epithelium; an inner wall that was covered by germinal epithelium; and a connective tissue layer between the inner and outer wall (Figure 3.2A). Oocyte development took place along and within the inner wall, with oocytes developing in cavities in the connective tissue. Previtellogenic oocytes developed up to a diameter of 105 µm; they had a large nucleus and uniform cytoplasm. At oocyte diameters greater than 105 µm, the cytoplasm became vacuolated suggesting that vitellogenesis was taking place (Figure 3.2B). Vitellogenesis involved the laying down of yolk granules in the cytoplasm and during this process the cytoplasm became granular in appearance. Vitellogenic oocytes reached a maximum diameter of 357 µm. It appeared that the largest vitellogenic oocytes did not retain their cytoplasm during the histological process. In these cases the cytoplasm appeared empty, with areas of vacuolated cytoplasm

remaining around the edges of the oocyte or as patches in the centre suggesting dissolution of lipid during processing. Both previtellogenic and vitellogenic oocytes were surrounded by a thin layer of accessory cells during their development (Figure 3.2 B). Many of the large vitellogenic oocytes were misshapen or appear crenulated; this was either as a result of the fixative process or the dehydration that occurred during the histology procedure.

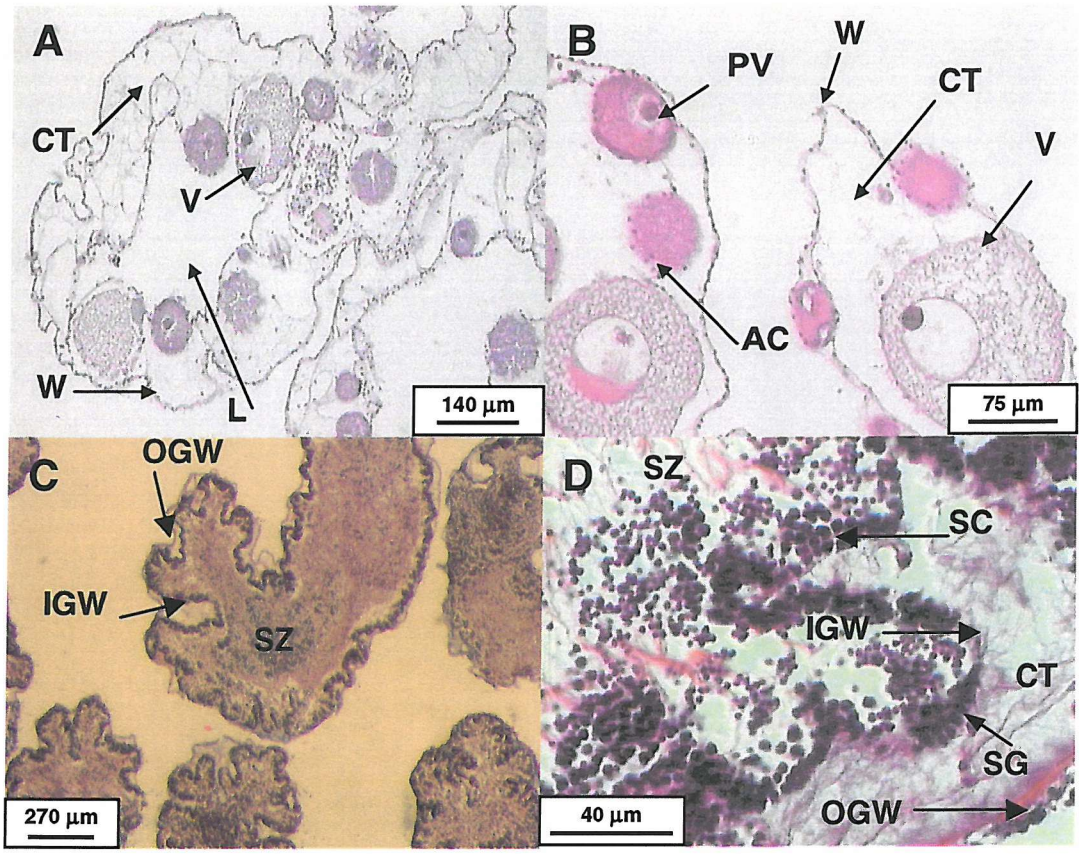

Figure 3.2. Light histology sections of the ovary (**A** and **B**) and testes (**C** and **D**) of *Protelpidia murrayi* stained with haematoxylin and eosin. AC = accessory cells; CT = connective tissue; IGW = inner gonad wall; L = lumen; OGW = outer gonad wall; PV = previtellogenic oocyte; SC = spermatocytes; SG = spermatogonia; SZ = spermatozoa; V = vitellogenic oocyte; W = ovary wall.

Sperm development. The testes wall was similar to that of the ovary and comprised an outer wall, connective tissue layer and an inner wall (Figure 3.2D). The inner wall had many infoldings that extended into the lumen of the testes. Spermatogonia (mean diameter 4.28 ± 0.1 µm) and spermatocytes (mean diameter 3.67 ± 0.32 µm) lined this inner sac (Figure 3.2D). The spermatocytes divided by meiosis and gave rise to spermatids (mean diameter 2.06 ± 0.18 µm). These spermatids subsequently differentiated into spermatozoa (mean head diameter 2.16 ± 0.14 µm) and filled the

lumen of the testes (Figure 3.2C). Spermatozoa were present in the testes lumen during all of the sampling dates, with the exception of June 2000 where testes were part or fully spawned.

Reproductive output

Gonad indices

Gonad indices were not calculated for *Protelpidia murrayi* because the nature of the sampling meant that the single gonad had to be divided in two in order to retain part for CHN analysis (Chapter 4). Division of the gonad into two portions did not always yield two equal portions, and weighing one section (the other being frozen for CHN analysis) would therefore give meaningless estimates for gonad indices analysis.

Fecundity

The division of the gonad into two portions also affected the accuracy of fecundity measurements as the two portions were not always of equal sizes. Estimates of fecundity were made for one portion of the gonad and multiplied by two to give an estimate of the whole gonad. This method resulted in a high within-sample variation most likely caused by the inaccuracy of splitting the gonad in half. Values ranged between 2, 076 and 30,998 with an overall mean of 13,584 oocytes per individual.

Mean oocyte size

Mean oocyte sizes for the five samples at sites A and C were presented in Figure 3.3. Data for previtellogenic and vitellogenic oocytes were plotted separately. There was a significant difference in mean vitellogenic oocyte sizes over the sampling period (Kruskall-Wallis site A, H=10.61, 4df, P=0.031; site C, H=19.13, 4df, P=0.001). Peak values of mean vitellogenic oocytes occurred during March of each year and minimum values occurred during October 2000. Previtellogenic oocytes exhibited a relatively constant value throughout the year, in the range of ~90 μm, with the exception of November/December 1999 where mean values were as low as 60 μm.

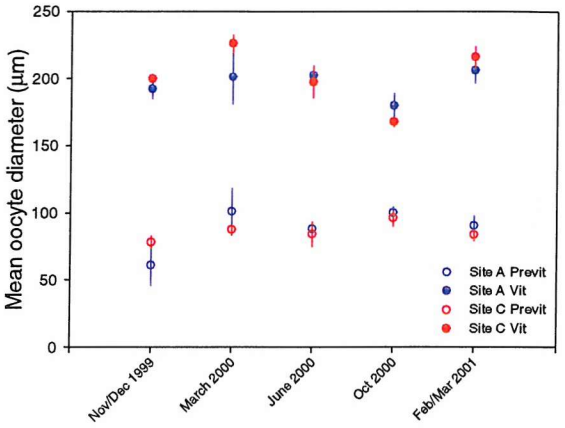

Figure 3.3. Seasonal variation of mean oocyte diameter of previtellogenic and vitellogenic oocytes for *Protelpidia murrayi*.

Oocyte size-frequency distributions

Spatially there were few differences in oocyte size-frequency distributions between sites A and C (Figure 3.4). On a temporal basis there were changes in the oocyte size-frequency distributions throughout the sampling period. The distribution of oocytes during November/December was almost exclusively (95%) comprised of previtellogenic oocytes (<140 µm) (Figure 3.3 and 3.4). However, by March 2000 the proportion of previtellogenic oocytes had significantly decreased and a new cohort of vitellogenic oocytes accounted for 35% of the oocytes. This value of vitellogenic oocytes halved during June and October 2000, but did not get as low as the previous November. A new cohort of vitellogenic oocytes was present in February/March 2001 and proportions reached as high as 43% of total oocytes. These data suggested that vitellogenic oocytes reached maturity during February/March. Spawning may have commenced some time between March and June, with minimum values of vitellogenic oocytes recorded during November/December. Examination of individual plots of oocyte size-frequency for each sampling period indicated synchrony of oogenesis between individuals of *Protelpidia murrayi* within each sample (Figure 3.5, Appendix 1). It is important to note these observations were made from relatively low sampling frequencies typical of deep-sea research; a monthly sampling programme would have given much higher resolution results.

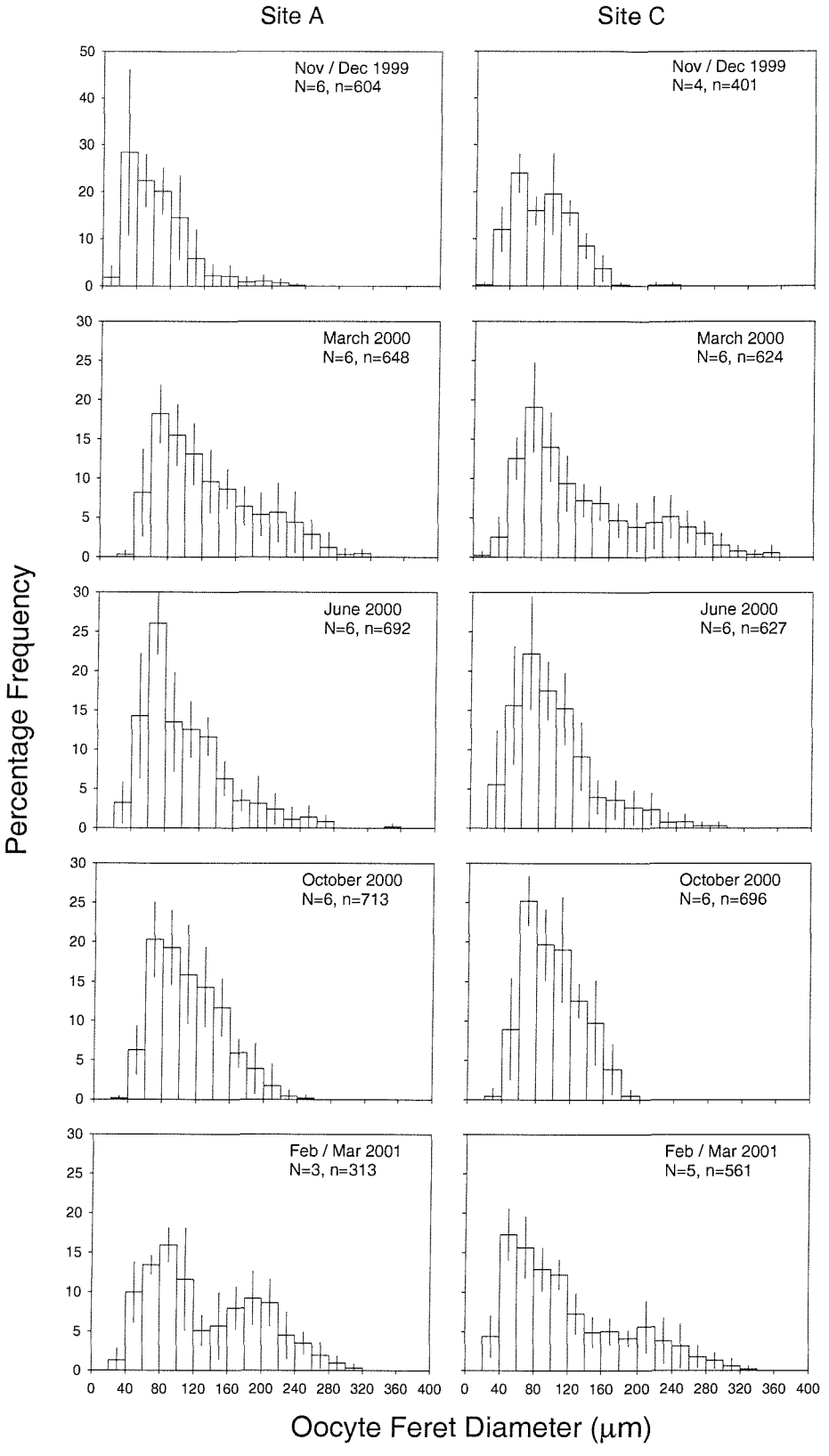

Figure 3.4. Oocyte size-frequency distribution for *Protelpidia murrayi*. Spatial and temporal variability from November/December 1999 – February/March 2001. Mean percentage frequency ± St Dev. N = number of females; n = number of oocytes. Note different scales for some months; scales match on adjacent graphs.

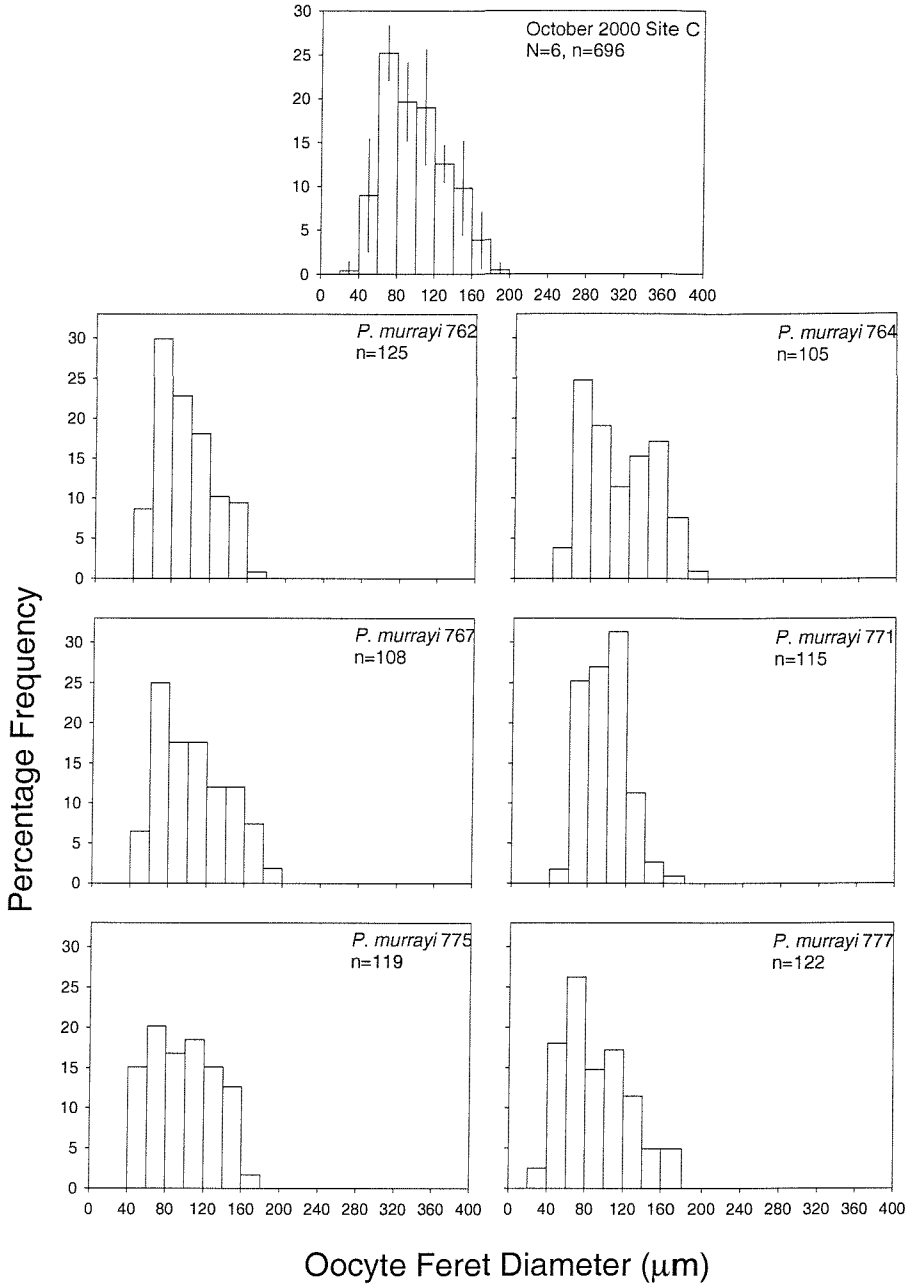

Figure 3.5. Mean oocyte size-frequency distribution for *Protelpidia murrayi* October 2000 Site C (top centre), and individual oocyte size-frequency distributions for October 2000 Site C. *P. murrayi* 696-777 = individual reference numbers. N = number of females; n = number of oocytes.

Population Size-Frequency Distributions

Population size-frequency distributions for *Protelpidia murrayi* were only determined during the February/March 2001 cruise. However, the two trawls allowed assessment of the spatial variability in the population size distribution between sites A and C on the Antarctic continental shelf. Specimens ranged in size from 10.0 to 110.0 mm, with a distinctly bimodal distribution (Figure 3.6). At site A modal body length peaks were in the range 20-30 mm and 55-60 mm. The positions of the two peaks at site C were

similar to that of site A, with modal body length peaks occurring at 25-30 mm and 55-65 mm. The most striking difference between the two distributions was the proportion of individuals that fell into each of the two size classes. The population at site A was dominated by individuals from the smaller size class (>87%), where as the population at site C was dominated by individuals from the larger size class (~63%). At site A only two individuals recorded lengths exceeding 75mm, and at site C the largest individual measured 80 mm.

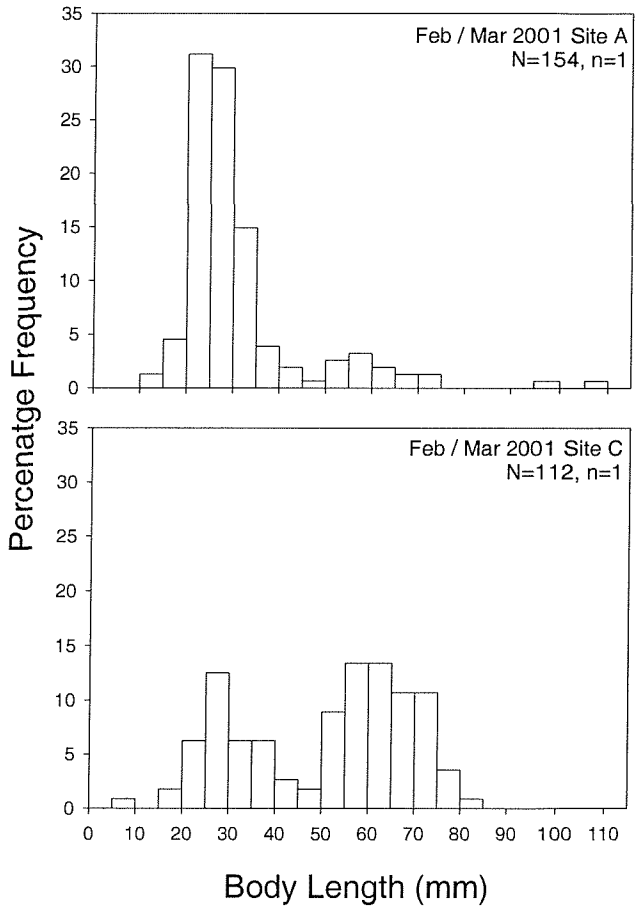

Figure 3.6. Body length size-frequency distributions for *Protelpidia murrayi*. Spatial variation in February/March 2001.
N = number of specimens measured; n = number of trawls.

Peniagone sp. (Holothurian)

External gonad morphology

The gonopore of *Peniagone* sp. was situated on the mid-dorsal line of the ventral inter-radius. From the gonopore the gonoduct proceeded through the mesentery into the coelom where the gonoduct divided into two, forming two gonad tufts. The gonoduct ran down each of the gonad tufts, branching at regular intervals. Each branch gave rise to a nodule of branched tubules in which either sperm or oocytes developed. The tubules of the ovary were rounded at the ends and developing oocytes could been seen through the transparent ovary wall (Figure 3.7A). Oocyte pigmentation gave the ovary an orange appearance. The testis was also a branched structure, which appeared more defined than the ovary as the tubules were more slender in shape. The testis looked white in colour when packed with spermatozoa (Figure 3.7B).

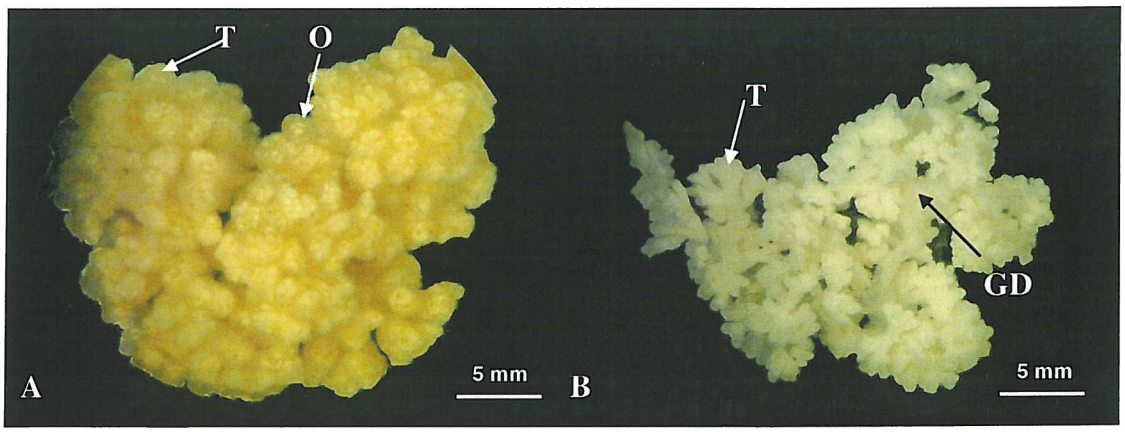

Figure 3.7. External gonad appearance of *Peniagone* sp. (**A**) Female, (**B**) Male. GD = gonoduct; O = oocyte; T = tubule.

Gametogenesis in *Peniagone* sp.

Oocyte development. In *Peniagone* sp. the ovary wall was similar in structure to that of *Protelpidia murrayi*. The ovary wall comprised three components; the outer wall covered by coelomic epithelium; the inner wall that was covered with germinal epithelium; and a layer of fibrous connective tissue between the outer and inner wall that varied in thickness. Oocyte development appeared to take place inside the gonad wall; oocytes of varying sizes developed in cavities within the wall. Previtellogenic oocytes had a large nucleus with eccentric nucleolus and a smooth basophilic cytoplasm, which stained purple with haematoxylin (Figure 3.8B). At sizes of 130 µm

diameter previtellogenic oocytes became less basophilic and stain a lighter colour and the cytoplasm became vacuolated suggesting the onset of active vitellogenesis. At diameters of ~220 µm the oocyte cytoplasm became smooth acidophilic staining pink with eosin. These vitellogenic oocytes reached maximum diameters of 570 µm (Figure 3.8A). Developing oocytes embedded in shallow cavities in the ovary wall were covered with a cellular layer continuous with the inner lining of the wall. Those oocytes not spawned appeared to undergo phagocytic breakdown; numerous phagocytes were found within these oocytes and the cytoplasm degenerated whilst the membrane remained intact.

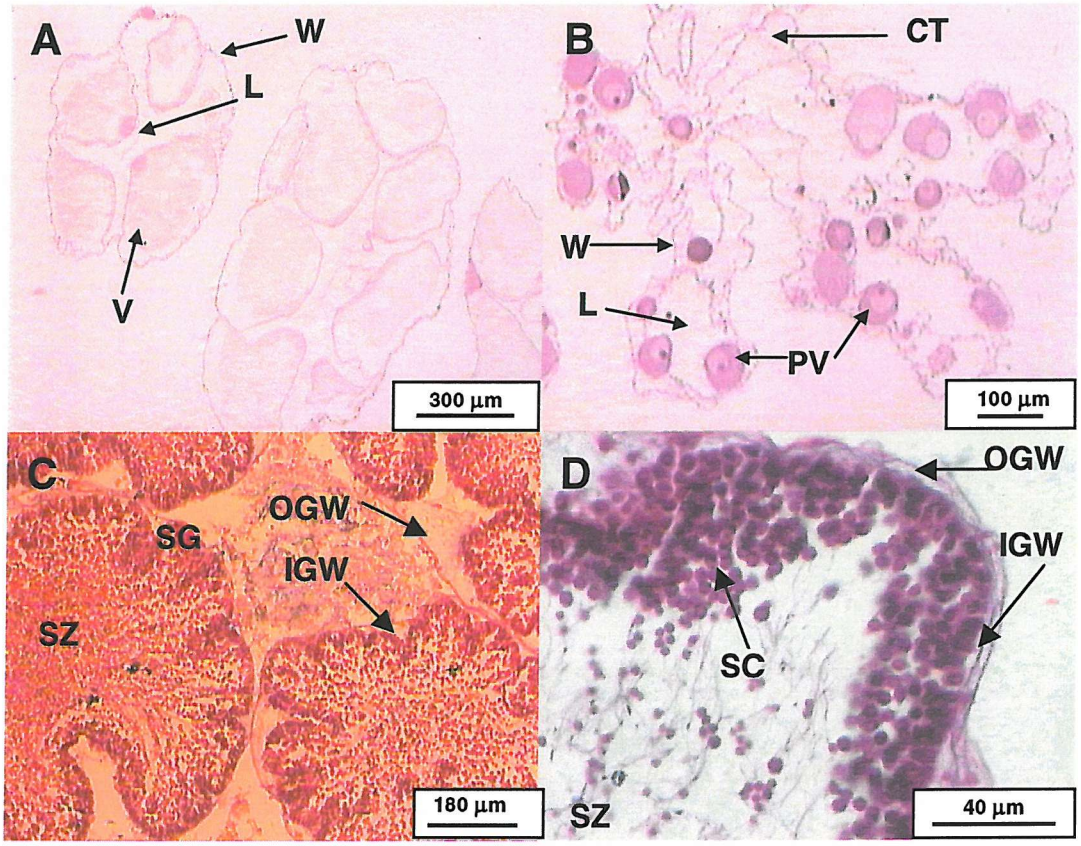

Figure 3.8. Light histology sections of the ovary (**A** and **B**) and testes (**C** and **D**) of *Peniagone* sp. stained with haematoxylin and eosin. CT = connective tissue; IG = inner gonad wall; L = lumen; OG = outer gonad wall; PV = previtellogenic oocyte; SC = spermatocytes; SZ = spermatozoa; V = vitellogenic oocyte; W = ovary wall.

Sperm development. The structure of the testis wall was similar to that of the ovary, comprising an inner and outer sac separated by a connective tissue compartment in between (Figure 3.8 C and D). The inner sac of the testes made infoldings into the testes lumen; these infoldings were lined with spermatogonia (mean diameter 5.02 ±

0.3 μm) and spermatocytes (mean diameter 4.46 ± 0.26 μm). These spermatocytes gave rise to spermatids via meiosis (mean diameter 2.86 ± 0.22 μm), which in turn differentiated into round-headed spermatozoa (mean head diameter 2.47 ± 0.12 μm) typical of holothurians. Spermiogenesis continued until spermatozoa filled the lumen of the testes, at this point gametes were either spawned or maintained within the testes.

Reproductive output

Gonad indices

The gonad indices for *Peniagone* sp. showed a very high within-sample variation, indicated by the wide error bars (Figure 3.9A). Mean values for male and female gonad indices showed variation both spatially and temporally. However, the considerable overlap of the error bars within these samples indicated that none of the variation between samples was of biological significance. This overlap suggested that there was no synchrony of gonad development amongst individuals.

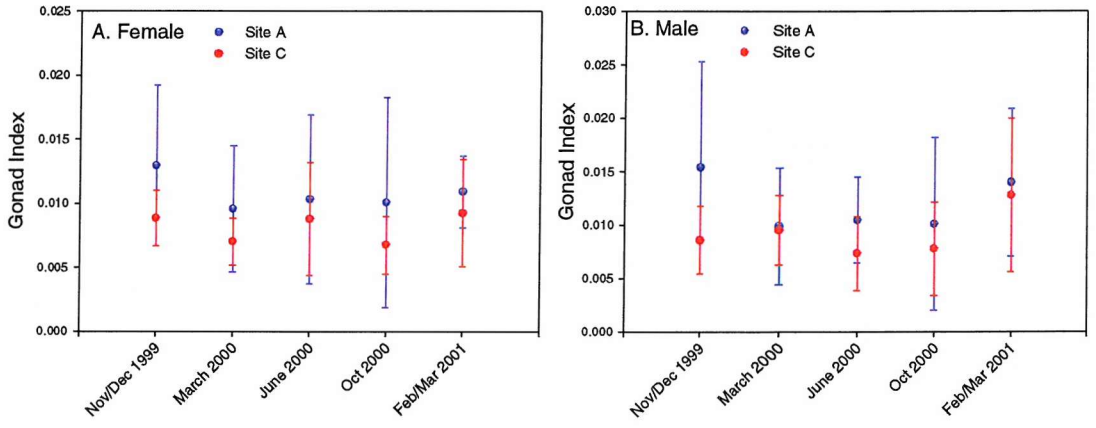

Figure 3.9. Seasonal variation of (**A**) female and (**B**) male gonad indices for *Peniagone* sp.; data are standardised to an animal length of 74.4 mm and presented as mean ± SD.

Fecundity

There was no relationship between fecundity and animal size for *Peniagone* sp., therefore data could not be size-corrected and were presented as mean ±SD (Figure 3.10A). The fecundity for *Peniagone* sp. ranged from 1498 to 9986 oocytes per individual. The mean fecundity at site A ranged from 4954 in October 2000 to 6881 in February/March 2001. However this temporal increase in fecundity between October and February/March was not significant (Mann-Whitney site A, W=32.0, n=6,

P=0.298) because the within sample variation was high. Spatially there was no significant difference in fecundity between site A and site C (Mann-Whitney, W=48.0, n=6, P=0.174).

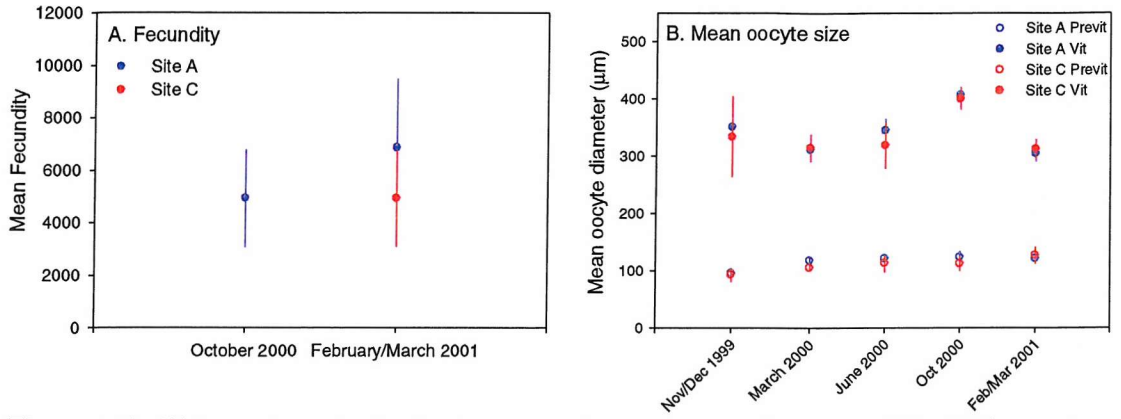

Figure 3.10. (**A**) Mean fecundity for *Peniagone* sp., data are presented as mean ±SD. (**B**) Seasonal variation in mean oocyte diameter of previtellogenic and vitellogenic oocytes for *Peniagone* sp.

Mean oocyte size

Mean oocyte sizes for previtellogenic and vitellogenic oocytes were plotted separately as mean ± SD (Figure 3.10B). The mean previtellogenic oocyte size for site A and site C showed little temporal or spatial variation from the mean value of 120 µm. Mean vitellogenic oocyte sizes between sites A and C were very similar within each sampling period, and followed the same pattern throughout the sampling duration. There was a significant difference in mean vitellogenic oocyte size over the study period (ANOVA site A, F=37.29, 4df, P=0.000; ANOVA site C, F=7.40, 4df, P=0.001). This significant difference was as a result of an increase in mean vitellogenic oocytes size in October 2000, compared to oocytes sizes in June 2000 and February/March 2001.

Oocyte size-frequency distributions

Spatially there were few differences between the oocyte size-frequency distributions of site A and site C (Figure 3.11). The main difference between sites occurred during March 2000, where the proportion of previtellogenic oocytes was 15% higher at site C than site A. Conversely site A had developed a 15% higher proportion of vitellogenic oocytes than site C. On a temporal basis there were changes in the oocyte size-frequency distributions throughout the sampling period. During November/December 1999 the oocyte distributions of sites A and C were dominated by previtellogenic

oocytes (>90%). During the subsequent two sampling periods the proportion of previtellogenic oocytes decreased to ~75%, as oocyte maturation occurred and vitellogenic oocytes developed. The proportion of vitellogenic oocytes in the ovary reached a maximum of 33% by October 2000. However a detailed examination of the individuals from October 2000 (Figure 3.12) showed in the case of individual 759 that vitellogenic oocytes were starting to be spawned. Four months later in February/March 2001, the proportion of vitellogenic oocytes in the ovary was high, 25-30%, however the maximum oocyte size fell from 570 μm in October to 428 μm in February/March. Evidence from individual oocytes size frequency distributions indicated that individuals were at the same stage of development within each sample (Figure 3.12; Appendix 2). The distinct lack of vitellogenic oocytes in November/December 1999 indicated that a spawning event had recently taken place. During subsequent sampling events the proportion and size of vitellogenic oocytes increased to a peak during October 2000. An overall drop in oocyte size by February/March 2001 intimated the occurrence of a second spawning event, which was probably similarly timed to that of the previous year. Despite the drop in overall oocyte size during February/March 2001 both sites exhibited high proportions of small vitellogenic oocytes.

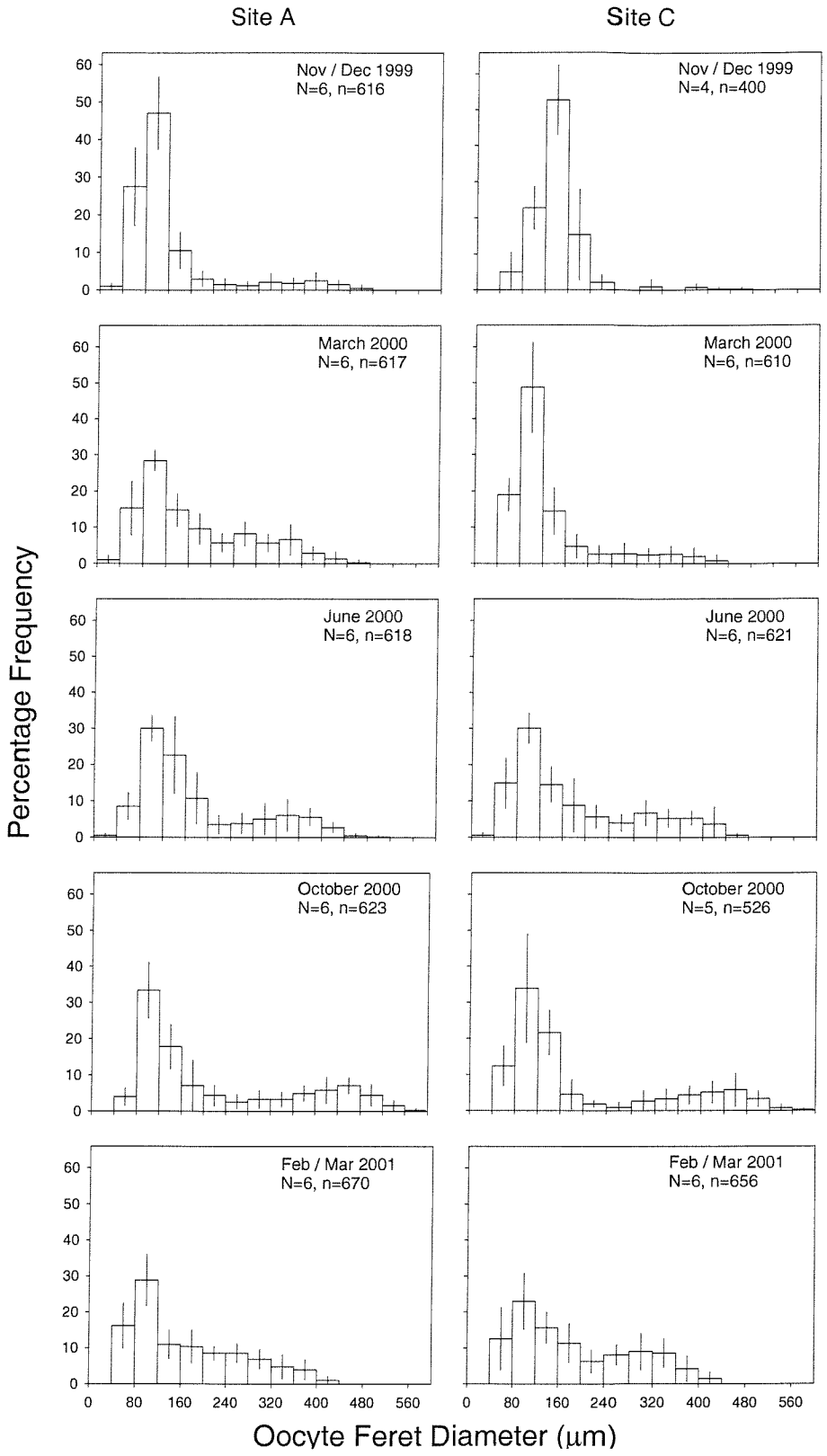

Figure 3.11. Oocyte size-frequency distributions for *Peniagone* sp. Spatial and temporal variability from November/December 1999 – February/March 2001. Mean percentage frequency ± St Dev. N = number of females; n = number of oocytes.

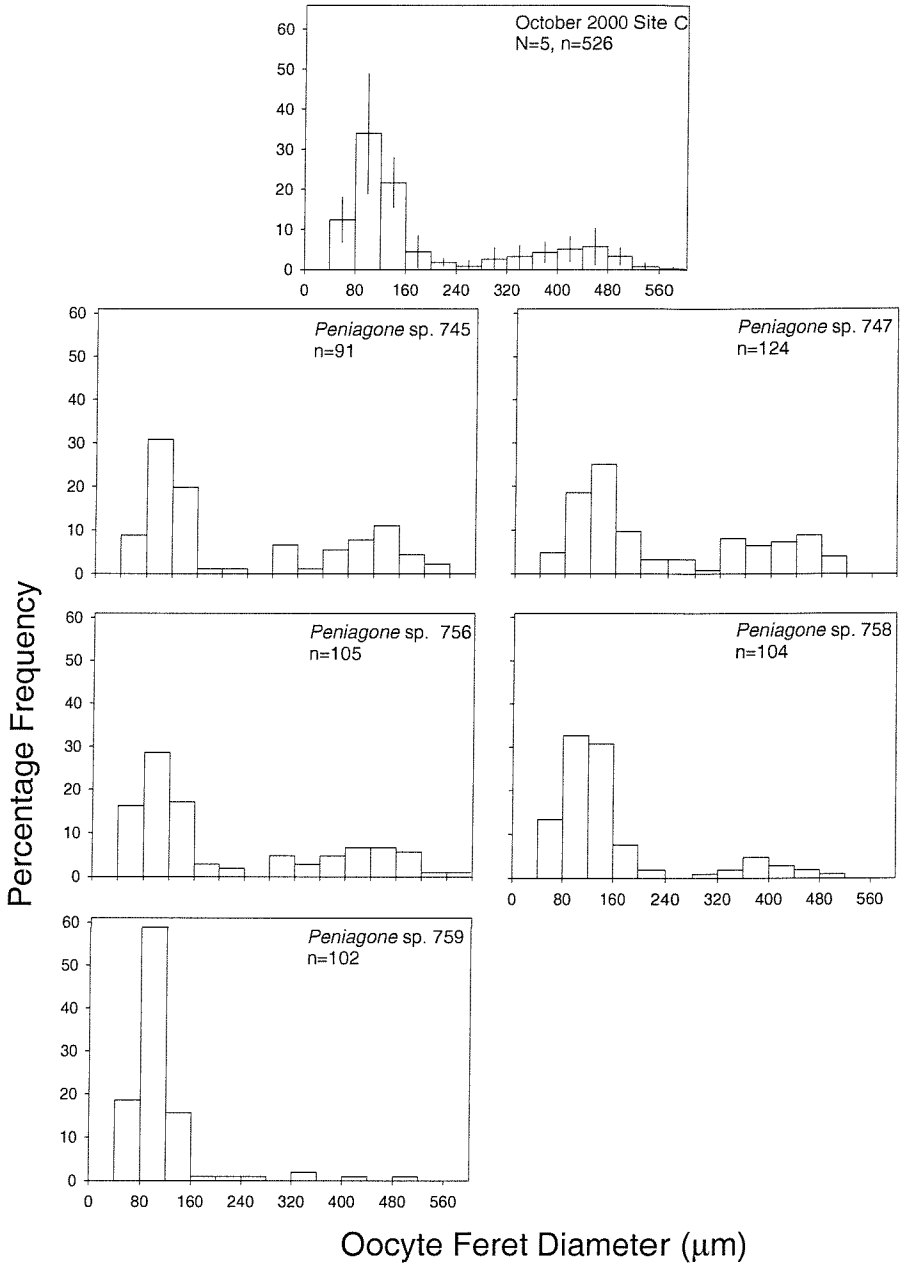

Figure 3.12. Mean oocyte size-frequency distribution for *Peniagone* sp. October 2000 Site C (top centre), and individual oocyte size-frequency distributions for October 2000 Site C. *Peniagone* sp. 745-759 = individual reference numbers. N = number of females; n = number of oocytes.

Psilaster charcoti (Asteroid)

External gonad morphology

The reproductive system of *Psilaster charcoti* comprised ten gonads, one pair in each arm. The gonads were attached to the body wall by a short gonoduct that lead to the gonopore. Each gonad consisted of numerous digitate tubules united basally into one tuft. The ovary wall was translucent and developing oocytes could clearly be seen through it (Figure 3.13A). The tubules of the testes were white and distinctly nodular in shape (Figure 3.13B).

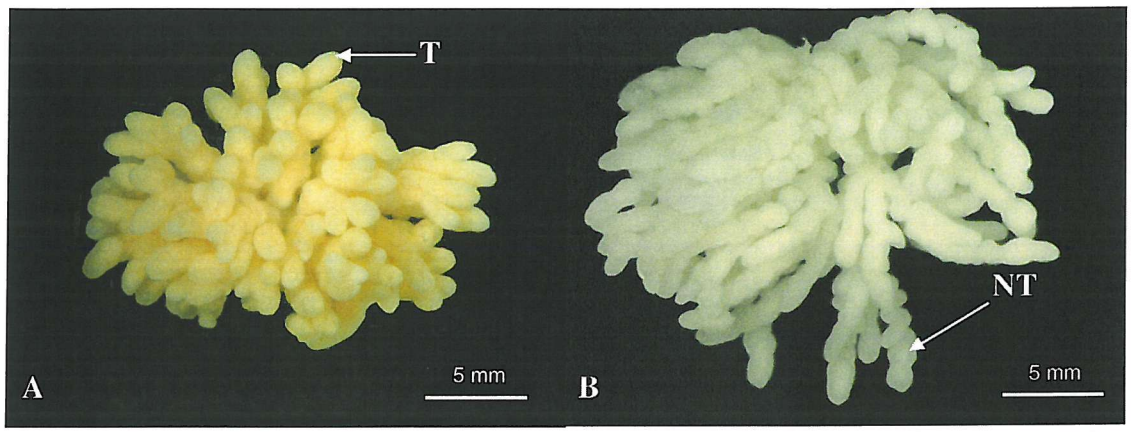

Figure 3.13. External gonad appearance of *Psilaster charcoti* (**A**) Female, (**B**) Male. NT = nodular tubule; T = tubule.

Gametogenesis of *Psilaster charcoti*

Oocyte development. Developing and mature oocytes were found in the ovaries of *Psilaster charcoti*. The ovary wall was composed of an outer and inner layer; the outer layer was lined with coelomic epithelium, and the inner layer was lined with germinal epithelium. The oocytes of *P. charcoti* developed along the germinal epithelium and at sizes less than 280 µm diameter the cytoplasm was basophilic and stained purple with haematoxylin (Figure 3.14B). These basophilic oocytes were previtellogenic and had a relatively large nucleus to cytoplasm ratio, the nucleus contained an eccentric nucleolus. Previtellogenic cells were also prolific at the tips of tubules where they may be several cells deep. At diameters greater than 280 µm the cytoplasm became acidophilic and stained pink with eosin, this suggested that vitellogenesis was occurring. These vitellogenic oocytes extended out into the lumen and moved away from the ovary wall into the centre of the lumen when the ovary was full of mature oocytes (Figure 3.14 A and B). As the vitellogenic oocytes developed

the volume of cytoplasm to nucleus increased. Oocytes developed up to a maximum feret diameter of 578 µm. Oocytes in all stages of development were surrounded by numerous accessory cells. Internal phagocytosis of oocytes was observed in a few individuals.

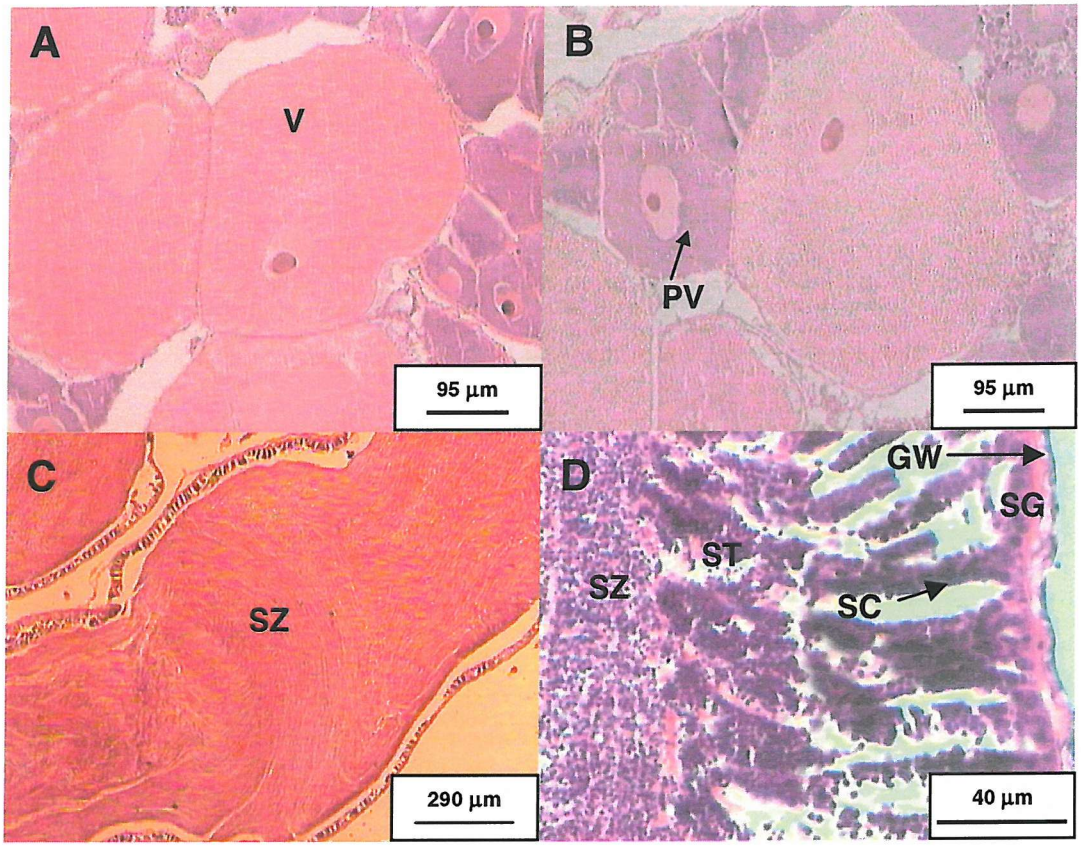

Figure 3.14. Light histology sections of the ovary (**A** and **B**) and testes (**C** and **D**) of *Psilaster charcoti*. GW = gonad wall; PV = previtellogenic oocytes; SC = spermatocyte; SG = spermatogonia; ST = spermatids; SZ = spermatozoa; V = vitellogenic oocytes.

Sperm development. The testes of *Psilaster charcoti* consisted of inner and outer sacs that were separated by the coelomic (perihaemal) sinus as described by Walker (1974) for *Asterias vulgaris*. Spermatogenesis was initiated by the mitotic division of spermatogonia cells (mean diameter 4.28 ± 0.1 µm), these cells lay within the germinal epithelium (Figure 3.14D). Spermatocytes (mean diameter 3.67 ± 0.32 µm) resulting from this division were organised into columns that extended into the lumen. Spermatocytes at the tips of these columns gave rise to spermatids via meiosis (mean diameter 2.06 ± 0.18 µm). In turn spermatids differentiated into spermatozoa (mean head diameter 2.16 ± 0.14 µm), the spermatozoa packed the lumen when the testis

was mature (Figure 3.14C). The testes of *P. charcoti* were packed with spermatozoa during all sampling seasons, only varying in the presence and absence of spermatogenic columns, which were present in March 1999 and February/March 2001.

Reproductive Output

Gonad Indices

The mean gonad index and standard deviation for females of *Psilaster charcoti* (Figure 3.15A) indicated little variation among seasons. The mean gonad index for males at site A was significantly higher than that of the mean gonad index of the specimens from site C (Mann-Whitney, W=422, n=15, 27, P=0.0094) (Figure 3.15B). The greatest difference in gonad indices between sites A and C was recorded during March and June 2000. The highest variability in the mean gonad index was seen at site A during March 2000 and June 2000, and this decreased during October 2000 and February/March 2001. The decrease in variability was accompanied by a decrease in the mean gonad index values between June 2000 and October 2000. This high variability in within and among seasons at site C was not seen in samples from site C.

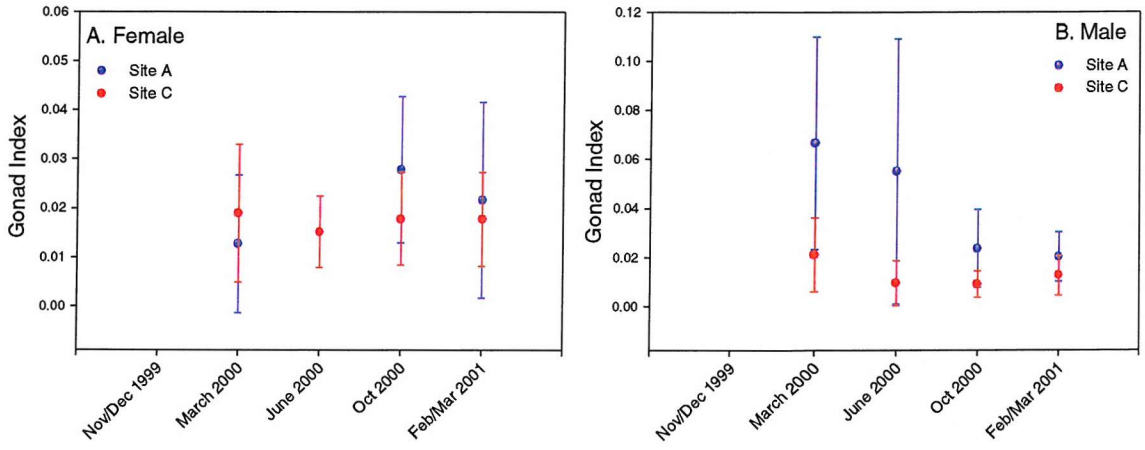

Figure 3.15. Seasonal variation in (**A**) Female and (**B**) male gonad index for *Psilaster charcoti*. Data are standardised to an animal of 109.45 mm test diameter and presented as means ± SD.

Fecundity

The mean actual fecundity of *Psilaster charcoti* was ~ 10,000 vitellogenic oocytes per female. This mean value did not vary significantly between cruises or sites (Figure 3.16A) (mean fecundity: site A, October 2000 = 10,443 oocytes; site A,

February/March 2001 = 9,539.9 oocytes; and site C, February/March 2001 = 8,634 oocytes). However the within site variability was high and values ranged from 879 to 24,750 oocytes at site A in October 2000, and 599 to 33,301 oocytes at site A in February/March 2001. Values of actual fecundity varied to a lesser extent at site C than site A, ranging from 3,557 to 13,970 in February/March 2001 at site C.

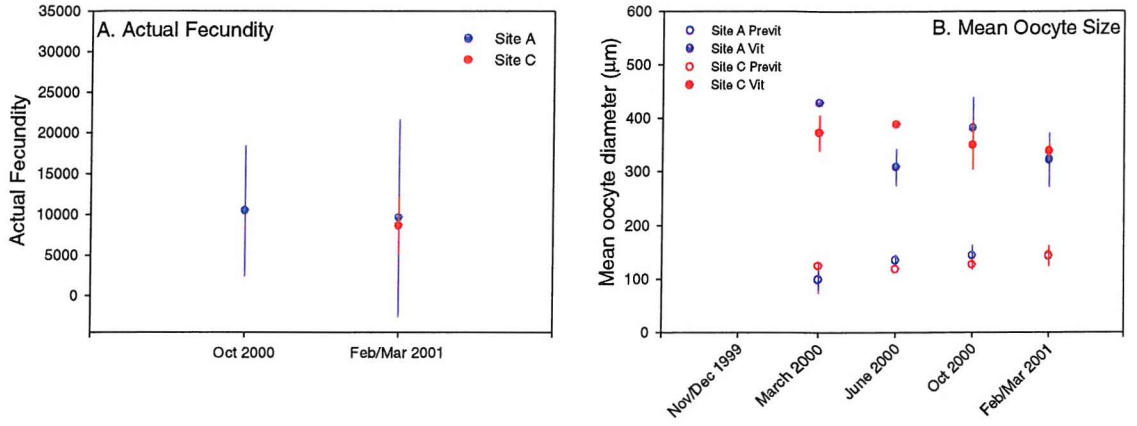

Figure 3.16. (**A**) Mean fecundity for *Psilaster charcoti* data are standardised to an animal of 109.45 mm test diameter and presented as means ± SD. (**B**) Seasonal variation in mean oocyte diameter previtellogenic and vitellogenic oocytes for *Psilaster charcoti*.

Mean oocyte size

The mean oocyte sizes (± standard deviation) for the four temporal samples at sites A and C were shown in Figure 3.16B. Previtellogenic and vitellogenic oocytes were plotted separately. Statistically there was no significant difference in the mean previtellogenic and vitellogenic oocyte sizes over the study period (previtellogenic oocytes site A Kruskall-Wallis, H=6.13, 3df, P=0.105; vitellogenic oocytes site A Kruskall-Wallis; H=3.77, 2df, P=0.152; previtellogenic oocytes site C Kruskall-Wallis, H=2.77, 2df, P=0.25; vitellogenic oocytes site C Kruskall-Wallis, H=2.89, 2df, P=0.236).

Oocyte size frequency distribution

Oocytes of many sizes were present throughout the year (Figure 3.17). In individual specimens from each sample the oocyte size-frequency distribution showed a dominance of oocytes <240 µm (Figure 3.18). These small oocytes had a strong reaction with haematoxylin suggesting that they were previtellogenic. Previtellogenic oocytes underwent vitellogenesis increasing their cell diameter from 240 µm to a

maximum size of ~578 μm. In the majority of cases the individual size-frequency distributions (Appendix 3) suggested that the growth of vitellogenic oocytes was a continuous process. There were, however, two individuals that had a bimodal distribution (in October site A and March site A). The secondary peak was at 500 μm, this suggested that oocytes in these individuals were developing in cohorts and underwent oogenesis at the same time. However, the development of the secondary cohort did not occur in two individuals at the same time of year. This suggested that although development of vitellogenic oocytes may be periodic within an individual there was no synchrony of oocyte growth among individuals.

The proportion of vitellogenic oocytes in the site A samples increased from 6.4% in March 2000 to 29.6% in October 2000. By February/March 2001 the proportion of vitellogenic oocytes had dropped to 11.9%. At site C there was also an increase in the percent of vitellogenic oocytes from 12% in March 2000 to 19 % in October 2000. However, unlike site A samples the proportion vitellogenic oocytes continued to increase up to 26% by February/March 2001, despite the 43 μm drop in maximum vitellogenic oocyte size.

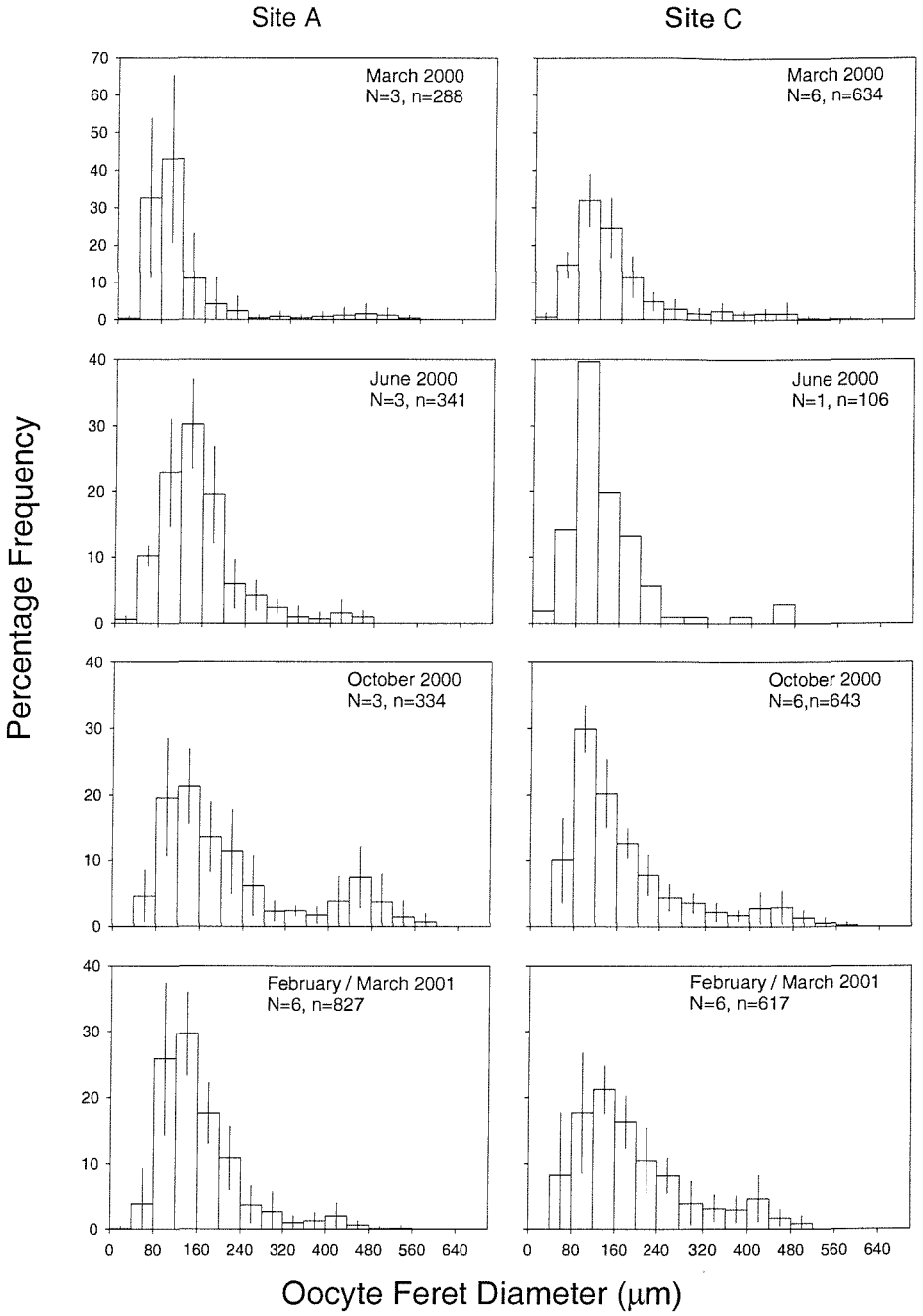

Figure 3.17. Mean oocyte size-frequency distributions for *Psilaster charcoti*. Spatial and temporal variability from March 2000 – February/March 2001. Mean percentage frequency ± St Dev. N = number of females; n = number of oocytes. Note different scales for some months; scales match on adjacent graphs.

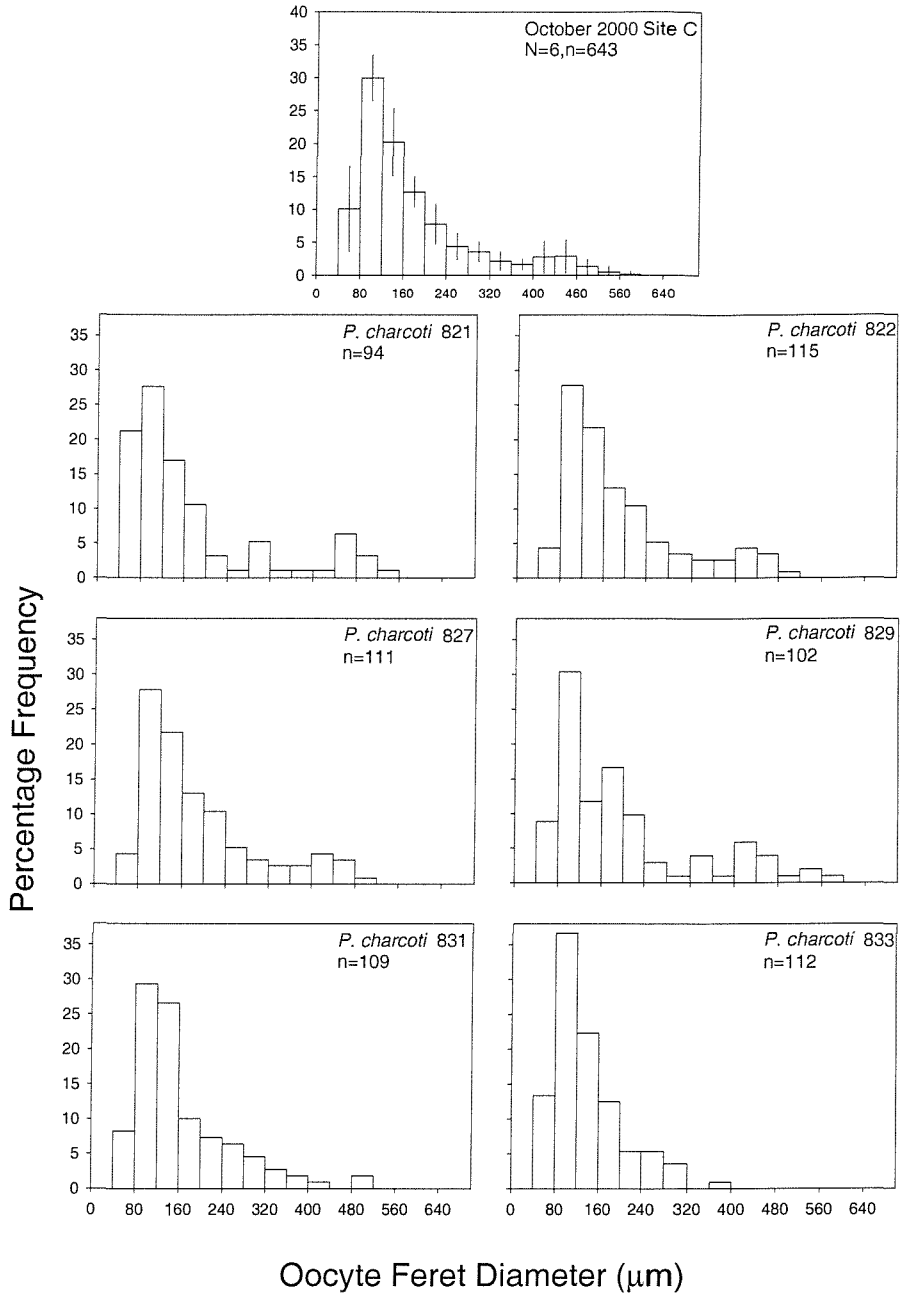

Figure 3.18. Mean oocyte size-frequency distribution for *Psilaster charcoti* at Site C cruise IV (top centre), and individual oocyte size-frequency distribution for *Psilaster charcoti* at Site C cruise IV. *P. charcoti* 821-833 = individual reference numbers. N = number of females; n = number of oocytes.

Sterechinus antarcticus (Echinoid)

External gonad morphology

S. antarcticus had five gonads that were suspended from the inner surface of the interambulacrum. The five gonads developed as discrete organs (Figure 3.19) and later fused into each other laterally as gametogenesis occurred (Hyman, 1955). A gonoduct extended the full length of the fused organs, branching at each gonad. The five gonads formed a C-shape on the inside of the test circling the apical system. The gut dissected the gonad at the opening of the C where the gut travelled from the apical system circling the inside of the test to connect to the top of the Aristotle's lantern. Externally the ovary appeared orange/red in colour from the pigmentation of the oocytes (Figure 3.19A). If the gonad pigmentation was particularly strong the ovary could be seen from outside the specimen test. The testes were white/cream in colour when full of spermatozoa (Figure 3.19B).

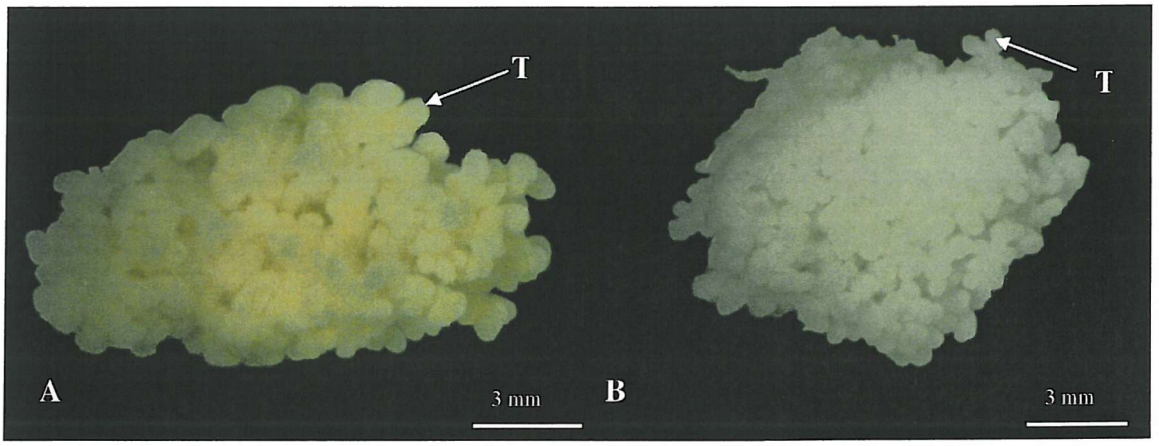

Figure 3.19. External gonad appearance of *Sterechinus antarcticus* (**A**) Female, (**B**) Male. T = tubule.

Gametogenesis in *Sterechinus antarcticus*

Oocyte development. Within the ovary, oocytes commenced development along the germinal epithelium of the inner gonad wall. Oocytes developing from 10 µm to 90 µm were young previtellogenic oocytes and were surrounded by nutritive phagocytic tissue. These young previtellogenic oocytes (having not yet laid down any yolk) were characterised by a large nucleus with an eccentric nucleolus, and a basophilic cytoplasm that stained purple with haematoxylin. At ~90 µm the oocytes began to undergo vitellogenesis and became vacuolated (Figure 3.20) as yolk reserves were

deposited within the oocyte. During this transition to a mature oocyte the proportion of cytoplasm to nucleus increased and the cytoplasm became acidophilic and granular in appearance. Vitellogenic oocytes were still found along the germinal epithelium or may have migrated to the lumen of the gonad, although still surrounded by nutritive tissue. As oocytes grew most remain spherical whilst some became elongated or misshapen, possibly as a result of spatial constraints within the gonad. Vitellogenic oocytes reached a maximum feret diameter of 258μm.

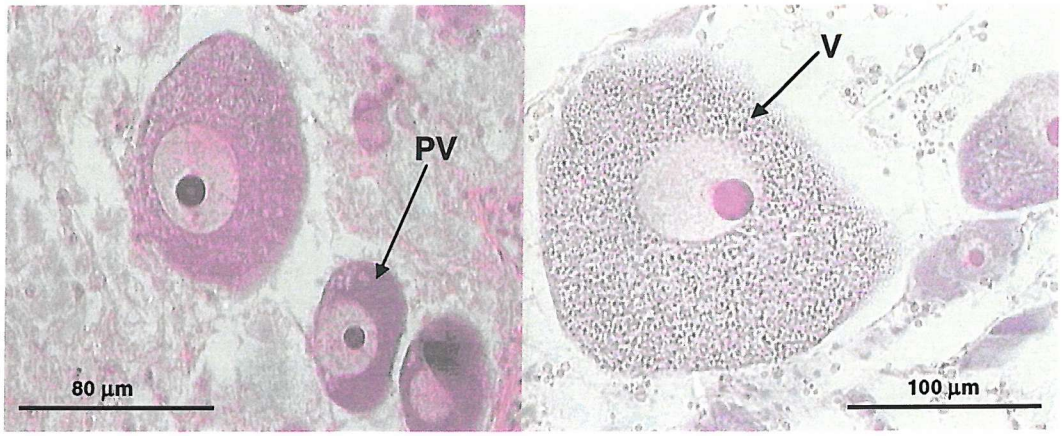

Figure 3.20. Light microscope sections of developing ovaries of *Sterechinus antarcticus*. PV = previtellogenic oocyte; V = vitellogenic oocyte.

Sperm development. In the testes the germinal epithelium gave rise to a thin layer of spermatogonia cells (mean diameter of 5.24 ± 0.9 μm). The spermatogonia underwent mitosis and developed into spermatocytes (mean diameter of 3.34 ± 0.3 μm). These spermatocytes formed colonettes that extended into the lumen of the testes. The spermatocytes at the tips of the colonettes underwent spermiogenesis and developed into spermatids (1.77 ± 0.2 μm diameter) via meiotic division.

The development of the testes was examined for each month sampled (Figure 3.21) and was classified according to the five maturity stages for *S. neumayeri* described by Brockington (2001). Samples examined in November/December 1999 and October 2000 were in a combination of stage 1 and stage 5 of development. Stage 5 was the spawning phase and the density of spermatozoa was greatly reduced. Stage 1 was the recovery stage where a thin layer of spermatogonia lined the testes wall and nutritive phagocytes formed a meshwork around the testes wall and lumen. Samples from

March 2000 and February/March 2001 were a combination of stage two and three maturity. Stage two was the initial development of the testes, nutritive tissue was of medium thickness and spermatocytes were visible on the germinal epithelium. Stage three was the developing stage, the testes contained columns of spermatocytes along the testes wall and spermatozoa began to accumulate in the lumen. Full maturity was achieved in June 2000, at this stage the lumen was full of densely-packed mature spermatozoa and nutritive tissue was reduced.

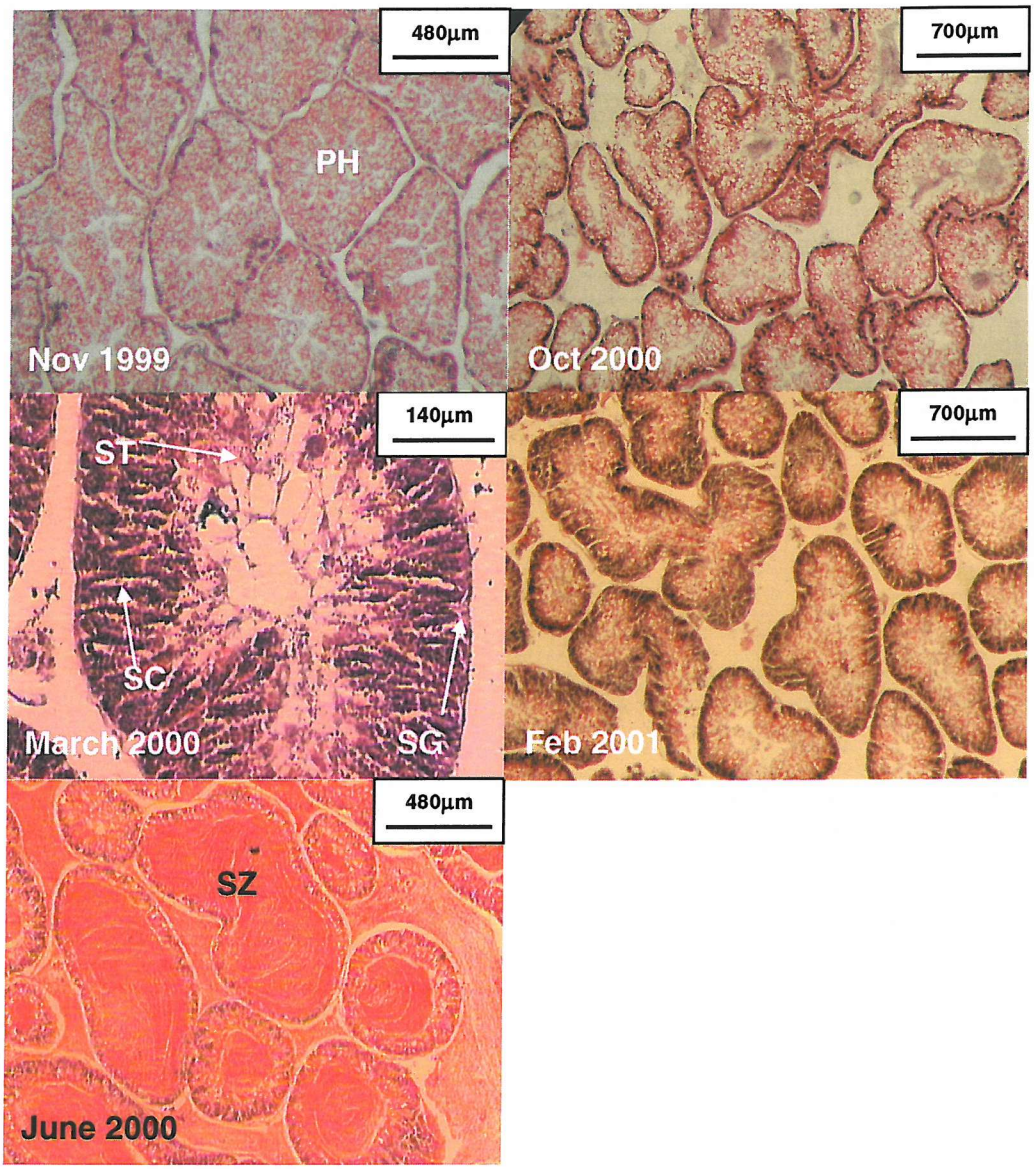

Figure 3.21. Light microscope sections of developing testes of *Sterechinus antarcticus*. PH = nutritive phagocytic tissue; SC = spermatocytes; SG = spermatogonia; ST = spermatids; SZ = spermatozoa.

Reproductive output

Gonad Indices

Figure 3.22A showed the gonad index for both female and male *Sterechinus antarcticus* from November/December 1999 to February/March 2001. Statistically the female gonad index showed a close to significant variation amongst the seasons (General Linear Model, F=2.62, 4df, P=0.056). Males exhibited a significant between-season variability in mean gonad index (General Linear Model, F=8.05, 4df, P=0.000). There was a clear pattern in the mean gonad index over the study period for both females and males. The peak values for both male and female gonad indices were in samples from June 2000 (austral winter). The lowest values of gonad index occurred in November/December 1999 and October 2000. This pattern was more pronounced in the males than in the females.

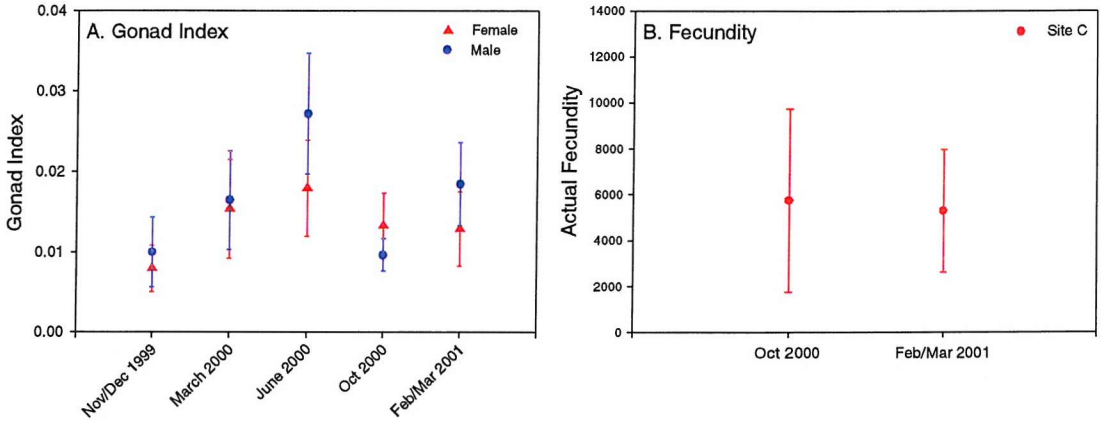

Figure 3.22. (**A**) Seasonal variation in male and female gonad indices for *Sterechinus antarcticus* at site C. (**B**) Seasonal variation in fecundity of *Sterechinus antarcticus* at site C. Data are standardised to an animal of 38 mm test diameter and presented as mean ± SD.

Fecundity

Actual fecundity was estimated as the number of vitellogenic oocytes in the ovaries of *S. antarcticus* (Figure 3.22B). In the specimens from October 2000 fecundity ranged from 2,361 to 12,694 oocytes per female with a mean fecundity of 5,786. In the specimens sampled in February/March 2001 fecundity ranged from 3,160 to 9,559 oocytes per female, with a mean fecundity of 5,341. There was no significant difference in mean fecundity between October and February/March (Student's t-test, t=0.24, 8df, P>0.82).

Mean oocyte size

The mean oocyte size for the five temporal samples at Site C was shown in Figure 3.23. To present meaningful results with regards to gametogenic development, the previtellogenic (0-120 μm) and vitellogenic (120-260 μm) size classes were plotted separately. Between November/December 1999 and March 2000 the mean vitellogenic oocyte size increased. Mean vitellogenic oocyte size decreased slightly by June 2000, the error bars greatly increased in size compared to the other months. The data point for June 2000 however, consisted of two individuals with the highest mean vitellogenic oocyte size of the whole sampling period (182 μm and 186 μm), and two individuals with no mature vitellogenic oocytes present. The results for June indicated that vitellogenic oocytes were reaching maturity and that spawning had commenced. By October the spawning period was completed, as there was no evidence of large vitellogenic oocytes in the October sample. The low mean vitellogenic oocyte size in October indicated that a new cohort of vitellogenic oocytes was starting to develop. All five individuals in October 2000 had a mean vitellogenic oocyte size between 137 μm and 142 μm, just lower than the value from November/December the previous year. The mean vitellogenic oocyte size increased in February/March 2001 following the pattern from the previous year. The mean previtellogenic oocytes were at the lowest in November/December 1999 and June 2000.

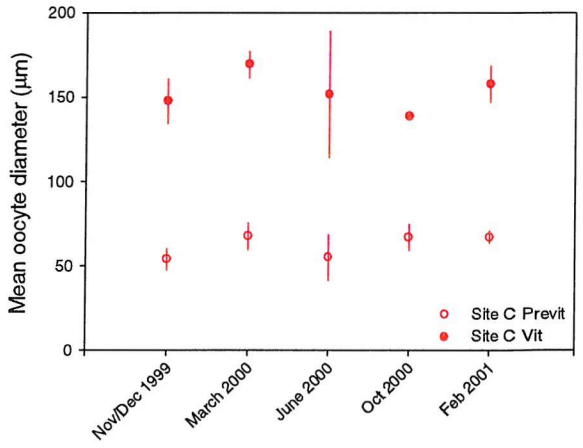

Figure 3.23. Seasonal variation in mean oocyte diameter (±SD) of previtellogenic and vitellogenic oocytes for *Sterechinus antarcticus*.

Oocyte size frequency distributions

Oogenesis in *Sterechinus antarcticus* was synchronous, as indicated by individual plots of oocyte size frequency (Appendix 4). Oocytes of many sizes were present (Figure 3.24) throughout the sampling period. The oocyte size-frequency distribution suggested that the gametogenic cycle of *Sterechinus antarcticus* took approximately 24 months, with a spawning period commencing in June 2000 (Figure 3.24).

In November/December 1999 there was a large cohort of previtellogenic oocytes (20-120 μm) and a small cohort of maturing oocytes (120-220 μm). By March the percentage of oocytes in the maturing cohort had increased by 15%. In June, oocytes in the larger cohort had either completed maturation (max oocyte size 258 μm) or disappeared from the gonad entirely (Figure 3.25). In addition the appearance of a new cohort of oocytes (~20-40 μm) was evident in the June distributions. In Figure 3.25, the presence of the new cohort could be explained by the recruitment of new oocytes into the ovaries of the two spawned individuals. Growth of each generation of oocytes was indicated by the progressive shift of peaks (and lines) across the frequency histograms between June and October 2000. By February/March 2001 there was little change in size of the new cohort, however the maximum oocyte size in cohort 2 had increased by 40 μm. It was clear that despite the long development period, oocytes underwent the majority of size increase (100 to 240 μm) in the last 6-12 months of the gametogenic cycle.

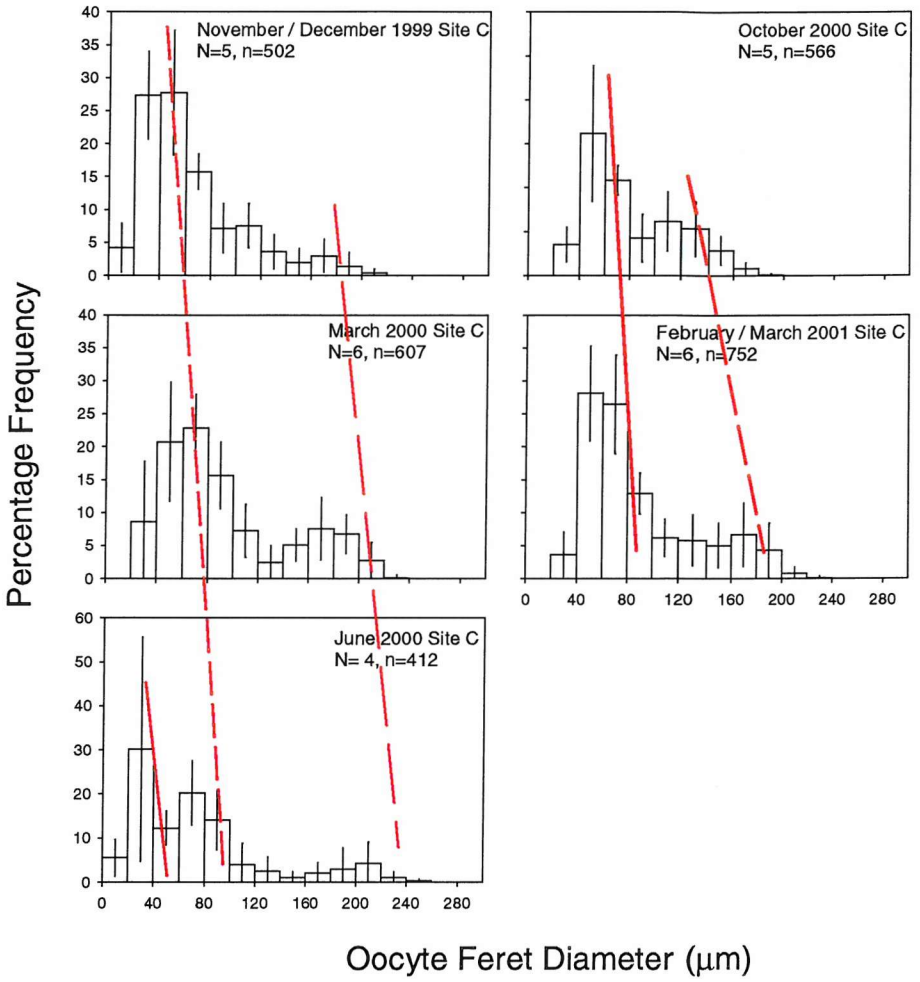

Figure 3.24. Oocyte size-frequency distributions for *Sterechinus antarcticus*. Spatial and temporal variability from November/December 1999 – February/March 2001. Mean percentage frequency ± St Dev. N = number of females; n = number of oocytes. Red diagonal lines connect indicate the progression of a cohort of oocytes through development. The long dashed line follows a cohort of vitellogenic oocytes through to spawning in June 2000. The short dashed line follows a cohort of previtellogenic oocytes from November 1999 through to vitellogenic oocytes in February 2001. The solid line indicates a new cohort of previtellogenic oocytes in June 2000. Note different scales for some months; scales match on adjacent graphs.

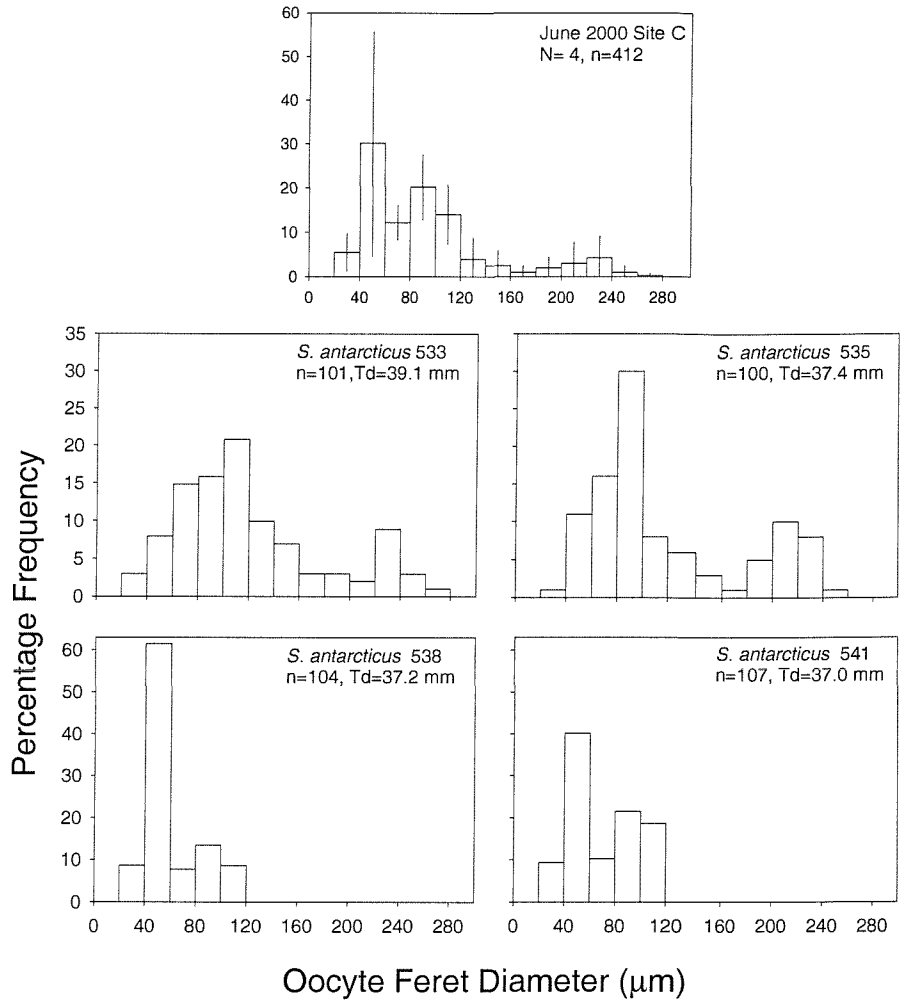

Figure 3.25. Mean oocyte size-frequency distribution for *Sterechinus antarcticus* June 2000 Site C (top centre), and individual oocyte size-frequency distributions of June 2000 Site C. *S. antarcticus* 533-541 = individual reference numbers. N = number of females; n = number of oocytes; Td = test diameter (mm). Note different scales, scales match horizontally.

Ctenocidaris perrieri (Echinoid)

External gonad morphology

Ctenocidaris perrieri had five gonads that lay within each interambulacrum near the apex of the test. The gonads were attached by the gonoduct to the aboral gonopore and extended down the inside of the test attached to the coelomic epithelium by mesenterial strands. Each gonad consisted of numerous branched tubules united basally at the gonoduct, which ran the length of the gonad. Oocytes developing in the rounded end tubules were clearly visible through the gonad wall, the oocyte deepening in colour as it laid down yolk and matured (Figure 3.26A). The oocytes grew in excess of 1 mm diameter, which was typical of brooding echinoderms. The

testes had more nodular tubules than the ovaries and were generally brown or cream in colour (Figure 3.26B).

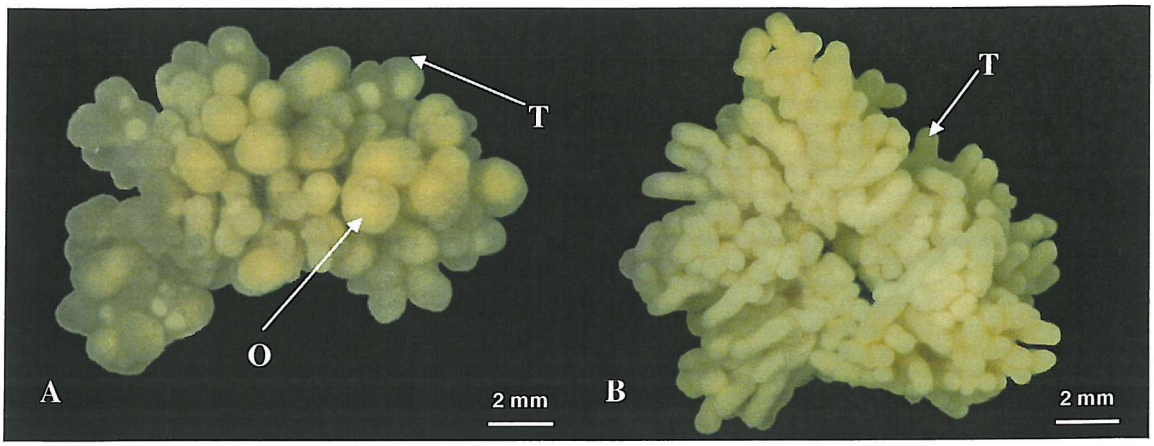

Figure 3.26. External gonad appearance of *Ctenocidaris perrieri* (**A**) Female, (**B**) Male. O = oocyte; T = tubule.

Gametogenesis in *Ctenocidaris perrieri*

Oocyte development. The oogenic cycle commenced with the development of small oogonia in the nutritive tissue, close to the germinal epithelium. These oogonia developed into previtellogenic oocytes that were characterised as having a basophilic cytoplasm that was smooth in texture (Figure 3.27A). The previtellogenic oocytes continued to grow up to a size of 600 µm where the nature of the cytoplasm changed and became granular in texture. At this stage the oocytes underwent vitellogenesis and laid down yolk/lipid in the cytoplasm. The lipid granules in the cytoplasm were acidophilic and stained pink with eosin. During vitellogenesis the volume of the cytoplasm greatly increased, and the cytoplasm/nucleus ratio increased correspondingly. The oocytes reached a maximum feret diameter of 1750 µm and thus filled the width of the tubule (Figure 3.26A and 3.27B). Nutritive tissue occupied most of the remaining space in the gonad not occupied by oocytes.

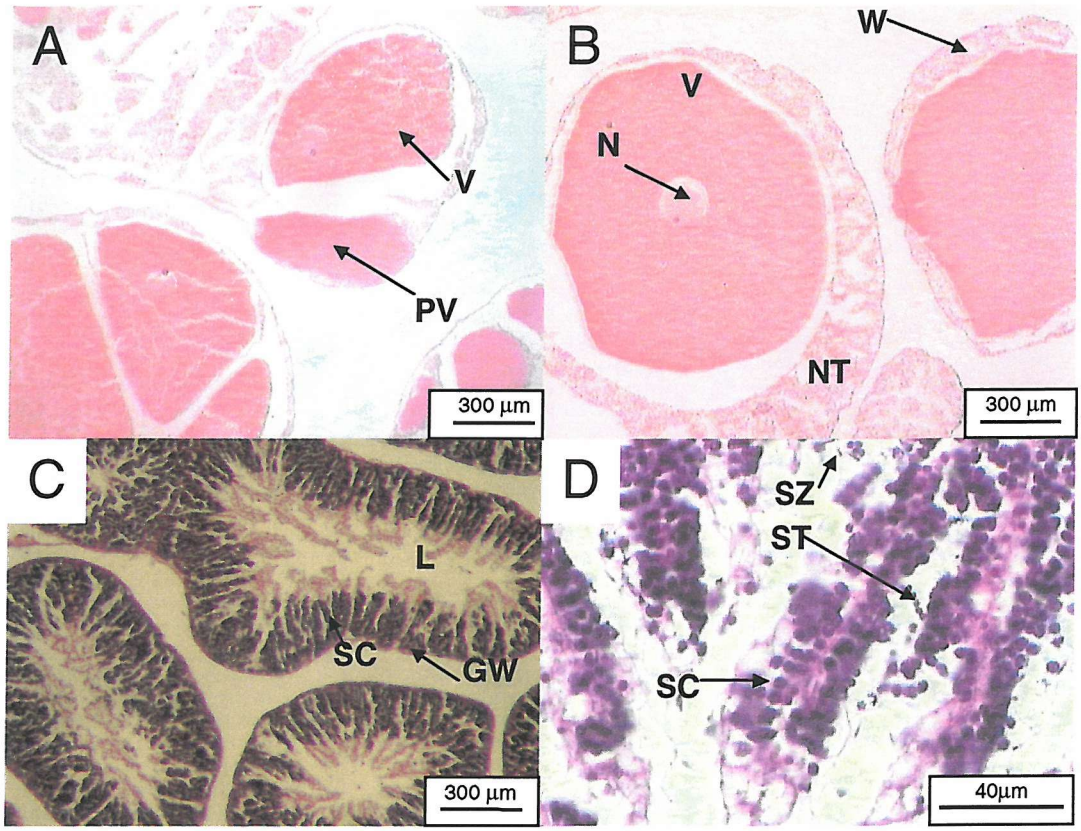

Figure 3.27. Light microscope sections of developing ovary (**A** and **B**) and testes (**C** and **D**) of *Ctenocidaris perrieri*. GW = gonad wall; L = lumen; N = nucleus; NT = nutritive tissue; PV = previtellogenic oocyte; SC = spermatocytes; ST = spermatids; SZ = spermatozoa; V = vitellogenic oocyte; W = ovary wall.

Sperm development. Spermatogonia (mean diameter of 5.87 ± 0.68 µm) developed along the germinal epithelium of the testes; the spermatogonia underwent mitotic division and gave rise to spermatocytes (mean diameter 3.75 ± 0.28 µm). In young developing testes the inner sac formed folds into the lumen that were stretched out as the testes developed (Figure 3.27C). As the spermatocytes proliferated they developed into colonettes that extended into the lumen of the testes (Figure 3.27D). At the end of the colonettes the spermatocytes underwent meiosis and produced spermatids (mean diameter of 2.22 ± 0.23 µm). These spermatids then differentiated into spermatozoa that began to fill up the lumen of the testes. The spermatozoa had elongated conical heads as was typical for echinoids (mean length of 4.51 ± 0.47 µm). During the time period sampled the majority of individuals examined were at the same stage of sperm development, in most individuals spermatocyte colonettes were well developed and few spermatozoa had entered the lumen. There did appear to be a greater proportion

of the lumen filled with spermatozoa during June 2000, however there appeared to be no change in development during the other months.

Reproductive output

Gonad Indices

The mean gonad index for *Ctenocidaris perrieri* was illustrated in Figure 3.28 A and B. The male gonad index illustrated a variation across the sampling period; this variation was also evident in the females when only considering the mean points. The pattern consisted of an increase in mean gonad index from November/December 1999 to June 2000. The peak in June was followed by a sharp decrease in October 2000. The gonad index then increased from October to February/March, mirroring the pattern of the previous year. There was no significant difference in female gonad index between seasons (cruise Kruskall-Wallis: H=9.21, 4df, P=0.056). Mean female gonad indices were greater at site C than at site A throughout the sampling period, this difference was most pronounced in February/March 2001 (site Mann-Whitney W=519.0, 1df, P=0.0065). The pattern in gonad index was most clearly shown in the male data. There was a significant difference in male gonad index between seasons, but the mean gonad indices for the two stations were very similar (cruise general linear model: F=11.38, 4df, P=0.000; site general linear model F=0.25, 1df, P=0.620).

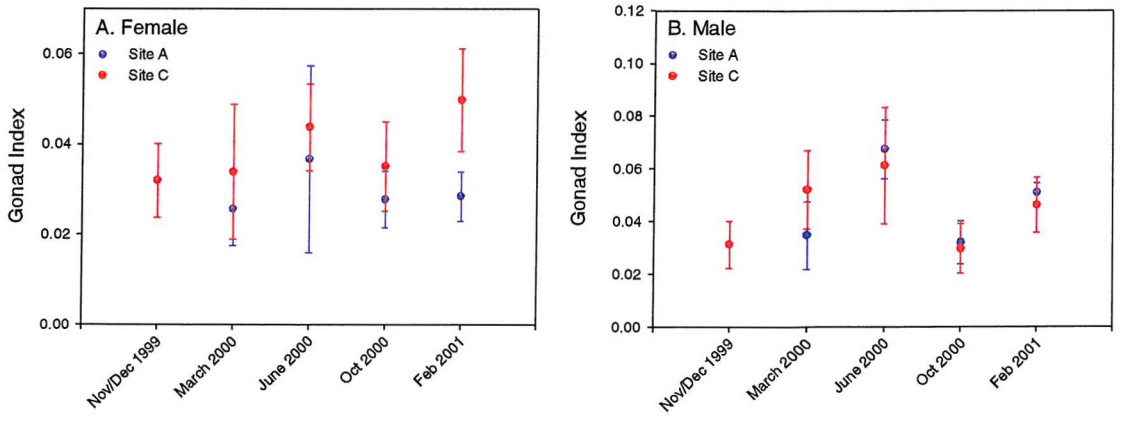

Figure 3.28. Seasonal variation in mean gonad indices for *Ctenocidaris perrieri*, (**A**) Female and (**B**) Male at sites A and C. Data are standardised to an animal of 46.7 mm test diameter and presented as means ± SD.

Fecundity

The temporal and spatial variation in fecundity for *Ctenocidaris perrieri* was represented in Figure 3.29A. It was clear from the error bars on the plot that there was no significant difference in fecundity at site A between October 2000 (mean fecundity 495) and February/March 2001 (mean fecundity 517). There was a clear spatial difference in fecundity between sites A and C during February/March 2001; the mean fecundity differed by 481 oocytes. However, variation about the mean was high, site C had a much higher variation about the mean than site A, a range of 970 compared to 320.

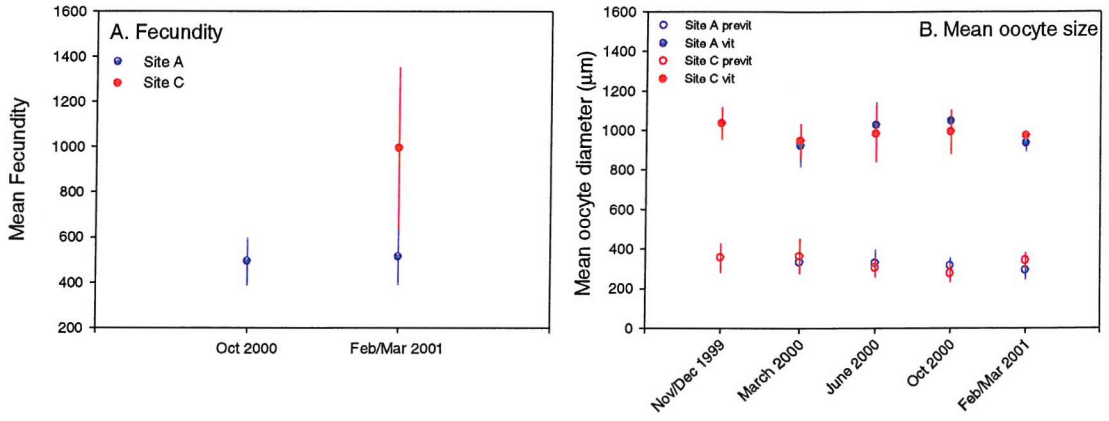

Figure 3.29. (**A**) Fecundity of *Ctenocidaris perrieri* at sites A and C between October 2000 and February/March 2001. (**B**) Seasonal variation in mean oocyte diameter of previtellogenic and vitellogenic oocytes for *Ctenocidaris perrieri.*

Mean oocyte sizes

There was no significant change in the mean previtellogenic oocyte size between November/December1999 and February/March 2001 (Figure 3.29). Mean previtellogenic oocytes were in the size range of 273 – 346 µm. There was no significant difference in the mean vitellogenic oocyte size from November/December to February/March, as indicated by the overlapping error bars. Mean vitellogenic oocytes were in the size range of 925 – 1049 µm.

Oocyte size-frequency distributions

Mean oocyte size-frequency distributions were been constructed for five seasons between November/December 1999 and February/March 2001 (Figure 3.30), at stations A and C. No specimens of *Ctenocidaris perrieri* were collected from station A during November/December 1999. The oocyte size-frequency showed a bimodal

distribution in both the mean and individual distributions (Figures 3.30 and 3.31). The first peak (~300 μm) represented previtellogenic oocytes. Previtellogenic oocytes accounted for between 6% in November/December 1999 and 40% in February/March 2001 of the total oocytes. Once oocytes reached 600 μm they underwent vitellogenesis increasing their cell diameter to its maximum size of 1750 μm. Vitellogenic oocytes accounted for the majority (>60%) of total oocytes in the ovary and were represented by the second peak (~1000 μm) on the size-frequency distributions. The large error bars on the mean oocyte size-frequency distribution suggested that there may be no synchrony between individuals. However, all individuals exhibited a bimodal distribution (Figure 3.31), and during November/December 1999 the proportion of previtellogenic oocytes was <8% in all six individuals (Appendix 5). This suggested that there was synchrony amongst individuals.

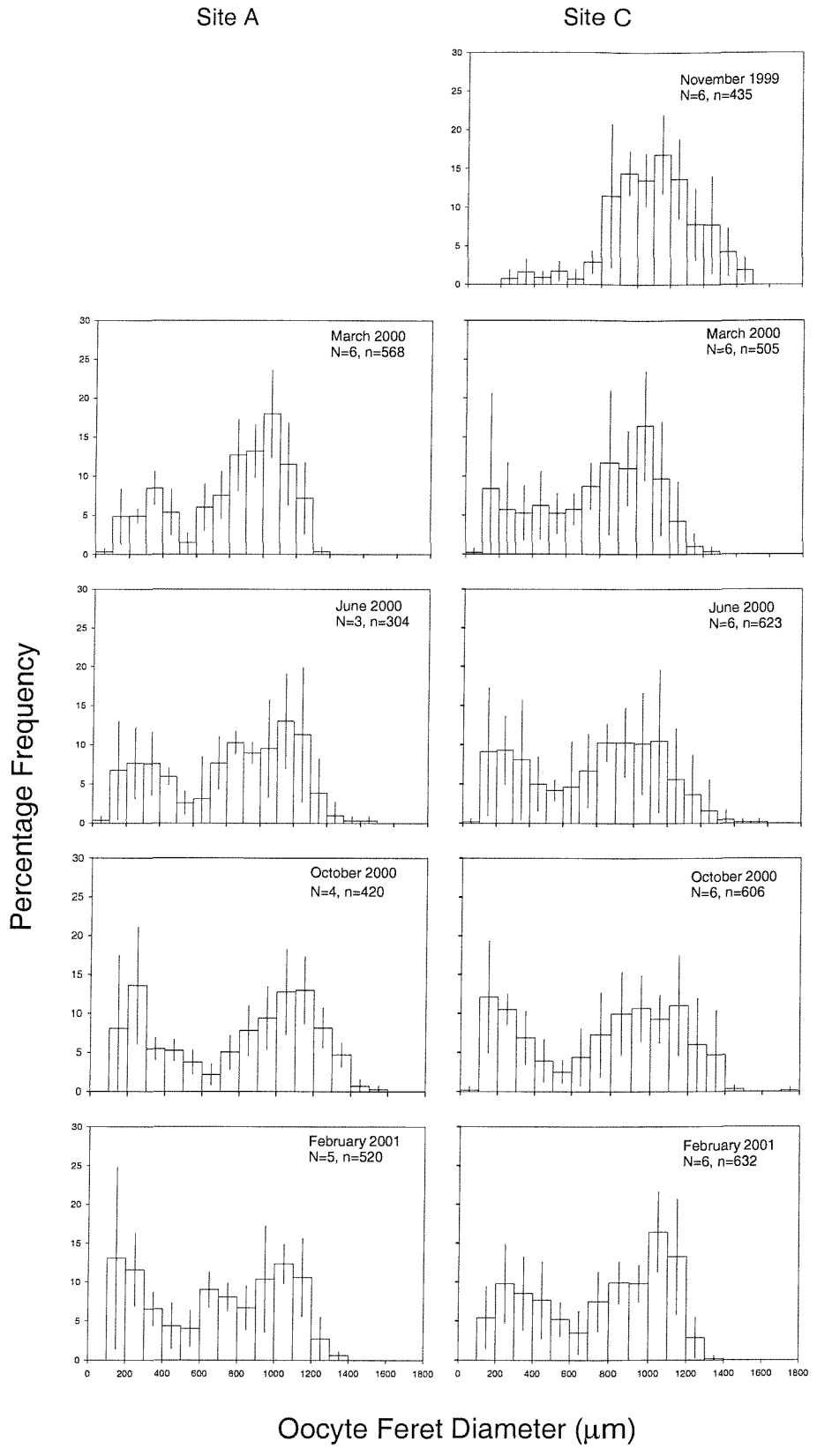

Figure 3.30. Oocyte size-frequency distributions for *Ctenocidaris perrieri*. Spatial and temporal variability from November/December 1999 – February/March 2001. Mean percentage frequency ± St Dev. N = number of females; n = number of oocytes.

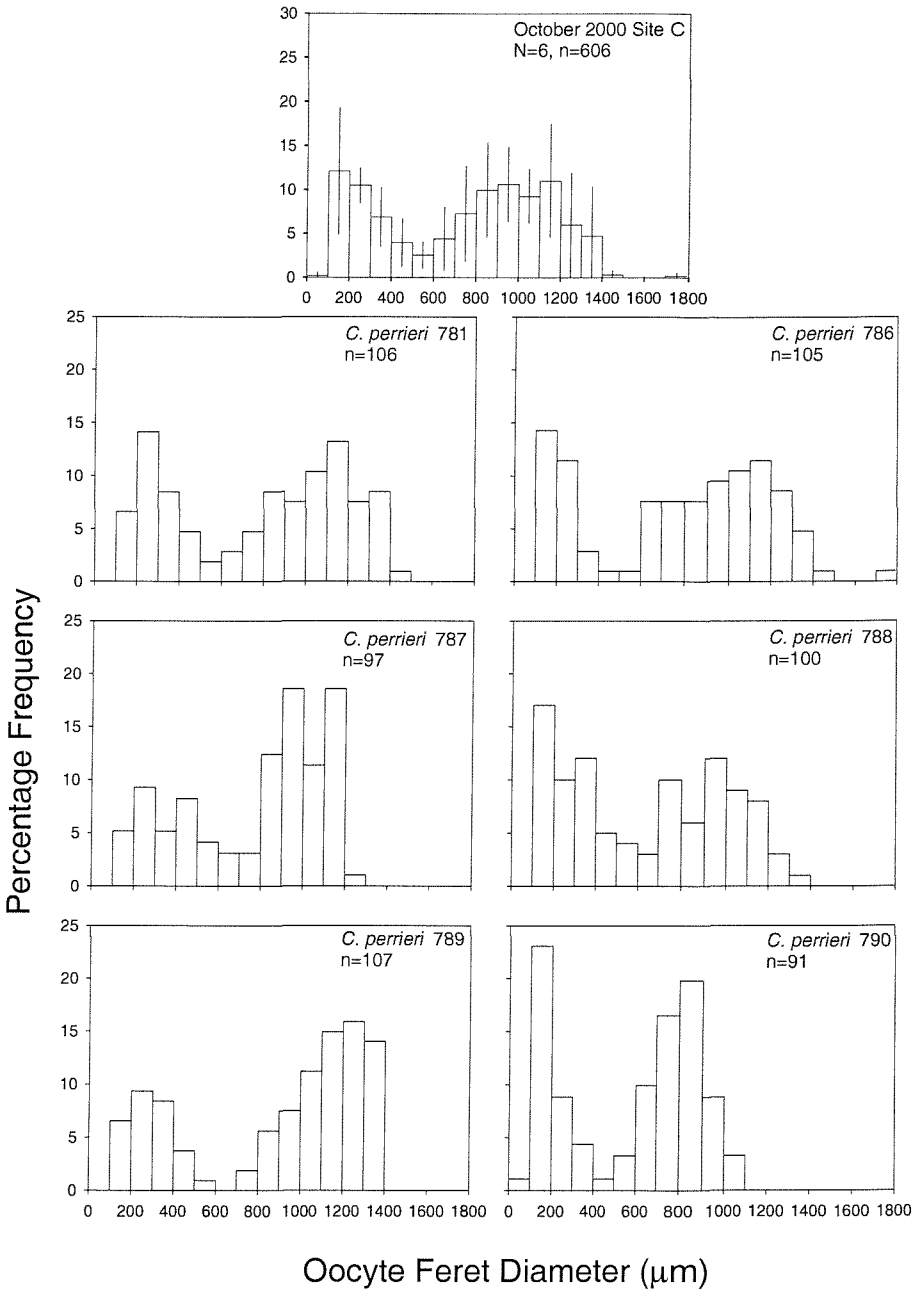

Figure 3.31. Mean oocyte size-frequency distribution for *Ctenocidaris perrieri* October 2000 Site C (top centre), and individual oocyte size-frequency distributions for October 2000 Site C. *C. perrieri* 781-790 = individual reference numbers. N = number of females; n = number of oocytes.

Amphipneustes lorioli (Echinoid)

External gonad morphology

As an irregular spatangoid urchin, *Amphipneustes lorioli* had only three gonads; the retreat of the periproct along the interradius AB inhibited the gonad developing in that interradius; the gonad of the interradius CD was also absent (Hyman, 1955). The gonads were suspended from the aboral gonopores in the top of the test by the gonoduct and mesenteries. The gonads consisted of numerous branched tubules, which united basally at the gonoduct (Figure 3.32). Developing oocytes in the tubules could be seen through the ovary wall and were very large in size owing to the brooding nature of the species. The testes had numerous blunt ended tubules, which were distinctly cream in colour when full of spermatozoa.

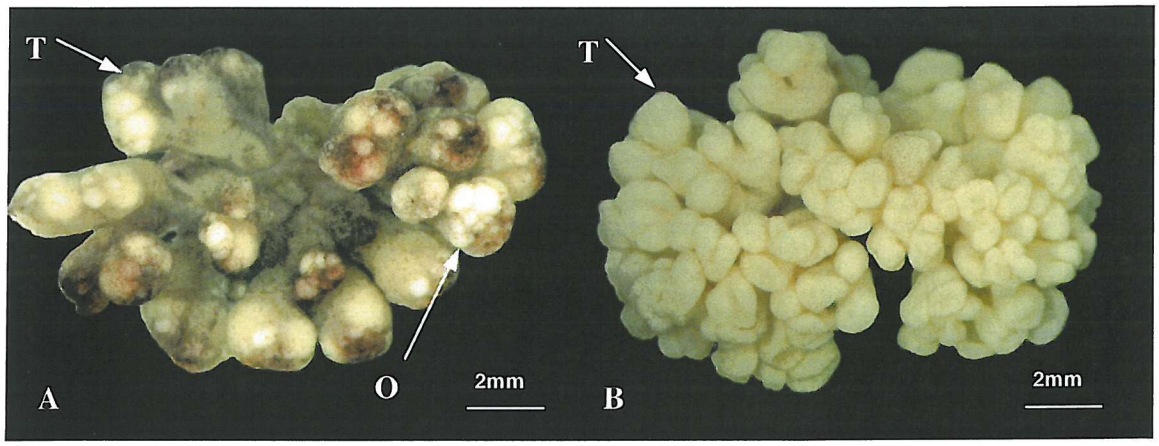

Figure 3.32. External gonad appearance of *Amphipneustes lorioli* (**A**) Female, (**B**) Male. O = oocyte; T = tubule.

Gametogenesis in *Amphipneustes lorioli*

Oocyte development. Previtellogenic oocytes commenced their development in the nutritive tissue along the germinal epithelium of the gonad wall. These oocytes could be recognised by their smooth cytoplasm that stained purple with haematoxylin (Figure 3.33 A and B). As these oocytes increased in size the volume of cytoplasm increased disproportional to the nucleus. Vitellogenesis commenced when oocytes reached ~520 µm in feret diameter. The cytoplasm of vitellogenic oocytes became acidophilic with the deposition of lipid, and was granular in structure (Figure 3.33A). Vitellogenic oocytes reached a maximum oocyte feret diameter of 1971 µm at which point part of the oocyte was still be in contact with the ovary wall and the rest of the oocyte expanded out into the lumen of the tubule and occupied the entire width of the

tubule. Phagocytosis was only observed in one individual where phagocytes produced a mass of breakdown material from vitellogenic eggs.

Sperm development. The testes wall of *Amphipneustes lorioli* comprised an outer gonad wall and an inner gonad wall. The inner wall was folded at regular intervals; these folds were often elongated and stretched far into the testes lumen (Figure 3.33C). Spermatogonia (mean diameter 5.8 ± 0.63 µm) and spermatocytes (mean diameter 4.7 ± 0.32 µm) lined the germinal epithelium of the inner sac and folds. The spermatocytes underwent meiosis to form spermatids (mean diameter 2.7 ± 0.2 µm), which were found in the lumen around the folds of the inner sac (Figure 3.33D). The spermatids then differentiated into spermatozoa with an elongated head (head length 8.83 ± 0.07 µm).

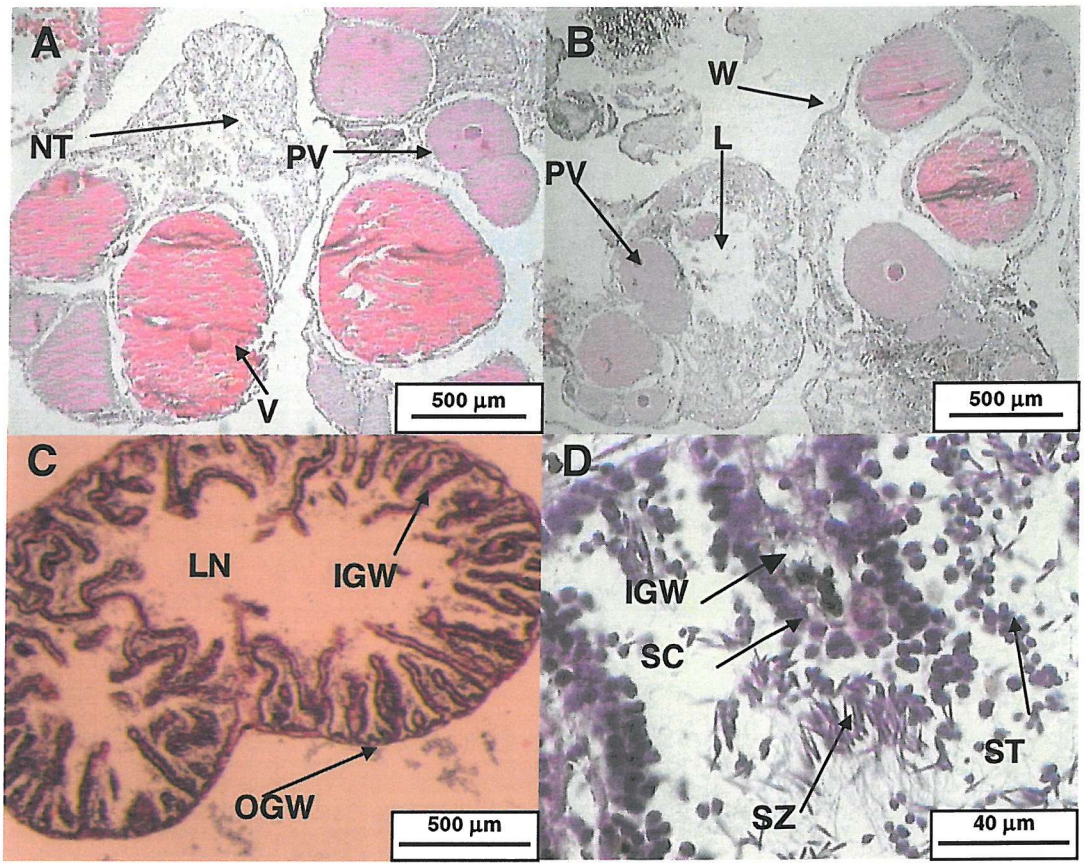

Figure 3.33. Light histology sections of the ovary (**A** and **B**) and testes (**C** and **D**) of *Amphipneustes lorioli*. IGW = inner gonad wall; LN = lumen; NT = nutritive tissue; OGW = outer gonad wall; PV = previtellogenic oocyte; SC = spermatocytes; ST = spermatids; SZ = spermatozoa; V = vitellogenic oocyte; W = ovary wall.

Reproductive output

Gonad indices

There was no significant difference in gonad indices among samples (Figure 3.34A) for either males or females (Two-way ANOVA: Sex F=16.09, df1, P= 0.000; Season F=1.11, df3, P=0.355). Figure 3.34A indicated a non-significant increase in female gonad index in October 2000, which decreased again in February/March 2001. A similar non-significant increase in the male gonad index was seen in June 2000, with indices decreasing again in October 2000 and a subsequent rise in the indices in February/March 2001. The within sample variability was high, indicating that there was no synchrony between individuals.

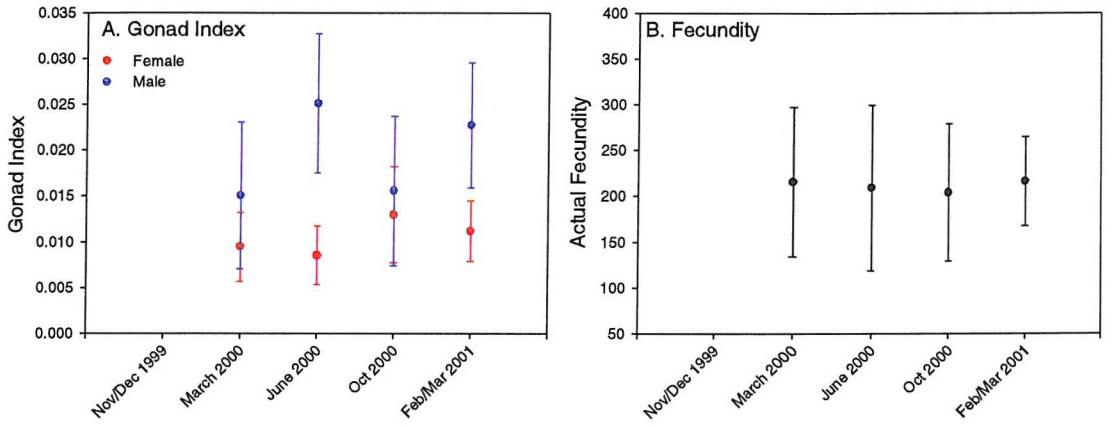

Figure 3.34. (**A**) Seasonal variation in male and female gonad indices of *Amphipneustes lorioli* at Site C. (**B**) Seasonal variation in fecundity of *Amphipneustes lorioli* at site C. Data are standardised to an animal of 65.7 test length and presented as means ± SD.

Fecundity

Figure 3.34B showed the actual fecundity for *Amphipneustes lorioli* from March 2000 to February/March 2001. There was no significant difference in mean fecundity during the sampling time period for *Amphipneustes lorioli*. All seasons showed a high within-sample variability. The mean actual fecundity was in the range of 215 vitellogenic oocytes per female throughout the sampling period, with a maximum of 372 and a minimum of 81 oocytes per individual.

Mean oocyte sizes

The mean oocyte size (± standard deviation) for the four temporal samples at site A were shown in Figure 3.35, previtellogenic and vitellogenic oocytes were plotted

separately. Statistically there were significant differences between the temporal samples of the vitellogenic oocytes (ANOVA, F=4.12, 3df, P=0.023). The mean vitellogenic oocyte sizes for March 2000, June 2000 and February/March 2001 were very similar (850.5 μm ± 73, 862.3 μm ± 44 and 842 μm ± 34.7 respectively) compared to the mean size for October 2000 (962 μm ± 88.5). It seems that there was a significant decrease in oocyte size between October 2000 and February/March 2001, as these samples showed no overlap. There was no significant difference in previtellogenic oocyte size over the sampling period as illustrated in Figure 3.35.

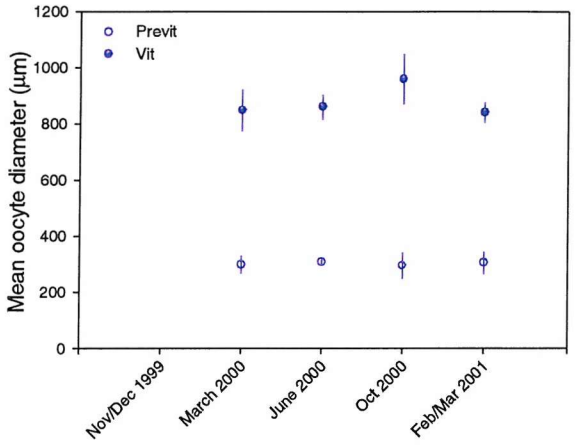

Figure 3.35. Seasonal variation in mean oocyte diameter of previtellogenic and vitellogenic oocytes for *Amphipneustes lorioli.*

Oocyte size-frequency distributions

Mean oocyte size frequency distributions were constructed for each of the four sampling periods during the study (Figure 3.36). Although there was variability between individuals, indicated by the error bars, the oocyte size-frequency distributions exhibited a similar general pattern. In all cases there was a broad range of oocyte sizes, from 45 μm to 1971 μm. Each individual exhibited a relatively balanced distribution of 50-56% previtellogenic oocytes (<550 μm) and 44-50% vitellogenic oocytes (>550 μm) (Appendix 6). The peak of previtellogenic oocytes was at ~300 μm, a second peak of early vitellogenic oocytes was sometimes present at either 600 μm or 800 μm. In the individuals 965, 993 and 996 from October 2000 (Figure 3.37), there was also a small peak of mature vitellogenic oocytes at 1400 μm. The maximum oocyte sizes for individuals in March 2000, June 2000 and February/March 2001 ranged from 1400 to 1540 μm. Two individuals from October

2000 had maximum oocyte sizes as great as 1650 μm and 1971 μm (Figure 3.37). The remaining four individuals from October had maximum oocyte sizes in the range of 1302 μm to 1450 μm.

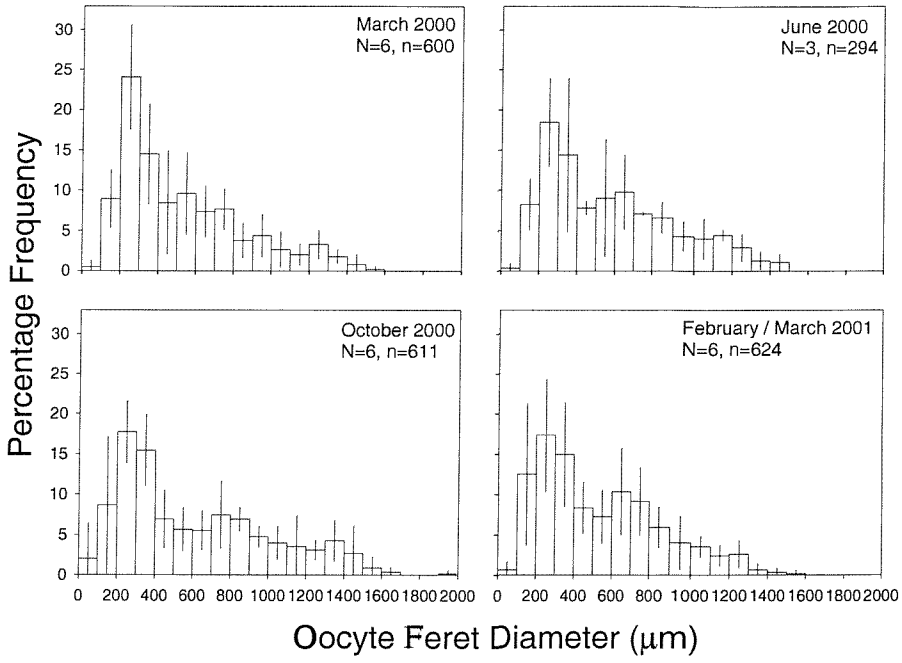

Figure 3.36. Mean oocyte size-frequency distributions for *Amphipneustes lorioli*. Temporal variability from March 2000 – February/March 2001. Mean percentage frequency ± SD. N = number of females; n = number of oocytes.

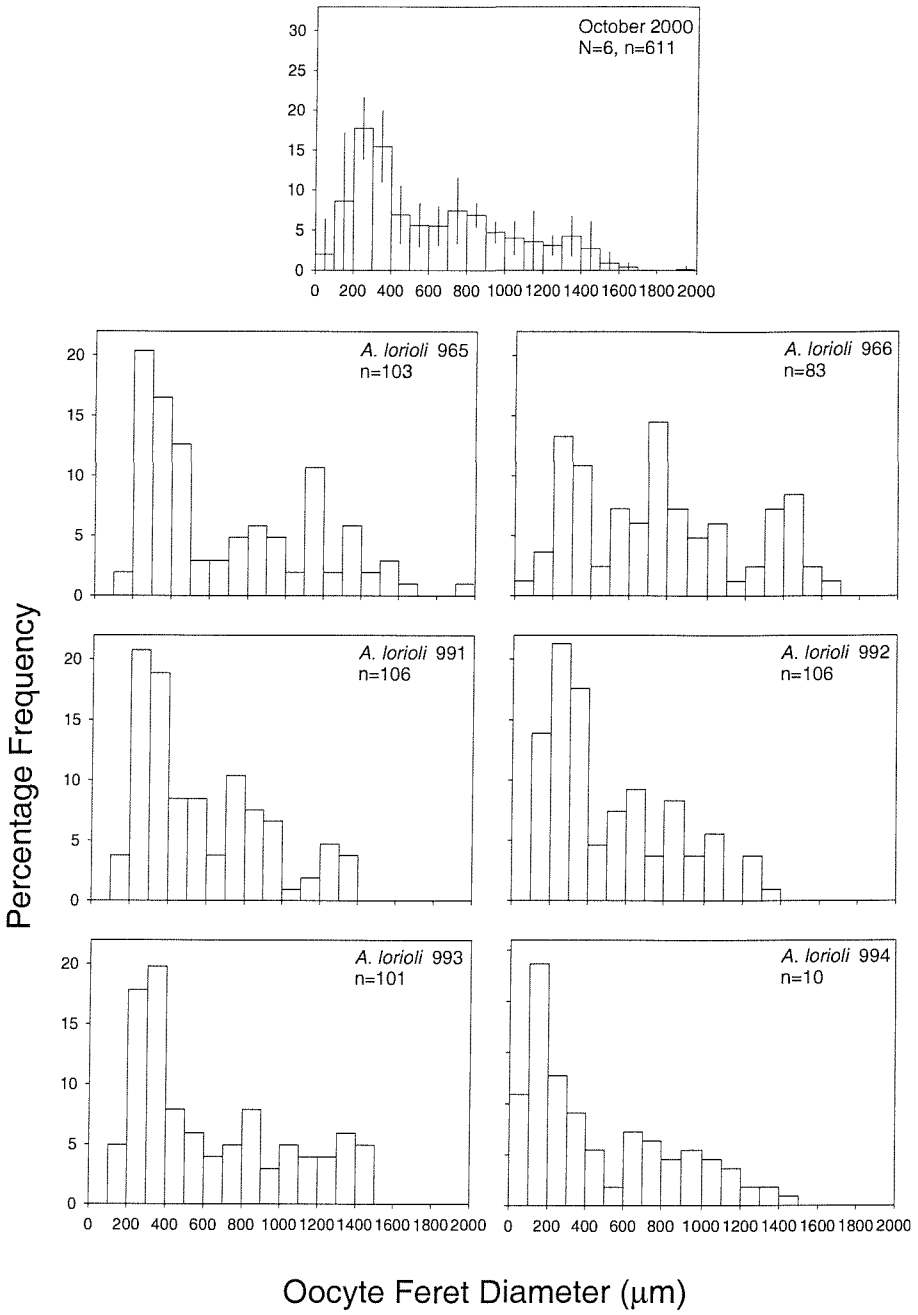

Figure 3.37. Mean oocyte size-frequency distribution for *Amphipneustes lorioli* October 2000 Site C (top centre), and individual oocyte size-frequency distributions for October 2000 Site C. *A. lorioli* 965-994 = individual reference numbers. N = number of females; n = number of oocytes.

Brood protecting in *Amphipneustes lorioli*

The test was modified in all species of the Antarctic genus *Amphipneustes* to enable brooding of the young. The lateral petaloid ambulacra were deeply sunken as marsupia; adjacent spines crisscrossed over the brooding area helped to keep the young in place. All of the brooding pouches examined contained embryos or juveniles of different stages of development, which occurred in approximately equal numbers in

each brooding pouch. Six embryo and juvenile stages could be identified from within the brooding pouches of the individuals over the whole sampling time period (Table 3.4, Figure 3.38). At stage 1 the embryo was smooth and spherical, the feret diameter ranged from 1.5 mm to 17.6 mm. Stage 2 embryo was no longer perfectly spherical and had increased diameter to 1.66 mm-2.1 mm. The primary juvenile stage was characterized by a complete exteriorisation of both oral and aboral sides. The five-ambulacral areas and tube feet rudiments had developed. Juvenile stage 1 was oblong in outline (diameter 2.5-3.33 mm) but became spherical as development proceeded. The advanced stage 2 juvenile had the appearance of a miniature urchin, pentagonal in shape, with short stout spines (test diameter 2.18-3.0 mm). As development continued the spines became longer and more numerous. At stage 3 the juvenile had retained the pentamerous symmetry. The final observed juvenile, stage 4, was slightly elongate in shape, similar to the irregular shape it would adopt as an adult. From juvenile stage 1 the test had almost doubled in size by the time it has developed to stage 4 (diameter 4.04-5.4 mm).

The embryo stages developed in the deep recess at the top of brooding pouch that was curved back under the test as it joined the apical system (Figure 3.39). The slightly more developed juvenile stages 1 and 2 had moved down from the recess to the back of the brooding pouch near the mouth, and stages 3 and 4 juvenile tended to cluster amongst the spines covering the mouth of the pouch.

	Embryo stage 1 (170)	Embryo stage 2 (277)	Juvenile stage 1 (132)	Juvenile stage 2 (89)	Juvenile stage 3 (151)	Juvenile stage 4 (60)
Minimum and maximum test diameters (mm)	1.5-1.76	1.66-2.1	2.5-3.33	2.18-3.0	2.8 - 4.0	4.04-5.4

Table 3.4. Minimum and maximum embryo and juvenile test diameters (mm). Values are calculated from 879 juveniles, taken from 21 adult females from March 2000 to February/March 2001. The value in brackets, for each category of juvenile, indicates the number of individuals measured.

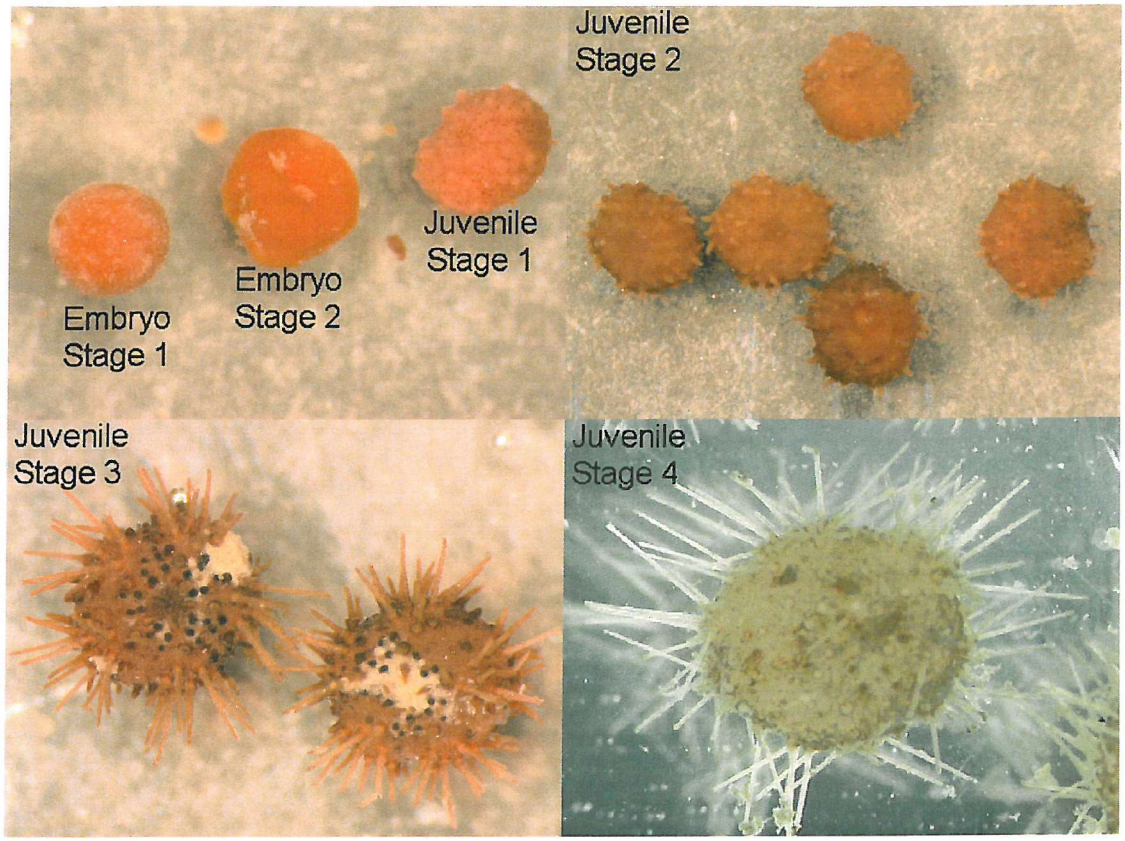

Figure 3.38. The six stages of embryo and juvenile development in *Amphipneustes lorioli* brooding pouches. For sizes see Table 3.4.

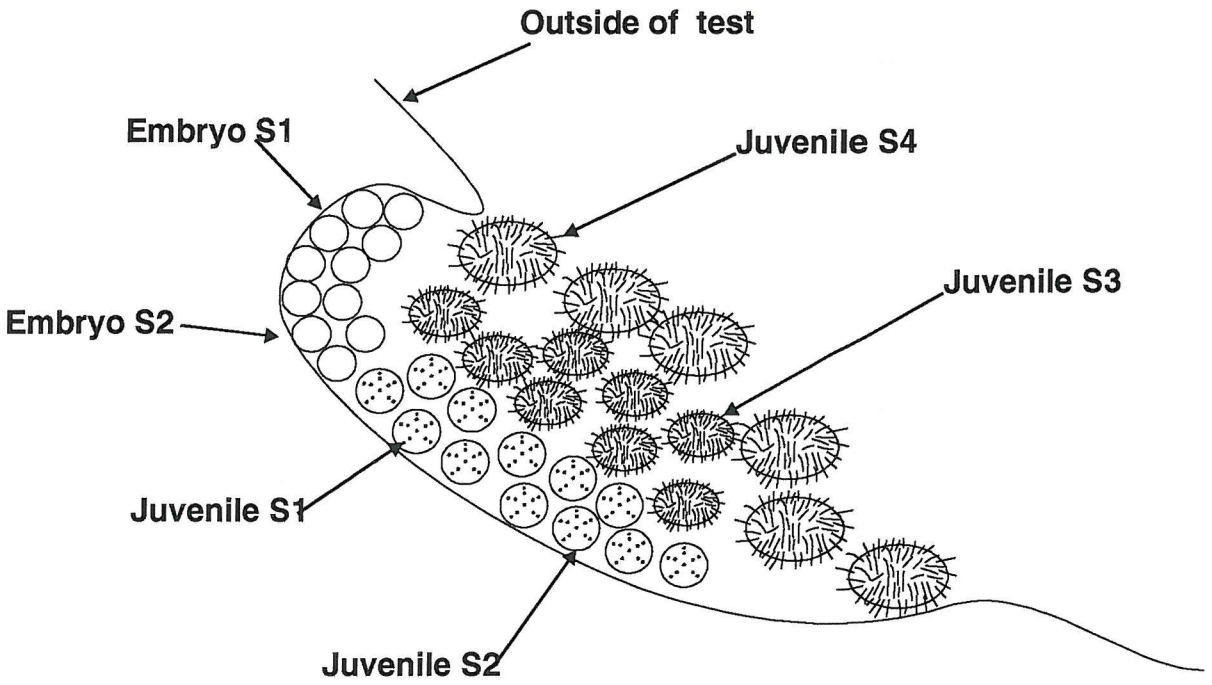

Figure 3.39. A cross-section of a brooding pouch from *Amphipneustes lorioli* showing the position of embryo and juvenile stages. Not to scale.

Population structure of juveniles in the brooding pouches

There were two cohorts of juveniles developing in the brooding pouches of *Amphipneustes lorioli* throughout the whole duration of the sampling period, indicated by the bimodal size frequency distribution in Figure 3.40. One cohort of the smallest embryo diameter (~1800 μm) was present throughout the sampling period. During March 2000 the larger cohort of juveniles had a mean of 3000 μm, and was present in all individuals. This cohort continued to grow and develop, attaining a mean diameter of 3600 μm by June 2000. During October 2000 the cohort of juvenile urchins had increased in size to 5600 μm maximum diameter in all individuals where it was still present. Several individuals no longer contained a cohort of large juveniles. Adults, which no longer contained this cohort, had a high proportion of small eggs that had a bimodal distribution, indicating the development of a new cohort of juveniles. Brooding pouches of only one individual were retained during February/March 2001. The cohort of mature juveniles was not present in this specimen, however there was a bimodal distribution. One peak was in the size range of mature oocytes (1800-2200 μm), the other cohort was somewhat more developed at sizes ranging 3000-4000 μm.

Given the pattern shown in Figure 3.40, it appeared that juveniles grew in specific cohorts. A cohort began to develop in October and was clearly distinguishable from the egg cohort by March the next year. The juveniles continued to grow and reached a maximum size of 5600 μm by October. The fully-grown juveniles appeared to be leaving the pouches at this time as several individuals lacked mature juveniles. This gave a mean brooding period of an individual, from newly laid egg to advanced juvenile, of 8-12 months.

There was a strong relationship between the adult test length and the length of the brooding pouch (Regression: R^2= 0.72, 20 df, P=0.000). However there was no relationship between brood pouch length and the number of brooded embryos and juveniles. The mean number of embryos/juveniles per individual is 57.5, however this figure was not very meaningful as the variation between individuals was so great. The minimum number of brooded embryos/juveniles was 4 (test diameter 75.5 mm), and the maximum number was 146 (test diameter 79.6 mm). The mean test diameter of

females sampled was 67.4 mm. The numbers of embryos/juveniles were unevenly distributed within the four brooding pouches as shown in Table 3.5. The position of the pouch on the female was not noted prior to dissection; therefore the numbers 1-4 only refered to the order of dissection.

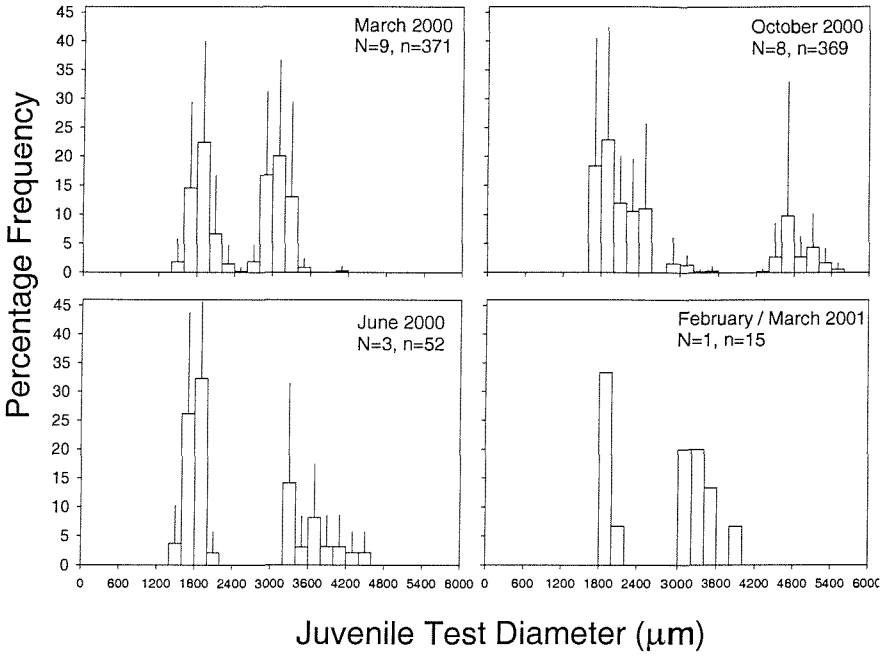

Figure 3.40. Size structure of juvenile *Amphipneustes lorioli* found in brooding pouches on the adult females. N= number of females; n= number of juveniles.

Brooding pouch - Individual number	1	2	3	4
397	4	5	3	
400	7	11	8	6
402	7	6	10	2
471	26	25	16	25
476	2	1		2
478	27	33	25	25
964	41	34	33	38
992	13	19	17	11
994	3	7	6	6

Table 3.5. Distribution of total number of embryos and juveniles in each of the four brooding pouches. Brooding pouch number does not relate to any position on the adult.

Discussion

Reproductive cycles of six species of echinoderm from five different taxonomic families are described in this study. Sampling intervals were specifically designed to cover several consecutive seasons, with the repetition of two seasons the subsequent year to include an inter-annual component. This strategy is highly beneficial to reproductive studies as gametogenic cycles can be followed throughout a set of seasons, and between years to check for repeatability and predictability. The reproductive patterns of *Protelpidia murrayi, Peniagone* sp., *Psilaster charcoti, Sterechinus antarcticus, Ctenocidaris perrieri* and *Amphipneustes lorioli* at 600m on the Antarctic continental shelf show a variety of gametogenic cycles and development modes. All species were found to be gonochoric.

Primary production in Antarctic coastal waters is highly seasonal. Extremely low production levels occur during the dark, ice-bound winter months and high levels occur during the austral spring/summer (Dec-Jan) phytoplankton bloom (Clarke, 1988; Knox, 1994). It has been shown by several sediment trapping programmes that this extreme seasonality in primary production produces dramatic variations in the magnitude of the vertical flux of organic matter from the surface waters to the seabed (reviewed by Karl *et al.*, 1996). These intense phytoplankton blooms could result in a high proportion of the organic matter export arriving on the Antarctic continental shelf floor. Mechanisms such as the production of faecal pellets through high levels of zooplankton grazing and the formation of aggregates are known to accelerate the sinking of fine particles (Honjo, 1990). This intense seasonality in the flux of labile organic matter to the Antarctic shelf floor may influence the growth, feeding and life history of Antarctic benthos. It has been shown for benthos in the NE Atlantic, where there is a distinct seasonality to the deposition of phytodetritus, that the reproduction of some invertebrate fauna is closely coupled to this flux (Tyler, 1986). In both deep-sea and shallow water Antarctic environments some species have been found to respond to the flux of organic matter with a seasonal reproductive periodicity (Table 3.1), whilst others show no such response. Prior to the FOODBANCS programme little was known about the fate of the flux of surface derived organic material to the seabed on the deep continental shelf of the western Antarctic Peninsula, and even less was known about the impact and subsequent responses of the organisms subject to this flux.

***Protelpidia murrayi* (Holothurian)**

The elasipod holothurians are unique to the deep sea and are considered to have evolved in deep water (Hansen, 1975). At 600m depths on the Antarctic continental shelf *Protelpidia murrayi* is living at the upper limits of its depth range. It is likely that the elasipodids had invaded the bathyal waters by the end of the Tethys Sea era, and that they moved along the South American continental slope into Antarctic waters (Gebruk, 1994), and onto the unusually deep continental shelf. The most diverse family of Elasipoda are the Elpidiidae. The reproductive biology of *P. murrayi* varies in detail compared to other elpidiid holothurians, although the maturation of gametes follows a similar pattern. Oocyte development of *P. murrayi* takes place within pockets in the connective tissue of the ovary wall between the coelomic epithelium and the internal epithelium, whereas oocyte development in other species of the family Elpidiidae takes place along the germinal epithelium where the connective tissue layer is thin (Tyler *et al.*, 1985; Wigham *et al.*, 2003).

Oocyte size-frequency distributions of *P. murrayi* show few differences between sites A and C. Site A is situated on the inner continental shelf close to the Antarctic Peninsula, the proximity to the coast and retreating spring ice edge would expose site A to comparatively high levels of surface phytoplankton production. In comparison, site C is situated on the outer continental shelf close to the shelf break where surface phytoplankton production is lower and the subsequent phytodetritus flux to the shelf is also reduced. At site A surface sediment phytodetritus chloropigment and phaeopigment concentrations ranged from 64.9 – 395.1 μg g^{-1}, and 202.5 - 1260.7 μg g^{-1} respectively, compared to site C where chloropigment and phaeopigment concentrations ranged from 12.7 - 18.9 μg g^{-1} and 20.7 - 53.5 μg g^{-1} respectively (Smith and Minks unpublished data). These values were recorded during February/March 2001 and were the highest values recorded throughout the sampling period, although values of chloropigment and phaeopigment were higher at site A than site C throughout the study period (Smith and Minks unpublished data). Evidence provided by the oocyte size-frequency distributions during February/March 2001, shows a higher proportion (12%) of vitellogenic oocytes present in the ovaries of females at site A than at site C. It is possible that this increase in proportion of vitellogenic oocytes is associated with a higher fecundity at site A, and it is tempting

to link this to the higher pigment levels at site A. Research carried out by the Palmer LTER group showed that phytoplankton community composition and abundance in the Palmer LTER study area was highly variable (Garibotti *et al.*, 2003). A gradient in chlorophyll *a* concentration was found across-shelf, with maximum concentrations in coastal waters and a gradual decrease of phytoplankton standing stock seaward. Garibotti *et al.* (2003) suggest that this may be a stable pattern for the area, as it has also been found throughout successive years by Smith *et al.* (1996). This suggests that this is a predictable gradient in phytoplankton availability from year to year and that organisms on the continental shelf are subject to different levels of organic matter depending on their location that may affect their reproductive productivity.

Eckelbarger and Watling (1995) propose that the duration of oogenesis in each species is genetically determined and therefore unaffected by exogenous factors such as an increase in food quantity or quality. It is the utility of different mechanisms of yolk synthesis (vitellogenesis) that determines the speed in which an organism can convert nutrients into egg production (Eckelbarger, 1994). Species that predominantly utilise autosynthetic yolk production (oocytes synthesizing their own vitellin) undergo slow oogenesis. Conversely, species that utilise heterosynthetic yolk production (yolk proteins synthesized by other cells and incorporated into the cell) are associated with rapidly growing oocytes. Eckelbarger and Young (1992) suggest a mixed method of yolk synthesis for holothurians and other echinoderms, although the precise extent of contribution that heterosynthetic production plays is unknown. However, fecundity is not a highly constrained parameter within a species, and can vary in response to an increase or decrease in food supply (Eckelbarger, 1986). Ramirez-Llodra *et al.* (2002) have shown how a variable food supply can affect the reproductive output of abyssal asteroids by comparing a station with a low organic input (Madeira Abyssal Plain: MAP) and one with a higher organic input (Porcupine Abyssal Plain: PAP). Specimens of *Styracaster horridus* taken from the MAP had a significantly lower fecundity than those individuals taken from the PAP.

The higher percentage of vitellogenic oocytes at site A compared to site C may reflect a higher individual fecundity at site A. The higher flux of organic material to the continental shelf at site A may have been utilised by the ovaries of *Protelpidia murrayi* to increase the proportion of heterosynthetic yolk production, which in turn

contributed to the increase in number of developing oocytes. Analysis of the population size structure of *P. murrayi* also suggests a greater recruitment of small individuals to the population at site A in comparison to site C. Population size frequency distributions represent the outcome of past reproductive events. Therefore if this gradient in phytoplankton from maximum concentrations in coastal waters were a stable pattern from year to year (Garibotti *et al.*, 2003) then the population distributions would reflect the greater proportion of vitellogenic oocytes produced at site A than site C from previous years.

Deep-sea elpidiid species have some of the smallest eggs recorded for any deep-sea holothurians. A maximum oocyte diameter of 300 μm was recorded for both *Peniagone azorica* and *Peniagone diaphana* (Tyler *et al.*, 1985), ~200 μm for *Amperima rosea* and *Ellipinion molle* (Wigham *et al.*, 2003), 180 μm for *Kolga hyalina* (Billett and Hansen, 1982), and ~200 μm for *Elpidia gracialis* (Hansen, 1975). A maximum egg size of 357 μm in *Protelpidia murrayi* is somewhat larger than those found in other Elpidiid species. Tyler *et al.* (1985) suggested that development in *Peniagone azorica* and *P. diaphana* is lecithotrophic, with an abbreviated larval stage (Hendler, 1975). Based on the egg size of *Protelpidia murrayi* and the inferred development mode of the confamilial species, *Peniagone azorica* and *P. diaphana*, lecithotrophic development is likely. The fecundity of *Protelpidia murrayi* ranged from 2,000 to 35,000 eggs per female, and such values are consistent with a species exhibiting a lecithotrophic development (Table 3.1). Emlet *et al.*, (1987) discussed echinoderm egg size in relation to the type of larval development, and noted that whilst the smallest known lecithotrophic egg is ~280 μm (*Peronella japonica*), at least two species with eggs in the 280 – 350 μm size range have planktotrophic larvae. Therefore despite the lecithotrophic larval development suggested for other elpidiid species, planktotrophy also remains a possibility.

Despite the large egg size of *Protelpidia murrayi*, oocyte size frequency distributions indicate synchronous development and an associated seasonal reproductive pattern. However, synchrony between individuals and seasonal periodicity is very unusual in deep-sea echinoderms possessing eggs of a large size (Tyler, 1986). Generally, in the deep-sea, seasonal reproductive strategies are associated with the small egg size (<200

μm) and high fecundity of planktotrophic developers. However, in Antarctic shallow-water environments a lecithotrophic development is the most prevalent, and many species exhibit a seasonal gonad cycle (Table 3.1).

On a temporal scale the oocyte size-frequency distributions, at both sites A and C, showed distinct changes throughout the sampling period. During November 1999 previtellogenic oocytes dominated the ovaries, with few early vitellogenic oocytes (105-240 μm) present. During the following 4½ months a second cohort of oocytes developed, being formed from vitellogenic oocytes >160 μm. By June 2000 the peak of vitellogenic oocytes had reduced and the testes of the male urchins were either part or fully spawned. As a result of the low sampling frequency throughout the sampling period it was difficult to determine when the oocytes reach fully maturity and exactly when spawning took place. However, it is likely that oocytes were in the final stages of maturity between March and June 2000, and that spawning commenced sometime close to June. This indicates that the oocytes undergo the greatest size increase during the last 6-7 months of development, with vitellogenesis commencing sometime after November. Samples taken during October 2000 replicate those of the previous year and ovaries contain only one cohort of previtellogenic oocytes, all mature gametes have either been spawned or reabsorbed. The spring/summer phytoplankton bloom in Antarctic waters occurs between November and February (Smith *et al.*, 1995) coinciding with the onset of vitellogenesis in *Protelpidia murrayi*. Eckelbarger and Watling (1995) proposed three patterns to clarify the correlation between seasonal phytodetrital pulses and seasonal reproductive patterns in some species. These patterns include: (1) species initiate gametogenesis immediately in response to organic input and undergo spawning soon after; (2) species spawn when seasonal pulses coincide with conditions favourable for their planktotrophic larvae; (3) seasonal organic input initiates and synchronises gametogenesis, producing a future spawning episode after an extended period of vitellogenesis. It appears that gametogenic cycles of *Protelpidia murrayi* are prompted by the arrival of the seasonal flux of organic material. In particular vitellogenesis is initiated and synchronised by this pulse. It is likely that vitellogenic processes continue into early winter, as spawning is suspected to take place sometime close to June. Deposited material from the spring/summer phytoplankton bloom may persist for long enough periods on the

continental shelf, as suggested by the FOODBANCS hypothesis, to fuel vitellogenesis into the early winter months. Alternatively *Protelpidia murrayi* may have developed mechanisms for storing nutrients, such as in the gonad or gut tissues (Chapter 4), to allow vitellogenesis to continue.

Size distributions of *Protelpidia murrayi* from sites A and C both show evidence of two modal size classes (Figure 3.6). Whilst the distributions at both sites are distinctly bimodal, the proportion of the two size classes varies greatly between the two sites. The smaller size class accounts for the majority of individuals at site A, compared to a more balanced distribution slightly biased towards the larger size class at site C. It is likely that each of the two size groups contains several year classes, owing to the slow growth typical of high latitudes (Arntz *et al.*, 1994). The population pattern of *P. murrayi* provides evidence of the heterogeneity of the shelf environment as discussed for the oocyte size frequency distributions. The larger proportion of smaller individuals at site A may be indicative of either a higher reproductive effort by the adult holothurians or an increased juvenile survival rate. Given that vitellogenesis is initiated by the pulse of phytodetritus to the shelf, a greater quantity of phytodetritus at site A (resulting from higher surface phytoplankton biomass in coastal waters (Garibotti *et al.*, 2003)) may account for a substantially higher reproductive output at site A than that from site C.

***Peniagone* sp. (Holothurian)**

The gametogenic biology of *Peniagone* sp., in terms of gamete production, differs slightly from that of the northeast Atlantic species *Peniagone azorica* and *Peniagone diaphana* (Tyler *et al.*, 1985). Oocyte development takes place along and within the ovary wall with a layer of accessory cells surrounding the oocytes. Vitellogenesis starts at an oocyte diameter of about 100-140 μm indicated by the vacuolated appearance of the cytoplasm. At oocyte sizes in excess of 300 μm the cytoplasm of the oocyte was damaged during the histology process and only remained around the egg wall. *Peniagone* sp. produces a maximum oocyte diameter of 570 μm. nearly double that of the two temperate deep-sea species, *P. azorica* and *P. diaphana*, which have a maximum oocyte diameter of 300 μm (Tyler *et al.*, 1985). Based on the correlation of egg size and fecundity with reproductive strategy, Tyler *et al.* (1985)

suggested a pelagic lecithotrophic development with an abbreviated larval stage (Hendler, 1975) for both *P. azorica* and *P. diaphana.* It is therefore likely that *Peniagone* sp. with a somewhat larger egg size and maximum fecundity of ~5000 also produces pelagic lecithotrophic eggs (Emlet, *et al.*, 1987).

Oogenesis in *Peniagone* sp. is synchronous. All individuals from a particular sample exhibit a similar pattern of oocyte size-frequency distribution (Appendix 2) and individuals from two different samples exhibit different distributions. Synchrony in gamete production is usually associated with a particular reproductive periodicity such as seasonality. However, in the deep-sea, a lecithotrophic larval development is generally associated with continuous production of oocytes. Within the echinoderms it is currently thought that all known species with seasonal breeding will produce planktotrophic larvae (Young, 2003). In support of this the two congeneric species, *P. azorica* and *P. diaphana,* both exhibit continuous production of gametes (Tyler, *et al.*, 1985). Similarly the confamilial species *Amperima rosea,* and *Ellipinion molle* show aseasonal reproduction despite their relatively small maximum oocyte diameter ~200 μm (Wigham *et al.*, 2003). However, these species of elpidiid holothurians are all from ~4800m depth in the northeast Atlantic. Interestingly, the confamilial species *Protelpidia murrayi* investigated in this study, from the same Antarctic stations as *Peniagone* sp., shows a seasonal periodicity in oocyte development. Lecithotrophy is not always associated with continuous reproductive patterns and it should be noted that there are many examples of lecithotrophic development with an associated seasonal reproductive strategy in the shallow water Antarctic environment (Table 3.1).

A seasonal gametogenic cycle may be inferred for *Peniagone* sp. from the oocyte size-frequency distributions. Given that the development of oocytes is synchronous between individuals a spawning event would occur throughout the population. During November 1999 oocyte size-frequency distributions were dominated by previtellogenic and very early vitellogenic oocytes (>90%). The absence of mature oocytes suggests that individuals had recently spawned, releasing all of the vitellogenic oocytes ready for fertilization. In most temperate invertebrate species with a seasonal reproductive cycle all of the oocytes within the ovary would be

released at one time. However, many polar species, such as *Sterechinus neumayeri*, *Nacella concinna*, *Laternula elliptica* and *Odontaster validus* (Table 3.1) have much slower oogenic cycles that can take up to 18-24 months to complete. The evidence of a cohort of previtellogenic oocytes remaining whilst the mature oocytes have been spawned suggests that oogenesis in *Peniagone* sp. also takes more than a year to complete. Oogenesis is likely to take up to 24 months to complete, as there appears to be only one spawning period per year. This slow rate of oocyte production in *Peniagone* sp. in comparison to species in temperate latitudes indicates that the species has only limited temperature adaptation (Pearse *et al.*, 1991).

Evidence suggests that a spawning period in *Peniagone* sp. commenced during October/November and that the mature oocytes had been spawned by the following February/March. Larval development rates also tend to be slower in polar species in comparison to temperate water species. Pelagic lecithotrophic larvae of shallow water Antarctic asteroids can take 2 to 3 months before settling and metamorphosing (Bosch and Pearse, 1990) in comparison to less than 1 month for most temperate and tropical lecithotrophs. Smiley *et al* (1991) noted that the pelagic period, from spawning to settlement for indirectly developing temperate and tropical holothurians generally ranges from 2 weeks to more than two months. It is likely; therefore, that development rates for polar lecithotrophic holothurian larvae are in excess of 2 to 3 months. Larvae of *Peniagone* sp. may start to settle as early as February/March. Sediment trap and megacore samples indicate that higher concentrations of fresh organic material have been deposited on the continental shelf during March than at other times of year. The presence of greater quantities of organic material coincides with possible juvenile settlement times and may provide a rich food source for newly settled *Peniagone sp.* Chlorophyll-*a* (chl-*a*) values taken from the sediment cores show an inter-annual variability between March 2000 (2.3μg g^{-1} chl-*a*) and March 2001 (4.7μg g^{-1} chl-a). The difference in quality i.e. freshness, of available organic matter between years may affect the success of recruitment to the population.

In addition to inter-annual variability, oocyte size-frequency distributions indicate differences in oocyte production between sites A and C. This difference is most evident during March 2000. At site A a distinct cohort of vitellogenic oocytes had started development. However, a cohort of larger oocytes did not develop at site C

until June 2000 (within the limitations of the sampling frequency). A greater proportion of vitellogenic oocytes in the ovaries of individuals at site A compared to those at site C was also evident in *Protelpidia murrayi*. In *Protelpidia murrayi* it was suggested that the higher proportion of vitellogenic oocytes was linked to a higher actual fecundity at site A. It has been shown that individuals can have a significantly lower fecundity at a site of low organic input when compared to individuals located at a site of higher organic input (Ramirez *et al.*, 2002). During March 2002 sediments from site A contained 2.4 $\mu g\ g^{-1}$ chl-*a*, compared to 1.2 $\mu g\ g^{-1}$ chl-*a* from site C. Using chl-*a* as an indication of fresh organic material it may be suggested that site A receives larger quantities of fresh phytodetritus than site C. The subsequent utilization of this resource may result in a more rapid development of vitellogenic oocytes at site A than site C. However there are no differences in oocyte size-frequency distributions between sites A and C during the remainder of the sampling period. Conversely, there are differences in surface sediment chl-a concentrations between sites A and C, specifically during February/March 2001. Chl-*a* concentrations of 4.7$\mu g\ g^{-1}$ chl-*a* were recorded from site A and 2.2$\mu g\ g^{-1}$ chl-*a* were recorded at site C. It is possible that a minimum food quality is required for energy to be channelled through to reproductive growth. These values of chl-*a* indicate a high inter-annual variability in quantity/ quality of phytodetrital flux between March 2000 and March 2001. This may account for both sites A and C showing a development of 30% vitellogenic oocytes by March 2001.

The initiation of vitellogenesis occurs between November and March, whilst the flux of phytodetritus is at a maximum. However, final maturation and increase in oocyte diameter to maximum of 570 μm occurs between June and October when the detrital flux is at a minimum (see Figure 1.5). This suggests that either there is sufficient food available in shelf sediments during the winter months to compete vitellogenesis, the hypothesised "FOODBANC", or that during the summer month's food is stored in body tissues for later utilisation during the winter.

Repeated sampling in the deep northeast Atlantic led to the discovery of dense aggregations of the elpidiid holothurian *Kolga hyalina* (Billett and Hansen, 1982). These aggregations comprised dense numbers of specimens, all of which were small and of a similar size at any one station. The profusion of these small, similarly sized

K. hyalina over a wide range of the Porcupine Seabight suggests a highly synchronised reproductive strategy (Billett and Hansen, 1982). It was suggested that in the formation of large aggregations *K. hyalina* may also be a response to periodic accumulations of organic matter brought about in an unstable sedimentary environment by turbidity currents. Similar responses to an increase in organic matter have been observed in another elpidiid holothurian, *Amperima rosea*. In this case oocyte size frequency distributions were not found to exhibit any classic patterns of synchronous development associated with seasonally breeding, free spawning invertebrates. However, *A. rosea* does exhibit temporal trends that indicate an opportunistic reproductive strategy (Wigham *et al.*, 2003). These trends suggest an increased reproductive output under conditions that are energetically favourable for vitellogenesis and spawning large numbers of eggs. The results of the oocyte size-frequency distributions for *Peniagone* sp. also suggested an increased reproductive output in more favourable conditions. Unlike *Protelpidia murrayi*, *Peniagone* sp. did not appear to spawn all of the vitellogenic oocytes at one time. Instead *Peniagone* sp. exhibited a seasonal intensity in the production of vitellogenic oocytes, followed by spawning events.

However in contrast to these opportunistic species, Tyler *et al.* (1985) found no evidence of any reproductive seasonality based on mean oocyte diameter in two elpidiid holothurian species *Peniagone azorica* and *P. diaphana*. However, a more detailed examination of the reproductive biology of both *Peniagone* species was not possible as insufficient oocytes were available for assessment of oocyte size-frequency distributions. Tyler *et al.* (1985) found that the population size frequency data of *P. azorica* only indicated the presence of juveniles in one sample collection. These results are similar to those of Billett and Hansen (1982) for *Kolga hyalina*, and may also suggest that recruitment to the population is infrequent, occurring only after irregularly spaced breeding events. However the authors deemed this an unlikely explanation owing to the planktonic larval phase, which is unlikely to result in such a locally restricted settlement.

In addition to species of the genus *Kolga*, species of *Peniagone, Ellipinion, Amperima* and *Elpidia* can all occur in dense populations (Barnham *et al.*, 1967; Rowe, 1971; Hansen, 1975; Billett and Hansen, 1982; Smith and Hamilton, 1983; Gutt and

Pippenburg, 1991; Wigham *et al.*, 2003). Evidence from previous work on the elpidiid holothurians coupled with the present results of both *Protelpidia murrayi* and *Peniagone* sp. would suggest that the elpidiid holothurians as a group have an opportunistic reproductive pattern. In the context of the highly opportunistic nature of the elpidiid group to which both *Protelpidia murrayi* and *Peniagone* sp. belong, it seems feasible to suggest that the apparent seasonality in reproductive cycle is a highly tuned opportunistic response to the supply of phytodetritus to the continental shelf. Surface primary production during the Antarctic summer months is amongst the highest recorded in the world's oceans and results in a very high export production to the continental shelves. The arrival of the phytodetrital flux to the Antarctic Peninsula shelf may be more reliable in seasonal terms than to deeper waters (~4800m). Such opportunistic species may have a direct response to this food resource with a resulting seasonal reproductive output, which is not necessarily truly seasonal and thereby phylogenetically constrained (Ramirez-Llodra, 2002). This enables such species to thrive in physically unstable conditions by allowing them to take advantage of an unpredictable/non-continuous food supply to promote gamete production.

***Psilaster charcoti* (Asteroid)**

The gametogenic cycle of *Psilaster charcoti* on the Antarctic continental shelf would appear to be similar to that of *Psilaster andromeda* and *Bathybiaster vexillifer* (Tyler *et al.*, 1982; Tyler and Pain, 1982) from the deep northeast Atlantic. There is a large pool of small oocytes less than 300μm lining the germinal epithelium. As these oocytes mature and undergo vitellogenesis they migrate to the centre of the tubules. Those oocytes that reach maximum size are either spawned or undergo degeneration by internal phagocytosis. The maximum oocyte diameter reached in this study was 578 μm, somewhat smaller than recorded for *Psilaster charcoti* in McMurdo Sound (950 μm) and *P. andromeda* in the northeast Atlantic (950μm). Egg diameters of *P. charcoti* recorded at Arthur Harbor (770μm) are also smaller than at McMurdo Sound. It is possible that this difference in egg size between the shallow water populations and ~600m depth population of *P. charcoti* is a result of different sampling techniques. The histological techniques used in this study involve dehydration of the gonad by gradation of alcohol concentrations leading to some shrinking of tissue. However the egg diameters of the Arthur Harbor population of *P. charcoti*, which are 180μm smaller than those of McMurdo Sound, were determined

from spawned oocytes with the same methods used for the McMurdo population (Pearse and Bosch, 1994). This suggests that the differences in egg size are a real biological factor and not as the result of differing sampling techniques. In other studies inter-population differences in egg size have been attributed to differences in the adult environment, differences in nutrient availability, and to differential demands on resource allocation (George, 1990; Jaeckle, 1995). *Psilaster charcoti* has been observed to feed on *Euphausia superba* and sometimes mud on the Antarctic continental shelf (Chapter 2) although its guts were often observed to be empty (pers. obs., 1999-2001). Shallow water populations have been recorded to feed on sediment, faecal pellets, and also penguin and fish meat from baited traps (Jangoux, 1982). Greater food availability in shallow waters may explain the difference in egg size; there may also be a difference in food availability between sites in shallow waters to account for the difference in egg size between the McMurdo and Arthur Harbor sites.

Psilaster charcoti has a wide bathymetric distribution and is geographically constrained to Antarctic and sub-Antarctic waters. *P. charcoti* has been the subject of previous research at a shallow water station (<33m) in McMurdo Sound (Bosch and Pearse, 1990). The mode of reproductive development and the timing of ripe females were ascertained by attempts to induce spawning on freshly collected females. The females of the shallow water population were found to be ripe during late October; producing maximum egg diameters of 950 μm. Embryos of *P. charcoti* hatched as large (900-1000μm diameter), buoyant, ciliated blastulae and developed into non-feeding lecithotrophic larvae. Despite the difference in oocyte diameter, *P. charcoti* at ~600m depth on the continental shelf is expected to produce a lecithotrophic larvae as indicated by the large maximum egg size and the relatively low fecundity.

There is no evidence of gametogenic synchrony among individuals from one sample (Figure A1.21-27, Appendix 3) or among samples; this suggests that different members of the population are of breeding condition and are ready to spawn throughout the year. Gonad index data for a standard animal size (R=109.45mm) shows a high variation within each sample suggesting that the ovaries are all at different stages of development. In addition there is little difference between mean gonad index between samples intimating that production of gametes is asynchronous.

This is again in contrast to the shallow water population of which individuals only showed a competence to spawn during October (Bosch and Pearse, 1990).

Individual specimens had a large proportion of small oocytes at all times of year. Some specimens contained an even distribution of oocytes throughout the size range above 300μm, suggesting a slow continuous release of oocytes. Others however showed a bimodal distribution with a second peak of vitellogenic oocytes, suggesting that oocyte release within individuals may be periodic. Further evidence for periodic spawning is provided by the total absence of oocytes >320μm in several adult individuals. The continuous presence of small oocytes throughout the sampling period, whether a cohort of vitellogenic oocytes are present or not, suggests that these oocytes undergo a long maturation period. Oocytes of several shallow-water Antarctic species have been shown to undergo development periods of 18-24 months prior to spawning (Table 3.1). The males, once developed, appear to remain constantly mature, possibly being stimulated to spawn by the presence of a mature female (Tyler and Pain, 1982). It is clear that the reproductive strategy of the population of *Psilaster charcoti* on the Antarctic continental shelf differs from that of the seasonally reproducing shallow water population. Instead the reproductive strategy of the deeper water population appears to approximate that of deep-sea echinoderms. All known deep-sea echinoderms with seasonal breeding produce planktotrophic larvae, whilst all known species of continuous breeders produce non-planktotrophic larvae (Tyler and Young, 1992) like the deeper population of *P. charcoti.*

Different reproductive periodicities and differences in egg size between isolated populations, as discussed above, provide evidence that the life history variables of *Psilaster charcoti* are polytypic. This species thus displays phenotypic plasticity in relation to both egg size and gametogenic timing. Variation in the life history traits of populations of *P. charcoti* occurring at different depths and geographic localities may ultimately be related to environmental factors, such as temperature and food quality and quantity (Ramirez-Llodra *et al.*, 2002). *Psilaster charcoti* is known to have a circumpolar distribution and is also found in shallow waters close to the FOODBANCS sampling sites (Fisher, 1940), although the reproductive biology of this population is not known. It is possible that the different populations of *Psilaster charcoti* that have evolved different phenotypes with relation to egg size and

gametogenic timing are undergoing speciation. Doebell and Dieckmann (2003) showed that a gradual environmental gradient facilitates speciation. This is because the environmental gradient initially induces gradual spatial differentiation due to local adaptation along the gradient, which increases the degree of frequency dependence in spatially localised ecological interactions, and hence phenotypic clusters become reproductively isolated. In the case of *Psilaster charcoti* these environmental gradients could include the presence of different water masses between sites and the pressure gradient between the shallow water environment and the deep continental shelf. Tyler *et al.* (2000) discovered that early embryos of *Sterechinus neumayeri* taken from Antarctic shallow waters were able to tolerate pressures of up to 150 atm at +2.5 °C and +0.9 °C, and 100 atm at –120 °C. The authors suggested from these data that the larvae of *Sterechinus neumayeri* were capable of penetrating the deep sea through formation of Antarctic Bottom Water in the Weddell Sea. It is therefore clear that larvae are capable of surviving over a certain pressure range and may have used this tolerance to invade the deeper waters in stages. However it may also mean that as a species increases its depth range that populations living at the lowest extreme may have become reproductively isolated from those at the top of the pressure range.

It is important to point out that ecological conclusions discussed above are based on only four time periods with few replicates in each case. To achieve a fuller picture on the reproductive biology of *Psilaster charcoti* it would be preferable to have more replicates and more frequent sampling dates. To compare samples from shallow water successfully with those from ~600m depth it is also important to use the same methods.

***Sterechinus antarcticus* (Echinoid)**

The internal gonad morphology of *Sterechinus antarcticus* is similar to that of *Sterechinus neumayeri*. The duration of oogenesis and spermatogenesis in *Sterechinus antarcticus* are similar to those recorded for the shallow water population of *Sterechinus neumayeri* (Pearse and Giese, 1966; Brockington, 2001). The whole gametogenic cycle of *Sterechinus antarcticus*, from initial production of previtellogenic eggs to mature oocytes ranged from 18 to 24 months. Maturation of previtellogenic to vitellogenic oocytes via vitellogenesis took ~ 8 to 12 months.

Oogenesis in shallow water Antarctic fauna is generally a slow process and often takes more than a year to complete (Pearse *et al.*, 1991). In *S. antarcticus* two cohorts of oocytes may be seen in the ovary at any time of the year. The cohort of largest egg size will be spawned within the first year; the cohort of smallest egg sizes will proceed through maturation and be spawned the following year. Therefore only a proportion of oocytes within the ovary will go though maturation and be spawned at one time. This pattern is unique to Antarctic echinoids as deep-sea echinoids complete their gametogenesis in ~ 15 months (Tyler and Gage, 1984).

During November 1999 there was a high proportion of previtellogenic oocytes compared to a small peak of vitellogenic oocytes. By March 2000 the cohort of vitellogenic oocytes had increased in size. Spawning occurred during June 2000. Two individuals had already spawned and showed evidence of a new cohort of previtellogenic oocytes developing, whilst two individuals still contained ovaries with fully mature oocytes. October 2000 and February/March 2001 saw the development of a new cohort of vitellogenic oocytes.

Spermiogenesis in *Sterechinus antarcticus* exhibits a distinctly seasonal cycle that takes 10-12 months. Testes are packed with mature spermatozoa during June, coinciding with the female winter spawning period. Some testes still contain relict spermatozoa in October; and by November relict spermatozoa are reabsorbed by phagocytic tissue in the testes. Spermatogonia in the testes wall give rise to new columns of spermatocytes during February and March, these spermatocytes mature and differentiate into spermatozoa by June. This spermatogenic cycle follows the same development mode of the congeneric species *S. neumayeri* (Brockington, 2001). The two species only differ with respect to time of sperm maturity, fully mature spermatozoa being present in the testes of *S. neumayeri* during December (Brockington, 2001).

Gonad indices data also indicate that there is a significant increase in gonad size in both the ovaries and the testes between November and June. A large drop in the gonad index in October indicates that mature gametes have been spawned by this time. The more pronounced gonad index cycle in the testes compared to the ovaries may reflect

the total spawning of male gametes compared to the partial spawning of only one cohort of oocytes per year in the female.

The reproductive pattern of *Sterechinus antarcticus* differs in some respects to that of its congener, *S. neumayeri*. The maximum oocyte size of *S. neumayeri* is 120 μm, just less than half the maximum oocyte size of *Sterechinus antarcticus*. *Sterechinus neumayeri* is known to have to planktotrophic development, releasing large numbers of clear negatively buoyant eggs (Bosch *et al.*, 1987; P. A. Tyler pers. comm.). In comparison, *Sterechinus antarcticus* spawns bright red positively buoyant eggs (pers. obs.), and this has led to both a planktotrophic (Brey, 1991) and lecithotrophic (Mortensen, 1909) mode of development being inferred. Determining development mode based on egg size can be ambiguous. According to literature, species with eggs sizes <250 μm probably undergo planktotrophic development, whilst the smallest known lecithotrophic egg has a diameter of 280 μm (Emlet *et al.*, 1987). Evidence is available to support both planktotrophic and lecithotrophic modes of development. A lecithotrophic mode of development may be inferred from observation of the freshly spawned egg. Emlet *et al.* (1987) notes that many non-feeding larvae develop from positively buoyant eggs and remain positively buoyant prior to metamorphosis. Also, lecithotrophic larvae tend to be large, yolky and colourful, whereas planktotrophic larvae are generally smaller and transparent (Young, 2002). From these descriptions, it would suggest that the bright red buoyant eggs produced by *Sterechinus antarcticus* are in fact lecithotrophic, in comparison to the clear negatively buoyant eggs produced by *Sterechinus neumayeri*, which is known to exhibit planktotrophic development.

The picture is further complicated by data from the shallow-water Antarctic genus, *Porania*, which show that two congeneric species with the same maximum egg size (550 μm) can have differing modes of larval development (Bosch, 1989). Female adults of *Porania antarctica*, broadcast spawn large numbers (3-4 x10^4) of buoyant eggs that measure 550 μm and develop into unusual yolky planktotrophic larvae. In contrast, adults of the undescribed Antarctic *Porania* sp.studied here have a low fecundity (100-310 eggs), the few eggs produced also measure 550 μm, are heavier than seawater, and develop into demersal lecithotrophic larvae. Given this differing

mode of larval development in two congeneric Antarctic species, it is possible that the larger (257 μm) buoyant eggs spawned in large numbers (35,400 eggs from a single mature female) by *Sterechinus antarcticus* will develop into planktotrophic larvae. Therefore whilst a lecithotrophic development is the most likely, confirmation of development mode is still required from direct observations of reared larvae.

The timing of spawning in *Sterechinus antarcticus* is not synchronous with that of the shallower species *Sterechinus neumayeri*. Spawning has been found to occur during November for populations of *S. neumayeri* at Rothera Point and Signy Island (Brockington 2001, Stanwell-Smith and Peck 1998); and during May-December for populations at McMurdo Sound (Pearse and Giese, 1966) with the main spawning period occurring in November (Pearse *et al.*, 1986). The spawning period for *Sterechinus antarcticus* at 600m depths on the west Antarctic continental shelf thus commences nearly 5 months prior to that of *S. neumayeri*. The spawning period of *Sterechinus neumayeri* and the presence of feeding planktotrophic larvae in the water column during the austral spring/summer have been linked to the summer phytoplankton bloom when food is abundant (Pearse and Giese, 1966). However the deeper water *Sterechinus antarcticus* has a different development strategy. Oocyte size-frequency distributions indicate an austral winter spawning period coinciding with minimum values of surface primary production and therefore also of phytodetritus on the continental shelf. This disparity in spawning time of these two congeneric species may be accounted for by the differing reproductive mode. Should *Sterechinus antarcticus* prove to have lecithotrophic development, the larvae would be freed from dependence on the flux of particulate food, and hence development would be uncoupled from the spring/summer phytoplankton bloom (Pearse *et al.*, 1991). However, if the unusual planktotrophic development of *Porania antarctica* (Bosch, 1989) is assumed, a winter spawning period may require an alternative source of food for the developing larvae. In the Antarctic, echinoderm larvae have been found to ingest bacteria (Rivkin *et al.*, 1986; Pearse *et al.*, 1991), moreover, invertebrate larvae feed throughout the year on a relatively high and constant bacterial population (Rivkin *et al.*, 1986; Rivkin, 1991). This may be sufficient to promote development in planktotrophic larvae with a large yolk reserve, whilst a phytodetritus food supply is at a minimum.

Lecithotrophic eggs are typically larger than eggs that develop into planktotrophic larva, and the juveniles produced are also generally much larger (see refs in Pearse *et al.*, 1991). Subsequently, under conditions where juvenile mortality is high and restrictive and is size dependent, lecithotrophy might be favoured (Pearse *et al.*, 1991). A further consequence of lecithotrophic development is the reduced development time in the plankton. In polar conditions where development rates are slow, lecithotrophic development may be seen as a positive adaptation to lower larval mortality by reducing the time exposed to predators in the plankton. The modified planktotrophic development of *Porania antarctica* described by Bosch (1989) has certain advantages over the lecithotrophic development of its congeneric species *Porania* sp. The planktotrophic development profits from a shortened embryonic and larval phase (65 days *vs.* 78 days) and a larger juvenile size at metamorphosis (0.8 *vs.* 0.6 mm). Whether the larvae of *Sterechinus antarcticus* have lecithotrophic or an unusual planktotrophic development the benefits of a shortened larval life span and a larger juvenile size at metamorphosis appear to remain the same. This permits a winter spawning time in *S. antarcticus*, in contrast to that of *Sterechinus neumayeri* that spawns during the spring/summer with larvae that feed on the phytoplankton bloom.

***Ctenocidaris perrieri* (Echinoid)**

Ctenocidaris perrieri is known to brood it offspring on the peristomal membrane (Mortensen, 1928). This is typical of cidarid urchins in which, with the single exception of *Austrocidaris canaliculata*, the brood is always found on the peristome (Mortensen, 1928). In this study no specimens were found to be brood protecting. It is possible that individuals did not have young on the peristome as a result of the highly destructive sampling method that is likely to have dislodged any juveniles that were present. It is also possible that no individuals were brooding young during the sampling period.

Oocyte size frequency distributions are bimodal. The peak of vitellogenic oocytes (>600μm) accounts for 60-70% of total oocyte composition during each month with the exception of November/December 1999. During November/December

vitellogenic oocytes were responsible for ~90% of the total oocytes within each individual. This dominance of large oocytes is seen in each of the six individuals in November 1999 suggesting that individuals show synchrony of development. By March 2000 there had been an increase in the percentage of previtellogenic oocytes (~30%) and a decrease of 200μm in the maximum oocyte diameter. Some individuals exhibited a maximum oocyte diameter of 900-1200 μm in March 2000, suggesting that at some point between November 1999 and March 2000 the largest mature vitellogenic oocytes had been spawned. Maximum oocyte diameters can be seen to increase slightly between March 2000 and October 2000. By February/March 2001 many individuals exhibit maximum oocyte diameters <1300μm. The evidence from the oocyte size-frequency distributions thus suggests that the spawning may occur after November 1999 and October 2000. Gonad indices data show a clear pattern increasing to a maximum in June 2000 followed by a sharp decrease in October 2000. The female gonad index has high variability, but the mean values follow the same pattern of the male gonad index. Therefore the gonad index data support an austral spring spawning. It is very difficult to tell whether a drop in the maximum egg size during March is the result of a spawning event, given the frequency of sampling events. The presence of any brood juveniles on the peristome would help clarify the gametogenic cycle. However the presence of two cohorts of oocytes does suggest that instead of being spawned continuously oocytes are released periodically. The synchrony of development with individuals from a particular sample suggests that the periodic release will involve the whole population.

In the deep-sea, seasonality of reproduction in echinoids with a direct development is unknown (Tyler and Young, 1992). However examples of brooding cidarid urchins with a seasonally defined reproductive cycle exist in shallower waters (Barker, 1984). For example *Goniocidaris umbraculum* occurring on the outer continental shelf off Otago has a clearly defined reproductive cycle. Oocytes take two years to complete development to a maximum diameter of 950μm in June to July, and spawning is completed by August to September (Barker, 1984). After spawning the developing eggs are retained on the peristome and up to 60 embryos can be accommodated for a period of four months. Barker (1984) noticed that the urchins do not feed whilst brooding eggs or embryos and this may be because movement of the Aristotle's lantern may be sufficient to dislodge eggs from the peristome area. A lack of feeding

whilst brooding would indicate that urchins could only brood periodically as continuous brooding would lead to starvation of the adult. Furthermore, observations of four species of Antarctic cidarids with brooded juveniles show that all juveniles within a brood are at the same stage of development (Lockhart *et al.*, 1994). Should there be a continuous release of oocytes then broods would contain juveniles at many stages of development. This evidence suggests that cidarids develop oocytes in discrete cohorts within the ovary; these oocytes are simultaneously released to form a single brood on the adult. Given the nature of many Antarctic echinoderms in which oogenesis takes up to two years to complete, and the large maximum size of the oocytes (1750 μm), it is likely that oocyte development takes at least two years to complete in *Ctenocidaris perrieri*. The cohort of mature oocytes may be spawned later that season, and the cohort of previtellogenic oocytes may be ready for spawning the following year. The synchronous oocyte development shown in *C. perrieri* suggests that adult females would spawn and brood protect at the same times of year.

***Amphipneustes lorioli* (Echinoid)**

Histological analysis of the gametogenic cycle of *Amphipneustes lorioli* indicates no obvious synchrony between individuals. There are large variations in gonad index at each station, this is also indicative of a lack of synchrony between individuals. Oocytes of all sizes are present in the ovary during all sampling occasions, in some cases the oocyte size frequency distributions appear to be bi- or tri-modal. This is perhaps a result of the slow oogenic processes in Antarctic echinoderms, whereby oogenesis may take more than a year to complete, so that two or even three annual cohorts of growing oocytes can be found in one individual at the same time (Pearse *et al.*, 1991). Despite the apparent lack of synchrony between individuals there was a significant decrease in mean oocyte diameter between October 2000 and February/March 2001. However when examining oocyte frequency distribution there is no evidence to suggest that this decrease is as a result of a large spawning event of mature oocytes.

Fecundity, calculated from the presence of mature oocytes within the ovary, is ~ 200 oocytes per individual. This is low in comparison to species with a planktotrophic or lecithotrophic development, but is typical of other brooding echinoids (e.g., brood

sizes of up to 60 in *Goniocidaris umbraculum*, Barker, 1984). There is no change in the average fecundity value throughout the sampling period. However there are large variances in fecundity between individuals from the same sample. These data also suggest that there is no synchrony between individuals, and that spawning occurs throughout the year.

The gametogenic cycle of *Amphipneustes lorioli* appears to be similar to that of the Antarctic species *Abatus shackletoni* and *Abatus nimrodi* (Pearse and McClintock, 1990), which do not show any change in gonad indices with time and are therefore likely to reproduce throughout most or all of the year. The most comprehensive reproductive work has been carried out on *Abatus cordatus* at the Kerguelen Islands (Magniez, 1980, 1983; Schatt and Féral, 1991). *A. cordatus* has a highly synchronised reproductive cycle, in contrast to that of *Amphipneustes lorioli*, *Abatus shackletoni* and *Abatus nimrodi*. The timing of spawning of *Abatus cordatus* may differ within different locations depending on the availability of trophic resources where the animals live (Schatt and Féral, 1991). Juveniles of *A. cordatus* remain in the brooding pouches for ~8.5 months (Magniez, 1980; Schatt and Féral, 1991, 1996) until they are sufficiently developed to leave. Evidence from *Amphipneustes lorioli* of juvenile development within the pouches suggests that juveniles develop in discrete cohorts despite the apparent lack of oogenic synchrony. Between March 2000 and October 2000 it was possible to follow the development of a cohort of juveniles. The developing cohort comprises two sequential stages of juvenile throughout the development. This indicates that the development of juveniles is not entirely synchronous and that some individuals will commence the next stage of development prior to others, as observed in *A. cordatus* (Magniez, 1980; Schatt and Féral, 1991). Throughout the three sampling periods, a cohort of eggs was present in addition to the cohort of developing juveniles. This was the case within each individual and in some cases the egg cohort may account for up to 50% of the brood. Magniez (1980) also recorded the presence of eggs throughout the entire brooding cycle of *A. cordatus*. He noted that when the young left the brooding pouches the protective spines opened to let them out. Whilst the spines were open sand was able to enter the pouch causing the remaining eggs to degenerate. It is likely that the eggs start development in the deepest recesses of the pouches for greatest protection against sand or sediment particles. Once the juveniles develop their protective test they are able to move

towards the opening of the pouch in preparation for release. The effect of sand exposure on developing embryos provides certain problems for brooding urchins with a continuous development. If the spines open to release a fully developed juvenile there is a high risk that sand will be let into the pouch and cause degeneration of developing embryos. However it is possible that echinoids with a continuous production of oocytes have adapted mechanisms of opening their spines to admit newly fertilised oocytes without permitting sand to damage those already within the pouch. Similar mechanisms may also be used to release juveniles. In the case of *Amphipneustes lorioli* it is likely that the oocytes within the pouches throughout the duration of the developing cohorts are oocytes that did not successfully fertilize during the transfer from the gonopore to the pouch.

Several authors describe the presence of different stages of juvenile within the brooding pouch at one time (Magniez, 1980; Lockhart *et al.*, 1994; Pearse and McClintock, 1990). With maximum brood sizes in the range of 102-129 for *A. cordatus, A. shackletoni* and *A. nimrodi* each pouch may contain a maximum of 25-30 juveniles. Such numbers of individuals growing to a maximum test diameter of 5mm (Lockhart *et al.*, 1994) before leaving the pouch would be too great in total volume to fit into a brooding pouch (with dimensions 8mm width, 8mm depth and 20-38mm length) if all individuals matured at the same time. An adaptation to crowding is the development of individuals within a pouch being slightly staggered to prevent overcrowding.

The observations concerning the number of eggs in each brooding pouch indicated that eggs were not distributed in equal numbers within the four brooding pouches. Each adult female has four brooding pouches on the outside of her test and three gonads and gonopores through which eggs may be supplied. It follows that a gonad may have to supply eggs to more than one brooding pouch. Eggs are transported from the gonopores to the brooding pouches by the spines and pedicellaria (Schatt and Féral, 1996). Therefore in order to ensure that all of the eggs reach the brooding pouches it is likely that they are transported to their closest pouch to reduce the time that they may be dislodged from the spines. Magniez (1980) found a good correlation between the distance of the brooding pouch from the gonopore, and the number of eggs incubated in *A. cordatus*. From this relationship he suggested that the position of

the pouch relative to the gonopore determines how many eggs each brooding pouch receives. Unfortunately the position of the brooding pouch on the test was not recorded for *Amphipneustes lorioli* so the hypothesis cannot be tested in this study. However, Pearse and McClintock (1990) did not find any significant difference in either numbers or stages of embryos among the 4 brood chambers of either *A. shackletoni* or *A. nimrodi*, which they felt reflected an overall lack of spawning synchrony. This shows that although three species of *Abatus* may have similar morphological characteristics, they may have very different reproductive modes that cannot be classified by egg size or location.

In the case of *Amphipneustes lorioli* the evidence suggests that gametogenesis is not synchronous and that spawning may occur throughout the year. There may however be an external selective pressure that ensures the survival or development of juveniles in a discrete cohort. The percentage of eggs in the brooding pouches throughout the sampling period is high (Figure 3.40), generally $\geq$ the number of juveniles. Is it possible that once mature, eggs are transported from the gonad to the brooding pouches where they are stored until the female encounters a male urchin? If this were the case then one would expect the developing juveniles from different females to be at different stages of development, which they are not (Appendix 7). Another possibility is that the adult female releases the eggs from the gonopores when triggered by the presence of a ripe male, and that only some of the eggs are fertilized during the transit to the pouches. The eggs that did not get fertilized remain in the brooding pouches until the developing juveniles depart. At this point the unfertilised eggs are broken down following the admittance of sand to the pouch. Should this be the case this would prove to be an energetically costly strategy for a species contributing a lot of energy into each egg.

Reproductive strategies of echinoderms on the Antarctic Continental Shelf

Of the six species of echinoderm examined from the Antarctic continental shelf a variety of reproductive modes were discovered. These modes include three seasonal spawning lecithotrophs, a quasi-continuous lecithotroph and two seasonal brooders. All species exhibited slow oocyte development with times in the range of 18-24 months. This is in keeping with rates of oocyte development described for shallow-water Antarctic invertebrates (Pearse *et al.*, 1991; Table 3.1). These rates are

somewhat slower than those described for many deep-sea invertebrates, in which oogenesis often takes 8-12 months to complete. In those Antarctic species that do exhibit seasonality of oogenesis, spermatogenesis also follows a seasonal cycle. In contrast to oogenesis, spermatogenesis only takes up to a year to complete. This is because females will grow two cohorts of oocytes within the ovary at the same time, one to be spawned this year and one that will mature to be spawned the following year. Therefore males must produce mature spermatozoa each year. In species that do not exhibit any seasonality of reproduction, spermatozoa develop when the individual urchin reaches maturity and the testes remain full of sperm throughout maturity.

The developmental mode of *Sterechinus antarcticus* has not been directly determined from larval cultures, however both planktotrophic (Brey and Gutt, 1991) and lecithotrophic development (Mortensen, 1910) have been suggested. This species exhibits a seasonal reproductive cycle, with spawning taking place in June. The timing of spawning in *Sterechinus antarcticus* is out of synchrony with the spring bloom, and would suggest that the developing larvae do not rely on the flux of phytodetritus for nutrition. This evidence would suggest that lecithotrophy is the most likely mode of development for this species. Seasonal spawning periodicities have been seen in deep-sea echinoderms with a planktotrophic development (Tyler, 1986), and in shallow-water Antarctic echinoderms with both planktotrophic and lecithotrophic development (Table 3.1). Therefore it appears that the reproductive biology of *Sterechinus antarcticus* more closely approximates that of shallow-water Antarctic species than of deep-sea species. With a depth range of 100 to 1080m, *S. antarcticus* may be considered a true shallow water species with regard to the limits of the Antarctic deep-sea set by Clarke (2003). It is likely that the reproductive strategy of *S. antarcticus* is adapted to its environment as depth down the continental slope increases.

The elasipod holothurians, *Protelpidia murrayi* and *Peniagone* sp., appear to exhibit the same opportunistic reproductive strategy as employed by their deep-sea relatives. In this case the holothurians appear to be responding to the highly seasonal supply of fresh phytodetritus to the continental shelf. The spawning periods of both species are out of synchrony with the phytodetrital flux that occurs between November and March each year. *Protelpidia murrayi* spawns between March and June, and

Peniagone sp. spawns around October/November. With estimated larval development times of 2-3 months it is possible that *Peniagone sp.* has adopted a winter spawning time to avoid the higher levels of larval predation during the summer bloom and so that the juveniles settle during the phytodetrital flux when food on the sea-floor will be at its most nutritious. In both of these elpidiid species the maximum egg growth and addition of nutrients to the eggs (vitellogenesis) occurs whilst the phytodetrital flux is at a maximum. This is in keeping with the opportunistic nature of this group described previously in this chapter. These two species are the dominant megafaunal species at the two FOODBANCS sites A and C, occurring in comparable numbers during February/March 2001. Such an opportunistic reproductive strategy would enable this group of invertebrates to have a wide distribution, and to thrive in a wide variety of habitats.

Chapter 4 – Nutritional Status of Antarctic Benthic Echinoderms

Introduction

The biochemical composition of an organism and its individual body components are basic to understanding its physiology (Giese, 1966). Differences in energy content of the body components are related to differences in the proportions of basic organic materials (e.g., protein, lipid, carbohydrate, nucleic acids and mineral ash) (McClintock, 1989). The relative contribution of these organic constituents in different tissues dictates the amount of energy invested in somatic and reproductive growth, and provides important information on the functional roles of the tissues and the nutritional status of the organism.

The analysis of the organic composition of the major echinoderm body tissues has been reported for species from tropical, temperate and polar environments (Giese, 1966; Moss and Lawrence, 1972; Sibuet and Lawrence, 1981; Lawrence and Guile, 1982; Magniez, 1983; McClintock and Pearse, 1987; Walker *et al.*, 1987; McClintock *et al.*, 1988; McClintock, 1989; McClintock *et al.*, 1990; Lares and McClintock 1991; Bishop and Watts, 1992; Brockington *et* al., 2001; David and MacDonald, 2002). Early studies predicted a latitudinal cline in the energy content of tissues, suggesting that polar species would accumulate large amounts of lipid during the summer to provide an energy reserve that could be utilised during the period of winter starvation (Lawrence, 1976). It has since been recognised that the biochemical composition of Antarctic echinoderms is similar to species from other latitudes and that no such cline exists (Lawrence and Guile, 1982; McClintock and Pearse, 1987, McClintock, 1989). Despite the period of winter starvation experienced by some polar benthic species, the lack of difference in energy reserves from temperate or tropical benthic species may be a result of the lower metabolic rates reported for polar marine invertebrates (Peck *et al.*, 1987), which reduce the demand for food reserves as fuel for over winter survival. In spite of the lack of a latitudinal cline in energy reserves, there is a distinct separation in the biochemical composition between polar benthic and pelagic invertebrates. Some polar pelagic crustaceans may contain greater amounts of energy-rich tissues than temperate or tropical species. This increase in energy content may reflect the higher metabolic rate of the planktonic species than the benthic species, which is probably caused by the energetic cost of swimming actions used to maintain a position in the water column (Clarke and Peck, 1991).

Most of the studies carried out to date have found a significant difference in the biochemical composition between the ovaries and the testes. Generally the ovaries contain higher levels of lipid than the testes. This reflects the higher energetic contribution of lipid to the development of an egg/embryo and the production of a juvenile when compared to the sperm (McClintock and Pearse, 1987). Within females of different species of echinoderm it has been recognised that levels of lipid may vary in the gonad depending on the mode of development. Specifically, echinoderm species with larger eggs contain more energy per egg than those with smaller eggs (McEdward and Morgan, 2001), and this is particularly clear in species with non-feeding larvae. This is because larger eggs contain more reserves (lipid and protein); so in absolute terms (joules per egg) lecithotrophic eggs contain more energy than smaller planktotrophic eggs. Echinoderms that produce either lecithotrophic eggs or brood their young, e.g. *Acodontaster hodgsoni* (lipid = 62% gonad dry mass) and *Abatus nimrodi* (lipid = 33.6 % gonad dry mass), have very high levels of lipid in their gonads, compared to species that produce a planktotrophic larvae, *Odontaster validus* (13% gonad dry mass) (McClintock and Pearse, 1987) and *Sterechinus neumayeri* (16-24% gonad dry mass) (Brockington *et al.*, 2001).

It is important to distinguish between the amount of energy per egg and the amount of energy per ovary; differences between egg types may not necessarily translate into differences in composition of the overall gonad. A gonad from a planktotroph and from a lecithotroph may package the same materials differently (so composition at the tissue level remains the same); or the composition of large and small eggs may differ and hence affect the overall gonad composition. When comparing the overall composition of gonads from planktotrophic species with gonads of lecithotrophic species it is also important to remember that whilst lecithotrophs have larger eggs, they also have much lower fecundities than planktotrophic species.

In contrast to the ovaries, the testes contain much higher levels of protein. In some cases where insoluble protein was measured, it is suggested that the high levels of insoluble protein reflect the presence of nucleic acids associated with sperm (McClintock and Pearse, 1987). Although DNA counts as a form of non-protein nitrogen (NPN) it is not really insoluble protein, and these differences probably

reflected total nitrogen not accounted for by soluble protein, and thus represent the RNA and DNA in the testes.

Several studies have shown that the gut tissue of echinoids and holothurians, in addition to its digestive and absorptive functions, may act as a nutrient storage organ (Lawrence *et al*, 1965; Klinger *et al*, 1988; McClintock *et al*, 1990; Bishop and Watts, 1992). Bishop and Watts (1992) found that both the stomach and intestine tissues experienced specific changes in the concentration and amount of lipid and protein. While the stomach appears to function as a lipid storage organ the intestines only appear to store lipids temporarily until they are translocated for other uses. Klinger *et al* (1988) suggested that the nutrient storage abilities of the gut tissue generally only function on a short-term basis, and that they have little capacity for long-term storage.

In asteroids the pyloric caeca serves as the main nutrient storage organ (Lawrence, 1985) and consequently it contains high levels of lipid and protein. Lipids contain more energy per unit mass than protein or carbohydrates, therefore by storing large quantities of lipid in the pyloric caeca asteroids are maximising the amount of energy stored in a limited space. In some shallow water seastars studies have linked the decrease in size of the pyloric caeca to the increase in size of the gonads, and suggested that the pyloric caeca provides nutrition and energy towards reproductive development (Giese, 1966). Nevertheless, it has been shown that whilst the pyloric caeca may be necessary for maximal gonadal development it is not actually responsible for the complete development of the gonad, nor essential for gonad development if the asteroid can feed (Lawrence and Lane, 1982). The biochemical composition of the pyloric caeca appears to show no difference between male and female asteroids, suggesting that there is no sexual difference involved in the nutrition of reproduction as far as the type of nutrient reserves is concerned (Lawrence and Lane, 1982). In addition, studies have found that the size of the pyloric caeca may decrease during times of starvation or brood protection (in species that brood their young orally) (Bosch and Slattery, 1999).

One of the basic characteristics of echinoderms is their sensitivity to the level of nutrients available; this is associated with an ability to resorb any tissue during conditions of low nutrient availability, and for rapid growth during conditions of high

nutrient availability (Lawrence and Lane, 1982). Under conditions where food levels are sufficiently limiting, growth may be restricted to the extent that dwarf populations result. In conditions when food levels are above the minimum for the maintenance level, most of the energy acquired is primarily allocated to somatic production, and then to gonadal production, food permitting (Lawrence, 1986). Nevertheless, species that have an annual reproductive cycle may also have seasonal changes in the allocation of energy to gonadal and somatic production (Lawrence, 1985). In this case there appears to be the presence of two thresholds for the energetic requirements of somatic and gonadal growth. The first threshold is the minimal amount of energy (food) necessary for maintenance, and below which gonadal growth does not occur. Above this threshold the energy available can support maintenance and gonadal growth. The second threshold of energy can support both active gonadal growth and somatic growth (Lawrence, 1985).

Organisms that have an intermediate storage organ, such as the pyloric caeca, start to deposit nutrients in the organ when energy intake is above the first threshold. During periods of gonadal development the pyloric caeca will decrease in size despite the nutrient deposition, as it will be supplying energy to the gonads to promote gametogenesis. Above the second threshold of energy availability large amounts of nutrients are deposited in the pyloric caeca and the caecae do not decrease in size during gonadal growth (Lawrence *et al.*, 1986). Many species of echinoderm tend to be herbivores and feed on bottom sediments. The deposition of these sediments is partly related to the seasonal phytoplankton bloom, and hence the availability of high quality food is also seasonal. Therefore the seasonal availability of nutritious food may dictate the amount of energy available to echinoderms, and to which of the two threshold levels of energy is met. In some cases as a result of seasonal energy availability echinoderms may have periodic requirements of nutrients for reproduction, and may exhibit periodic deposition of nutrient reserves in their nutrient storage organs (Lawrence and Lane, 1982).

Studies on the suspension-feeding dendrochirotid holothurian *Cucumaria frondosa* indicate that the holothurian has both a seasonal reproductive cycle and seasonal feeding activity, with a non-feeding period occurring each year (David and MacDonald, 2002). Biochemical analyses of the body tissues of *C. frondosa* indicate

an increase in the protein and glycogen content of the gonad tissues during the active feeding period, and a subsequent decline once feeding ceased. David and MacDonald (2002) predicted that this decline in protein and glycogen reserves was probably a result of energy utilization to support body maintenance. There was also an increase in the protein and glycogen content of both the gonad and body wall tissues that was associated with the post-spawning period, and is probably the result of an accumulation of energy reserves prior to the non-feeding period. Recent studies on the shallow-water Antarctic holothurian *Heterocucumaria steineni* showed that the holothurian has a seasonal feeding periodicity with a variable winter starvation pattern that always covered a four-month period. Fraser *et al.* (in press) have demonstrated that changes in metabolism, protein dynamics and body composition are associated with this feeding cycle.

The degree of variation in biochemical composition of the different body components will depend on several factors. The nature of the tissue in question will dictate the boundaries to its response; for example, gonad tissues are likely to change in composition as gametes mature, and the reproductive strategy will determine whether gametes develop continuously or synchronously and if they produce a high lipid-content lecithotrophic egg or a lower lipid-content planktotrophic egg. The mode of feeding of the species will determine how reliant on fresh material from the spring bloom an organism is, or if it is higher up the food web and removed from the seasonal dependence. Inter-annual variability in the phytoplankton bloom may also affect how much material is available to the benthos and therefore how high the difference in food quality is between seasons.

The purposes of this study were to examine the nutritive and energetic condition of six species of echinoderm with particular regard to their reproductive and nutrient storage organs. The species include two holothurians (*Protelpidia murrayi* and *Peniagone* sp.), and asteroid (*Psilaster charcoti*) and three echinoids (*Sterechinus antarcticus*, *Ctenocidaris perrieri* and *Amphipneustes lorioli*). These species encompass a variety of feeding mechanisms (Chapter 2) and reproductive strategies (Chapter 3), and therefore provide evidence on how different species store and utilise nutrients given the same broad environmental background. With *Psilaster charcoti* being a scavenger and feeding mainly on dead krill (pers. obs.) it is not directly

affected by the phytoplankton bloom, whereas the remaining species are all deposit feeders and responses may be directly linked to the phytodetrital flux. In addition, samples were collected over four different seasons so that biochemical composition of gonadal and body tissues from male and female specimens could be compared to establish the relationships between energy reserves, feeding and reproductive activities.

Methods

Sample Collection

Specimens of six echinoderms (*Protelpidia murrayi*, *Peniagone* sp., *Psilaster charcoti* *Sterechinus antarcticus*, *Ctenocidaris perrieri* and *Amphipneustes lorioli,* for descriptions see Chapter 2) were collected from three locations (sites A, B and C, Figure 1.1) at ~600m depth on the continental shelf west of the Antarctic Peninsula. Samples were obtained during the FOODBANCS cruise programme (Chapter 1) on four different cruises to the Antarctic Peninsula in November/December 1999, March 2000, October 2000 and February/March 2001. The specimens were dissected into individual body components and frozen separately at -80°C onboard ship. Gonad tissues were collected from all species from all cruises and sites where possible. Gut tissue was collected for both holothurian species (*Protelpidia murrayi* and *Peniagone* sp.), and all three species of echinoid (*Sterechinus antarcticus*, *Ctenocidaris perrieri* and *Amphipneustes lorioli*). Initial attempts at freezing the gut and contents intact for later dissection in the lab were not successful, therefore the gut was dissected onboard the ship from the third cruise (June 2000) onwards. Care was taken to ensure that all gut contents were removed, and that the gut was rinsed clean prior to freezing. Body wall tissues were collected from both species of holothurian and pyloric caecae were collected from the asteroid *Psilaster charcoti*. Further analysis of all samples from the June 2000 cruise could not be undertaken because delays to shipping the samples from South America to Southampton resulted in the samples thawing before arrival at the laboratory.

Elemental Analyses

Samples were freeze-dried or oven dried at 60°C until they maintained a constant weight (24hrs+); both wet and dry masses of the tissue were recorded. The tissue was

then ground with a pestle and mortar and homogenised to reduce variability between replicates. Elemental (CHN) composition of the tissues was determined using a Carlo Erba 1108 CHN auto analyser at the British Antarctic Survey in Cambridge. The pre-dried samples of gonad, gut lining, body wall and pyloric caeca tissue were re-dried at 60°C for 24 hours prior to processing. Care was taken to ensure that dried samples were transferred from the oven to the CHN analyser in a desiccator over silica gel to prevent re-absorption of water.

Sub-samples of powdered tissue weighing 1-2mg were sealed in clean, pre-dried tin capsules and accurately weighed (0.001mg) on a microbalance. Samples were then immediately transferred to the autoanalyser cartridge. The autoanalyser cartridge may hold up to 50 samples, each of which took 10 minutes to be processed through the machine. A vial of silica gel was placed into the cartridge and changed when necessary to keep the preloaded samples dry until completion of the sample run. The exact mass of the sample and the sample details were entered into the sample spreadsheet, the results of which were then automatically appended to a summary file.

Prior to each sample run an air blank and an empty tin capsule were run through the machine to confirm zero carbon and nitrogen to set the zero standard. Subsequently three consecutive capsules containing increasing mass of a pure chemical standard were run through the machine to provide known values of CHN from which calibration factors for the machine were automatically calculated. The standard was acetanilide (C=71.09%, H=6.71% and N=10.36%). Finally a single standard was run as an unknown following the calibration to ensure that the calibration had been successful. Subsequently, after every 10 samples a standard was run as an unknown to ensure that there was no drift in the calibration during the sample runs.

Test samples of 10 replicates were carried out on each tissue type from the different species so that the number of replicates necessary for an accurate result at required precision could be determined. The whole tissue was ground to a powder and homogenised using a pestle and mortar. Tissues types varied between all of the species, some were relatively dry and others were oily. When grinding the dried gonad tissue it became apparent that species with lecithotrophic oocytes ground into a fine powder, and that species with a larger egg size and brooding mode of

development had very oily tissue that could not be ground to a fine homogenous powder. The numbers of replicates required thus varied depending on the tissue type. Having calculated the standard deviation for each tissue type with increasing number of replicates it was found that three replicates were required for oily tissues but only two for the dry tissues to achieve a reasonable precision.

Biochemical Analyses

Proximate biochemical composition (protein, carbohydrate and lipid content) was estimated from the elemental analysis using the stoichiometric algorithm developed by Gnaiger and Bitterlitch (1984). The algorithm was adapted by Prof. A. Clarke (BAS) (Appendix 8) to be run as an executable routine in Minitab. Error messages are given by Minitab when variables exceed boundary conditions. Negative values of either lipid or carbohydrate may occur if the amount of carbon explained by protein has either been overestimated or underestimated, respectively. The mass fraction of carbon in the non-protein organic matter (MFC) should lie between 0.776 and 0.444. If the MFC is >0.776 then lipid has been overestimated and carbohydrate values will be negative. The most likely explanation is that the fraction of non-protein nitrogen has been overestimated or that residual water values are too high. If the MFC is <0.444 then carbohydrate has been overestimated and lipid values will be negative. It is likely that residual water has been severely underestimated. If the results from the algorithm contained either negative lipid or carbohydrate values then the data were rerun with optimised residual water or non-protein nitrogen values until they conformed to the set boundary levels. In the event that an error message arises during an algorithm run it is accompanied by a set of instructions that recommend the adjustment of the default values of residual water and total non-protein nitrogen (NPN) to optimise the output variables. The algorithm is executed using the following steps:

1. Estimate dry matter from assumed residual water content (default value = 0.06)
2. Estimate organic carbon by subtraction of inorganic carbon from measurement of the carbon content in the ash (or assumed default value of 0.01)

3. Estimate fraction of total nitrogen explained by protein; non-protein nitrogen is (NPN) predominantly chitin or nucleic acid; default value in absence of direct measures = 0.10.
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.

Tissue energy content was estimated using an empirical relationship between enthalpy of combustion and the mass fraction of carbon in organic matter (derived by Gnaiger and Bitterlitch, 1984). The CHN auto-analyser could not discriminate between carbon in calcium carbonate and carbon in organic matter; 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. Samples were ashed between 450-500°C for 24-48 hours and then the ash was run through the autoanalyser to determine the carbon content of the ash. The stoichiometric CHN method for the determination of biochemical constituents was preferentially used in this study over more traditional techniques on account of the following advantages: 1) It is consistent with established ecophysiological concepts for biochemical and energy budget calculations; 2) It requires only small amounts of material (1 mg dry weight); and 3) Automatic analysers can perform measurements in a very short time (Gnaiger and Bitterlitch, 1984).

Unusual tissues, such as those of gelatinous zooplankton (Clarke *et al.*, 1992), have previously been shown to pose problems to the algorithm. This is an important consideration for the analysis of high water content holothurian tissues. These tissues have very high water and high ash contents, and in these species the sum of the protein, lipid, carbohydrate, chitin and ash contents explained only 61 to 85% of the measure dry matter in comparison to the 90 to 95% explained in crustacean zooplankton (Clarke *et al.*, 1992). It is generally assumed that much of the

unexplained loss on ignition is water that remained in the tissues after drying at 60°C, and therefore residual water contents are higher in gelatinous species. In addition it was also suggested that there is a missing organic component that contains nitrogen and is probably a carbohydrate that is not explained by the stoichiometric analysis (Clarke *et al.*, 1992).

Statistical Analysis

All statistical analyses were undertaken using Minitab13. The biochemical (protein, lipid and carbohydrate) and elemental (C, H, N) data were firstly analysed using principal component analyses (PCA). This was because there were a large number of variables for each tissue and species, and PCA acts to combine these variables into one value, therefore simplifying future analyses without losing the importance of each variable to the within sample variance. The principal component was then tested for differences between sexes using a one-way ANOVA. The gonad tissues from each species were significantly different and therefore further analyses of the gonads were made separately on each sex. Data for each species were then analysed for differences in elemental and biochemical composition among sites and among seasons. A two-way ANOVA was carried out on the gonad tissues (using season and site as factors) and on the body tissues of *Sterechinus antarcticus* and *Amphipneustes lorioli* (which were only collected from one site) (using sex and season as factors). A three-way ANOVA was performed on the principal component of the body tissues of *Protelpidia murrayi, Peniagone,* sp., *Psilaster charcoti* and *Ctenocidaris perrieri* (using sex, cruise, and site as factors). The crossed ANOVA design was used as all variables were fixed. Nevertheless, as there were unbalanced numbers of replicates for each site a crossed ANOVA was carried out using the general linear model function in Minitab13, which can cope with a data set that is deficient in one or more observations (Wardlaw, 2002). The original data were presented in table format with each species and season presented as a mean and standard deviation. Species that showed no significant difference in tissue composition between both site and sex were presented as the mean and standard deviation of all sites or both sexes for a particular tissue.

Results

The tissue compositions of the six species of echinoderm are very variable and therefore respond differently when run through the Gnaiger and Bitterlitch (1984) algorithm. Some tissues conform well to the boundaries set and give good results with the estimated default values, for example *Sterechinus antarcticus* in which all individual replicates gave good results and conformed to the set boundaries (Table 4.1). In contrast, some tissues gave low quality results and required large changes in the default values to optimise results and often still had individual specimens that did not give good results, for example *Protelpidia murrayi* male gonad tissues (Table 4.1). It appears that the Gnaiger and Bitterlitch (1984) algorithm works well for tissues that do not show a high variation in composition between seasons. *Protelpidia murrayi* had a high seasonal variation in composition and as a consequence did not conform well to the set boundaries until it was broken down by season and data individually optimised (Table 4.1).

The holothurian body wall tissues examined in this study did not conform to boundaries set by the algorithm (Table 4.1) and because of their gelatinous nature it may be suggested that they experience similar problems to those of the gelatinous zooplankton. In addition to problems arising from the various tissue compositions a certain amount of error may be as a result of the inherent sensitivity of the algorithm as on occasion different individuals with similar elemental and ash compositions would behave very differently when run through the algorithm. However on the whole the algorithm has been shown to give accurate results when compared with direct assays (Brockington *et al.*, 2001).

Species and Tissue Type	Ash (%DM)	C in Ash (%)	% organic matter explained by G-B	Residual Water %	Total NPN	Total no. of individuals	Errors (no. of individuals) discarded
Protelpidia murrayi							
Male gonad, cruises 1 & 2	33.97	0.13	99.99	9	5	27	4
Male gonad cruise 4 sites A & B	37.41	0.08	99.99	7.6	5	7	
Male gonad cruise 4 site C	60.57	0.12	99.99	16	5	3	
Male gonad cruise 5	24.45	0.06	99.99	6	5	9	
Female gonad cruise 2	22.21	0.12	100.00	7	5	10	1
Female gonad cruise 4 sites A & B	42.99	0.10	100.00	6	5	5	
Female gonad cruise 4 site C	56.12	0.12	100.00	10	5	3	1
Female gonad cruise 5	18.12	0.28	99.99	7	5	11	1
Male & female gut lining	36.26	0.05	100.00	8	5	33	2
Male & female body wall	59.03	0.06	99.99	9	5	31	12
***Peniagone* sp.**							
Male gonad	28.49	0.22	99.99	7	5	27	3
Female gonad	15.60	0.24	99.99	6	5	37	
Male & female gut lining	27.85	0.05	100	7.5	5	32	
Male & female body wall	59.70	0.07	100	11	5	34	25
Psilaster charcoti							
Male gonad	12.07	0.32	100.00	8	5	33	3
Female gonad	7.16	0.81	100.00	5	5	29	1
Male & female pyloric caeca	7.44	1.75	100.00	7	5	51	
Sterechinus antarcticus							
Male & female gonad	8.78	0.30	100.00	6	5	24	
Male & female gut lining	25.91	0.07	100.00	6	5	11	
Ctenocidaris perrieri							
Male gonad	11.70	0.16	100.00	7.5	5	29	
Female gonad	6.77	0.33	99.99	6	5	37	
Male & female gut lining	14.34	1.80	100.00	6	5	30	
Amphipneustes lorioli							
Male gonad	21.45	0.46	100.00	7.5	5	9	
Female gonad	9.89	0.42	100.00	6	5	11	
Male & female gut lining	41.51	0.07	99.99	7.3	5	11	1

Table 4.1. Table of values for residual water and total non-protein nitrogen (NPN) used to estimate proximate composition from elemental analysis based on stoichiometry (Gnaiger & Bitterlitch, 1984). The values of residual water and total NPN show the values that optimised the results (original default values = residual water (6%) and total NPN (5%)). All species and tissues had a default value of the fraction of NPN = 0.15. The negative value column indicates the number of individual specimens that had either negative lipid or carbohydrate values and were therefore eliminated from any further analyses. The final three columns indicate a summary of the overall output from the CHN data and Gnaiger & Bitterlitch (1984) algorithm.

Biochemical Composition of Gonad Tissues

The biochemical compositions of the body components of the species of Antarctic echinoderms are presented in Tables 4.5 to 4.10. Sexual differences in % composition of gonad tissues were found to be significant in all six species (Table 4.2).

Species	df	F	P
Protelpidia murrayi	1,68	17.65	P<0.01
Peniagone sp.	1,59	68.1	P<0.01
Psilaster charcoti	1,56	393.0	P<0.01
Sterechinus antarcticus	1,22	106.2	P<0.01
Ctenocidaris perrieri	1,66	198.5	P<0.01
Amphipneustes lorioli	1,18	95.4	P<0.01

Table 4.2. The results of principal component analysis and one-way ANOVA testing for differences between sexes in the gonad tissues of each species of echinoderm. df = degrees of freedom, F = the ratio of between-group variance to within-group variance, P = significance level.

The significant difference between male and female gonads was the result of higher levels of protein and ash in the testes than the ovaries; in contrast, levels of lipid were higher in the ovary than in the testis (Figure 4.2). The difference in biochemical composition between testes and ovary tissues was more pronounced in the echinoid and asteroid species than it was in the two species of holothurian. Mean protein and lipid values ranged from 48.2 to 61.2 % dry mass (DM), and from 1.8 to 18.2 % DM respectively in the male echinoid gonad, and from 32.7 to 41.0 %DM and from 9.9 to 45.8 % DM respectively in the female echinoid gonad. In the asteroid species mean protein and lipid values ranged from 62.8 to 66.3 % DM and from 3.6 to 8.1 % DM respectively in the male gonad, and from 31.9 to 43.4 % DM and from 28.3 to 47.0% DM respectively in the female gonad. These differences in protein and lipid composition are far greater than for the two holothurian species, where mean protein and lipid values of gonad tissues range from 34.4 to 52.0 % DM and from 1.0 to 14.7 % DM respectively in male gonads, compared to a range of 23.9 to 53.2 % DM and from 6.9 to 38.1 % DM in female gonad tissues. These results also suggested that there was a greater similarity in the biochemical composition within the species of echinoid and within the species of holothurian than there were between the two classes. This difference between the two classes of echinoderm is driven to a significant extent by variations in ash content. Holothurians contain calcareous spicules in all of their soft tissues and it is likely that they account for the higher ash content recorded in the holothurian gonads (Table 4.3).

Species	Ovary			Testis		
	C:N in AFDM	Protein:Lipid in AFDM	% Ash (%DM)	C:N in AFDM	Protein:Lipid in AFDM	% Ash (%DM)
P. murrayi	6.89	2.93	24.53	4.13	15.19	34.65
Peniagone sp.	7.35	1.33	15.60	4.95	4.56	28.49
P. charcoti	8.74	1.08	7.16	3.67	12.29	12.09
S. antarcticus	8.17	1.05	6.82	4.38	16.78	10.23
C. perreri	8.85	0.87	6.77	4.55	17.07	11.70
A. lorioli	7.72	1.31	9.89	4.38	15.37	21.48

Table 4.3. Comparison of C:N, protein:lipid, and % ash between the ovaries and tests of the six species of echinoderm.

The gonads of all species were primarily composed of protein, lipid, carbohydrate and ash, in varying proportions depending on the species (Figure 4.1). In the male gonad of *Protelpidia murrayi* protein and ash were the major constituents, comprising up to ~80 % to the dry mass, with lipid and carbohydrate making up the remainder in roughly equal proportions. The ovary contained a much higher proportion of lipid than the testes, protein accounted for ~23 – 53 %, with lipid values reaching a maximum of 25 – 32 %. Carbohydrate values were comparably low, but accounted for <14 % at times. Ash values were generally lower than 23%, however they did reached as high as 46.2 % during October. The composition of the testes in *Peniagone* sp. was similar to that of *Protelpidia murrayi*, although the ash content was slightly lower (20 – 35 %) and the lipid content was slightly higher (~ 9 – 14.7 %). The composition of the ovary showed less variability than for *Protelpidia murrayi* generally comprising ~35 % protein, 26 – 38 % lipid, 7 – 10 % carbohydrate and 12 – 17.5 % ash.

The testes of *Psilaster charcoti* were dominated by protein (~ 63 %), and had low levels of lipid (< 8 %), carbohydrate (<9 %) and ash (<12.7 %). In the ovary, lipid levels (~40 %) generally exceeded levels of protein (~ 38 %), except during November 1999. Both the ash (< 8.4 %) and carbohydrate (<12.4 %) contents were relatively low.

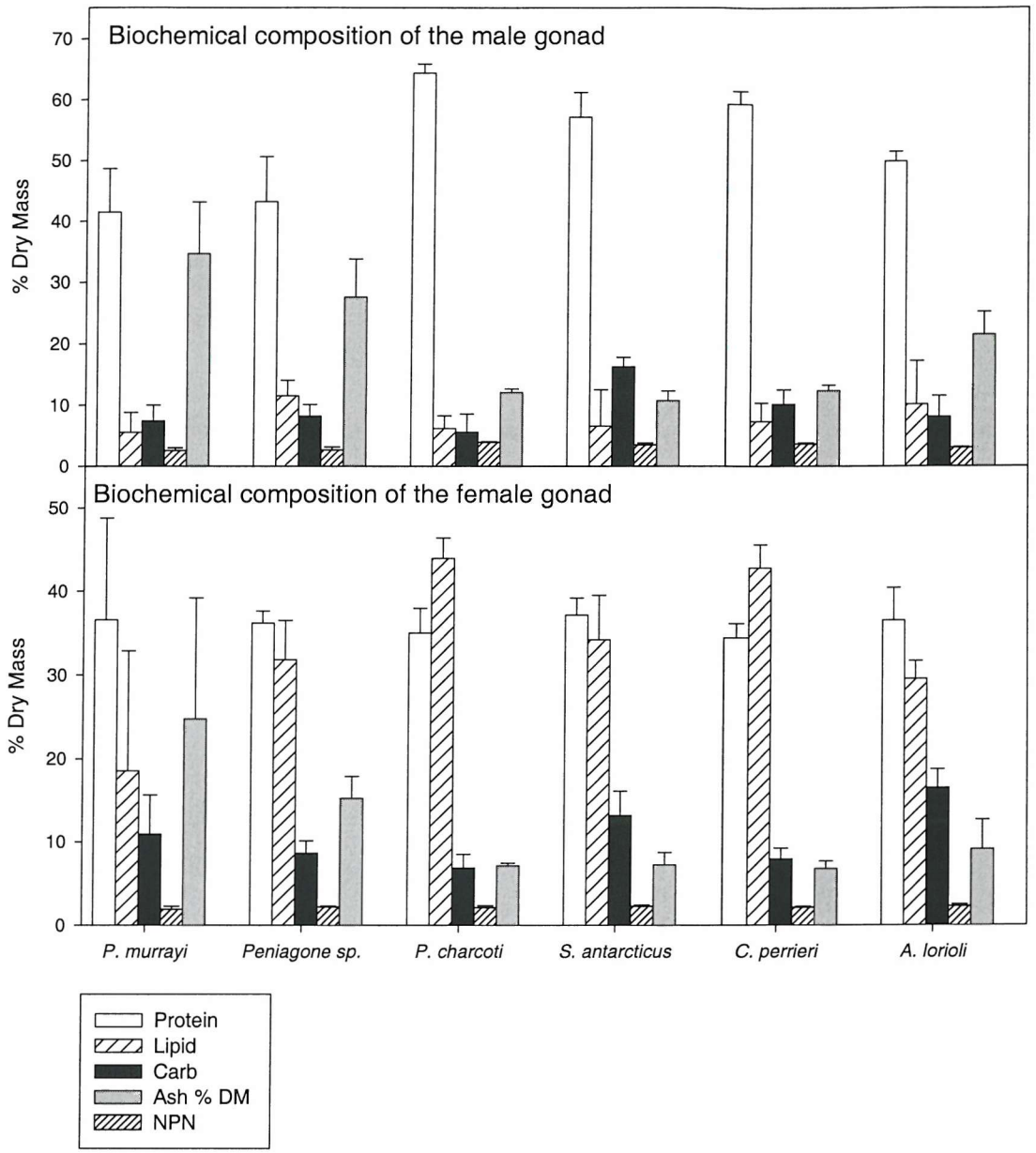

Figure 4.1. Biochemical composition of male and female gonad tissue from the six species of Antarctic echinoderm. Data are plotted as an overall mean and SD (mean of means from each season). The size of the error bars indicates the amount of seasonal variation.

Testes of the regular urchin *Sterechinus antarcticus* were dominated by protein (~60 %), and also contained relatively high levels of carbohydrate (15-18%). Levels of both lipid (<5.7 % DM; except during November 1999 – 15.1%) and ash (<12 %) were low throughout the study period. In contrast to the testes the ovaries consisted of higher levels of lipid (27 – 40 % DM) and a lower proportion of protein (34 – 39 % DM). Carbohydrate values remained relatively high (~15 %DM) and the ash content

was very low (~6 % DM). As a result of the higher lipid values the ovaries had a higher energy content than the testes.

The biochemical composition of the testes of *Ctenocidaris perrieri* was dominated by protein (~60 % DM). Levels of lipid, carbohydrate and ash all remained relatively low and comprised the remaining 40% DM in roughly equal proportions. The main component of the ovary was lipid (40 % DM), and together with *Psilaster charcoti* the ovary of *Ctenocidaris perrieri* had some of the highest lipid and energy values (~ - 29 kJ/g DM) of all of the 6 species investigated. Nevertheless, protein still remained an important component of the ovary (~ 35 % DM), whilst carbohydrate and ash were less abundant.

The composition of gonad tissues in *Amphipneustes lorioli* was similar to those of the other species of echinoderm studied. The major constituent of the testes was protein (~50 % DM). The second largest component was ash that accounted for 17 – 25 % of the total dry mass. Lipid and carbohydrate levels were generally quite low. The ovaries had much lower levels of protein (34 – 41 % DM), higher levels of lipid (28 - 30 % DM) and carbohydrate (14-18 % DM).

The biochemical compositions of the gonads tissues of the six species of echinoderm varied among species as well as sex. The two species of holothurian contained a much higher proportion of ash than the echinoid and asteroid species; this was most likely caused by the calcareous spicules found in many holothurian tissues. The ovaries and testes of *Protelpidia murrayi* both showed significant seasonal variation in biochemical composition. Seasonal variation of biochemical composition among the remaining five species was only exhibited in the testes of *Peniagone* sp. and *Psilaster charcoti*. In all species of echinoderm the ovary compositions were dominated by yolk, which contains protein and lipid, whereas the testis were dominated by sperm (protein and DNA) and the machinery for their production.

Biochemical Composition of Other Body Components

None of the remaining body components (gut lining, body wall and pyloric caeca) showed any significant variation in biochemical composition between male and female specimens (Table 4.4).

Species /Tissue	Sex			Season			Site			Season*Site		
	df	F	P<	df	F	P<	df	F	P<	df	F	P<
Protelpidia murrayi												
Testes				2,25	26.92	0.01	2,25	7.19	0.01	4,25	8.96	0.01
Ovary				2,17	30.05	0.01	2,17	0.05	ns			
Gut lining	1,19	0.11	ns	1,19	7.76	0.05	2,19	1.5	ns			
Body wall	1,15	1.96	ns	1,15	0.91	ns	-	-	-			
***Peniagone* sp.**												
Testes				2,8	7.53	0.05	2,8	1.30	ns			
Ovary				2,23	1.59	ns	2,23	0.02	ns			
Gut lining	1,21	0.00	ns	1,21	27.8	0.01	2,21	2.5	ns			
Body wall	1,23	0.54	ns	1,23	2.73	ns	2,23	0.80	ns			
Psilaster charcoti												
Testes				2,18	9.63	0.01	2,18	1.03	ns			
Ovary				2,16	1.74	ns	2,16	0.16	ns			
PC	1,34	2.18	ns	2,34	32.9	0.01	2,34	25.7	0.01	4,34	5.18	0.01
Sterechinus antarcticus												
Testes				3,16	0.25	ns						
Ovary				3,16	1.85	ns						
Gut lining	1,7	0.41	ns	1,7	0.18	ns						
Ctenocidaris perrieri												
Testes				3,21	2.85	ns	2,21	0.17	ns			
Ovary				3,23	1.55	ns	2,23	0.02	ns			
Gut lining	1,16	0.91	ns	1,16	5.18	0.05	1,16	1.4	ns			
Amphipneustes lorioli												
Testes				2,14	1.19	ns						
Ovary				2,14	2.57	ns						
Gut lining	1,6	0.08	ns	1,6	0.32	ns						

Table 4.4. Results of the two-way and three-way analyses of variance on chemical composition of different tissue types. df = degrees of freedom, F = the ratio of between-group variance to within-group variance, P = significance level ns = not significant. An interaction term (Season*Site) is only calculated when both factors show a significant difference, therefore no interaction terms were calculated for Sex*Season or Sex*Site.

The pyloric caeca had both high levels of protein (~50 % DM) and lipid (~30 % DM), and levels of carbohydrate ranged from 4.7 to 21 % DM (Figure 4.3). The proportion of ash in the pyloric caeca was low and of a similar magnitude to that of the ovary (<10 % DM). Ash was the major constituent of the body wall tissue in the two species of holothurian (57.7 - 59.8 % DM) as a result of the high spicule content (Figure 4.2). The remaining organic matter in the body wall tissue was dominated by protein (20.7 – 22.7 % DM), with lipid and carbohydrates contributing a very small amount (<5%) to the total dry weight. Owing to the high levels of ash, the body wall had the lowest energy content (< -15.3 ± 1.7 kJ/g DM) of any of the other body tissues measured in this study.

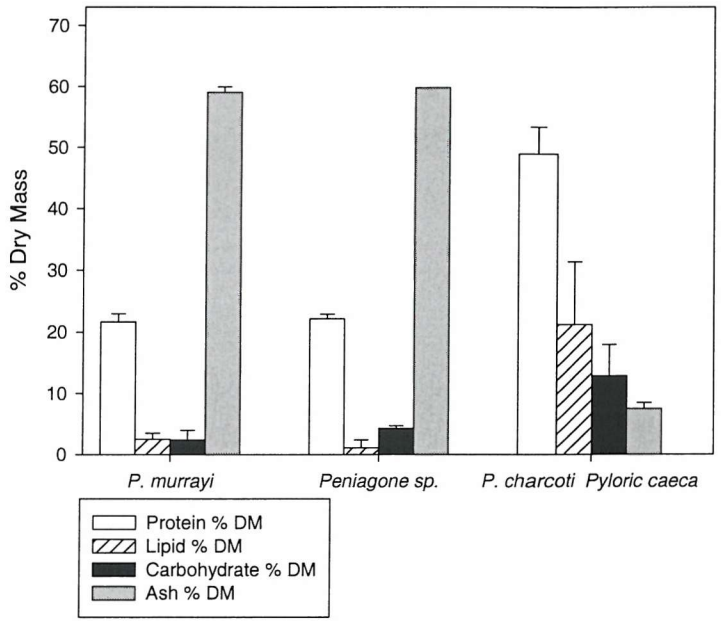

Figure 4.2. Biochemical composition of the body wall tissue from two holothurians, and of the pyloric caeca from the asteroid. Mean ± SD.

Gut lining tissues were relatively high in protein in both the echinoids and the holothurians (37.4 to 46.5 % DM). Generally lipid values in this tissue were quite low (<8.6 % DM) compared to those of the gonad tissues, with the exception of *Sterechinus antarcticus* and *Ctenocidaris perrieri* that had lipid values in the range of 11.7 to 21.0 % DM. The ash content of the gut lining tissue was the highest in *Amphipneustes lorioli* (~41.7 ± 4.0 % DM) and *Protelpidia murrayi* (~38.7 ± 10.2 % DM), and the lowest in *Sterechinus antarcticus* (~7.1 ± 1.2 % DM) and *Ctenocidaris perrieri* (15.2 ± 2.0 % DM).

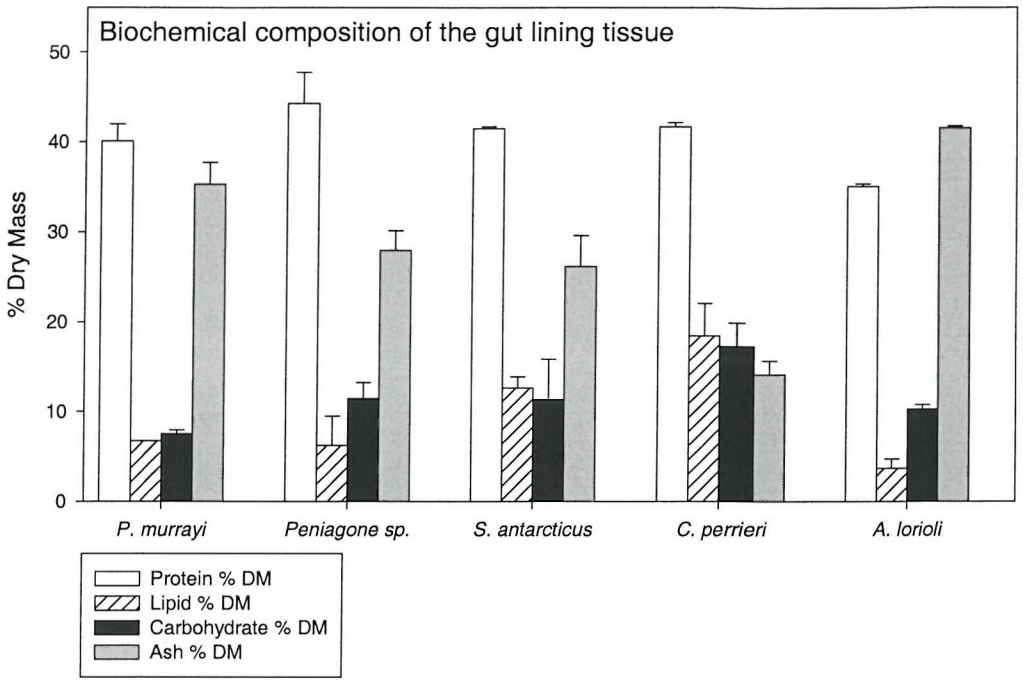

Figure 4.3. Biochemical composition of gut lining tissue from five species of Antarctic echinoderm. Mean ± SD.

Spatial and Temporal Variation

Of the six species of echinoderm studied only four were collected from more than one site. In all cases seasonal variation in biochemical composition tended to be more marked than spatial variation (Table 4.4). Of those four species found at both sites only *Protelpidia murrayi* and *Psilaster charcoti* showed a significant variation between sites in biochemical composition (Table 4.4). Variation in the biochemical composition of tissues among sites was not a feature of all of the tissues within a species, nor did it exist within the same tissues between species. *Protelpidia murrayi* only exhibited a significant difference among sites during October 2000, and only in the testes and the body wall tissues. Testes at sites A and B consisted of over twice the amount of protein (45.1 ± 5.1 % DM) than tissues at site C (16.1 ±0.4 % DM). Energy values were also double the value at sites A and B (-20.3 ± 0.9 kJ g^{-1} DM) than those at site C (-10.7 ± 0.7 kJ g^{-1} DM). Energy values are conventionally given negative units because it is released during measurement. *Psilaster charcoti* showed no variation in composition of gonad tissue among sites, however, it did exhibit a significant difference in composition of the pyloric caeca among sites. The pyloric caeca from both the male and female specimens generally showed the highest levels of protein at site B (except during February/March 2001 when site C had the highest protein levels) and the lowest levels of protein site A. Consequently, values of lipid

were highest at site A and lowest at site B (except during February/March 2001 when site C had the highest lipid levels), the energy content of the pyloric caeca was also highest at site A throughout the study.

Evidence of seasonal variation in the biochemical composition of the body components is indicated in Table 4.4. Notable variations in gonad composition over the seasons included the male and female gonad tissues of *Protelpidia murrayi*. Ovaries exhibited higher lipid and energy levels during March 2000 and February/March 2001 when compared to levels in November/December 1999 and October 2000. Despite having large variance the mean levels of protein and ash in the male gonad also showed a significant variation among seasons. The highest levels of protein occurred during March each year and the lowest levels occurred during November/December 1999 and October 2000. *Peniagone* sp. exhibited a similar pattern of protein variation within the testes, to that of *Protelpidia murrayi*, whilst the ovary did not show a significant variation in biochemical composition. Gut lining tissues of both *Protelpidia murrayi* and *Peniagone* sp. showed a significant increase in levels of protein and lipid between October 2000 and March 2001.

There was no significant variation in the composition of the ovary in *Psilaster charcoti*, (Table 4.4) but the testes did show significant variation among seasons. Table 4.7 indicates that the significant difference was caused by a decrease in lipid levels and an increase in carbohydrate levels during October 2000. This was followed by a subsequent increase in lipid during March 2001 and a decrease in the carbohydrate levels. Pyloric caeca tissue exhibited a significant difference in composition amongst the seasons. The major variation in tissue composition could be accounted for by changes in the levels of lipid (Table 4.7 and Figure 4.2). Pyloric caeca tissues at sites A and B showed a large increase in the proportion of lipid between October 2000 and February/March 2001. Specimens from site C showed a similar increase in the proportion of lipid between March 2000 and October 2000. There was a three-fold increase in the proportion of lipid at site A between November 1999 and October 2001, which then showed no subsequent decrease. Lipid proportions at site B declined slightly between March 2000 and October 2000 and then increased to a maximum during March 2001. Pyloric caeca tissues from site C

showed a large increase in lipid levels between March 2000 and October 2000, then fell slightly during March 2001.

Sterechinus antarcticus and *A. lorioli* showed no significant variation in gonad or gut lining tissues throughout the seasons and *Ctenocidaris perrieri* showed no significant variation in gonad tissues. *Ctenocidaris perrieri* did exhibit a significant variation in composition of gut lining tissues between the two seasons. This difference was caused by an increase in levels of lipid from October 2000 to February/March 2001, which was accompanied by a decrease in levels of carbohydrate between these seasons.

Species		N	% dry mass												kJ g^{-1} DM	
			Protein		Lipid		Carbohydrate		Ash		C		N		C:N		Energy	
Testis																		
Nov/Dec	1999	9	36.3	±10.7	6.3	±7.2	8.3	±2.9	37.9	±11.3	28.8	±6.2	6.6	±1.9	4.6	±1.3	-20.1	±2.2
March	2000	14	48.3	±5.1	4.1	±1.9	4.0	±2.6	31.7	±6.1	31.9	±2.9	8.8	±2.9	3.6	±0.2	-20.4	±0.6
October	2000	10	34.4	±14.0	2.0	±1.5	7.0	±3.4	44.4	±12.7	23.9	±8.8	6.3	±2.6	3.9	±0.3	-17.1	±4.5
Feb/Mar	2001	9	46.9	±11.9	9.6	±11.0	10.2	±6.3	24.5	±8.7	38.1	±6.1	8.6	±2.2	4.7	±1.4	-22.5	±2.6
Ovary																		
Nov/Dec	1999	1	53.2		5.5		15.4		15.7		40.7		9.7		4.2		-21.3	
March	2000	9	33.0	±4.2	29.4	±4.7	8.5	±3.6	20.2	±5.8	44.9	±3.6	6.0	±0.8	7.5	±0.5	-26.6	±1.0
October	2000	7	24.0	±3.9	6.9	±7.7	14.3	±4.0	46.2	±9.5	25.1	±7.3	4.4	±0.7	5.7	±1.2	-19.7	±3.5
Feb/Mar	2001	10	36.0	±4.1	32.4	±4.4	5.5	±2.4	16.9	±7.4	47.6	±4.4	6.6	±0.8	7.3	±0.6	-27.3	±0.9
Gut Lining (both sexes)																		
October	2000	16	38.7	±7.2	6.7	±3.1	7.2	±2.7	37.0	±9.6	29.9	±5.6	7.0	±1.3	4.3	±0.1	-20.8	±1.4
Feb/Mar	2001	15	41.4	±7.1	6.7	±2.0	7.8	±2.7	33.6	±9.7	31.7	±5.2	7.5	±1.3	4.2	±0.1	-21.0	±0.8
Body Wall (both sexes)																		
October	2000	8	20.7	±7.0	1.8	±1.5	3.5	±2.8	59.7	±9.0	14.5	±4.8	3.8	±1.3	3.8	±0.2	-13.3	±2.8
Feb/Mar	2001	11	22.6	±4.2	3.2	±1.6	1.3	±2.8	58.5	±5.1	15.7	±2.8	4.1	±0.8	3.8	±0.1	-14.8	±1.6

Table 4.5. Elemental and biochemical composition of body tissues of *Protelpidia murrayi*. Mean and standard deviation are given for sample sizes greater than 1.

Species		N	% dry mass												kJ g^{-1} DM	
			Protein		Lipid		Carbohydrate		Ash		C		N		C:N		Energy	
Testis																		
Nov/Dec	1999	6	38.8	±4.4	14.7	±6.6	8.3	±4.3	28.8	±5.4	36.8	±4.5	7.1	±0.8	5.2	±0.5	-23.4	±1.9
March	2000	8	46.3	±3.5	12.1	±2.2	6.7	±2.7	25.2	±5.4	38.1	±3.0	8.4	±0.6	4.5	±0.1	-23.0	±0.6
October	2000	7	35.6	±12.5	9.0	±6.6	10.8	±7.9	35.4	±10.6	31.7	±7.6	6.5	±2.3	5.6	±2.4	-21.3	±3.0
Feb/Mar	2001	3	52.0	±1.3	10.2	±1.5	7.0	±2.3	20.7	±4.0	40.0	±2.1	9.5	±0.2	4.2	±0.2	-22.7	±0.3
Ovary																		
Nov/Dec	1999	5	34.3	±5.7	38.1	±3.3	7.2	±2.9	12.5	±5.7	51.8	±2.3	6.2	±1.0	8.5	±1.2	-28.4	±1.1
March	2000	11	37.2	±4.6	31.1	±10.2	10.0	±2.1	13.5	±5.5	49.3	±5.4	6.8	±0.9	7.4	±1.4	-26.8	±1.9
October	2000	9	35.6	±3.1	31.3	±6.3	7.5	±3.3	17.5	±5.3	47.4	±4.5	6.5	±0.6	7.3	±0.7	-27.2	±1.3
Feb/Mar	2001	12	37.5	±4.1	26.8	±3.9	9.9	±1.3	17.5	±4.3	46.1	±2.6	6.8	±0.7	6.8	±0.8	-26.2	±0.9
Gut Lining (both sexes)																		
October	2000	17	46.7	±4.0	3.9	±2.1	12.7	±3.4	26.4	±5.3	34.6	±2.9	8.5	±0.7	4.1	±0.1	-20.5	±0.7
Feb/Mar	2001	15	41.8	±3.4	8.5	±2.8	10.2	±2.6	29.5	±4.2	34.4	±2.3	7.6	±0.6	4.5	±0.3	-21.7	±0.8
Body wall (both sexes)																		
October	2000	19	21.7		0.1		4.6		59.8	±6.5	13.7	±3.7	3.6	±1.1	3.9	±0.4	-14.1	
Feb/Mar	2001	15	22.7	±4.2	2.0	±1.5	4.0	±2.1	59.8	±5.7	15.2	±3.8	4.0	±0.9	3.8	±0.2	-15.3	±1.7

Table 4.6. Elemental and biochemical composition of body tissues of *Peniagone* sp. Mean and standard deviation are given for sample sizes greater than 1. Different numbers of replicates were used to estimate protein, lipid, carbohydrate and energy percentages than were used for measuring ash, carbon and nitrogen content, as not all individuals gave positive results using the Gnaiger macro. Values of ash, carbon and nitrogen were measured from using the number of individuals indicated in the table. Values of protein, lipid, carbohydrate and energy were estimated using 1 individual from October 2000, and 8 individuals from Feb/Mar 2001.

Species	N	% dry mass												kJ g^{-1} DM Energy	
		Protein		Lipid		Carbohydrate		Ash		C		N		C:N			
Testis																	
Nov/Dec 1999	3	64.3	±2.5	7.6	±2.3	4.4	±1.3	11.7	±1.0	43.7	±1.0	11.7	±0.5	3.7	±0.2	-22.2	±0.4
March 2000	10	63.9	±3.9	5.3	±3.2	6.7	±5.2	12.3	±1.6	42.7	±1.3	11.6	±0.7	3.7	±0.3	-21.6	±0.8
October 2000	8	62.8	±1.3	3.6	±2.3	9.0	±2.6	12.7	±1.2	41.8	±1.0	11.4	±0.2	3.7	±0.1	-21.1	±0.6
Feb/Mar 2001	9	66.3	±2.6	8.1	±1.2	2.2	±1.7	11.3	±0.7	44.2	±0.5	12.1	±0.5	3.7	±0.2	-22.4	±0.2
Ovary																	
Nov/Dec 1999	3	35.1		41.9		8.2		7.5		55.8		6.4		8.7		-29.0	
March 2000	8	34.1	±4.8	44.8	±9.1	7.1	±4.3	7.0	±1.6	56.9	±3.2	6.2	±0.8	9.4	±1.9	-29.6	1.8
October 2000	9	31.9	±3.2	47.0	±6.7	7.7	±2.7	6.7	±1.1	57.6	±2.3	5.8	±0.6	10.1	±1.5	-29.9	±1.3
Feb/Mar 2001	8	38.9	±3.2	41.9	±6.5	4.6	±2.6	7.3	±0.9	56.3	±2.3	7.1	±0.6	8.0	±1.1	-29.2	±1.3
Pyloric caeca (both sexes)																	
Nov/Dec 1999																	
Site B	5	52.3	±2.0	11.7	±3.6	18.2	±1.8	7.7	±0.3	46.3	±1.2	9.5	±0.4	4.9	±0.3	-22.5	±0.7
March 2000																	
Site A	4	41.6	±4.1	27.7	±7.3	14.3	±2.6	6.9	±0.6	51.0	±2.3	7.6	±0.8	6.8	±1.0	-25.6	±1.5
Site B	6	51.5	±3.6	12.4	±8.4	16.9	±4.9	9.0	±1.2	45.9	±2.8	9.4	±0.7	4.9	±0.6	-22.7	±1.8
Site C	6	50.8	±2.1	10.2	±4.7	20.9	±2.7	8.1	±0.6	45.4	±1.6	9.3	±0.4	4.9	±0.4	-22.1	±1.0
October 2000																	
Site A	6	43.0	±3.4	31.2	±9.0	9.2	±5.8	7.0	±1.0	52.3	±3.0	7.8	±0.6	6.7	±0.9	-26.5	±1.9
Site B	6	54.0	±2.6	12.0	±3.1	15.0	±2.0	8.7	±0.4	46.1	±0.9	9.8	±0.5	4.7	±0.3	-22.7	±0.6
Site C	5	46.8	±4.2	25.6	±7.5	10.5	±3.9	7.3	±0.7	50.7	±2.1	8.5	±0.8	6.0	±0.9	-25.5	±1.5
Feb/Mar 2001																	
Site A	6	41.3	±2.1	37.8	±4.6	5.2	±1.8	6.3	±1.0	54.9	±1.7	7.5	±0.4	7.3	±0.6	-27.9	±0.9
Site B	6	48.5	±1.7	26.6	±3.6	8.8	±2.0	6.2	±0.4	51.5	±1.1	8.8	±0.3	5.8	±0.3	-25.7	±0.7
Site C	6	50.1	±2.4	21.7	±5.2	10.9	±3.5	7.2	±0.4	49.7	±1.5	9.1	±0.4	5.5	±0.4	-24.7	±1.1

Table 4.7. Elemental and biochemical composition of body tissues of *Psilaster charcoti*. Mean and standard deviation are given for sample sizes greater than 1.

Species		N	% dry mass												kJ g^{-1} DM	
			Protein		Lipid		Carbohydrate		Ash		C		N		C:N		Energy	
Testis																		
Nov/Dec	1999	4	51.2	±8.2	15.1	±7.7	16.2	±3.6	8.4	±0.5	47.4	±2.1	9.3	±1.5	5.2	±0.9	-23.5	±1.4
March	2000	4	60.4	±2.7	3.5	±2.2	15.0	±3.0	11.4	±2.3	43.0	±1.5	11.0	±0.5	3.9	±0.3	-21.3	±0.3
October	2000	2	57.5	±4.6	5.7	±5.7	15.3	±0.7	12.1	±1.5	43.2	±2.2	10.5	±0.8	4.2	±0.5	-21.7	±1.1
Feb/Mar	2001	3	59.4	±0.5	1.8	±1.8	18.4	±1.3	10.8	±2.0	42.8	±1.3	10.8	±0.1	4.0	±0.1	-20.8	±0.4
Ovary																		
Nov/Dec	1999	1	39.2		27.5		15.5		9.4		50.1		7.1		7.0		-25.8	
March	2000	4	34.6	±4.9	40.3	±4.6	9.9	±2.4	7.1	±1.2	54.9	±1.7	6.3	±0.9	8.9	±1.5	-28.4	±0.7
October	2000	3	38.3	±0.9	35.6	±3.4	11.5	±3.7	6.3	±0.9	54.0	±1.2	7.0	±0.2	7.8	±0.6	-27.4	±0.8
Feb/Mar	2001	3	36.5	±2.5	33.4	±4.7	15.8	±2.9	6.2	±0.4	53.2	±1.2	6.6	±0.5	8.0	±0.7	-26.8	±1.0
Gut Lining (both sexes)																		
October	2000	5	41.3	±13.	13.5	±4.7	8.1	±2.6	28.6	±20.0	37.0	±11.5	7.5	±2.3	5.0	±0.1	-23.2	±1.8
Feb/Mar	2001	6	41.6	±5.8	11.7	±4.1	14.5	±1.9	23.7	±10.4	38.7	±6.2	7.6	±1.2	5.1	±0.3	-22.6	±1.2

Table 4.8. Elemental and biochemical composition of body tissues of *Sterechinus antarcticus*. Mean and standard deviation are given for sample sizes greater than 1.

Species		N	% dry mass												kJ g^{-1} DM	
			Protein		Lipid		Carbohydrate		Ash		C		N		C:N		Energy	
Testis																		
Nov/Dec	1999	10	56.6	±5.5	9.5	±6.3	11.9	±3.0	11.1	±1.7	44.1	±1.6	10.3	±1.0	4.3	±0.6	-22.3	±1.3
March	2000	6	60.7	±1.3	2.9	±1.7	12.3	±1.1	12.9	±0.4	41.5	±0.5	11.0	±0.2	3.8	±0.1	-20.9	±0.5
October	2000	7	58.3	±2.8	9.0	±2.9	8.7	±2.3	12.9	±1.7	43.3	±1.5	10.6	±0.5	4.2	±0.2	-22.3	±0.7
Feb/Mar	2001	6	61.2	±2.6	7.7	±2.7	7.5	±3.9	12.5	±1.8	43.3	±0.8	11.1	±0.5	3.9	±0.2	-22.2	±0.7
Ovary																		
Nov/Dec	1999	9	33.9	±0.7	43.9	±2.4	8.5	±2.3	5.5	±0.6	56.7	±0.9	6.2	±0.1	9.2	±0.3	-29.0	±0.5
March	2000	11	34.5	±3.2	42.1	±3.9	7.9	±2.7	7.5	±1.9	55.4	±1.4	6.3	±0.6	8.9	±0.9	-28.8	±0.7
October	2000	11	36.7	±4.5	39.2	±9.2	9.2	±3.9	6.7	±2.2	55.0	±3.3	6.7	±0.8	8.4	±1.3	-28.2	±1.8
Feb/Mar	2001	6	32.7	±1.4	45.8	±5.3	6.1	±3.3	7.4	±3.8	56.4	±3.1	6.0	±0.3	9.5	±0.8	-29.6	±1.0
Gut Lining (both sexes)																		
October	2000	18	41.3	±2.6	15.9	±3.8	19.1	±2.7	15.2	±2.0	43.9	±1.4	7.5	±0.5	5.9	±0.4	-23.4	±0.9
Feb/Mar	2001	12	42.0	±2.5	21.0	±4.4	15.4	±3.0	13.0	±1.9	47.0	±2.0	7.7	±0.5	6.2	±0.6	-24.6	±0.9

Table 4.9. Elemental and biochemical composition of body tissues of *Ctenocidaris perrieri*. Mean and standard deviation are given for sample sizes greater than 1.

Species		N	% dry mass												kJ g^{-1} DM	
			Protein		Lipid		Carbohydrate		Ash		C		N		C:N		Energy	
Testis																		
March	2000	3	50.2	±1.2	7.0	±5.7	6.9	±6.1	25.3	±0.2	36.4	±2.0	9.1	±0.2	4.0	±0.2	-21.7	±1.7
October	2000	3	51.3	±5.8	5.1	±4.6	11.9	±10.3	21.2	±0.0	37.9	±2.0	9.3	±1.0	4.1	±0.3	-21.1	±1.7
Feb/Mar	2001	3	48.2	±6.9	18.2	±5.2	5.5	±4.2	17.8	±3.9	43.3	±1.7	8.8	±1.2	5.0	±0.6	-24.3	±1.3
Ovary																		
March	2000	5	34.2	±3.1	28.1	±9.0	16.4	±3.9	13.2	±7.2	48.1	±6.1	6.2	±0.6	7.7	±1.0	-25.8	±1.9
October	2000	3	34.4	±1.0	32.0	±4.7	18.8	±3.6	6.7	±0.0	52.4	±1.5	6.3	±0.2	8.4	±0.5	-26.4	±1.0
Feb/Mar	2001	3	41.0	±0.7	28.7	±1.3	14.3	±1.1	7.5	±1.0	51.5	±0.6	7.5	±0.1	6.9	±0.2	-26.0	±0.3
Gut Lining (both sexes)																		
October	2000	4	35.3	±2.8	2.9	±1.8	10.6	±2.7	41.7	±4.0	26.6	±1.7	6.4	±0.5	4.1	±0.1	-19.6	±0.6
Feb/Mar	2001	6	34.9	±3.2	4.4	±3.2	9.9	±4.7	41.4	±3.8	27.3	±2.2	6.4	±0.6	4.3	±0.2	-20.1	±1.3

Table 4.10. Elemental and biochemical composition of body tissues of *Amphipneustes lorioli*. Mean and standard deviation are given for sample sizes greater than 1.

Discussion

The estimated biochemical compositions of the body components of the six species of Antarctic echinoderms are similar to those of other Antarctic (Pearse and Giese, 1966; McClintock and Pearse, 1987; McClintock *et al.*, 1988; McClintock, 1989) and temperate echinoderms (Giese, 1966; Lawrence and Guile, 1982;). While the levels of carbohydrate tended to be high (carbohydrate < 29.6 % DM) when compared with some studies of the biochemical composition of echinoderm tissues (<6 % DM carbohydrate) (Lawrence and Guile, 1982; McClintock and Pearse, 1987; McClintock *et al.*, 1988; McClintock, 1989), they were within the range of values found for tissues of the Antarctic echinoid *Sterechinus neumayeri* (Pearse and Giese, 1966, Brockington *et al.*, 2001) and for the echinoid *Mellita quinquiesperforata* (Moss and Lawrence, 1972). Brockington *et al.* (2001) suggested that while the cause of these higher carbohydrate values was not clear, it might be related to the quality of food available. Giese (1966) noted that echinoderms feeding on a food source that is rich in either fats or carbohydrates might lead to accumulation of these substances in the tissues. Although the food source of the echinoderms in this study was not analysed for biochemical composition, all species were feeding in the same environment and all showed relatively high (>6%) levels of carbohydrate in gut and gonad tissues. Pearse and Giese (1966) also hypothesised that specimens of *Sterechinus neumayeri* taken from Turtle Rock contained low levels of carbohydrate and were exposed to less favourable nutritional conditions than specimens taken from Cape Evans and Cape Royds that contained higher levels of carbohydrate in their gonads.

Nutritional Status and the Role of Echinoderm Body Components

Gonads

The mean biochemical composition of the gonad tissues indicated differences between the two species of holothurian and the echinoids and asteroid. Generally the male holothurians had a lower proportion of protein and a higher proportion of ash in their gonads than did the other species. The ovaries also exhibited a much higher ash content than those of the other species. This would suggest that the holothurians had a much higher structural component to their gonads than the other classes of echinoderm. There was also a distinct sexual differentiation in biochemical composition. The ovaries of all species contained much higher proportions of lipid than the testes (Figure 4.1). This was probably because it was either accumulated in

the oocytes (Pearse and Giese, 1966) or in the nutritive tissue of the ovary, and reflected the inherently greater energetic contribution of lipid to the development of an egg and the production of a juvenile when compared to sperm (McClintock and Pearse, 1987).

Among echinoderms, it has been shown that lecithotrophic eggs have a greater mass-specific energy content than planktotrophic eggs (Jaeckle, 1995) and that the relationship between egg energy content and egg volume show similar positive scaling patterns for both lecithotrophic and planktotrophic eggs. The relationship between egg size and energy content cannot be directly measured in this study as gonads were analysed whole, although total gonad energy content does vary among the six species. The ovaries of *Ctenocidaris perrieri* and *Psilaster charcoti* both contained the highest proportions of lipid (>40% DM) and had high maximum energy contents (-29.6 and -29.9 kJ g^{-1} DM respectively), similar to levels reported by McClintock and Pearse (1987). Both species produced large eggs, for which *Ctenocidaris perrieri* (<1750 μm diameter) brooded its embryos and *Psilaster charcoti* (<578 μm diameter) had lecithotrophic development. *Ctenocidaris perrieri* had a much larger egg size than *P. charcoti* and yet had a similar energy content; this was probably as a result of the much higher fecundity of *Psilaster charcoti* (<40,720 oocytes per individual) than *C. perrieri* (<1,505 oocytes per individual). The lowest levels of lipid and energy (<-27.3 kJ g^{-1} DM) were found in *Protelpidia murrayi*, which produced the smallest eggs (<357 μm).

The echinoid *Sterechinus antarcticus* had higher levels of lipid (<40% DM) in its ovaries than the congeneric species *Sterechinus neumayeri* (<29% DM (Giese, 1966)) as *Sterechinus antarcticus* produces a lecithotrophic egg (Chapter 3) unlike the planktotrophic egg produced by *Sterechinus neumayeri*. The difference in biochemical composition of the two species of *Sterechinus* supported the suggestion that *Sterechinus antarcticus* produces a lecithotrophic egg as the ovary contained a higher amount of lipid and energy than its planktotrophic counterpart. In contrast to evidence in support of an increase in lipid and energy levels with an increase in egg size, *Amphipneustes lorioli* had the largest egg size (<1971 μm) and yet also had lower levels of lipid and energy (<-26.4 kJ g^{-1} DM) than *Ctenocidaris perrieri*,

Psilaster charcoti and *Sterechinus antarcticus*. The discrepancy in lipid content in *Amphipneustes lorioli* may be in part as a result of the lower actual fecundity (<372 oocytes per individual) than the other species of Antarctic echinoderm (382 to 40,720 oocytes per individual). The ovaries of *Amphipneustes lorioli* also had higher levels of carbohydrate in the ovary than *Ctenocidaris perrieri*, *Psilaster charcoti* and *Sterechinus antarcticus*, which may be stored in the nutritive tissue surrounding the oocytes to be used as a readily metabolised source of energy. Direct comparisons of energy input into offspring by the female would require direct measures on eggs as the analysis of the entire gonad does not necessarily give an indication of the level of energy deposited in the spawned zygotes (Lawrence, 1985).

Pyloric Caeca

The pyloric caeca in asteroids is primarily a nutrient storage organ (Hyman, 1955, Lawrence and Lane, 1982) and levels of both protein and lipid recorded in the pyloric caeca of *Psilaster charcoti* were relatively high (Table, 4.7, Figure 4.3) with ash contents lower than any other tissue in this study. Mean levels of lipid (~22 % DM) in the pyloric caeca were similar to those reported for *Psilaster charcoti* by McClintock and Pearse (1987), while protein levels were slightly lower and levels of carbohydrate were often much higher. Nutrient reserves stored as lipid allow a higher energy per unit mass than either protein or carbohydrate, although these latter compounds may be metabolised more readily (Lawrence and Guile, 1982). Therefore, higher levels of carbohydrate in this study may have been as a result of varying food availability.

Gut

Gut tissues of all species were high in protein which is the major substrate used to fuel metabolic requirement (Peck *et al.*, 1987). This suggests that the gut lining tissue may play a role in providing material and energy for general metabolic function and gametogenesis. *Ctenocidaris perrieri* had relatively high levels of lipid and carbohydrate in the gut tissue, which would suggest a capacity for nutrient storage (Bishop and Watts, 1992). The ability to store nutrients in gut tissues may be particularly valuable to *Ctenocidaris perrieri*, which broods its young on its peristome, and possibly ceases feeding for the duration of brooding (Barker, 1984). During periods of starvation *Ctenocidaris perrieri* may require stored nutrients to fuel its metabolism.

The organic fraction of the body wall of both holothurian species was dominated by protein; lipid and carbohydrate were only present in small quantities. Protein is an important constituent of the body wall of holothurians, providing the building blocks of the connective tissue and muscle (Giese, 1966). The ash content of the body wall was the highest of any of the tissues measured and is likely to account for skeletal material such as calcareous spicules and deposits in the wall tissue.

Spatial Variability Across the Antarctic Continental Shelf

Of the four taxa where sufficient samples were available to examine patterns of spatial variability, significant variation across sites was detected only in the composition of the testes in the holothurian *Protelpidia murrayi* and the pyloric caeca of the asteroid *Psilaster charcoti* (Table 4.4). The organic input from surface waters to the continental shelf was variable with increasing distance from the coastline; this was shown in pigment analyses of sediment cores taken from the three FOODBANCS sites. At site A surface sediment phytodetritus chloropigment and phaeopigment concentrations ranged from 64.9 – 395.1 μg g^{-1} and 202.5 – 1260.7 μg g^{-1} respectively, compared to site C where chloropigment and phaeopigment concentrations ranged from 12.7 – 18.9 μg g^{-1} and 20.7 – 53.5 μg g^{-1} respectively (Smith and Mincks unpublished data). These values were recorded during February/March 2001 and were the highest recorded throughout the sampling period. It was expected that the biochemical composition of the body components would reflect such a great difference in food quality at the three sites. Of the six species examined in this study *Sterechinus antarcticus* and *Amphipneustes lorioli* were found at only one of the three sites on the continental shelf and were therefore excluded from analysis of spatial variation. Of the remaining four species that inhabit all three sites *Protelpidia murrayi* and *Psilaster charcoti* were the only species to exhibit any significant variation in biochemical composition of body tissues among the different sites.

The holothurian *Protelpidia murrayi* showed evidence of variation in biochemical composition of tissues among sites for the male gonad tissues. Protein is the major biochemical component of the testes and it was this component that showed high

spatial variation among sites. Interestingly, *Protelpidia murrayi* only exhibited variation in protein levels among sites during October 2000, and no other season. The variation in protein levels was accompanied by a significant variation in tissue energy levels. Histological examination of the testes throughout the seasons revealed that the testes were either full or part spawned during June 2000 and that they remained devoid of spermatozoa during October 2000. The specimens from sites A and B had much higher levels of protein in the testes than specimens from site C. It was possible that the variation may have reflected a delay in the gametogenic cycle of specimens from site C compared to those of sites A and B, whereby the testis were delayed in recovering from a spawning event and that the protein levels reflected lower levels of germ cells. Another possibility was that the biochemical composition of the tissue was affected by food availability at site C. If a low nutritional food availability were the cause for the lower values of protein in the testes at site C then similar low values would be expected in other body components, and also in the female gonads. It is unlikely that the variation in biochemical composition was a result of lower food availability at site A as pigment analyses of sediment from site A suggested that higher quantities of pigment rich detritus accumulated at site A than at site C (Smith and Mincks, unpublished data).

In contrast to the species of holothurian, the asteroid *Psilaster charcoti* did not exhibit any variation in biochemical composition of either male or female gonad tissue among the sites. Nevertheless significant variation in biochemical composition of the pyloric caeca was observed from both male and female tissues during each season. The male and female pyloric caeca had consistently higher levels of lipid at site A than sites B and C. Site B had the highest levels of protein throughout the study and the lowest lipid, except during March 2001 when site C had the highest protein levels. Levels of ash were the lowest of any tissue type in this study and were of a similar level at each of the sites. Owing to the high levels of lipid in the pyloric caeca at site A, tissues from this site also had the highest energy levels throughout the study, with site B having the lowest.

The pyloric caeca is generally accepted as playing an important role in nutrient storage (Giese, 1966; Lawrence and Lane, 1982; McClintock and Pearse, 1987, McClintock *et al.*, 1990), and evidence suggests that the utilisation of this resource

may occur in response to starvation as well as in support of gametogenesis (Lawrence and Lane, 1982). The differences in biochemical composition of the pyloric caeca among the sites thus most likely reflect the differing food availability at the three sites rather than a response to different gametogenic requirements.

The main food of *Psilaster charcoti* in this study was the Antarctic krill, *Euphausia superba*, which were frequently found in its stomach contents (Chapter 2). The highest proportions of asteroid specimens with stomachs containing krill were recorded at site A during October 2000, and during this season stomachs from other stations were empty (Galley unpublished data). Water column krill abundance is at a minimum during October and has been since June (Siegel, 1988) when spent females migrate into coastal shelf waters following the spawning season. During this time an increased mortality rate among the spawned krill stock may cause sinking krill to provide a food source for some of the benthos. It is unclear why asteroids from site B had such low levels of lipid compared to sites A and C, although it is likely that it is in response to a comparably limited food source. Perhaps the presence of the clockwise rotating gyre (Knox, 1994) on the continental shelf in the vicinity of the study sites influenced the distribution of zooplankton in that area and thus limited the deposition of *Euphausia superba* to the central site. Nevertheless, this is just speculation as there was not enough data on the stomach contents of the *Psilaster charcoti* to perform any statistical analyses.

Seasonal Variation in Biochemical Composition of Body Tissues

Of the six taxa examined in this study, two (the echinoids *Sterechinus antarcticus* and *Amphipneustes lorioli*) showed no significant seasonal variation in the chemical composition of any tissue. The third echinoid (*Ctenocidaris perreri*) showed significant seasonal variation only in gut tissues, whereas the two holothurians and the asteroid each showed significant seasonal variably in several tissue components. In addition to a spatial variation in sediment deposition, the Antarctic continental shelf experiences a distinct seasonal and inter-annual component to the phytodetrital flux (Chapter 1). The highest rate of particle flux is observed shortly after the spring phytoplankton bloom in surface waters, and the lowest rates are observed during the winter months when surface phytoplankton production is at a minimum and the ice cover stabilises the water column. Such variation in the quality of food supply may

have several effects on the biochemistry of the body components. It may have a direct effect on the amount of food available to be channelled into nutrient storage organs such as the pyloric caeca and the gut lining, or it may affect the timing of gametogenic cycles and hence how much energy can be allocated into producing mature gametes.

The most significant seasonal variation of tissue composition was recorded in the holothurian *Protelpidia murrayi*. The ovaries varied by six orders of magnitude in the proportion of lipid between the austral spring (October-November) and the austral autumn (March). There were also high fluctuations in protein, carbohydrate and ash levels between these seasons. The peak in lipid levels, during March, coincided with the ovaries containing high proportions of mature vitellogenic oocytes (Chapter 3). Conversely the minimum lipid levels coincided with the post spawn-out period when the ovaries contained a high proportion of previtellogenic oocytes and no mature vitellogenic oocytes. The testes also exhibited a seasonal variation in biochemical composition. The testes contained low levels of lipid and carbohydrate at all times, therefore the main variation was observed in the protein levels. Protein levels were highest during March when spermatozoa were present in the lumen of the testes (pers. obs.) and lowest during November 1999 and October 2000 when the testes appeared spent, which was particularly evident from the histological analyses of specimens during October 2000. The biochemical composition of both the male and female gonad tissues in *Protelpidia murrayi* thus shows distinct seasonality that strongly reflects the seasonal gametogenic cycles and maturity of the gonads.

There was no significant variation in the biochemical composition of the gut lining or body wall tissue of *Protelpidia murrayi*. This suggests that *P. murrayi* did not need to lay down extra energy stores in the body components during the summer to compensate for a lack of fresh phytodetritus during the winter, neither did it need to reabsorb any organic material from the existing tissues.

The testes of *Peniagone* sp. showed a significant variation in protein levels of similar proportions and during the same seasons as *Protelpidia murrayi*. In *Peniagone* sp. the peak values of protein during March do not correspond to an increase in gonad index, neither do they appear to correspond to an increase in spermatozoa concentration

(pers. obs.). The variation in the October 2000 samples was very high. This suggests that not all of the testes sampled during this month were at the same stage of development. Some of the testes may have been fully mature and some may have just spawned their gametes. The analysis of the oocyte size frequency histograms showed that some ovaries contained mature gametes and that some ovaries were part spawned (Chapter 3). This suggested that a spawning event was starting to take place during October 2000, which would account for the high variation in the protein levels of the testes.

The ovaries generally showed no variation in composition with season, with the exception of the lipid and energy contents. The average composition of bulk gonad tissues combined oocytes at all stages of development with surrounding gonad tissues, hence important small-scale changes may well be obscured. It does, however allow an assessment of overall investment to the gonad. Lipid levels decreased from November 1999 to March 2000 and then decreased again during February/March 2001. These fluctuations in lipid level did not appear to bear any relation to the gametogenic cycle of the ovaries. Ovaries contained few mature vitellogenic oocytes during November 1999, however the proportion of vitellogenic oocytes had increased by March 2000 and October 2000. During February/March 2001 ovaries contained a high proportion of small vitellogenic oocytes (Chapter 3). The change in ovary maturity was reflected in the non-significant fluctuations of protein and carbohydrate content, which were higher during March 2000 and February/March 2001 when the ovaries had higher proportions of young vitellogenic oocytes.

Of the remaining body components of *Peniagone* sp. the gut lining tissue also exhibited a slight but significant seasonal variation. The proportion of protein in the tissue decreased, whilst the proportion of lipid increased. This change in composition to higher levels of lipid may have reflected the change in quality of food available from October to February/March. The samples collected during October had been exposed to the lowest levels of phytodetrital flux during the previous 3-4 months (Chapter 1), whilst the specimens collected during February/March 2001 had been exposed to the highest flux values recorded throughout the study. Lipids contain higher energy levels per unit mass than proteins and therefore by changing the storage of energy from protein to lipid, the limited space available in the gut lining could

contain the highest possible levels of energy (Lawrence and Guile, 1982). Body wall tissues also showed an increase in both protein and lipid values during February/March 2001, however too few samples gave accurate results to enable statistical analyses.

Psilaster charcoti showed a significant variation in the biochemical composition of the testes between October 2000 and February 2001. The change in tissue composition resulted in higher levels of protein and lipid during March 2001 than were seen in October 2000. Histological examination of the testes of *P. charcoti* indicated that the lumen of the testes was packed with spermatozoa at both times of year but that during March 2001 long colonettes of spermatocytes extended into the lumen, indicating active production of spermatozoa. The presence of higher quantities of undifferentiated germ cells during March 2001 may have been responsible for the increase in protein levels within the testes. Within-season variation in biochemical composition of the ovaries was high throughout all of the study, suggesting a lack of synchrony in oocyte development between individuals as seen in Chapter 3.

The biochemical composition of the pyloric caeca appeared to be the most variable body component in *Psilaster charcoti*, showing differences in composition between sites and seasons. There did not appear to be a consistent pattern of variation among seasons or among the three sites. For example when the proportion of lipid increased between two seasons at site A, it decreased at site C. This suggested that the biochemical composition of the pyloric caeca in *P. charcoti* was very sensitive to the quality and quantity of food available, which is decoupled from season. The main source of nutrition observed for *P. charcoti* in this study was *Euphausia superba*, which must have had a very variable distribution across the shelf sites; this was reflected in the variation of biochemical composition of the pyloric caeca tissues. Similar patterns in the variation of biochemical composition occurred in both the male and female pyloric caeca tissues, supporting the theory that these variations are closely linked to food supply and not to gametogenic cycles.

There was no significant variation in composition of any of the tissues from *Sterechinus antarcticus*, including any variation that would be accounted for by the seasonal gametogenic cycles found in this species (Chapter 3). Pearse and Giese

(1966) and Brockington (2001) similarly found no variability for *Sterechinus neumayeri*, indicating that *S. neumayeri*, which ceases feeding during the austral winter, uses stored reserves in the same ratio that they are present in the tissues. There was no evidence that *S. antarcticus* ceased feeding during the winter (pers. obs. of gut containing sediment throughout the study). Therefore as the majority of gamete maturity occurs during the austral summer, *Sterechinus antarcticus* may be able to gain sufficient energy from the surrounding sediments during the winter to support the low metabolic rate observed in Antarctic benthic invertebrates (Clarke and Peck, 1991). In this case there would have been no need for *S. antarcticus* to store or utilise energy reserves in the soft tissues.

The brooding cidarid urchin *Ctenocidaris perrieri*, exhibited a significant change in both lipid and carbohydrate levels in the gut lining during March 2001. Lipid levels were at a minimum during October 2000, at the end of the winter low food availability period and they had increased by 5% by March 2001, following the input of phytodetritus to the shelf sediments. Lawrence *et al.* (1965) indicated, from laboratory experiments on *Strongylocentrotus purpuratus*, that the gut rather than the gonad can be affected immediately by starvation and that lipids are the biochemical component most utilized during starvation. The tissues of *Ctenocidaris perrieri* seem to fit this pattern as there was little change in the proportion of protein in the gut lining, and the gonad tissues show no significant variation throughout the seasons. The biochemical composition of the gut lining in *C. perrieri* has already indicated the possibility of a role as a nutrient storage organ, which appears to have been utilised during the austral winter in this study whilst either food quality was poor or the urchins may have been brooding their young. Although no specimens recovered were carrying broods, the nature of the sampling technique is very destructive and may have caused the broods to be released from the adult. Had any of the specimens been carrying a brood it is likely that they would have stopped feeding as brooding in cidarids occurs on the peristome (Barker, 1984).

No seasonal variation in the biochemical composition of any of the tissues was evident in specimens of *Amphipneustes lorioli*. This may be because *A. lorioli* showed no evidence of either seasonal gametogenic cycles or seasonal intensity of oocyte production that would cause a variation in biochemical composition of the gonad

tissue. In addition there was no evidence of any seasonal intensity of feeding that may lead to a nutrient shortage and the utilisation of stored reserves. In addition the metabolic rates of Antarctic benthic invertebrates are low when compared to tropical or temperate invertebrates, and therefore there is a reduced demand of energy.

Conclusion

Seasonal changes in biochemical composition of tissues caused by either a response to specific gametogenic cycles or by a difference in food availability could be seen within some of the echinoderm species. Nevertheless, this is not the rule for all of the echinoderms, two of the species showed no significant variation in the biochemical composition of their body components. The greatest variation in biochemical composition was seen in the gonads of *Protelpidia murrayi* (holothurian). This species has been shown to have a highly opportunistic reproductive strategy, whereby the production of mature oocytes is promoted by the increase in quantity and quality of food available (Chapter 3) and an increase in production of mature, vitellogenic oocytes would result in higher lipid and energy contents of the gonad tissues. Conversely a species such as *Amphipneustes lorioli*, that did not exhibit any seasonality in gametogenic cycle or feeding, showed no significant variation in biochemical composition throughout the seasons.

Interestingly the pyloric caeca of *Psilaster charcoti* exhibited a significant variation in composition among both sites and seasons, suggesting that the energy storage organ of this species responds rapidly to changes in food availability.

Chapter 5 – Overview and Summary

Responses of Echinoderms to a Seasonal Food Supply on the Antarctic Continental Shelf

Little is known of the ecology of benthic invertebrates on the Antarctic continental shelf. The majority of research carried out in Antarctic waters has been confined to depths accessible by SCUBA divers, and most of the multidisciplinary oceanographic projects carried out on the continental shelf have focussed on the pelagic environment. In this thesis I have examined the life history patterns of a variety of continental shelf echinoderms, which comprised a significant proportion of the megabenthos in the FOODBANCS study area.

Key findings:

The important results from this study are:

A) Holothuroidea

Protelpidia murrayi

- Seasonal spawning occurred in *P. murrayi*, the onset of vitellogenesis being initiated and synchronised by the arrival of the phytodetrital pulse.
- Suggested gametogenic cycle of 18-24 months, with spawning commencing during June.
- The biochemical composition of the ovaries exhibited a significant increase in the proportion of lipid during the main period of vitellogenesis.
- Gut tissues indicated a significant change in biochemical composition between October 2000 and March 2001, suggesting a role as a nutrient storage organ.

***Peniagone* sp.**

- *Peniagone* sp. exhibits a seasonal intensity of reproduction; the increase in production of vitellogenic oocytes is associated with an increase in food supply.
- Suggested gametogenic cycle of 18-24 months.
- Gut tissues also indicated a significant change in biochemical composition between October 2000 and March 2001, suggesting a role as a nutrient storage organ.

- The two species of holothurian exhibit an opportunistic reproductive strategy in response to a fluctuating food supply, which may be typically characteristic of the elpidiid holothurian family.

B) Asteroidea

Psilaster charcoti

- *Psilaster charcoti* had much smaller eggs and showed no evidence of a seasonal reproduction cycle when compared with those from the McMurdo population of *P. charcoti.*
- The ovaries showed no significant variation in biochemical composition between seasons, supporting the hypothesis of a continuous reproductive strategy.
- Specimens of *P. charcoti* on the continental shelf were found to feed mainly on *Euphausia superba.*
- The pyloric caeca showed significant variation in biochemical composition both among sites and among seasons, with no consistently greater nutritional state at one particular site. This suggested that the nutritional status of the pyloric caeca closely reflects the quality and quantity of food available.

C) Echinoidea

Sterechinus antarcticus

- *Sterechinus antarcticus* has much larger eggs than those of the shallower water congeneric *Sterechinus neumayeri*, 250 μm and 120 μm respectively.
- *Sterechinus antarcticus* exhibits a seasonal reproductive cycle like the congeneric species *S. neumayeri.* However, the timing of spawning differs by ~5 months to that of *S. neumayeri. Sterechinus antarcticus* commences spawning during June.
- The majority of oocyte development and vitellogenic processes occur after the main period of phytodetrital flux.
- Suggested gametogenic cycle of 18-24 months for the ovaries and 10-12 months for the testes.
- Despite a seasonal reproductive cycle *Sterechinus antarcticus* shows no seasonality in biochemical composition of tissues throughout the seasons. *Sterechinus neumayeri* also shows no significant variation in biochemical composition with season (Brockington *et al.*, 2001).

Ctenocidaris perrieri

- *Ctenocidaris perrieri* showed no seasonality of reproduction, or any evidence of brooding.
- There was no significant variation in the biochemical composition of the gonads of *C. perrieri.*
- Gut tissues exhibited a significant change in biochemical composition between October 2000 and March 2001, indicating a potential role as a nutrient storage organ.

Amphipneustes lorioli

- *Amphipneustes lorioli* showed no evidence of a seasonal gametogenic cycle based on analysis of oocyte size-frequency histograms. However, it did show a synchronous development of embryos and juveniles in the brooding pouches.
- The lack of evidence of seasonality in the gonads was supported by a lack of seasonality in the biochemical composition of body tissues, unlike the other urchin species *A. lorioli* did not exhibit any variation in the gut tissue.

Reproductive patterns of Antarctic Benthos, shallow water *vs.* deep sea

Shallow-water Antarctic and deep-sea environments have many important physical and biotic similarities. These have led to the formulation of conflicting hypotheses for faunal origins, ages and evolutionary processes (Lipps and Hickman, 1982). They include the suggestion that modern deep sea fauna originated in the shallow Antarctic, that the shallow Antarctic fauna originated in the deep sea, that both faunas migrated into their present sites from other regions, or that each fauna evolved independently in place (Lipps and Hickman, 1982). It is possible that the deep Antarctic continental shelf may provide an evolutionary stepping-stone for species migrating between these two environments. Gebruk (1994) has suggested that the elasipodid holothurians invaded the bathyal Antarctic waters by moving along the South American continental slope and that further invasion of the Antarctic abyssal waters occurred from there.

The life-history characteristics of the echinoderms presented in this study show parallels to both deep sea and Antarctic shallow water populations. Gametogenesis is generally regarded as a slow process in both environments when compared to tropical

shallow waters. Nevertheless, it has been shown that many polar species have extended oogenic cycles (estimated to last between 18 and 24 months), when compared to those in the deep sea (which are typically estimated to last between 8 and 14 months) (Table 3.1, Chapter 3). The gametogenic cycles of at least three of the species in this study have been predicted to take between 18 and 24 months to completion, timescales similar to those of shallow water Antarctic species.

In addition to the timescales of reproductive cycles, the periodicity and mode of development also differ. In the deep sea, seasonality of spawning is only associated with species that produce a planktotrophic larvae, whereas species from the Antarctic may exhibit seasonal reproductive cycles regardless of the mode of development (Figure 3.1, Chapter 3). Of the species examined in this study only two, *Protelpidia murrayi* and *Sterechinus antarcticus*, showed a seasonal gametogenic cycle in response to the deposition of organic material. These species possessed the smallest egg size of the six species examined and an unknown, but probably lecithotrophic, mode of development. It was suggested that *Protelpidia murrayi* had an opportunistic reproductive strategy, and that the distinct gametogenic response to the seasonal food supply shown in this environment may have readily adapted to a different food regime.

Such an opportunistic reproductive strategy has also been seen in other species of holothurian belonging to the elpidiid family (Billett and Hansen, 1982; Wigham *et al.*, 2003). Therefore despite the apparent seasonality of reproduction observed in *Protelpidia murrayi*, it was likely that its reproductive strategy was similar to those of other deep-sea elpidiids and that it showed such a pronounced seasonality as a direct result of a highly seasonal food supply. *Peniagone* sp. also exhibited an opportunistic reproductive strategy, responding to the seasonal food supply with a seasonal intensity of reproduction.

Psilaster charcoti showed no evidence of reproductive seasonality, either in the gametogenic cycle or the nutritional status of the gonad tissues. This was in contrast to the shallow water population of *P. charcoti* at McMurdo Sound that was found to be synchronous and sexually mature during October (Bosch and Pearse, 1990). In this case the deeper water population may be reproductively isolated, either by living in a

different water mass or by differences in hydrostatic pressure, from the shallow water population. This separation may eventually lead to speciation. Whilst the McMurdo population exhibits a typical reproductive behaviour seen in many shallow water asteroids, the deeper water population seems to behave much like true deep-sea species, in which no seasonality has currently been observed in species possessing lecithotrophic larvae (Tyler and Young, 1992).

The main source of nutrition for the deeper population was found to be *Euphausia superba*. The source of *E. superba* may be inconsistent across the shelf environment and lack the seasonal signal associated with the phytodetrital flux. This was highlighted by the nutritional status of the pyloric caeca (Chapter 4). The shallow water population may subsist on an entirely different food source that is coupled to the spring phytoplankton bloom. If this is the case then the seasonality seen in the shallow water population may be driven by a seasonal food supply, whereas the deeper population may have become uncoupled from the bloom as a result of dietary differences.

Of the remaining three species *Sterechinus antarcticus* is the only species to display a discrete reproductive seasonality. Although the mode of larval development is not known for *S. antarcticus*, a lecithotrophic development is inferred from the size of the egg, its colour and positive buoyancy. The Antarctic Echinidae are represented by only five species all within a single genus, *Sterechinus* (Pawson, 1969). The distribution of these species ranges from the east coast of South America and South Georgia (*Sterechinus antarcticus* and *Sterechinus agassizi*), to circum-Antarctic (*Sterechinus antarcticus* and *Sterechinus neumayeri*), a truly deep sea form (*Sterechinus dentifer*) that is only recorded from 1266 and 1565 m depth in the Southern Indian Ocean and one endemic to the Kerguelen Islands (*Sterechinus diadema*) (Mortensen, 1943; Pawson, 1969). Biermann (unpublished data) has shown that *S. neumayeri* is closely related to the north Atlantic species *Echinus esculentus,* which suggests a possible migration and speciation of *Echinus* southward through the Atlantic as suggested by Tyler and Young. (1998). The genus *Sterechinus* may then have migrated across from South America to the Antarctic Peninsula and then invaded the deep Antarctic Ocean in a similar manner to that suggested for the elasipodid holothurians (Gebruk, 1994).

Data from this study thus suggest that most of the species examined have reproductive strategies similar to those of related deep-sea echinoderms, but that the speed of physiological processes has slowed at the very cold Antarctic temperatures. The exception is *Sterechinus antarcticus*, which is the only species showing a true seasonality of reproduction, and exhibits similar traits to those of shallow water echinoderms. The differences in the reproductive strategies and physiology of the six Antarctic continental shelf echinoderms examined in this study may be a result of different evolutionary pathways that have brought these organisms together on the deep continental shelf. Given that the main hypotheses for the origin of deep sea fauna includes the invasion of the deep sea from shallow waters and the evolution of fauna in place, the origin of the continental shelf fauna may be either shallow water or the deep sea. The periodic flooding of the continental shelf by Circumpolar Deep Water may provide a pathway for the larvae of deep-sea organisms to infiltrate the shelf environment. Conversely, the formation of Antarctic Bottom Water close to the Antarctic continent may provide a pathway for the invasion of deeper water by shallow water Antarctic fauna.

Implications for the FOODBANCS hypothesis.

The main hypothesis of the FOODBANCS programme was that phytodetritus and/or faecal pellets accumulating on the continental shelf floor might persist for longer periods and provide a more predictable organic signal, relative to the sinking phytodetritus in the water column. This predictable benthic food source may persist long enough on the sea floor to serve as a nutritional resource throughout the austral winter when the flux of phytodetritus and faecal pellets are at a minimum.

Whilst reproductive pattern, in terms of seasonal and continuous gamete production, are generally phylogenetically constrained (Eckelbarger and Watling, 1995), the timing of spawning and the magnitude of fecundity may change in response to varying inputs of organic matter (Ramirez-Llodra, 2002). In *Sterechinus antarcticus* the gonads showed true seasonality of gamete development, and the timing of spawning differed by about 5 months from that of the congener *Sterechinus neumayeri*. In this species it appears that the arrival of the seasonal pulse of phytodetritus initiates the start of vitellogenesis and the production of mature oocytes.

However there is no evidence of an increase in fecundity associated with the arrival of the pulse, although timescales may be too short for this to be observed. In addition, there was no evidence of seasonal changes in the biochemical composition of the body tissues of *Sterechinus antarcticus*, perhaps as this species does not cease feeding during the austral winter (Galley pers. obs.), unlike its congener *Sterechinus neumayeri* (Brockington *et al.*, 2001). It appears that there is sufficient food available year round on the Antarctic continental shelf to support the population of *Sterechinus antarcticus* and that this species takes advantage of the particularly high quantity of labile organic matter to promote and possibly synchronise vitellogenic processes.

The two species of holothurian were shown to have variable reproductive patterns, in that *Protelpidia murrayi* exhibited a seasonal gametogenic cycle and *Peniagone* sp. exhibited a seasonal intensity of gamete production. Both species of holothurian continued feeding and producing gametes throughout the year, however the main period of vitellogenesis coincided with the seasonal phytodetrital flux. Significant increases in the proportion of protein and lipid occurred in the gut tissues of these two species between October 2000 and March 2001,which suggested a role as a nutrient storage organ that was replenishing its reserves after the winter period. These results suggested that the physiology of the holothurians was closely tied to the quality and quantity of food available. Moreover, the quality of food may have been sufficiently high during the austral summer to support gamete production and nutrient storage. Therefore, although these species continue to feed during the austral winter and may gain sufficient energy to maintain a basal metabolism, more energetically expensive activities, such as vitellogenesis, are reserved for the summer when a higher quality of food is readily available.

Psilaster charcoti mainly consumed *Euphausia superba* and therefore its physiological processes were not directly coupled to the summer phytoplankton bloom. No evidence was found of seasonality associated with either gamete production or the biochemical composition of the gonads. There was however, significant seasonal variation in the biochemical composition of the pyloric caeca. This may reflect the distribution and migration patterns of krill over the continental shelf and hence the availability of dead material to the benthos.

Of the two remaining echinoids examined in this study, there was no evidence of seasonality of reproduction and no variation in mean fecundity throughout the entire sampling period. Biochemical compositions of the body tissues also remained constant with the exception of the gut tissues in *Ctenocidaris perrieri*. The gut tissues exhibited a significant increase in the proportion of lipid and a decrease in the proportion of carbohydrate between October 2000 and March 2001. This suggests that whilst there was a slight shift in the allocation of energy in the gut tissues of *C. perrieri* there remained a sufficient "foodbanc" throughout the winter to support the physiological and metabolic needs of these two species of echinoid.

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Appendices

Individual oocyte size-frequency distributions for *Protelpidia murrayi*.

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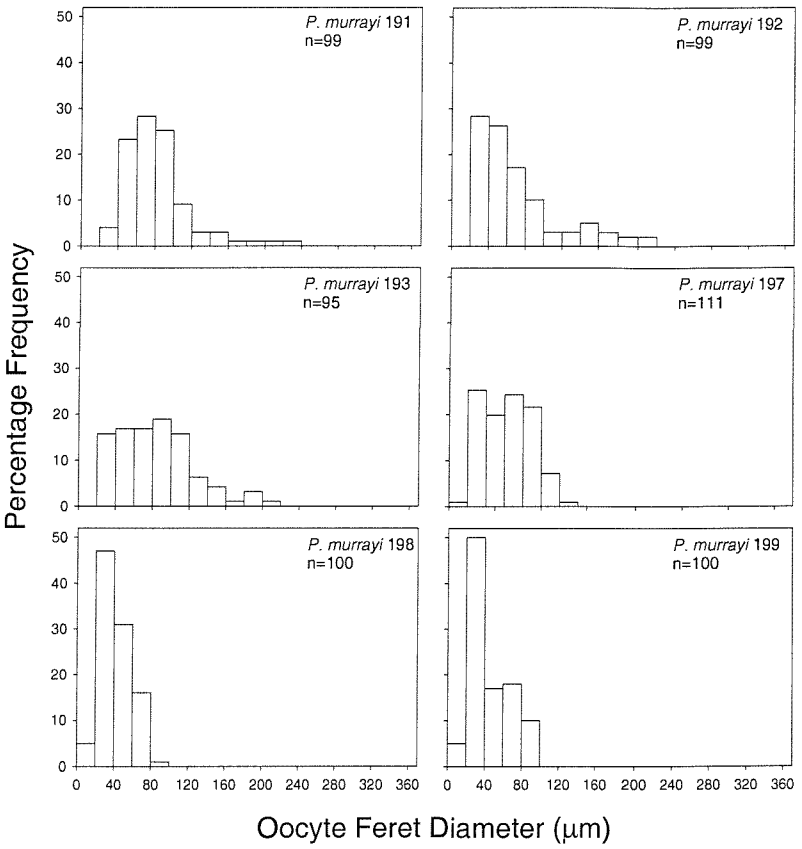

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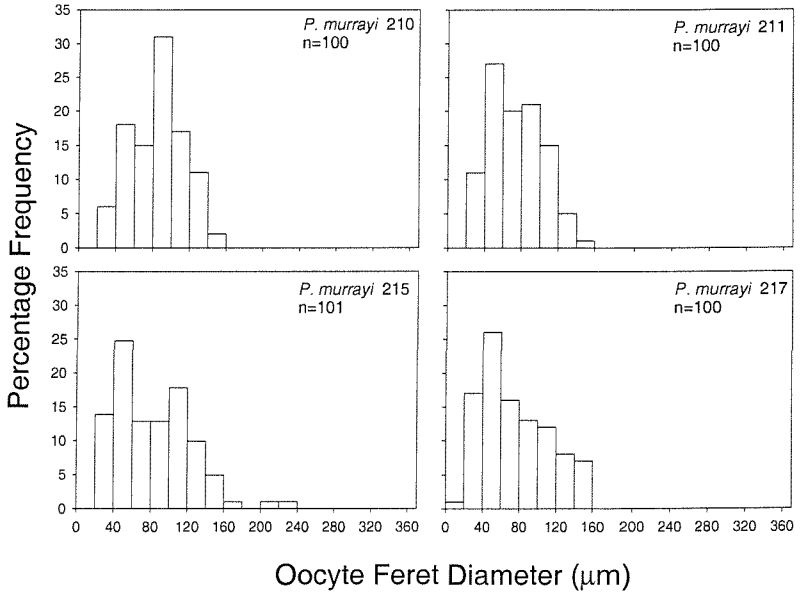

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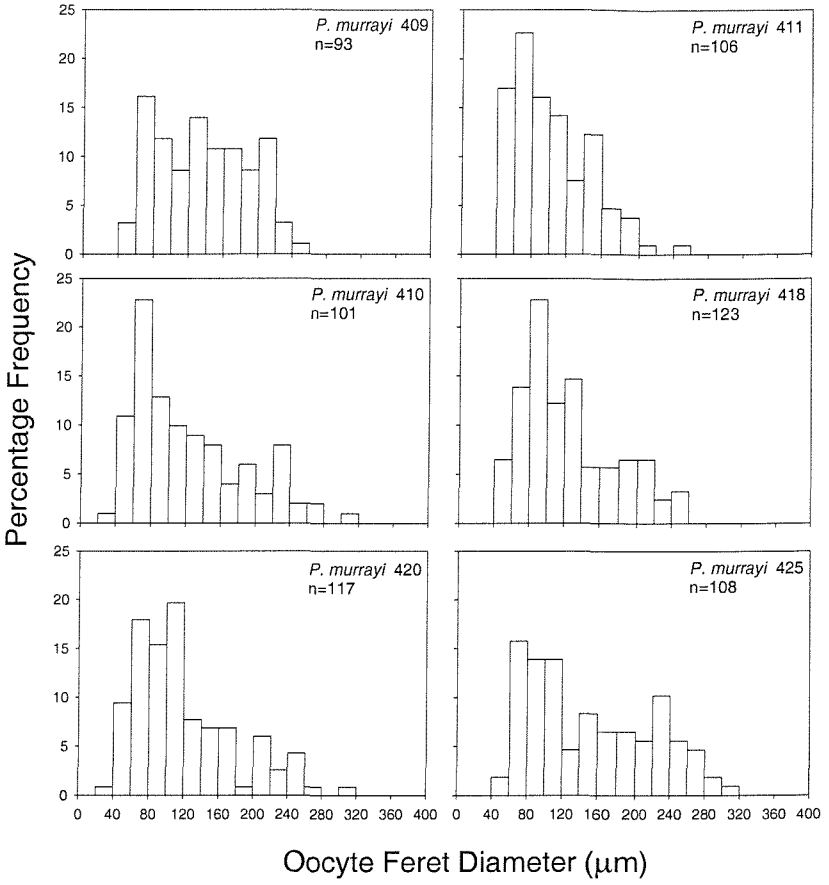

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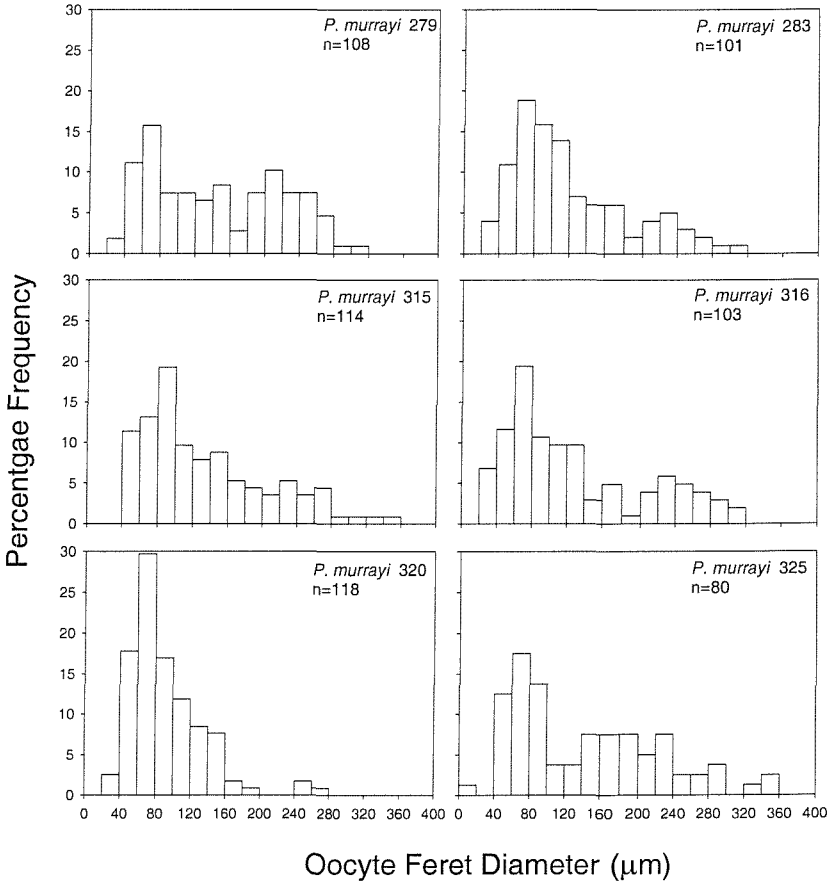

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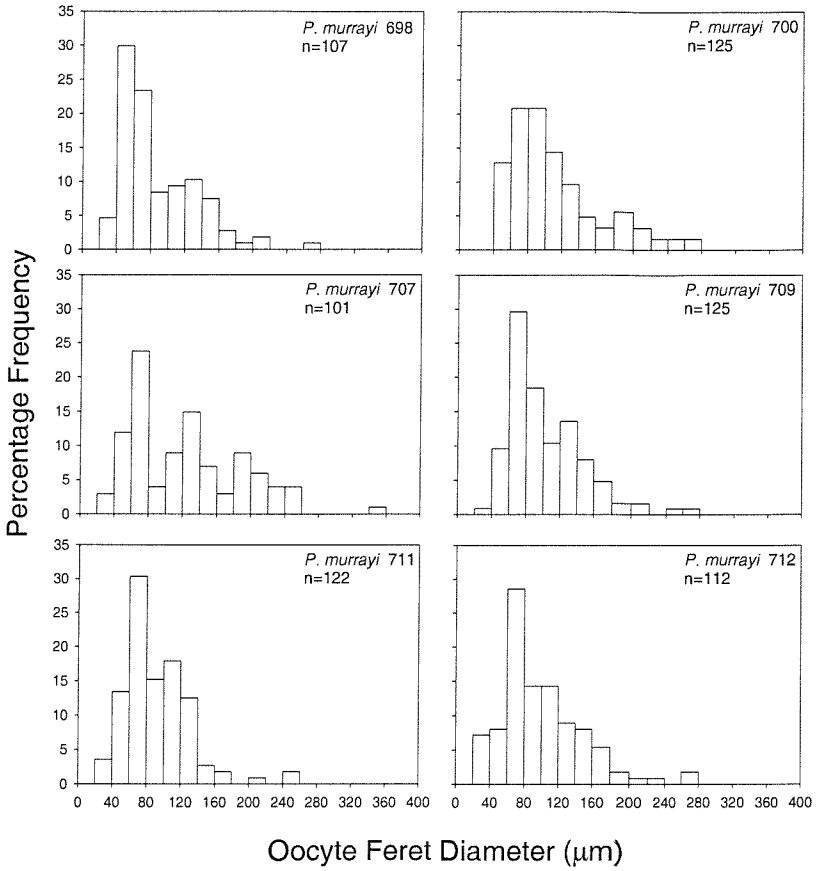

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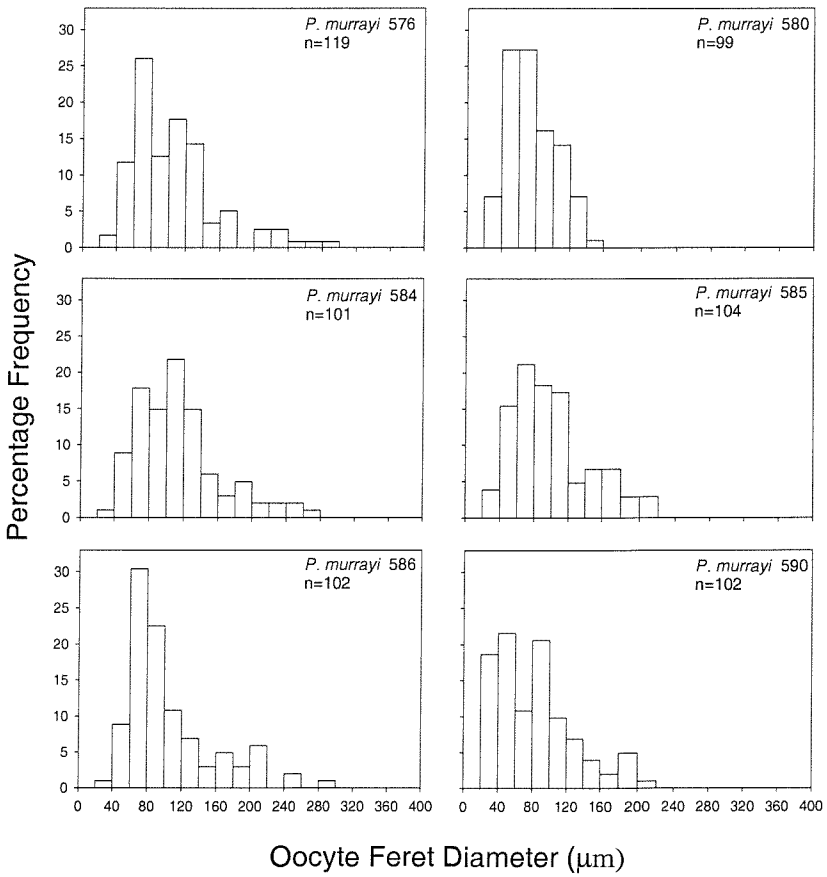

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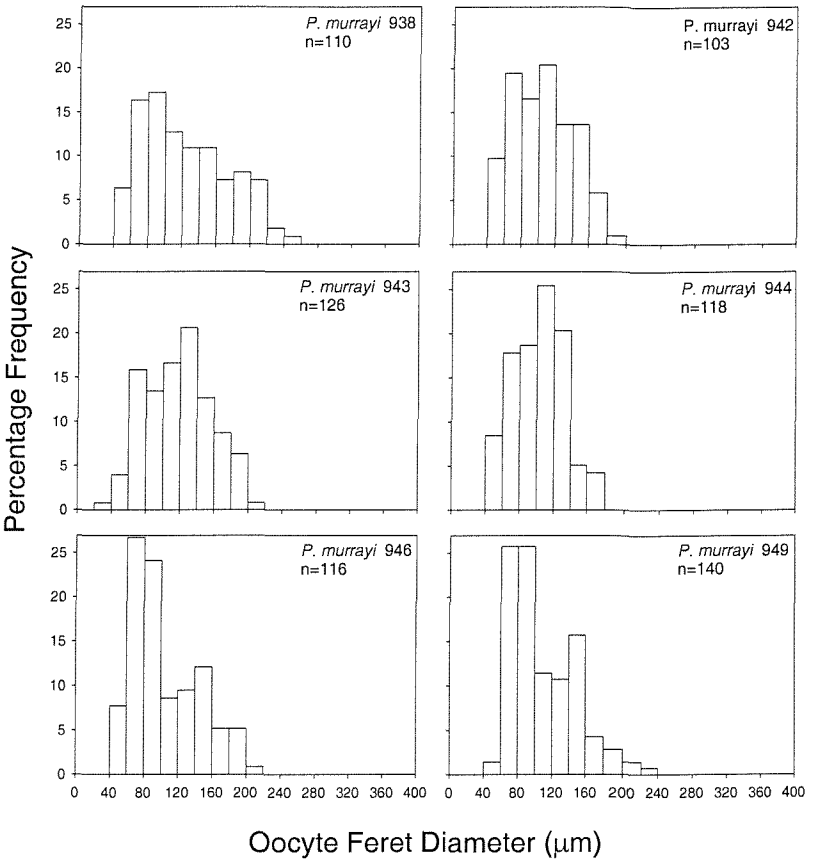

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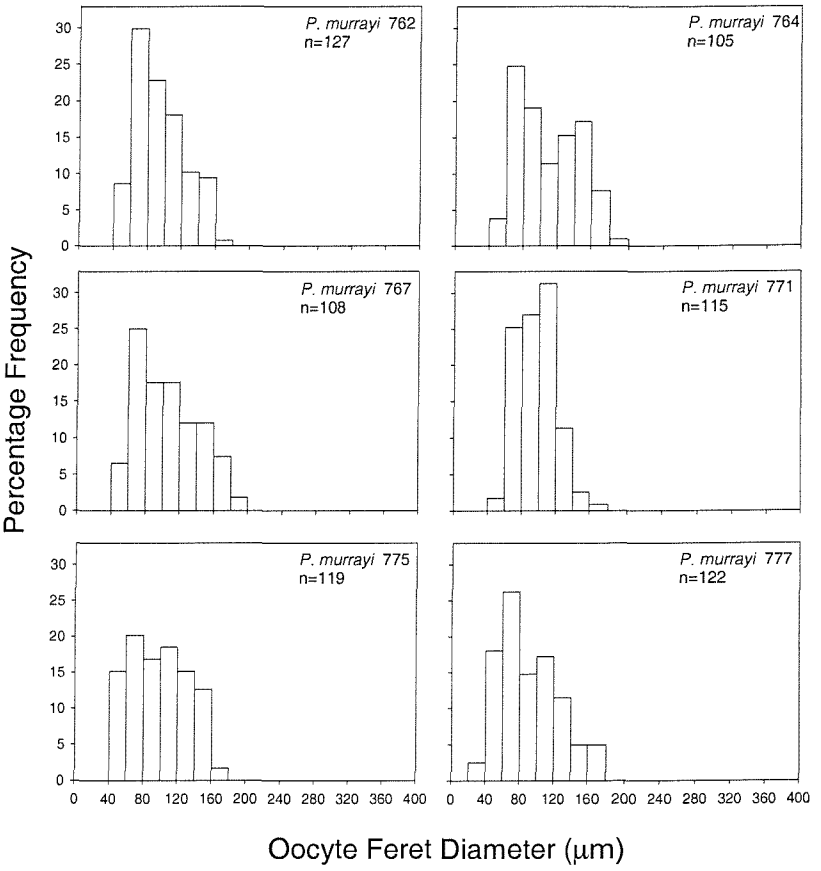

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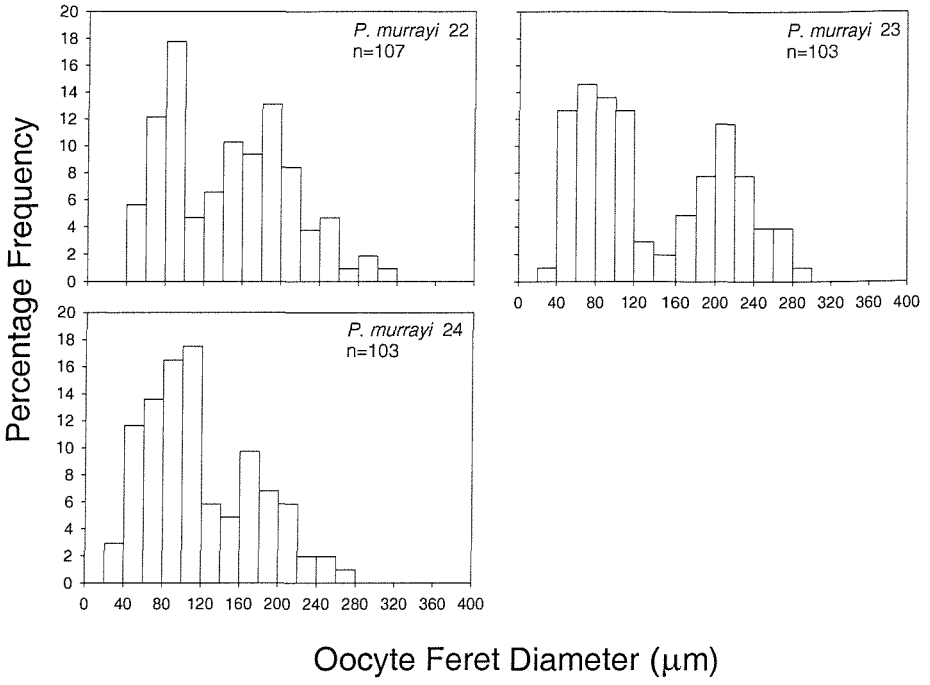

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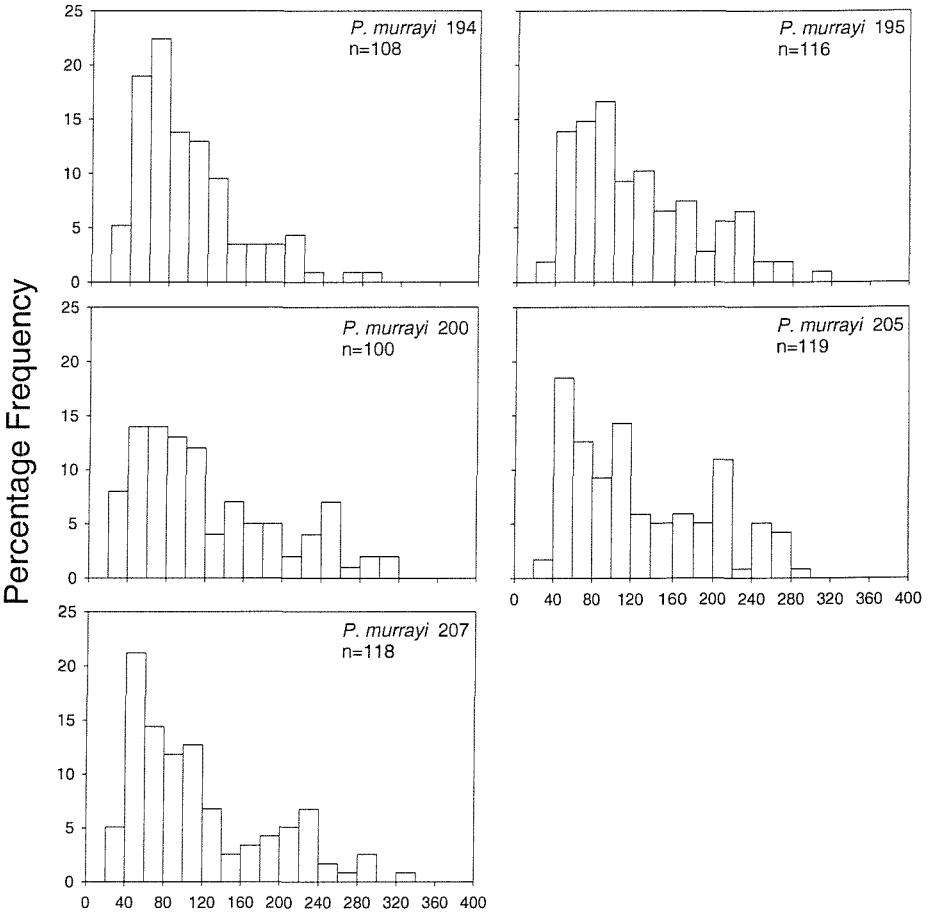

Individual oocyte size-frequency distributions for *Peniagone* sp.

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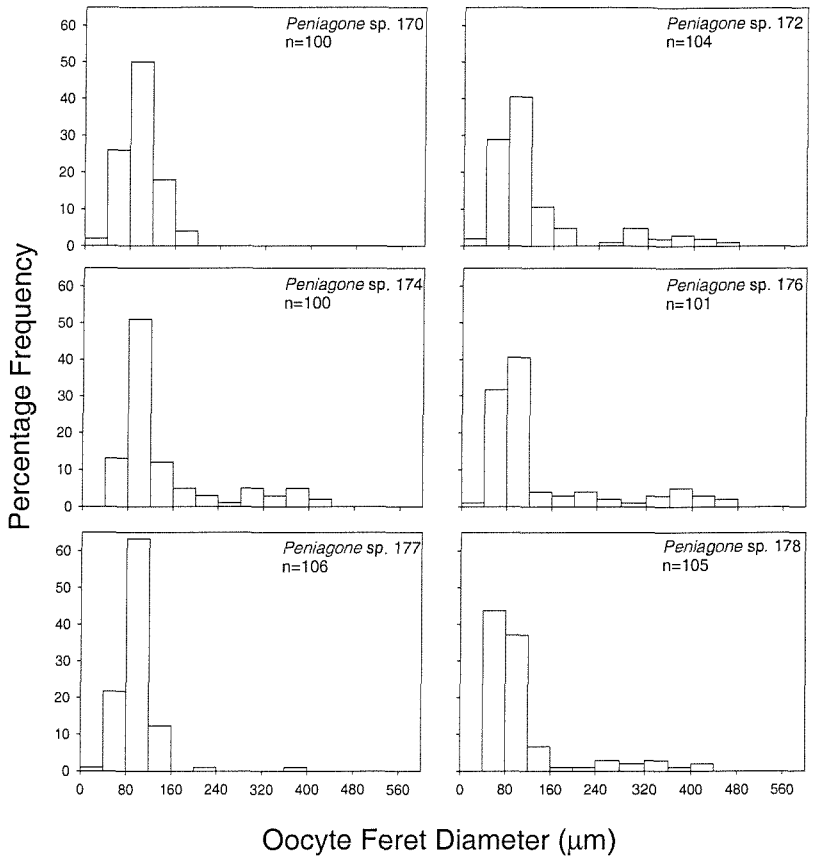

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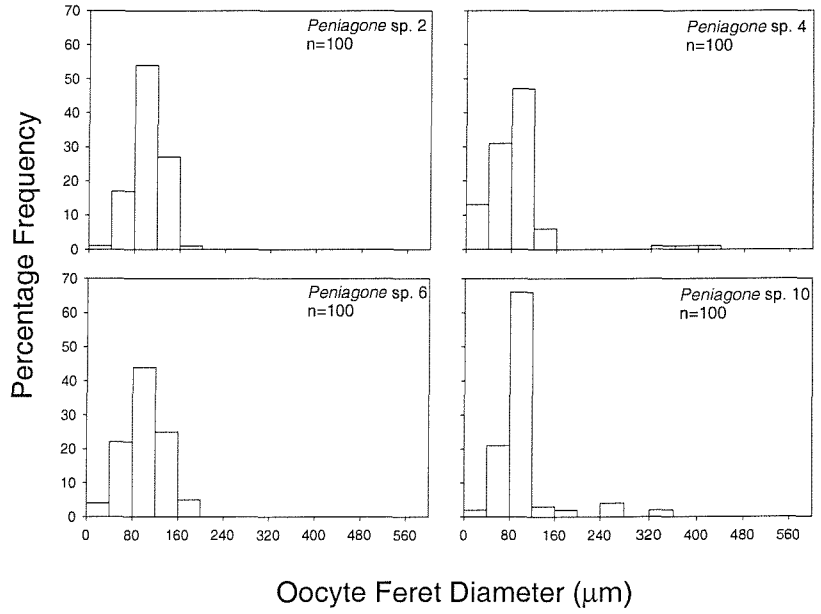

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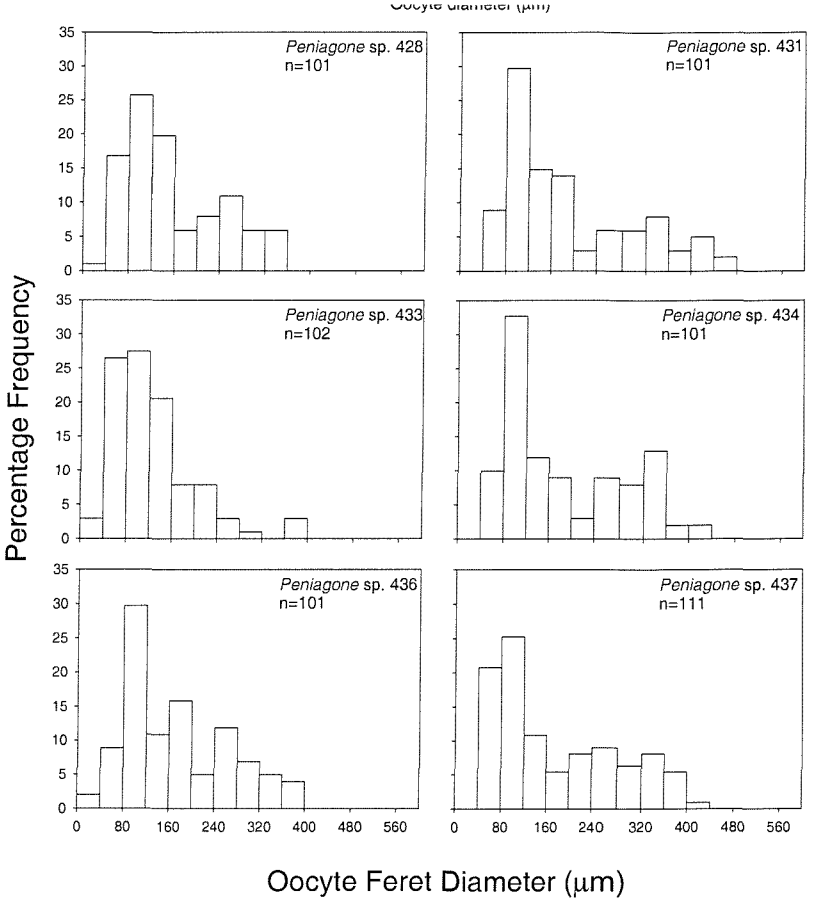

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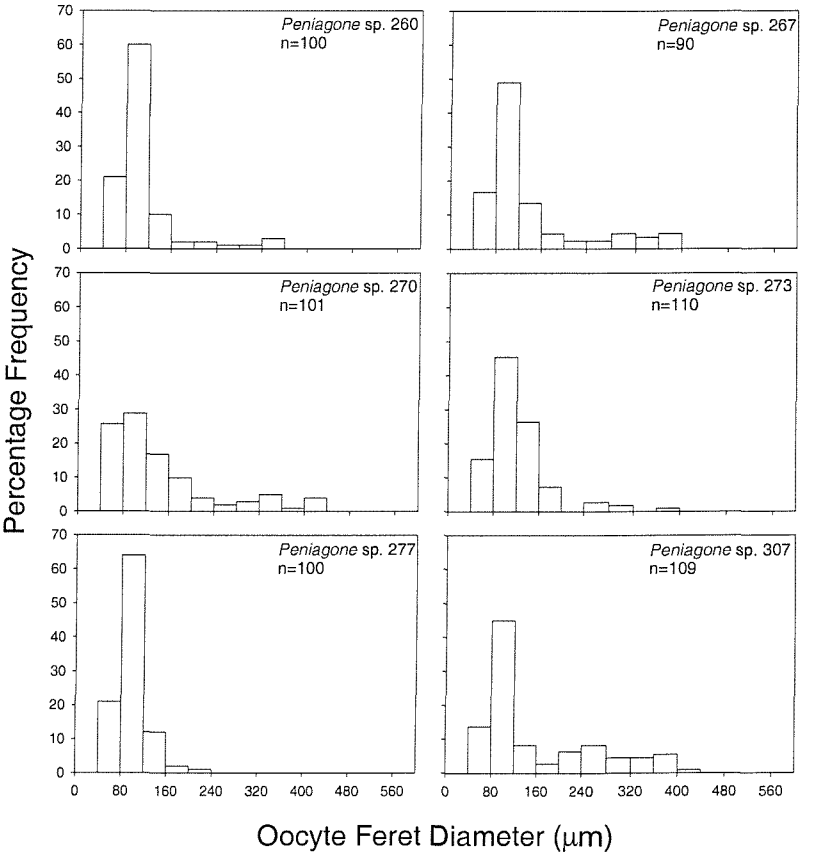

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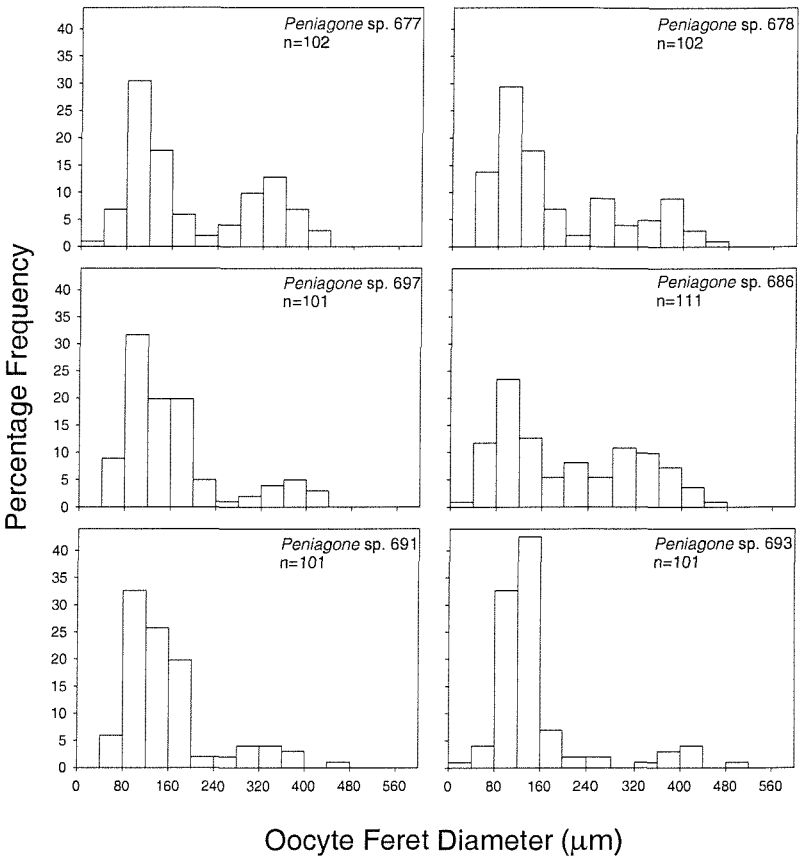

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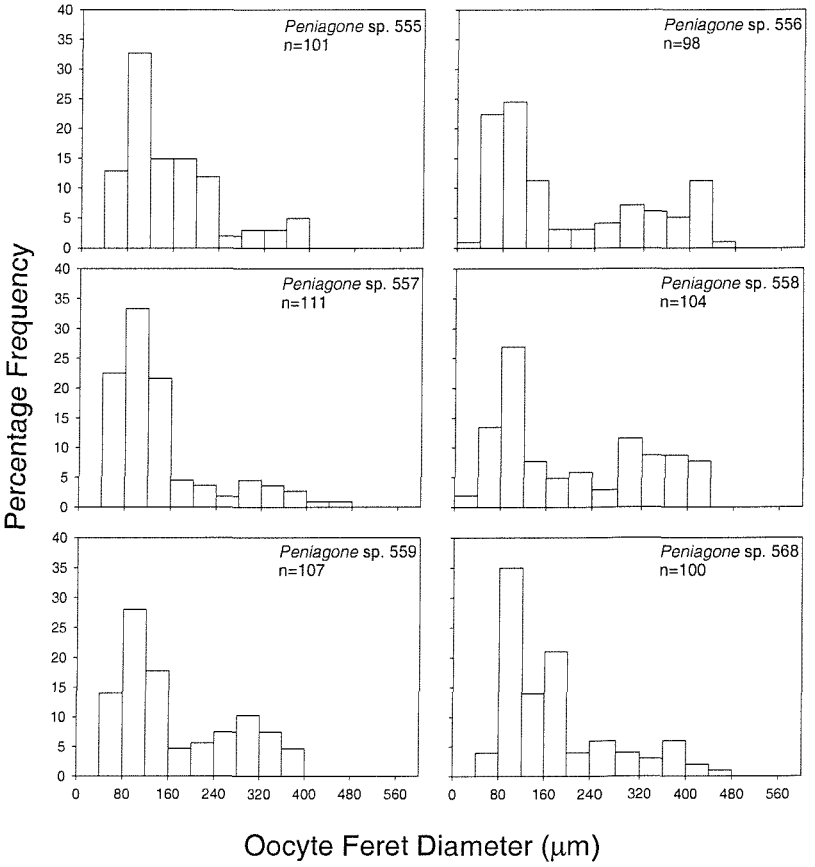

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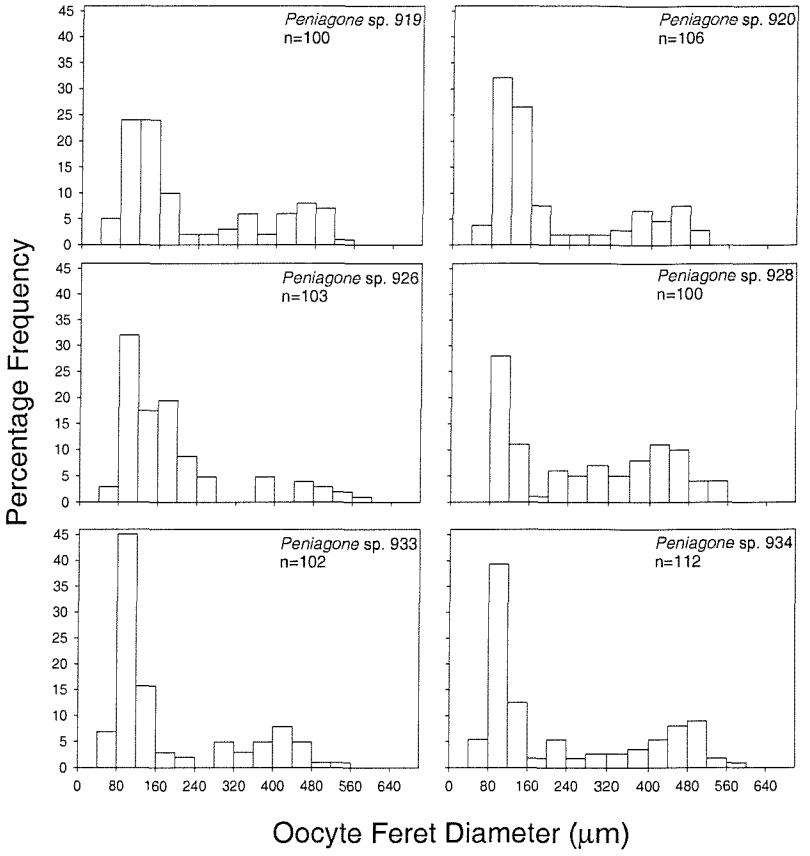

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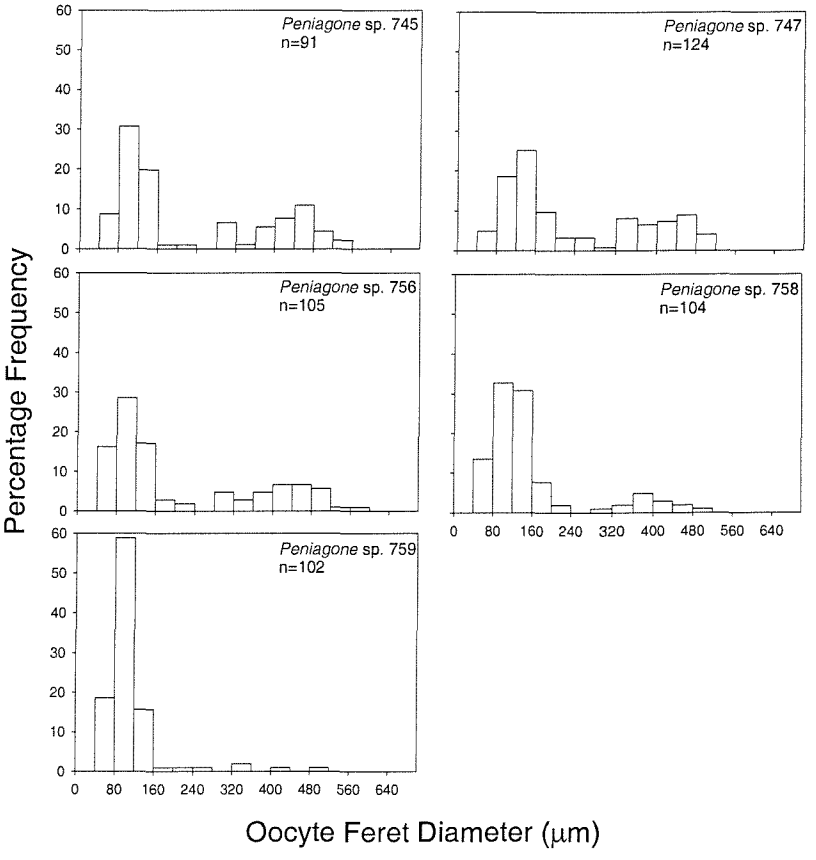

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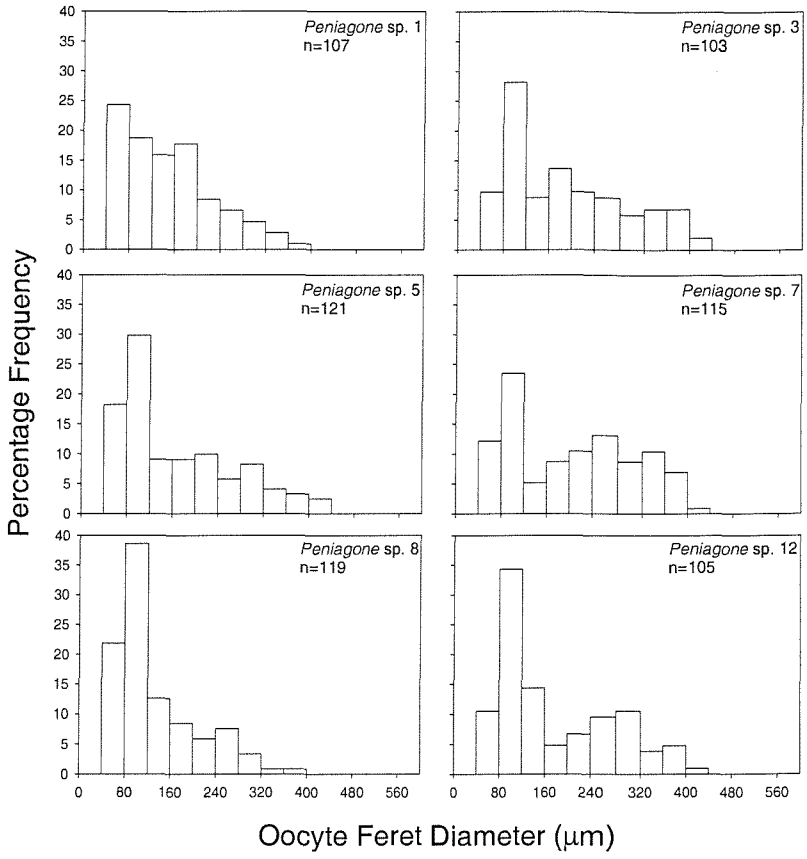

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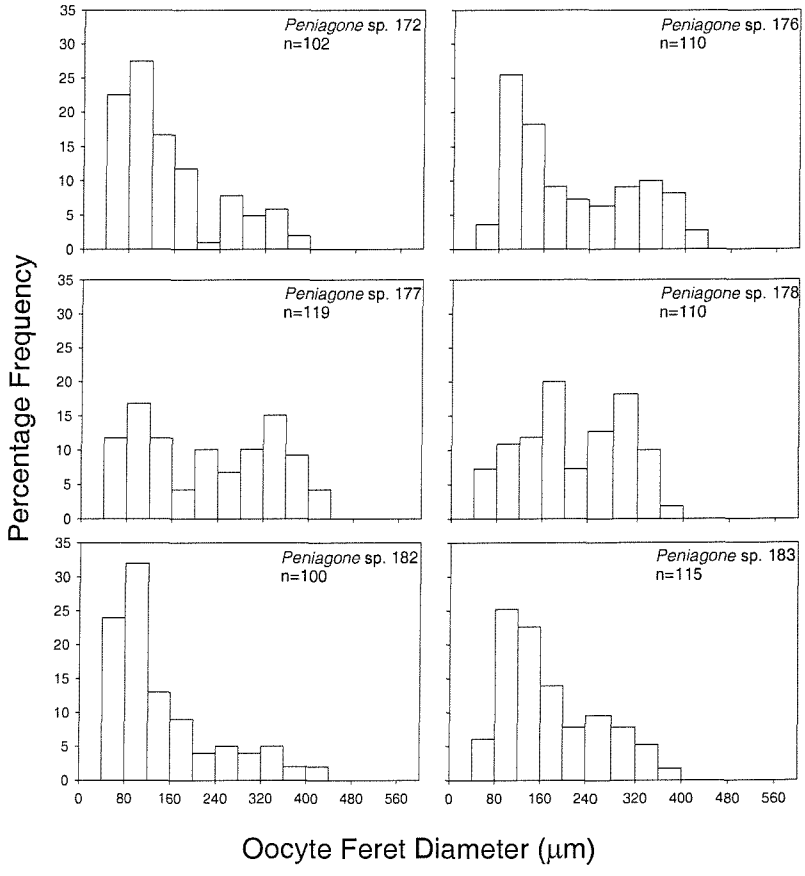

Individual oocyte size-frequency distributions for *Psilaster charcoti.*

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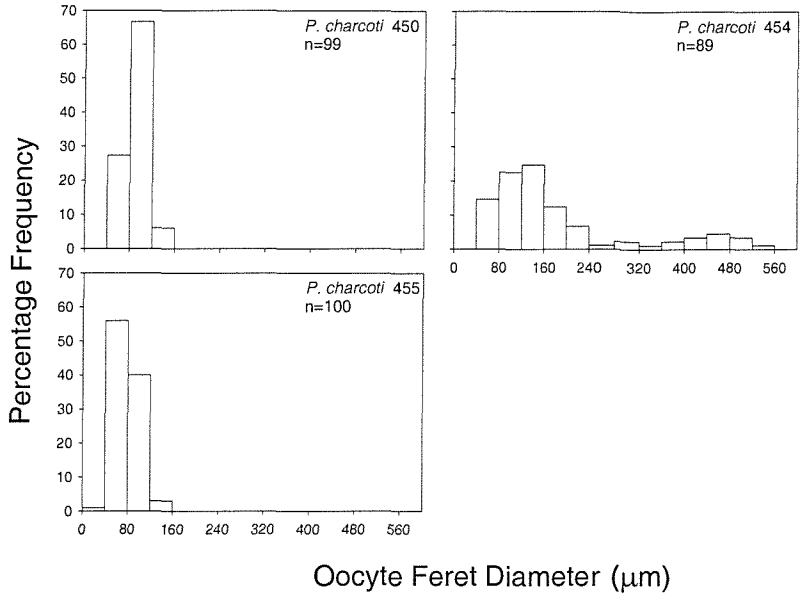

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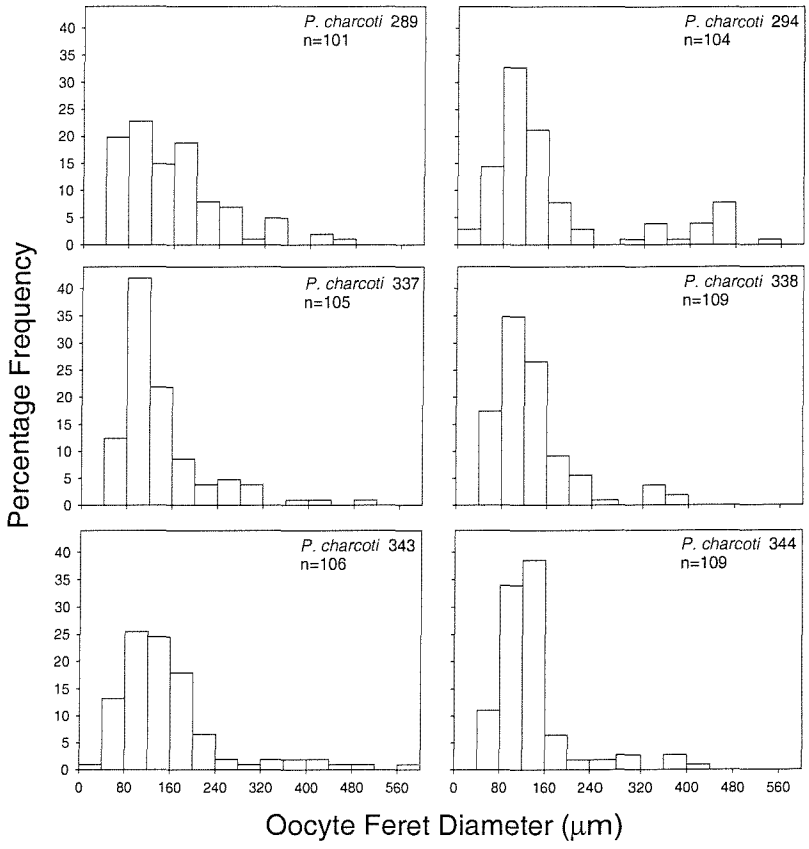

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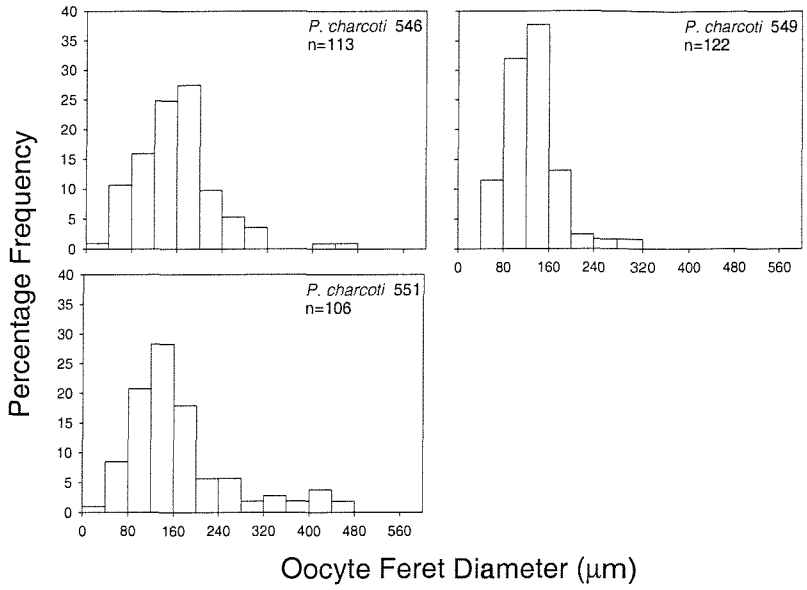

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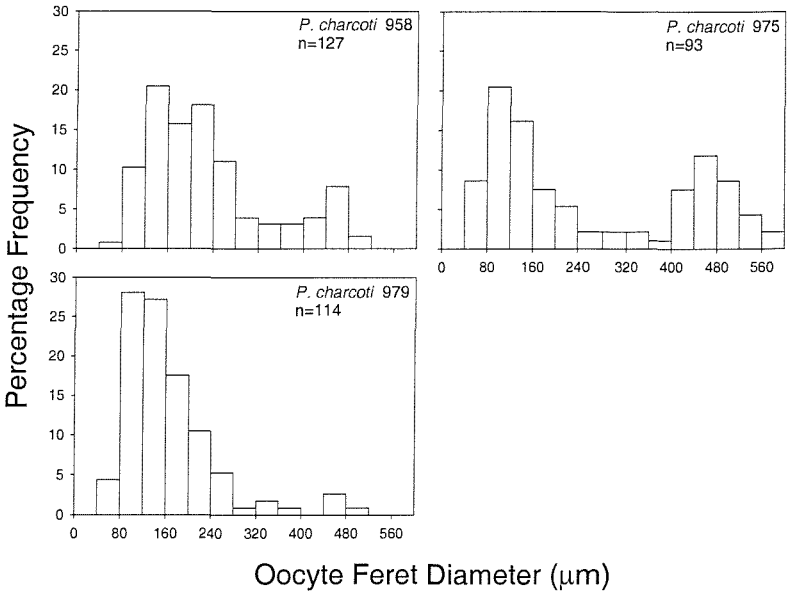

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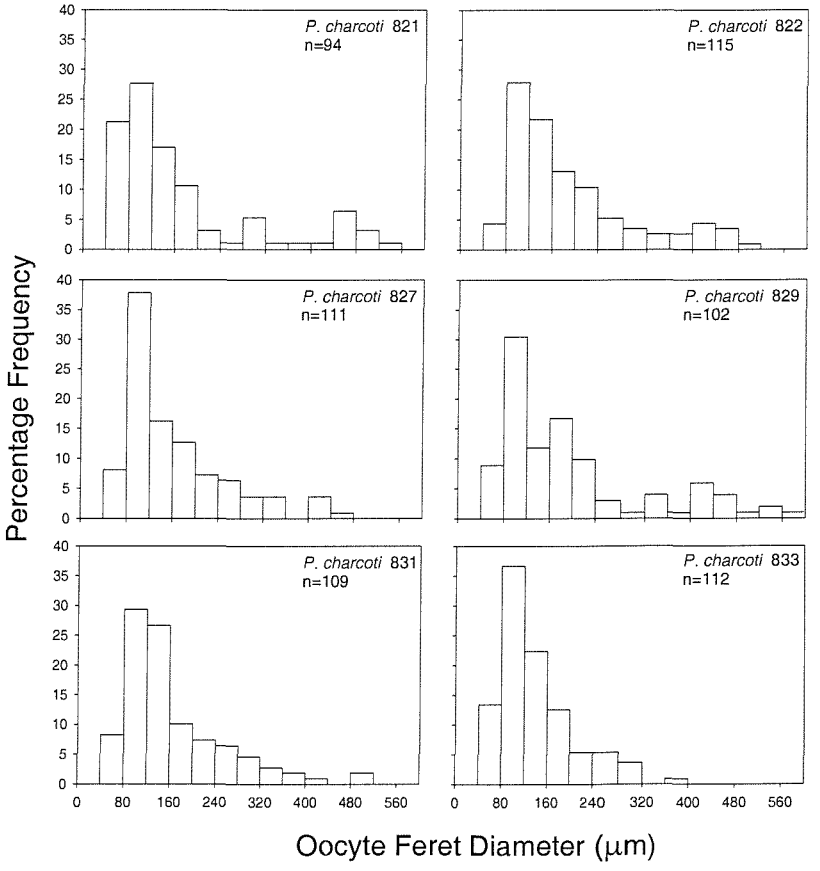

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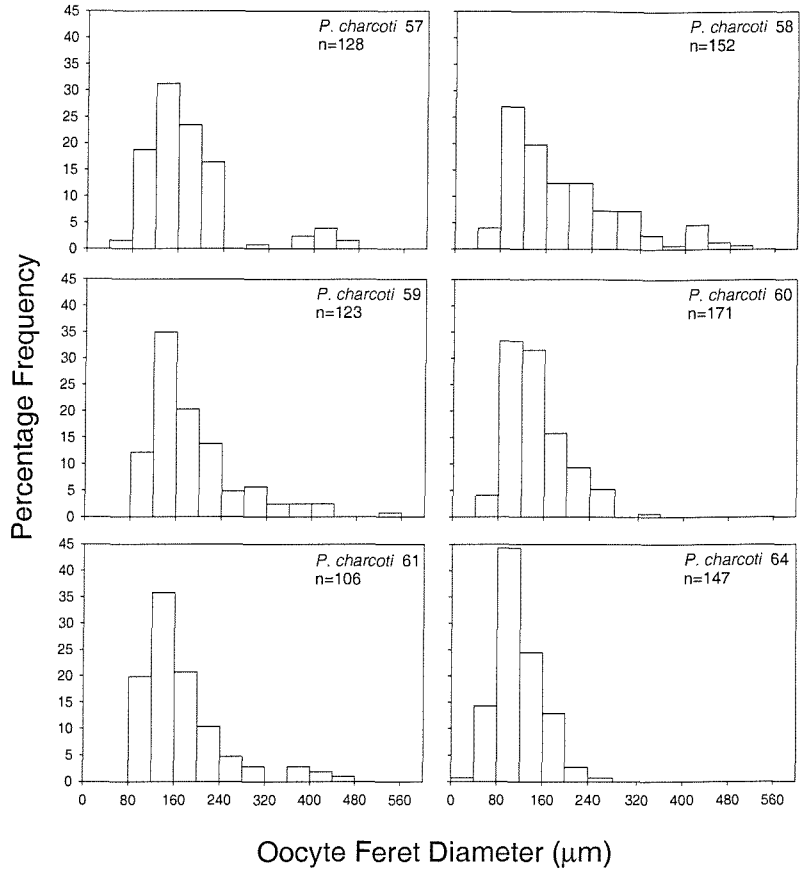

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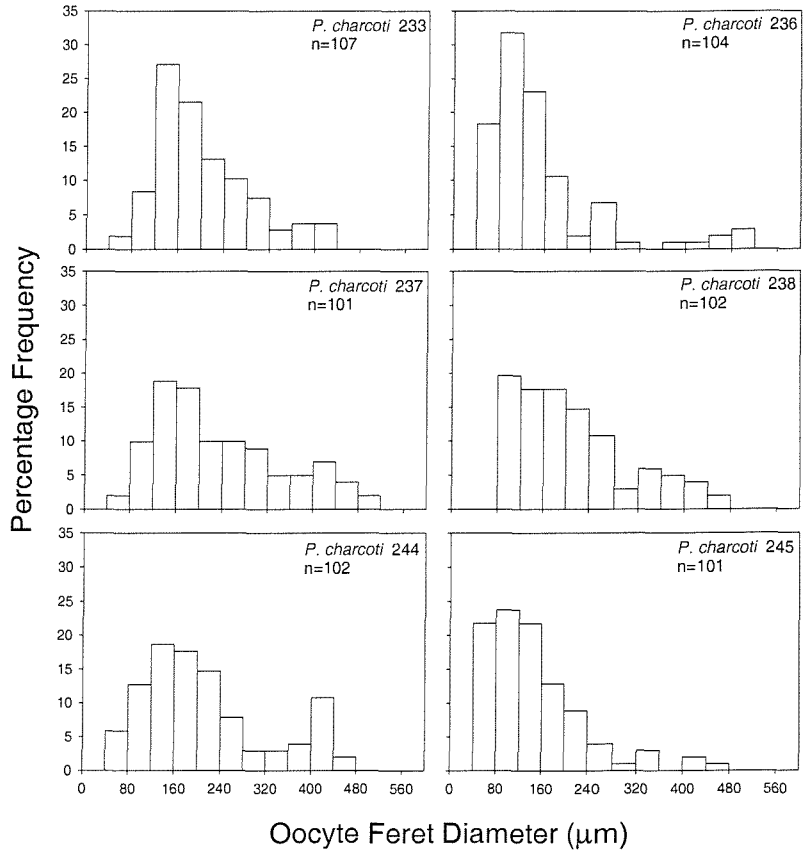
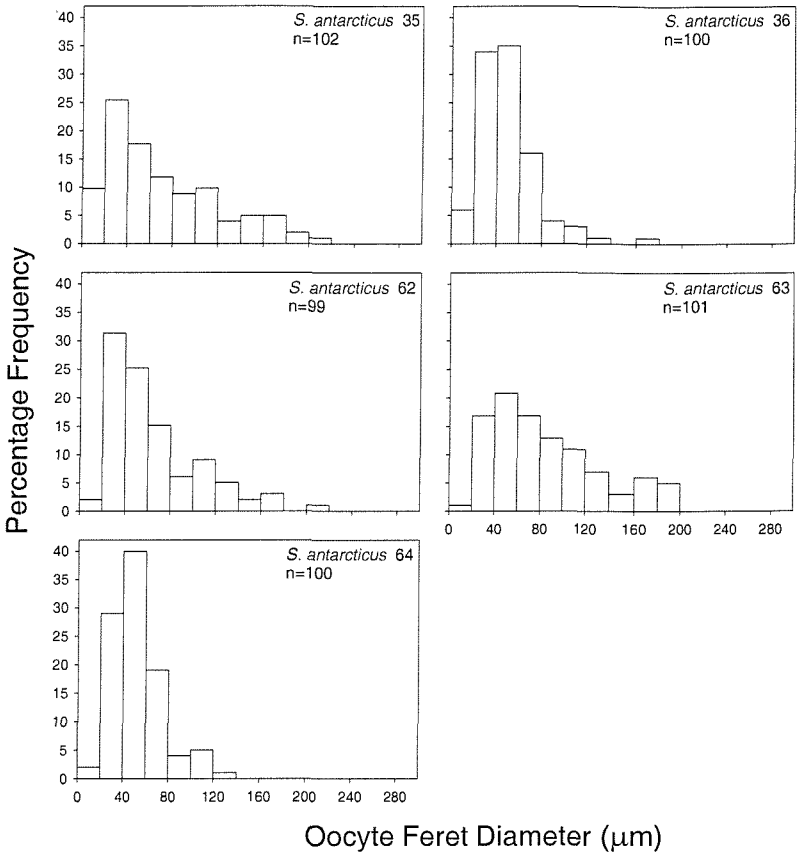


Individual oocyte size-frequency distributions for *Sterechinus antarcticus*. November 1999 site C.



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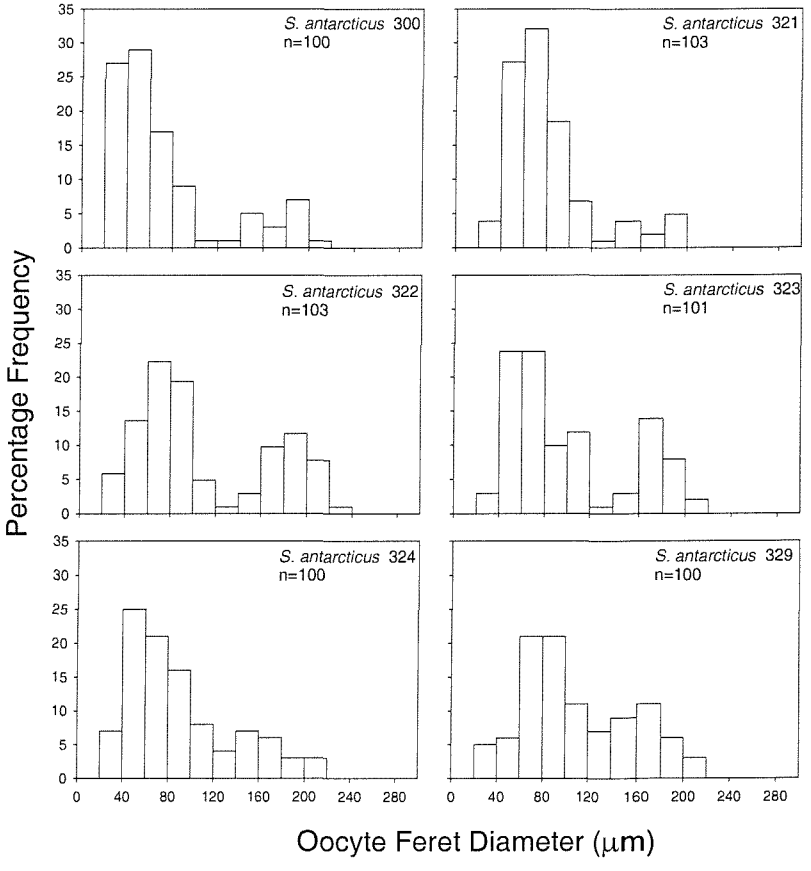

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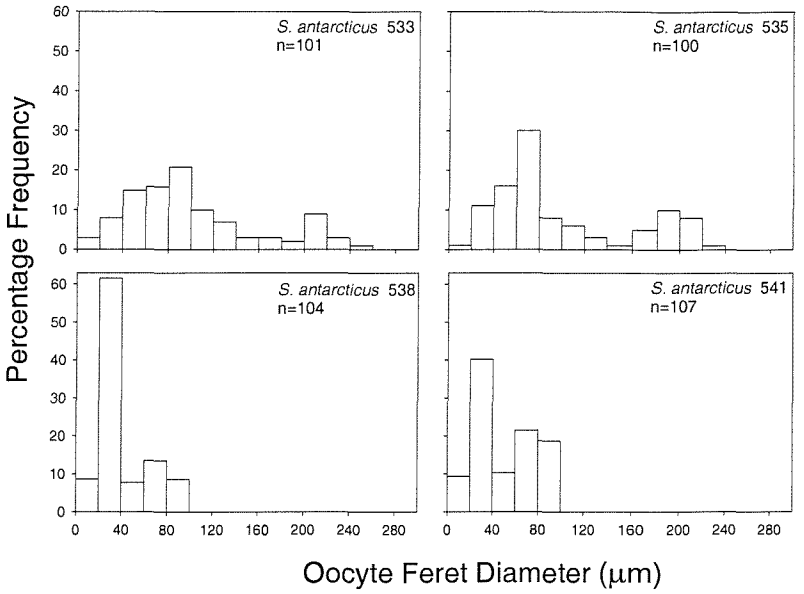

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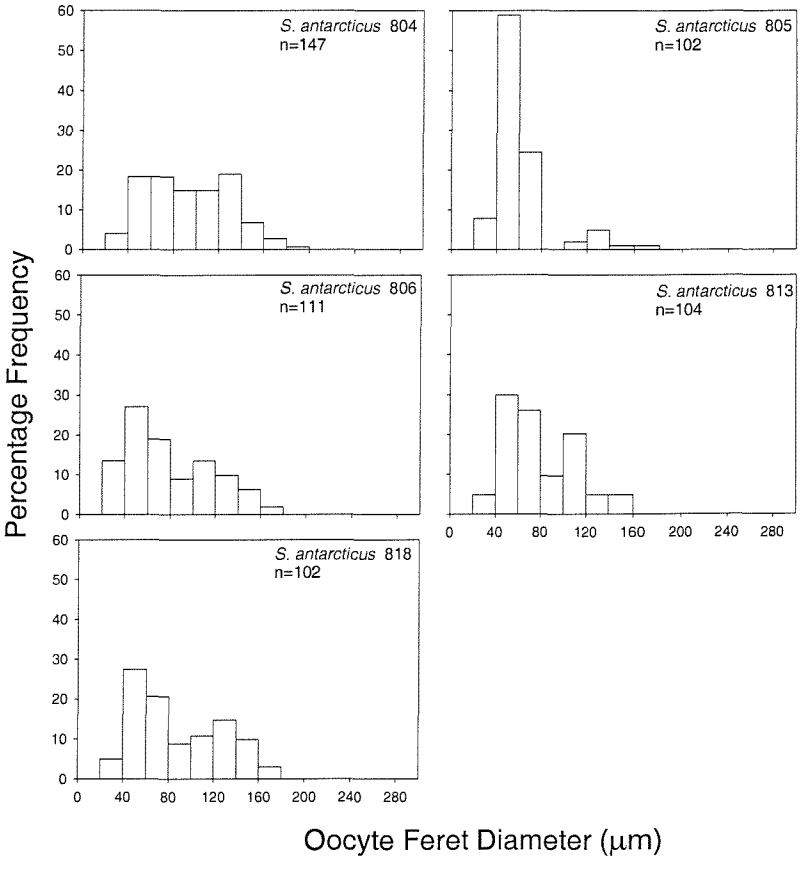

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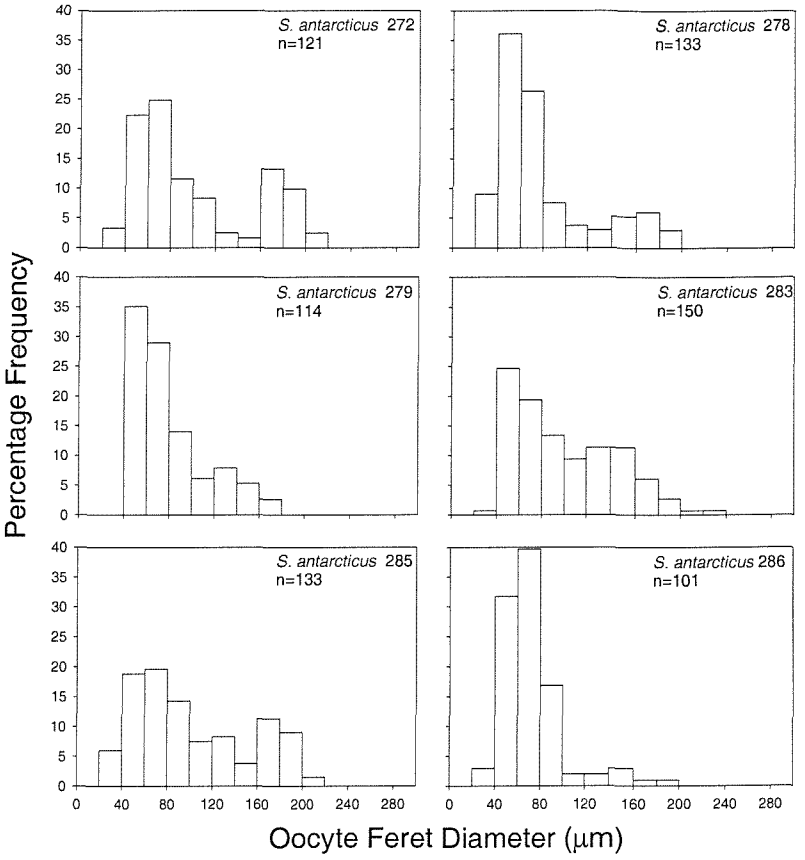

Individual oocyte size-frequency distributions for *Ctenocidaris perrieri.*

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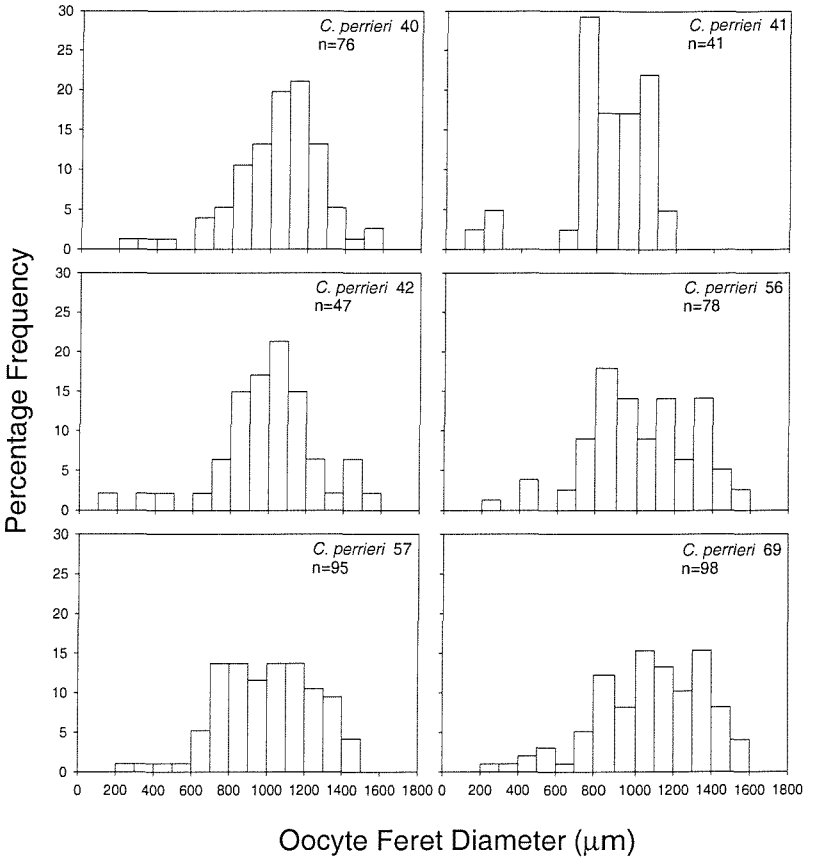

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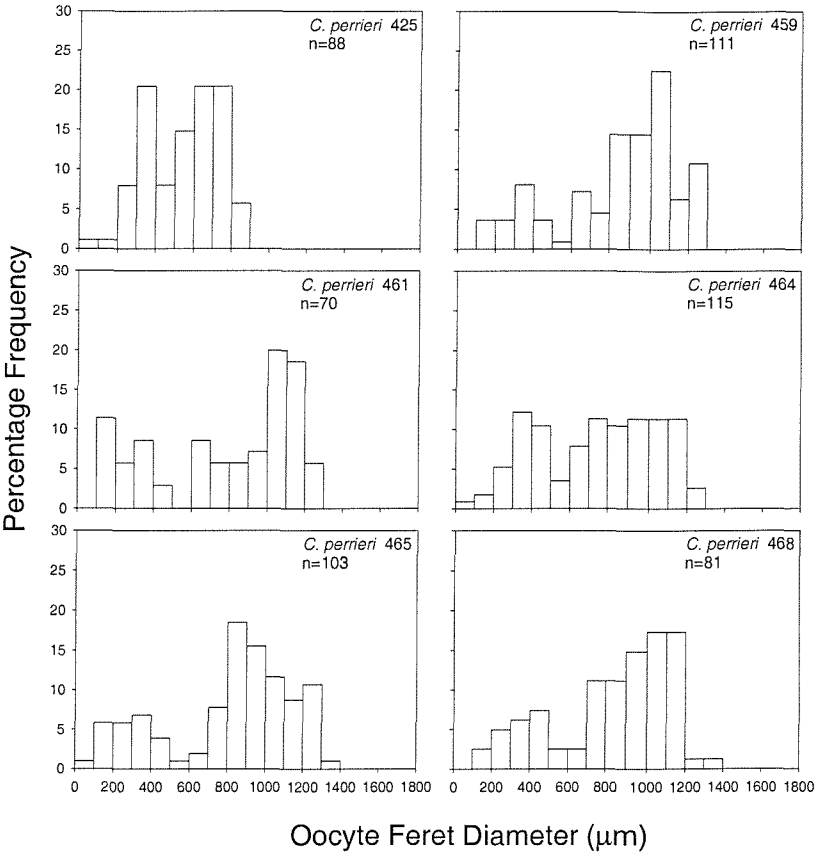

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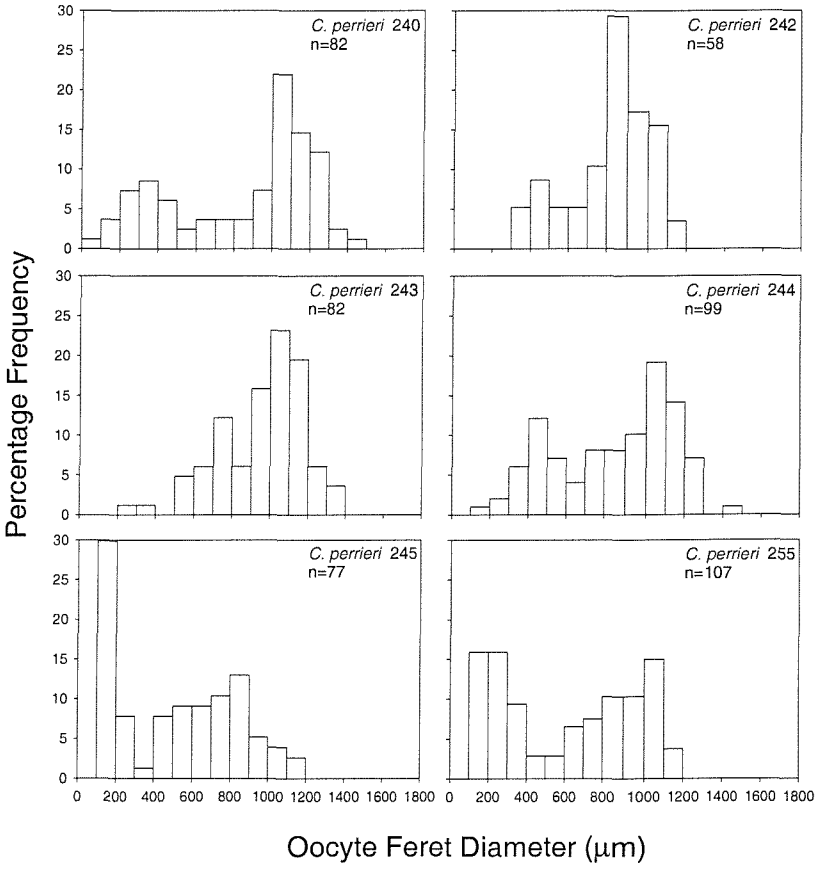

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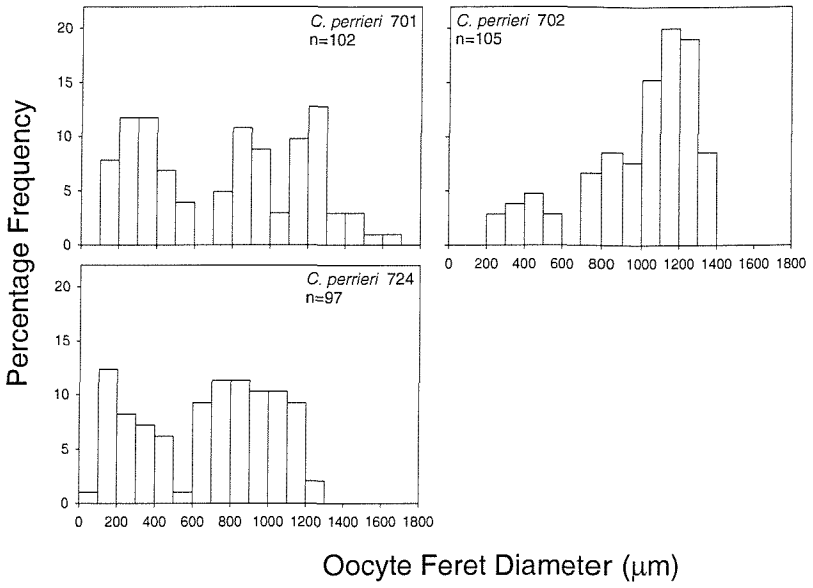

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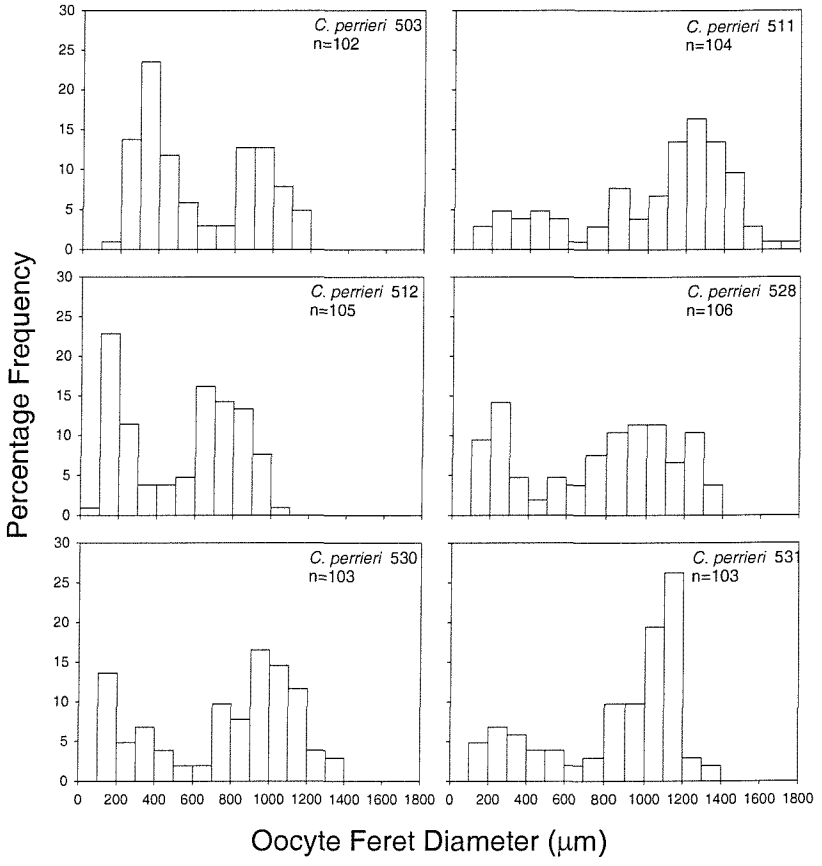

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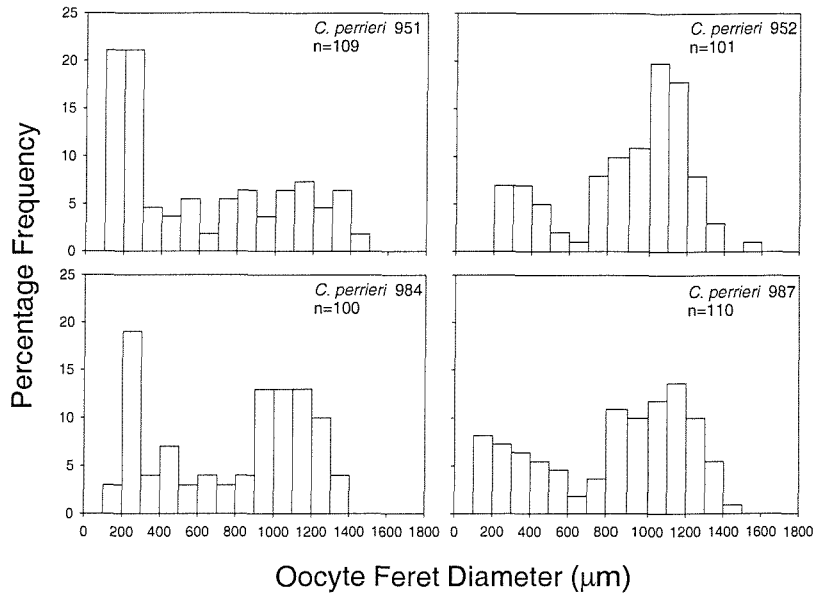

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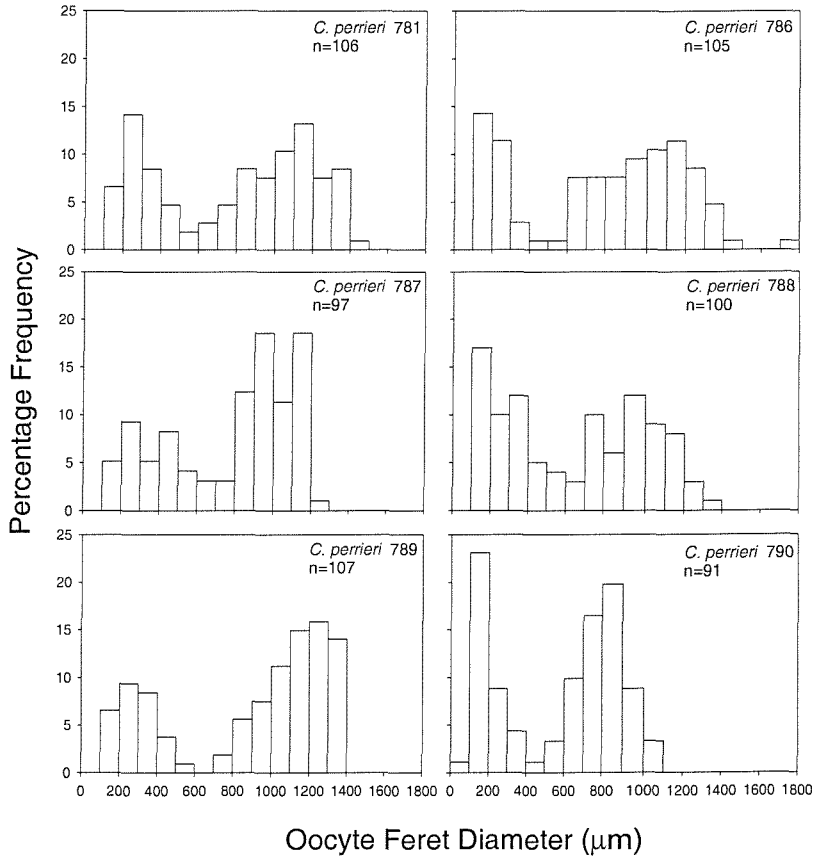

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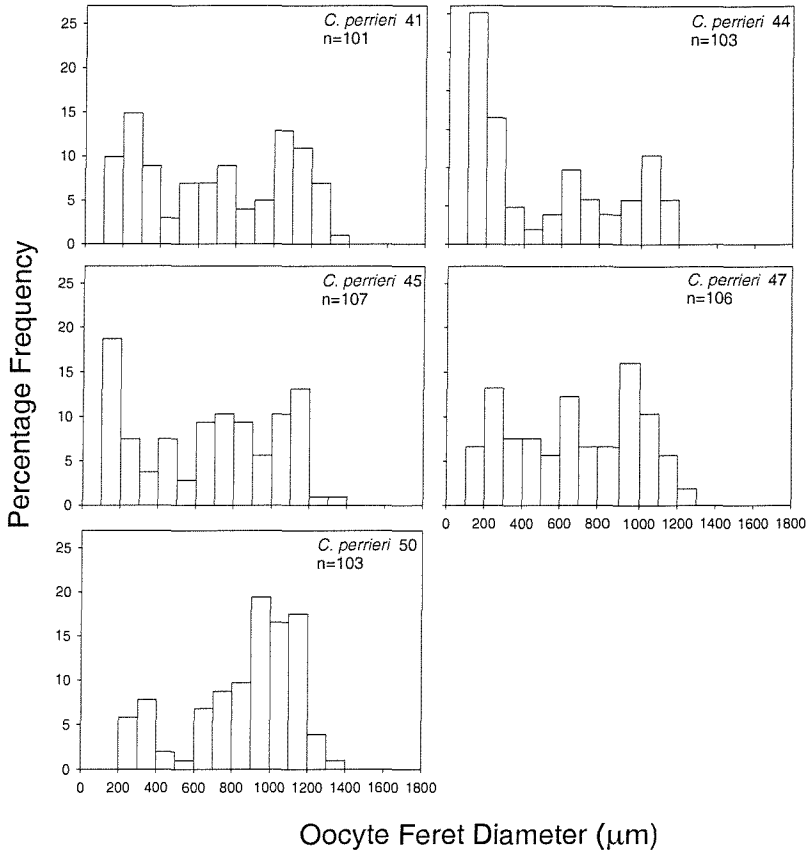

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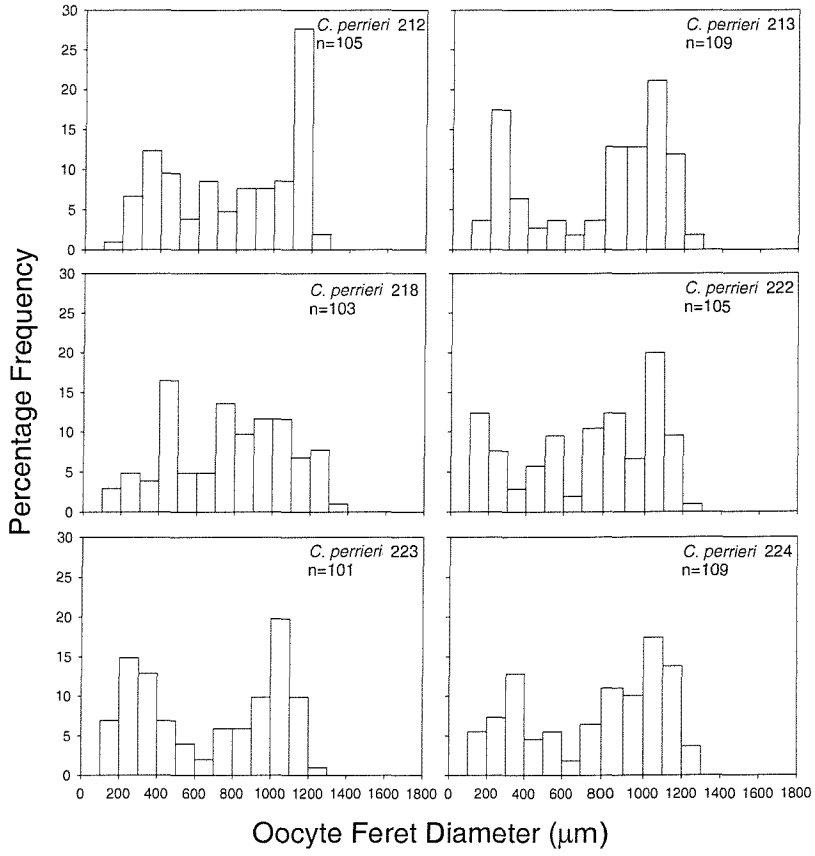

Individual oocyte size-frequency distributions for *Amphipneustes lorioli.*

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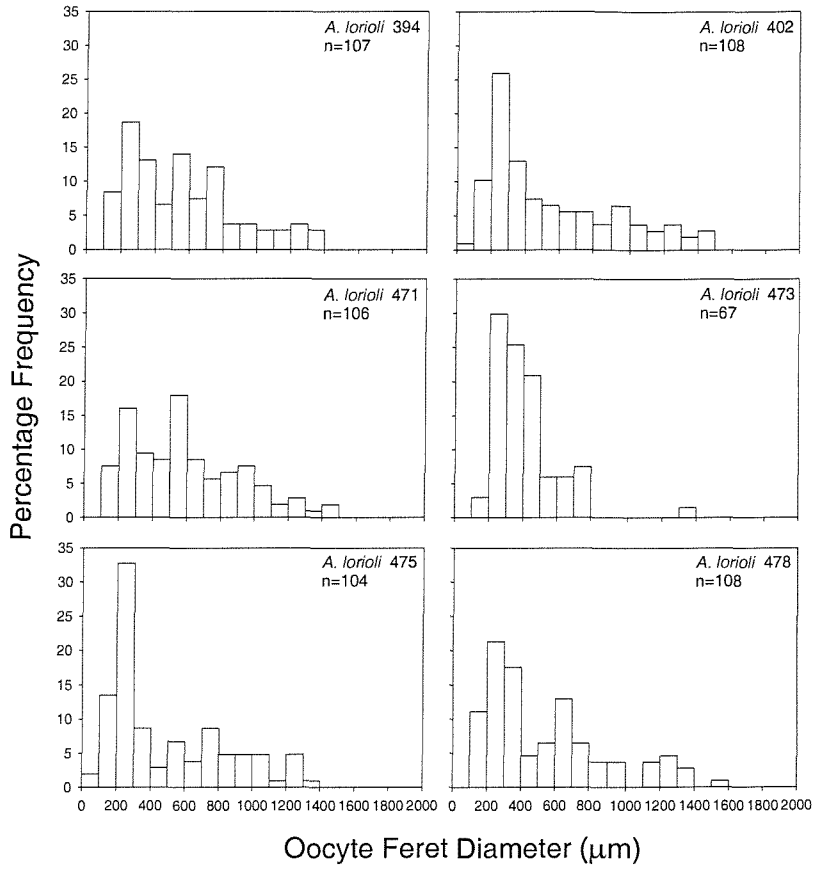

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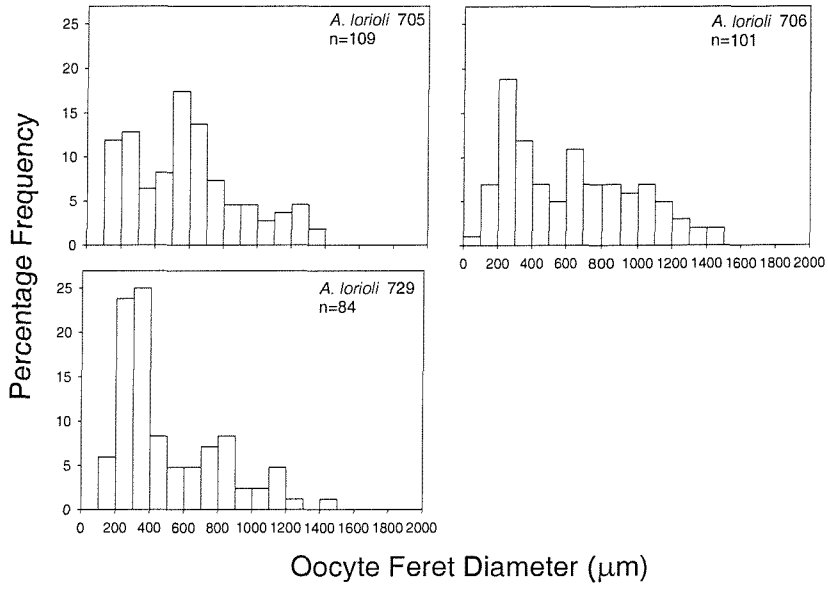

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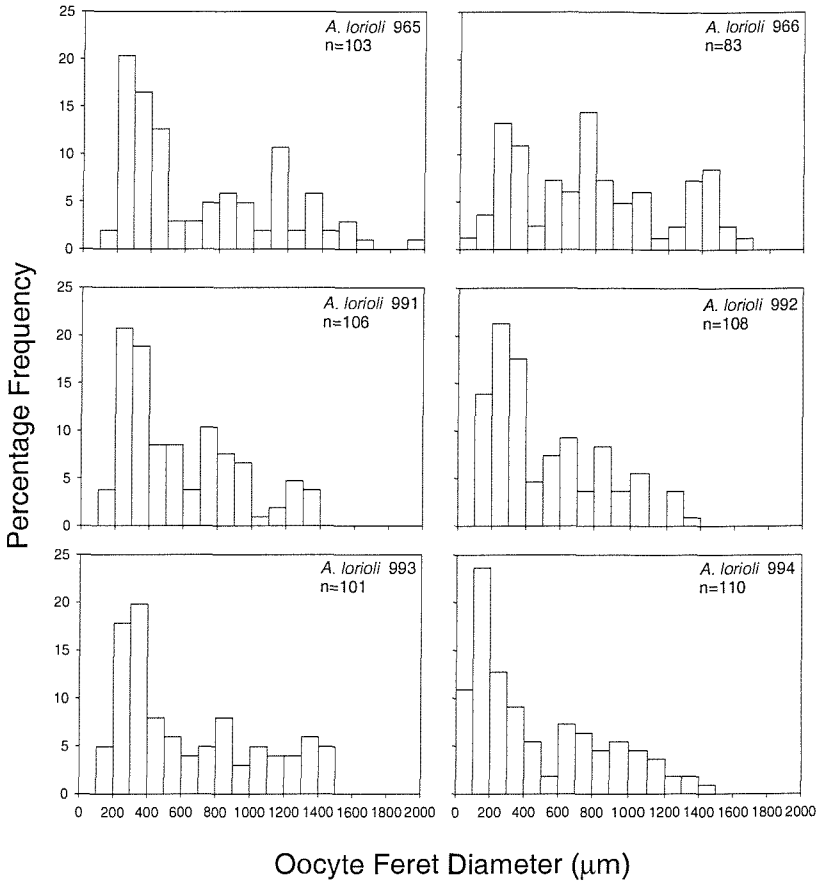

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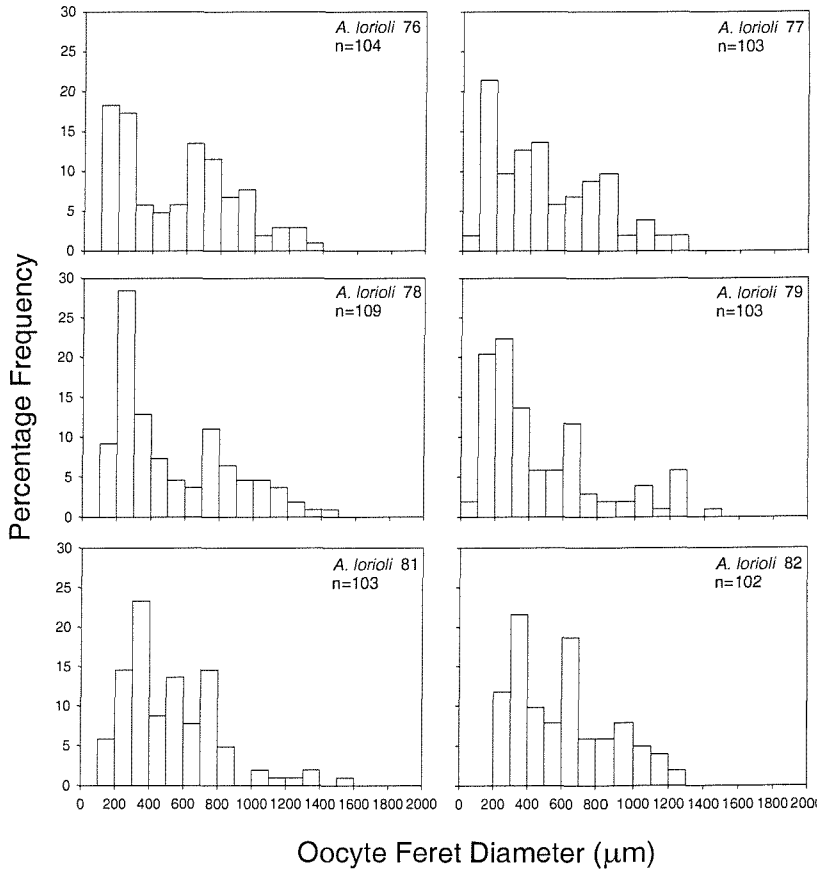

Individual size-frequency distribution for *Amphipneustes lorioli* juveniles.

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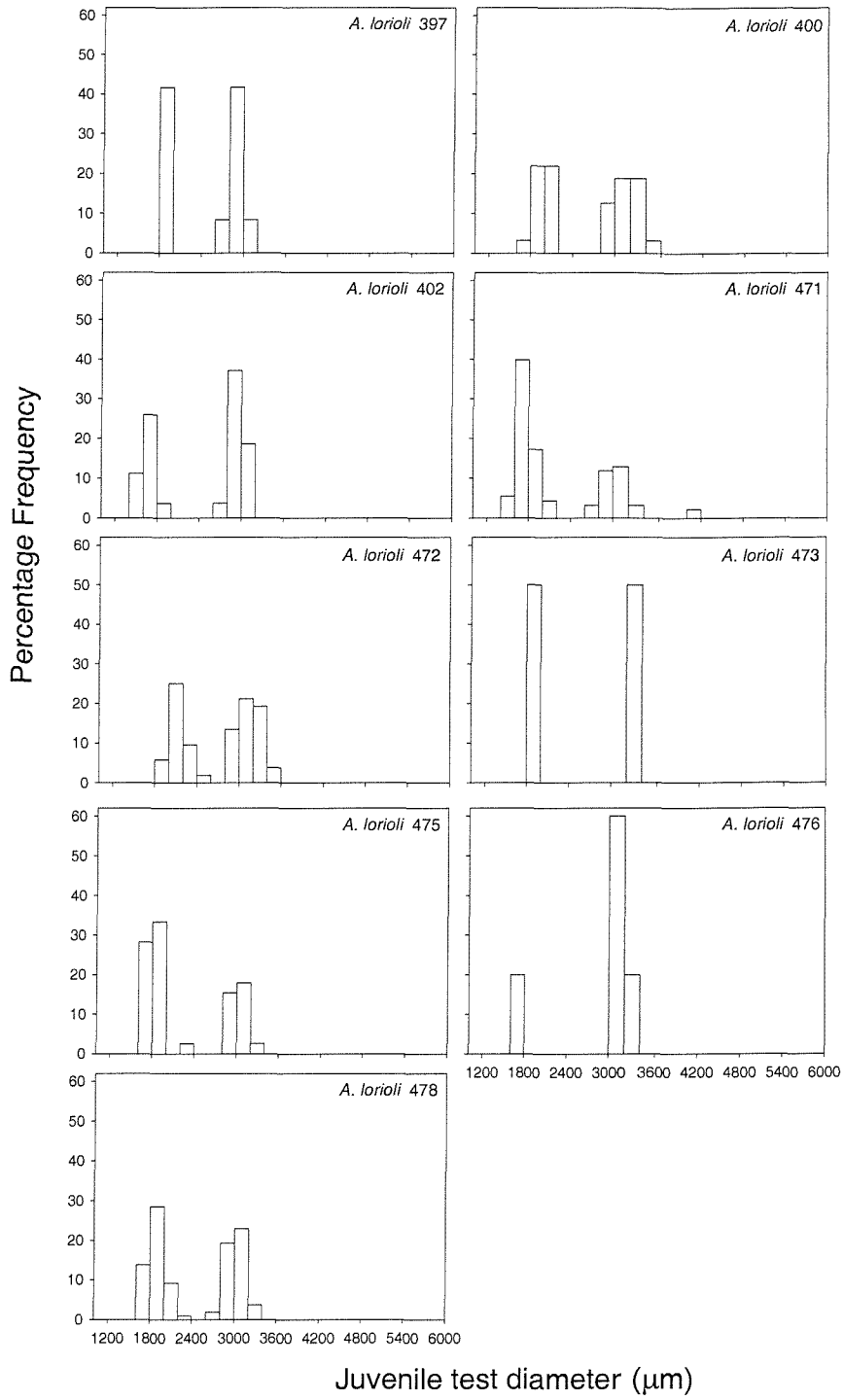

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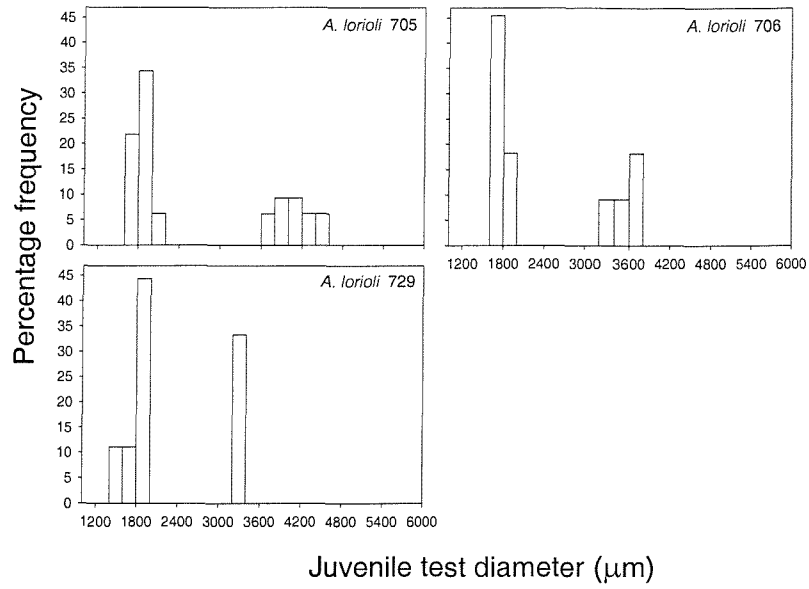

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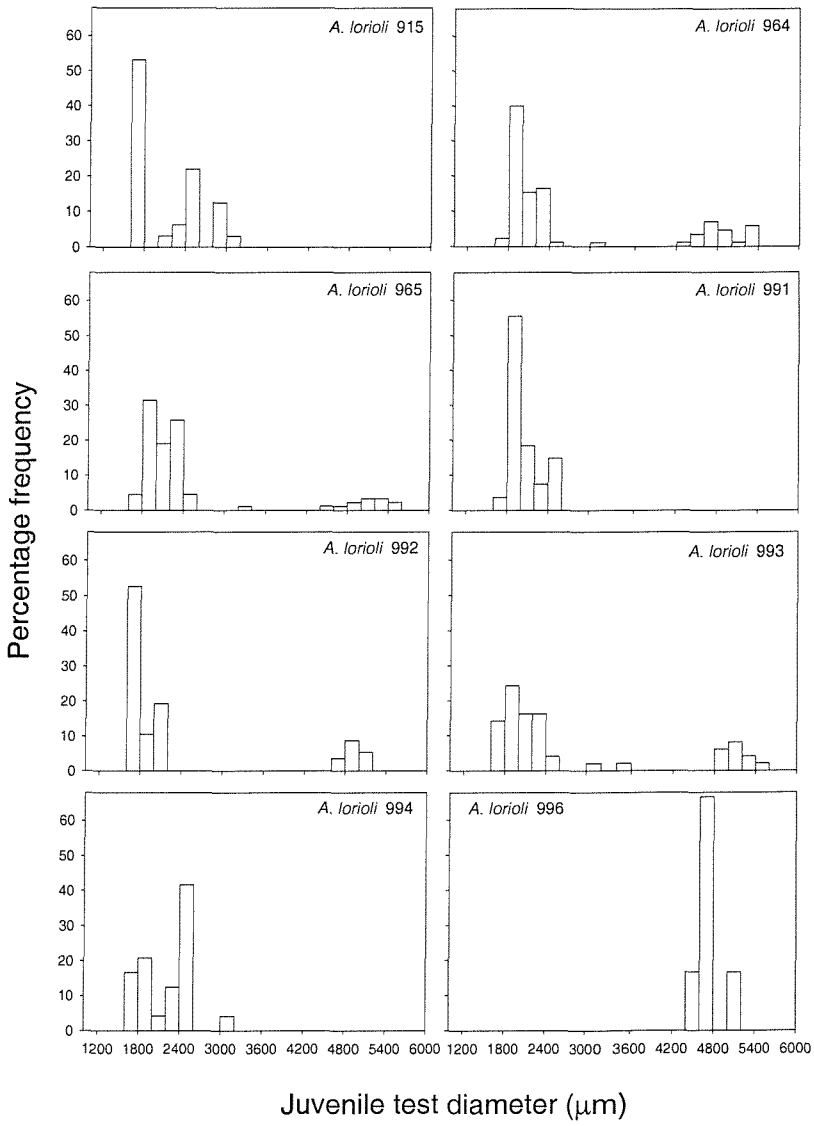

General Outline of CHN macro converted from Gnaiger and Bitterlitch (1984) by A. Clarke.

Calculate organic carbon – allowing for residual water and inorganic carbon.

1. Dry organic matter = Dry mass – (Residual water + ash)

Calculate carbon in ash.

2. Ash carbon/100g = mass of ash x $\frac{\text{inorganic carbon (\%DM)}}{100}$

3. Organic carbon = Total carbon – Ash carbon

Calculate absolute amount of protein from nitrogen allowing for NPN

4. Mass of Protein N = N %DM x (1 – (%N is NPN / 100))

5. Mass of Non-Protein N = N %DM x (%N is NPN / 100)

Calculate mass of Protein

Defined stoichiometric parameter for Protein

a) Mass fraction of N in protein = 0.173
b) Mass fraction of C in protein = 0.529
c) Mass fraction of H in protein = 0.070

6. Mass fraction of protein = $\frac{\text{Mass of Protein N (4)}}{\text{Mass fraction of N in protein (a)}}$ = Ptn %DM

Calculate C and H due to this protein.

7. Carbon protein = Ptn %DM x (b) – mass fraction of C in protein

8. Hydrogen protein = Ptn %DM x (c) – mass fraction of H in protein

Calculate Carbohydrate and Lipid

9. Calc remaining organic matter after protein = Actual dry organic matter – Ptn %DM

10. Mass fraction of Non-protein C = $\frac{\text{Total organic C – Organic C in protein}}{\text{Organic dry mass – protein}}$

Mass fraction of C in Lipid	= 0.776	(K7)
Mass fraction of C in Carbohydrate	= 0.444	(K8)
Mass fraction of H in Lipid	= 0.114	(K9)
Mass fraction of H in Carbohydrate	= 0.062	(K10)

11. Proportion of Lipid = (Mass fraction of N-Protein C – Fraction of C in Lipid)

12. Proportion of Non Lipid = 1 – Proportion of Lipid

13. Lipid %DM = Proportion of Lipid x Non-protein organic matter

14. Non Lipid = Proportion of non-lipid x non-protein organic matter

Now estimate C and H explained by lipid and non-lipid

15. Lipid carbon = Lipid %DM x (K7)

16. Lipid hydrogen = Lipid %DM x (K9)

17. Non-lipid carbon = Non-lipid x (K8)

18. Non-lipid hydrogen = Non-lipid x (K10)

Calculate non-protein nitrogenous component

19. Mass of NPN = $\frac{\text{Mass of NPN (5)}}{\text{Mass fraction of nitrogen in NPN component (0.15)}}$

Estimate Carbohydrate by subtracting mass of NPN component from mass of non-lipid protein free organic matter

20. Carbohydrate %DM = Non-lipid protein free DM (14) – Mass of NPN (19)

Calculate unexplained component

21. Unexplained organic matter = (Ptn% + Lipid% + NPN% + Carboh%)