The seasonal ecology and physiology of *Sterechinus neumayeri* (Echinodermata; Echinoidea) at Adelaide Island, Antarctica

Thesis

The seasonal ecology and physiology of *Sterechinus neumayeri* (Echinodermata: Echinoidea) at Adelaide Island, Antarctica

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Frontispiece

Sterechinus neumayeri; photographed at South Cove (15m depth), Rothera Point.

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Abstract

This study used an energy budget approach to record changes in the biology of the Antarctic sea urchin *Sterechinus neumayeri* in relation to environmental seasonality (i.e changes in chlorophyll standing stock and seawater temperature) over an unbroken two year period. Chlorophyll standing stock showed a brief but intense bloom each austral summer which contrasted with prolonged winter minima. Benthic chlorophyll standing stock, as recorded from sediment cores showed a similar cycle. Seawater temperature varied between -1.8°C and +1.2°C.

Feeding activity was highly seasonal and closely correlated to chlorophyll standing stock. Feeding ceased during the austral winter of 1997 and 1998 for 6 and 4 months respectively. Metabolism, as measured by oxygen consumption and also ammonia excretion showed strong seasonality, with relatively brief 3 to 4 month periods of elevated activity in the austral summer contrasting with prolonged winter dormancy. Laboratory studies indicated that only 10-15% of the 3 fold seasonal rise in metabolism was caused directly by temperature (Q_{10}=2.5) and that 80-85% was related to increased physiological activity associated with feeding.

Growth rate was measured over one year and was very slow. Comparison with other studies indicated that echinoid growth rate is strongly dependent on food availability, but that maximal growth rate is limited by seawater temperature, or by a co-varying factor. *S. neumayeri* is an annual spawner and histology was used to describe both the vitellogenic cycle and also to calculate reproductive output. Comparison with other published studies worldwide indicated that reproductive output is highly dependent on food availability, and that maximal reproductive output is not limited by temperature. Although the overall P:B ratio was low, the ratio of reproductive production to total production was higher than expected.

These results indicated that due to the low metabolic rate only 12-16% of total body energy levels were used to endure the prolonged non-feeding polar winter. The overall annual growth efficiency was greater than for warmer water species, due to the larger relative contribution to reproductive output.

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Acknowledgements

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Chapter 1 General Introduction

1.1 Project rationale

Antarctica formed during the breakup of Gondwana, a process which took roughly 150 Ma and culminated in the opening of the Drake Passage between 25 - 30 Ma BP. During this process Antarctica's position over the South Pole remained relatively unchanged. The climate at polar latitudes ~65 Ma BP was mild and humid, with mean surface water temperatures around 15°C. Oxygen isotope ratios from Antarctic foraminifera in sediment cores indicate a general trend of decreasing temperature and subsequent glaciation from this time to the present day (Kennett 1977). The evolution of the current polar marine fauna has therefore taken place under conditions of both increasing geographical isolation and gradual reduction in temperature (although relatively rapid temperature decreases have taken place: Clarke 1990). This prolonged isolation has resulted in a present day fauna which is highly characteristic and shows a strong degree of endemism (Arntz et al. 1994). Biomass is frequently high in the sublittoral below the most intense region of ice scour (Plate 1.1), and diversity of many groups is comparable with other regions of the globe. Some taxa however are poorly represented, the most notable being decapod crustaceans, bivalve and gastropod molluscs and teleost fish (Clarke and Crame 1992).

The initial biological surveys to visit Antarctic waters in the early 1900s were concerned mainly with taxonomy. These were highly successful, and the *Discovery* expeditions between 1925 and 1939 generated an extremely comprehensive collection of Antarctic species descriptions. Subsequent investigations into the general biology of the

marine benthos gained strength with the opening of various shore based research stations in the early 1960s. One of the first hypotheses to be erected for polar species was that of metabolic cold adaptation (M.C.A.) - see for example Dunbar (1960). This concept was based around initial experimentation by Krogh (1914), who showed that the metabolic rate of warm water ectotherms decreased with decreasing temperature. He therefore hypothesized that for polar ectotherms to be able to perform any activity their metabolic rates must be raised relative to levels expected by simple extrapolation of the warm water relationship. A number of early experimental studies appeared to lend credibility to this idea, but mounting evidence eventually established the contrary view that metabolic rates of polar marine ectotherms are indeed lower than those of comparable warmer water species. Clarke (1987) subsequently argued that an increased basal metabolic rate represents an increased demand for ATP, and this is a cost to the organism.

Plate 1.1 A vertical rock face sheltered from intense ice scour at Cheshire Island (29m). The dense benthic assemblage is dominated by ascidians and *S. neumayeri*.

However low temperatures are not the only characteristic of nearshore polar regions. At high latitudes incident light levels are extremely seasonal and this produces a brief but intense pulse of primary production which takes place over an 8-10 week period in the austral summer, leaving the remainder of the year essentially devoid of autotrophic production (Chapter 2). One of the first published reports of the polar benthos in winter (Gruzov 1977) described the community of the Davis Sea entering a state of reduced activity or diapause. Further studies (Barnes and Clarke 1994, 1995) demonstrated that many species suspended feeding for varying periods in winter, reinforcing the view of seasonal energy limitation at polar latitudes and the need for low metabolic rates to allow efficient utilisation of reserves over the austral winter.

Data on overwintering in Antarctic species are nevertheless extremely rare. In a comprehensive review of the Antarctic zoobenthos Arntz et al. (1994) commented that "complete energy budgets for Antarctic benthic animals, covering all aspects of population dynamics, activity budgets, excretion and secretion, faecal egestion and, where applicable, calcification costs have not yet been constructed". The underlying rationale in this thesis was consequently to achieve these aims and also to determine the severity of the polar winter in physiological terms. A reduction in metabolic rate under polar conditions may potentially have other consequences including a limited aerobic scope (Peck 1998) and a raised growth efficiency (Clarke 1987). The project was therefore also intended to test these aspects of adaptation to polar conditions.

1.2 The research facility at Rothera Point

Rothera Research Station is situated on a rock and raised beach promontory at the

southern extremity of the Wormold Ice Piedmont, south-eastern Adelaide Island (Fig 1.1). The station was first established on the 25th October 1975 as a replacement to the Adelaide Research Station, located approximately 60km further to the south at the southern extremity of Adelaide Island. A crushed rock / gravel runway was opened at Rothera Station in the 1991-92 austral summer which facilitates the landing of both Twin Otter and a DHC Dash-7 aircraft. The latter is regularly used to transport personnel and some cargo between the research station and the Falkland Islands. Both marine and terrestrial biological research at Rothera are based around the Bonner Laboratory. This facility was officially opened by John Krebs in January 1997, and provides scientific offices, a small library, relatively well equipped analytical facilities, a wet laboratory and also a scientific SCUBA diving facility. The total number of personnel based at Rothera is around 23 during the austral winter (April to October), and rises in the summer up to a maximum of 130.

Rothera lies roughly 120km within the Antarctic circle, and consequently the sun remains below the horizon for a period of between three or four weeks each year around midwinter (June 21st). Conversely, the station is bathed in 24 hour daylight for a similar period at the end of December. Air temperatures vary between 0 and +5°C in the summer, and between -10 and -20°C in winter, although winter lows of -40°C have been recorded. Strong and prolonged gales, normally accompanied by blowing snow are also very common in winter. Rothera Point is home to 6 species of breeding birds, although a total of 23 species have been recorded (Milius 2000). Weddell seals breed each year on Rothera Point, and leopard and elephant seals are frequently sighted. Non breeding male fur seals also visit Adelaide Island in increasing numbers each year.

1.2a The use of SCUBA for sampling

One of the major problems surrounding any investigation into the biology of marine benthos centres around gaining access to the environment. SCUBA has regularly been used by the British Antarctic Survey (B.A.S.) since the early 1960s, and over this time it has proved to be a reliable and flexible method of obtaining regular collections of specimens or data from under fast ice, where conventional trawls or grabs would be unusable. For reasons of reliability and availability, divers at the Rothera Research Station use equipment available to the ever growing sports diving market. 8mm neoprene dry-suits allow dives of around 30 minutes to be carried out in a reasonable degree of thermal comfort, and conventional open circuit *Poseidon Cyclon 300* and *5000* demand valves have been found to be reliable in the conditions and relatively resistant to free flows (the uncontrolled escape of air as a result of freezing in the pressure reduction valves). All diving is carried out under the guidelines of the U.K. Health and Safety Executive, and under ice at least one of the dive pair is attached to a line which is continuously monitored by a surface tender. Rothera Research Station is equipped with a recompression chamber

Plate 1.2 A diver collecting *S. neumayeri* at South Cove

to deal with any incidents of decompression sickness, and the Station doctor receives training in hyperbaric medicine before travel to Antarctica. The number of dives performed per year by members of B.A.S. based at Signy (1962 - 1994) and at Rothera (1996 onwards) has steadily increased, and a total of roughly 500 dives (1000 man dives) were performed each year during the period when the data for this project was collected.

1.2b Sampling locations

The sea urchins used for this study were collected by SCUBA from three sites (Fig 1.1). The main sampling location (North Cove) was an area of sediment at 29m depth

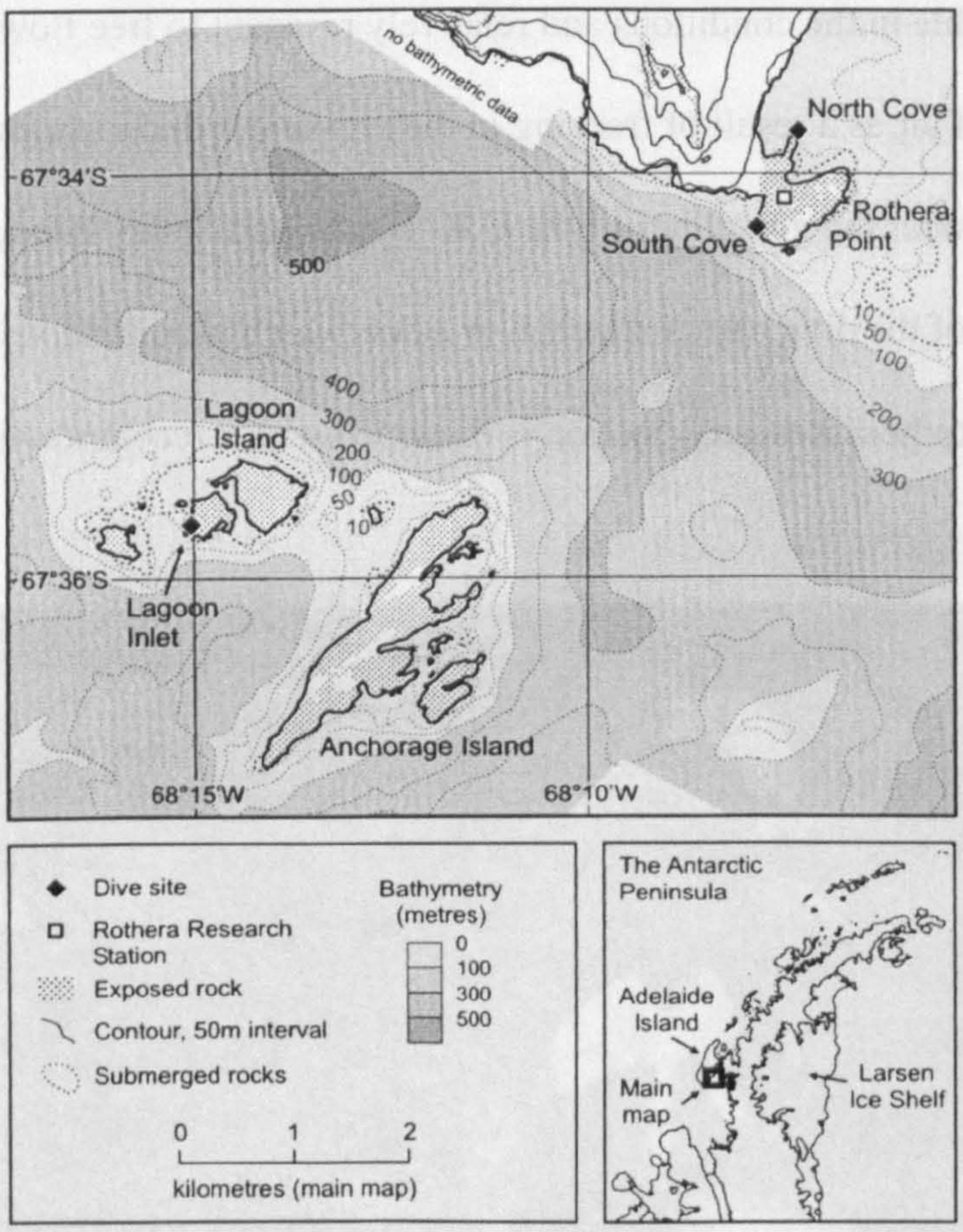


Figure 1.1 Map of Adelaide Island showing the northern section of Ryder Bay. The location of the three sampling sites used in this study are indicated. (Figure supplied by B.A.S. Mapping and Geographic Information Centre).

parallel with the Northern most extremity of the research station runway. The population density of *S. neumayeri* at this site was 80 individuals.m^{-2} (section 1.3), and monthly collections from this locality were used to investigate seasonal changes in feeding, tissue energy contents, metabolism, growth and reproduction. Two other sites, South Cove and Lagoon Island, were used to provide comparisons to North Cove. South Cove site was at the other end of the station runway and here *S. neumayeri* colonized a hard rock substratum between 6 and 10m depth. At Lagoon Island, a further 5km further south, sea urchins were collected at 4 to 6m depth from a cobble / boulder beach. At all sites annual formation of fast ice removed macrobenthos in the upper 1 - 2 metres of the sublittoral. Current flow around Rothera Point has not been described, although northerly currents to the east of Adelaide Island have been observed to create a localised anticlockwise circulation in North Bay.

1.3 *Sterechinus neumayeri*

Sterechinus neumayeri is a regular echinoid (order Echinoida, family Echinidae) and is endemic to Antarctica. It has a circum-polar distribution and has been recorded from most shallow water localities so far sampled, often in great abundance (Dell 1972). In the Weddell Sea below ~450m the density of *S. neumayeri* decreases and the density of the congeneric *S. antarcticus* increases (Brey and Gutt 1991). Previous autecological studies by other authors have documented growth and production (Brey et al. 1995), organic constitution and reproduction (Pearse and Giese 1967) and feeding (McClintock 1994). However none of these investigations assessed seasonal or interannual variations.

Initially, in this study, population densities and size frequency distributions were

established for the North Cove sampling site and for a depth profile at South Cove. At each site or depth the number of sea urchins occurring within replicate $0.25m^2$ quadrats

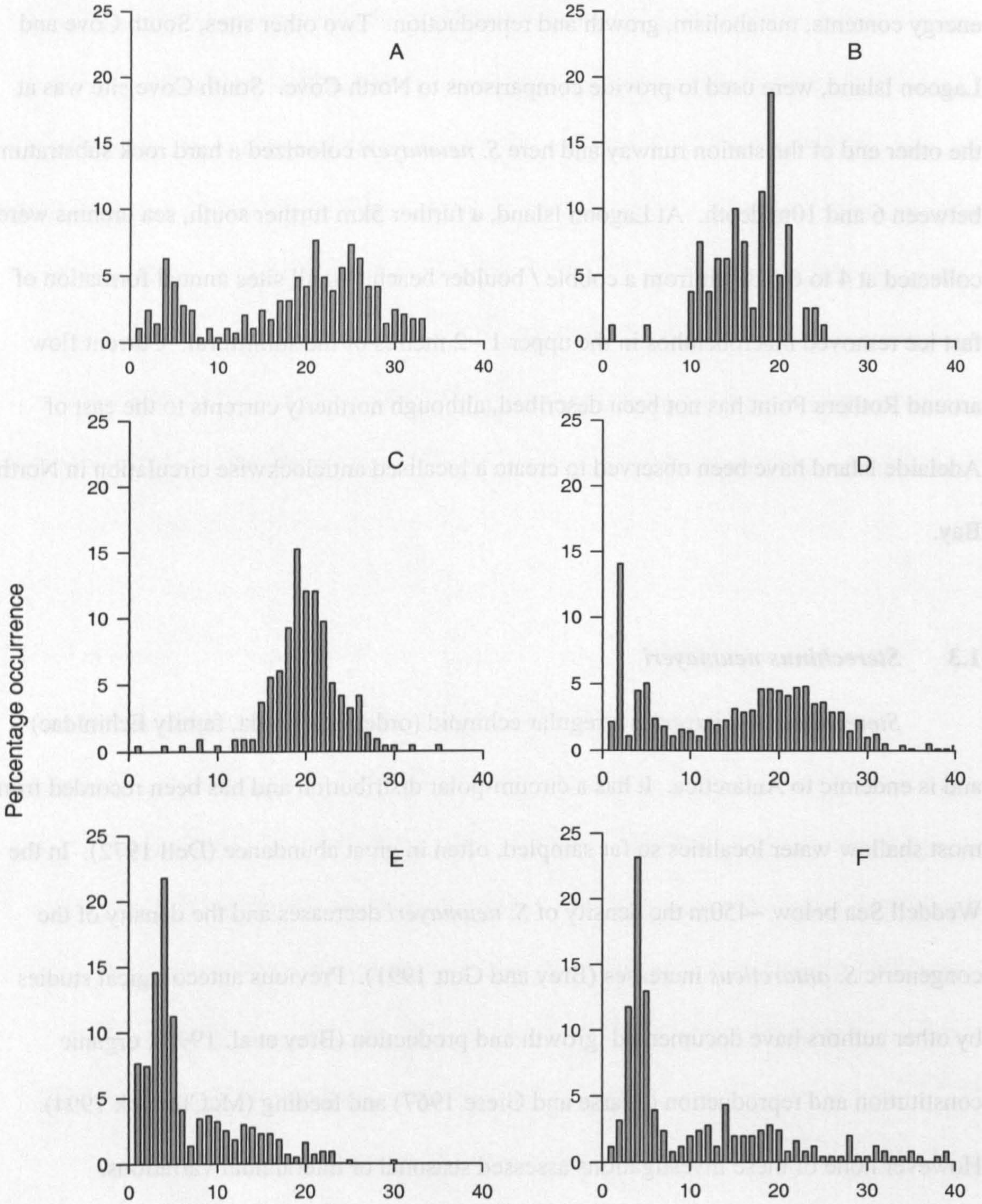


Figure 1.2 Size frequency distributions of *S. neumayeri* around Rothera Point. A) North Cove 30m, n = 288; B) South Cove 3m, n = 80; C) South Cove 6m, n = 216; D) South Cove 11m, n = 833; E) South Cove 23m, n = 446; F) South Cove 35m, n = 255. Sampling took place in June 1997.

were counted and placed into a mesh bag underwater. The urchins were returned to the laboratory, and horizontal test diameters were measured in triplicate using knife edged calipers, and a mean value calculated. For small urchins (< 3 mm) test diameters were measured by placing the urchins on a steel rule graded to 0.5mm and estimating diameter whilst viewing at ×20 with a binocular microscope. Replicate counts from the quadrats were meaned and multiplied by four to establish population density per m^2, and size frequency distributions were constructed from the test diameter measurements (Fig 1.2).

At North Cove the population density was 80 $ind.m^{-2}$. The size frequency distribution for this site showed that the two initial size classes were visible in the population with modal test diameter peaks at approximately 2 and 4mm (Fig 1.2, but see also Fig 6.1). Very few individuals were present in the range 8-14mm, and a large proportion of the population was >20mm test diameter. At South Cove the population density increased with depth from a mean of 36 $ind.m^{-2}$ at 3m to 223 and 170 $ind.m^{-2}$ at 23 and 35m respectively. Juvenile sea urchins (<10mm test diameter) were absent from the shallow sublittoral (i.e. shallower than 10m), although were found in great abundance at the deeper stations, particularly 23 and 35 metres. Relatively large urchins (>20mm test diameter) were common in the high sublittoral above 11m, but became increasing infrequent with depth. The high population densities of *S. neumayeri* around Rothera Point mean that this species is a prominent member of the benthic community, especially between 6 and 11metres. At this depth regular brash ice scour prevents the establishment of sessile benthos, and the depth band is instead inhabited by aggregations of mobile species, especially relatively large *S. neumayeri* and also the limpet *Nacella concinna*.

Chapter 2 Seasonality of seawater temperature, chlorophyll standing stock and fast ice formation

Data on pelagic chlorophyll standing stock and seawater temperature were collected by the Rothera Research Station marine assistant as part of the long term Rothera Time Series (RaTS) oceanographic monitoring programme. Sediment core data were collected by the Rothera Research Station marine assistant.

2.1 Introduction

In most nearshore marine ecosystems two environmental factors, i.e. food availability (phytoplankton standing stock) and seawater temperature change on a seasonal basis, and both have a profound effect upon the biology of benthic primary consumers. In polar regions phytoplankton production may take place either in the water column, at the sea-floor or associated with fast ice. The greatest level of production predominantly takes place in the water column (Nedwell et al. 1993). The importance of pelagic primary production to benthic communities was reviewed by Graf (1992), who emphasized that organic matter produced in the pelagic zone is the basis of many benthic food webs. Although pelagic phytoplankton may be grazed directly by zooplankton or benthic suspension feeders, remaining cells can settle rapidly and the coupling of benthic processes to sedimentation events can take place over a very short timescale. In this manner water column phytoplankton blooms can have an important influence on both the quantity and quality of food available to benthic herbivores and omnivores. Seawater temperature also affects the biology of benthic communities since many biological processes, most notably metabolic rate, decrease with decreasing temperature (although there is no evidence for direct thermal limitation of metabolism).

Annual cycles of both food and temperature change with latitude. Seasonal

changes in phytoplankton standing stock are minimal at the tropics where primary production may be almost constant on a year round basis, but seasonality progressively increases with latitude culminating with a brief but intense bloom which develops each summer in polar regions. Annual mean seawater temperature decreases with latitude, but variability about the mean decreases at both high and low latitudes. In most latitudes therefore annual variations in pelagic autotrophic standing stock and seawater temperature take place simultaneously. In polar regions however temperature is fairly constant (and low), while phytoplankton standing stock exhibits strong seasonality. Study of seasonal biological responses of benthic communities in polar regions therefore affords a natural opportunity to distinguish the different effects of food availability and temperature (Clarke, 1988). Data reported in this chapter describe seasonal changes in seawater temperature and pelagic phytoplankton standing stock (important to the deposit feeding benthos through vertical flux) at Rothera Point. Sediment chlorophyll concentrations (indicative of sedimentation of pelagic primary production and benthic in-situ primary production minus loses through remineralisation) are also reported.

2.2 Methods

Seawater samples for chlorophyll analysis were collected weekly from a depth of 15m using a National Institute of Oceanography (N.I.O.) type water bottle and returned to the laboratory in polythene bottles. After gentle agitation the samples were fractionated using a succession of filters (smallest pore size 0.2 μm) under gravity and in the dark. Chlorophyll was extracted from the retained particulate matter with a mixture of 2:1 v/v chloroform:methanol (Wood 1985), and subsequently assayed fluorometrically using a Turner fluorometer calibrated against a chlorophyll standard extracted from *Anacystis*

nidulans (supplied by Sigma-Aldrich). Records of sea-ice formation and thickness (assessed by drilling) were also taken at weekly intervals during the winter periods.

Benthic chlorophyll standing stock at the North Cove site was estimated from a series of sediment cores. Ten replicate cores of 26mm diameter were taken on a roughly monthly basis by SCUBA divers from the area used for sampling sea urchins in Chapters 3 - 8. The corer was pushed into the sediment to a depth of approximately 30 - 60mm and sealed at the protruding end with a bung. The core was then lifted clear of the sediment and the remaining open end sealed with a second bung. The cores were transported to the surface and back to the laboratory in a vertical position and were subsequently frozen at -20°C. The frozen core was loosened from the holder by running briefly under hot water and the frozen seawater at the top of the core broken away (any residual ice being removed with a razor blade). The top five millimeters of sediment were then sectioned from each of the ten replicate cores. Five of the replicates were dried to constant mass at 65°C, and organic content subsequently determined (as ash free dry mass, AFDM) by difference following ignition at 465°C for 22 hrs. Decomposition of $CaCO_3$ is minimal at this temperature (L. Peck, pers comm), and decomposition of Aluminium (from the weighing vessel) commences above 470°C. The remaining five of the top core sections were placed in test tubes containing 2:1 v/v chloroform:methanol and left overnight at +5°C in the dark to extract chlorophyll and phaeopigments. After incubation the supernatant was filtered, diluted and chlorophyll was assayed fluorometrically as described above. The data were expressed as mass of chlorophyll per square metre of seafloor.

2.3 Results

Fast ice formed at the sampling site during winter of 1997 and 1998, although it was on a much reduced scale in the second year (Fig 2.1). Ice formation started in early

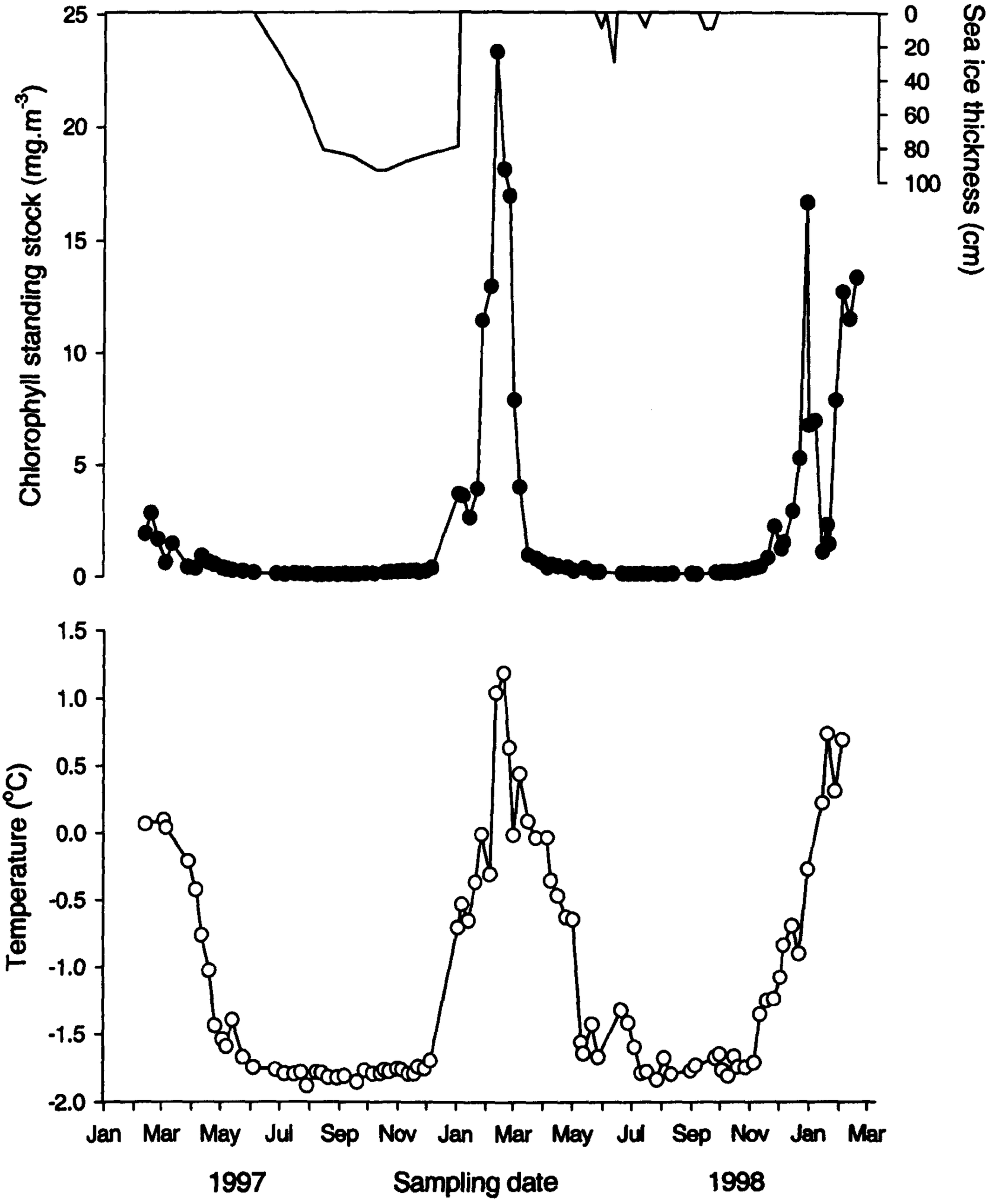


Figure 2.1 Seasonal cycles of fast ice thickness, chlorophyll standing stock (●) and temperature (○) in Ryder Bay. Data from RaTS oceanographic programme.

June 1997, and the thickness increased steadily to a maximum of 94cm by October. Build up of an algal mat on the underside of the ice started in early September 1997, and algal growth increased steadily until December. In late December sheets of the algal mat started to slough away from the underside of the ice and sank to the sea floor. By late December the ice had started to decay, and open water returned to Ryder Bay during a storm in early January 1998. The shading effect of the ice layer was increased by a layer of snow up to 20cm depth lying on top of the ice which persisted until breakout of the ice. The maximum thickness of fast ice recorded in the winter of 1998 was 30cm, and although fast ice started to form on four occasions between May and September strong winds destroyed the ice before any appreciable thickness formed.

Chlorophyll standing stock displayed the strong seasonal cycle characteristic of Antarctic nearshore locations (Fig 2.1). A single intense phytoplankton bloom developed in early January 1998, immediately after the breakout of the winter fast ice (peak value >20 $mg.m^{-3}$). Two blooms developed during the 1998 / 1999 summer. The initial increase in chlorophyll concentration took place in October 1998 and rose to a peak value of 16.7 $mg.m^{-3}$ in December 1998. A second peak of 13.4 $mg.m^{-3}$ was recorded in February 1999. The earlier development of the phytoplankton bloom in 1998 coincided with extra light penetrating the water column as a result of reduced fast ice formation. Winter chlorophyll concentrations (0.05 - 0.2 $mg.m^{-3}$) persisted for 142 days in 1997 and 159 days in 1998. A clear seasonal cycle was also apparent in the soft substrata of North Cove (Fig 2.2). Benthic chlorophyll standing stock varied from 5 to 90 $mg.m^{-2}$ during the annual cycle, and was lowest (< 30 $mg.m^{-2}$) between May & August 1997 and May & September 1998. The average winter microalgal benthic standing stock was lower during 1997 than in 1998. A

brief late winter rise was recorded in September and early October 1997 before standing stock again returned to low winter levels in November and December preceding the breakout in winter fast ice; this rise did not occur the following year. The percentage organic content of the sediment varied between 0.75% and 2.3% (Fig 2.2). No seasonal trend in sediment organic content was recorded.

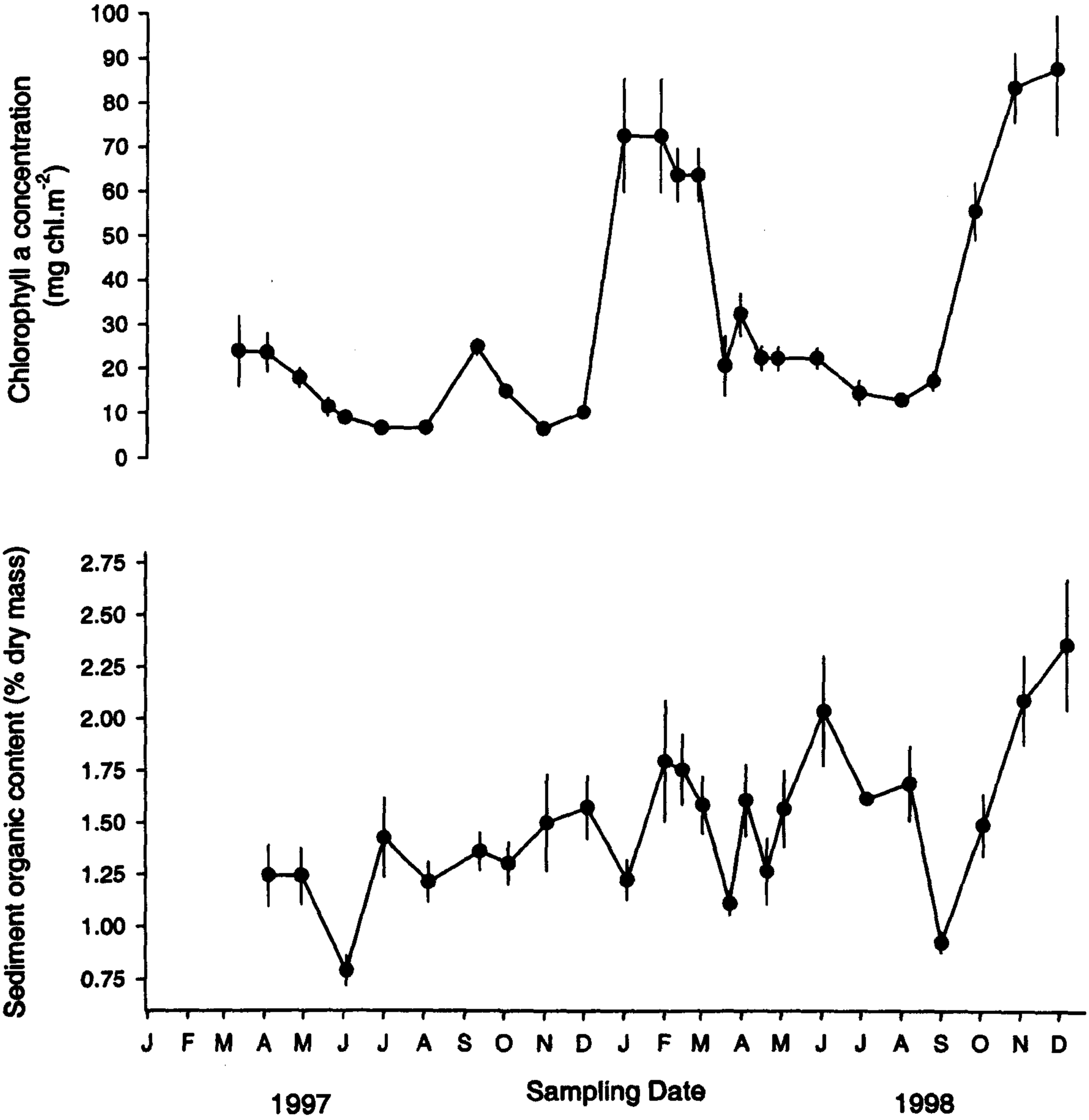


Figure 2.2 Benthic microalgal standing stock as measured by chlorophyll concentration from a soft sediment substratum at North Cove (30m depth). Data are presented as mean of five cores for both chlorophyll and sediment organic content determined by loss on ignition ± standard error.

Seawater temperature also showed a seasonal profile characteristic of Antarctic nearshore locations (Fig 2.1). Minimum values of -1.8°C were recorded between June and December of 1997 and July and November of 1998. Summer maxima of +0.1°C, +1.2°C and +0.74°C were recorded in March 1997, February 1998 and January 1999 respectively, indicating a maximal recorded temperature range of 3.0°C at this locality.

2.4 Discussion

In a 27 month study at Signy Island (60°S, 45°W) between 1972 & 1974 Whitaker (1982) showed that a rapid increase in chlorophyll standing stock took place each year in December following break out of sea-ice. The ensuing bloom lasted for 8-10 weeks, and ended with an abrupt decline in February. Low productivity was associated with very low phytoplankton standing crop at other periods of the year. This general pattern was confirmed by two subsequent longer term studies at the same locality, described in Clarke et al. (1988, data from 1972 to 1982) & Clarke and Leakey (1996, data from 1989 to 1994). Similar patterns at other nearshore Antarctic localities have also been recorded by Bunt (1964) at McMurdo, Krebs (1983) at Arthur Harbour and Satoh et al. (1986) near Syowa Station. The seasonal cycle of primary productivity at Rothera is very similar to that reported from Signy. The summer peak chlorophyll concentrations of 23 and 17 $mg.m^{-3}$ at Rothera fall well within the range of typical Signy values. The start of the bloom was retarded by a late ice breakout in the austral summer 1997-98, but a strong standing stock quickly developed in early January after departure of the fast ice.

The studies presented by Clarke et al. (1988) and Clarke and Leakey (1996) from Signy showed that although the abrupt rise in chlorophyll concentration is caused by a

rapid proliferation of large diatoms and colonial forms (>20 μm), this peak is superimposed upon a more prolonged and less intense nanoflagellate bloom (size range 2 - 20 μm). Although the chlorophyll concentrations attributable to the nanoflagellate bloom were typically an order of magnitude lower than those from the diatom bloom, the prolonged persistence of the smaller fraction may be important in allowing extended feeding activity by various suspension feeding groups. The size of the phytoplankton bloom in the 17 different summer seasons at Signy reported by Clarke et al. (1988) and Clarke and Leakey (1996) showed considerable interannual differences, with peak bloom chlorophyll concentration ranging from 2.2 to 41.3 $mg.m^{-3}$. Two seasons showed particularly small and brief blooms: 1977-78 and 1978-79. Analysis of results presented by these studies showed that no correlation could be found between bloom magnitude and ice duration, date of ice break out, summer seawater temperature or mean wind speed in October. However significant inverse correlations were found between wind speed and bloom size in November and January, indicating that water column stability is important in allowing the build up of a large pelagic autotrophic biomass in shallow coastal waters.

The decline of the bloom was always accompanied by a large increase in vertical flux of chlorophyll. Patterns of sedimentation of particulate matter from the water column from other locations in the Southern Ocean have been documented by Bodungen et al. (1985), Bathmann et al. (1991) and Karl et al. (1991). Benthic chlorophyll standing stock results from benthic production minus losses due to grazing and remineralisation, together with advected pelagic input and provides a more direct indication of food availability to benthic deposit feeders. Total benthic chlorophyll concentration per unit area was

generally lower at Rothera than found by Gilbert (1991a) at Signy Island, and this may be a consequence either of latitude or the increased depth of the sampling site at the more southerly location. Benthic standing stock and production have also been recorded at various times and depths for McMurdo (Dayton et al. 1986). Direct comparison with the McMurdo data are difficult because of the highly heterogeneous nature of the substratum (sponge spicule mats) at this locality, but it is clear that the data from all three studies fall within the same order of magnitude, and all show a clear seasonality.

Detection of a rise in sediment organic content during sedimentation events depends on the background levels of sediment organic matter (Graf 1992) coupled to the rate of remineralisation, which may be very rapid during the Antarctic summer (Nedwell et al. 1993). In a further study at Signy Island, Gilbert (1991b) found a rise in benthic chlorophyll without concurrent increase in sediment organic content, and this was attributed to a high carbon:chlorophyll ratio coupled with an AFDM:organic carbon ratio of 2:1. Together these suggested that only a small proportion of total sediment organic mass was attributed to advected microalgal production. The strong and erratic variability in sediment surface AFDM recorded by the Rothera study may be indicative of the highly disturbed nature of the seafloor due to frequent gouging by icebergs.

In winter 1997 a dense microalgal mat developed on the underside of the annual sea-ice (Chapman 1998). Chlorophyll settlement rates are low in winter (Nedwell et al. 1993), but a strong deposition of algal biomass took place in Ryder Bay during November associated with the onset of melting of the sea-ice. The vertical flux of autotrophic production was clear in the sediment core chlorophyll data at this time. Midwinter benthic

chlorophyll standing stock was lower in winter 1997 than winter 1998 and this may have been indicative of the combined shading effect of sea-ice and snow cover. The differences between winters suggest that trade-offs occur between sites of primary production between years, in that if sea-ice is not present to provide a substratum for attachment then algal growth will occur at the seafloor in the late winter period when solar radiation increases. Large interannual variation in sea-ice cover has been recorded in Antarctica (Clarke et al. 1988, Murphy et al. 1995) and data presented here indicate that ice cover may be an important factor in determining whether early summer autotrophic production is associated with either ice or sediment. Antarctic benthic microalgae are highly shade adapted and may be able to provide a food source for detritivores in early summer. However the strong shading effect of the intense pelagic bloom at the height of the austral summer reduces the light intensity reaching the benthic microalgae at 30 m in North Cove to almost zero. This suggests that most of the energy input available to the benthic community is of pelagic origin at the height of summer.

The annual seawater temperature regimes of nearshore Antarctic sites are often described as low and stable (Arntz et al. 1994). The most southerly seawater temperature records in existence are from McMurdo Sound (78°S) where the annual temperature range varies by ±0.07°C around an average of -1.89°C at 10m depth: this environment is almost permanently ice covered and is one of the most thermally stable on earth. A clear seasonal temperature cycle was observed at Signy Island (Clarke et al. 1988, Clarke and Leakey 1996) where temperature varied from <-1.86°C in winter to between +1.6 and +0.5°C in summer. The data reported here from three summers at Rothera suggest the annual temperature range at Rothera Point is very similar to Signy Island.

Chapter 3 Seasonality of feeding and nutritional status

Data also presented in: Brockington S, Clarke A & Chapman ALG (in press). Seasonality of feeding and nutritional status during the austral winter in the Antarctic sea urchin *Sterechinus neumayeri*. Mar. Biol.

3.1 Introduction

Changes in morphology as a response to variation in ecological conditions have been recorded for many members of the Echinodermata, and especially the Echinoidea. Such phenotypic changes have been suggested to enhance survival (Ebert 1996), and they include reduction of maximum body size, reduction in mass of gonad and gut tissue and a relative increase in size of the Aristotle's lantern under low food conditions (Lawrence and Lane 1982, Marcus 1983, Ebert 1996). The Echinodermata are prominent members of the Antarctic marine fauna, and it is possible that such morphological changes have been important in adapting to the unique conditions of life in the Southern Ocean.

The nearshore Antarctic marine environment exhibits an extreme but predictable variability in food availability (Chapter 2). Many species cease feeding during the austral winter and Clarke (1988) suggested that the effects of such starvation ought to be more heavily felt by suspension feeders and herbivores than by the omnivorous and carnivorous members of the benthic community, though this hypothesis has yet to be rigorously tested. Work by Barnes and Clarke (1994; 1995) based at Signy Island has shown that a marked heterogeneity is apparent between suspension feeding taxa in the length of time for which feeding ceases during the polar winter, and that ability to feed for these groups appears to be related to size of phytoplankton taken. Holothurians ceased feeding earliest in late summer and this group is believed to feed on the larger cells of the microplankton, whereas

some species of bryozoans (feeding on nano and picoplankton) were observed to feed throughout the year. It is not however clear from these studies how any class of Antarctic benthic marine invertebrate deals with winter starvation in physiological terms. Polar benthic invertebrates do not appear to lay down high energy metabolic stores before the onset of the austral winter (Clarke and Peck 1991). This is in strong contrast to polar zooplankton many of which lay down extensive reserves, typically of lipid. It is likely that this is a response to the extra energetic costs associated with maintenance of position within the water column (Clarke and Peck 1991). For benthic Antarctic echinoderms biochemical compositions have been shown to be very similar to those from more temperate and tropical regions (McClintock and Pearse 1987). Given the low metabolic rates reported for polar marine ectotherms (Clarke and Johnson 1999; Peck and Conway 2000), this may reflect a low demand for reserves to fuel overwinter survival.

Sterechinus neumayeri has been described as a generalistic feeder by McClintock (1994), and such a strategy has been suggested to increase fitness by allowing feeding over a much longer period than for specialist feeders (Dell 1972). Brand (1976) demonstrated a reduced occurrence of amphipods in the gut contents of *S. neumayeri* at Anvers Island (64°33'S, 63°35'W) during the winter months in comparison to two more specialized feeders that were able to maintain feeding on amphipods at this time. The data presented in this chapter firstly document the feeding period for *S. neumayeri* in relation to water column and sediment autotrophic standing stock, and secondly record seasonal changes in body morphology and energy content between two geographically close sites.

3.2 Methods

The direct measurement of feeding rate in free-living marine invertebrates is technically very difficult. Therefore in this study rate of faecal production was measured as a proxy for feeding activity. This technique was developed by Hargrave (1972) and Calow (1975) and subsequently adapted for polar marine invertebrates by Clarke (1990). The underlying assumption in this method is that all material being passed through the gut provides an energy gain instead of simply maintaining gut functionality. Assessment of *S. neumayeri* were made monthly, when 16 freshly collected sea urchins of approximately 30mm test diameter were divided into four groups of four and placed in 5 litre beakers of clean seawater. The total amount of material egested over a 24 hour period by each of the four groups was then collected and sorted to remove any extraneous debris (broken spines etc) before being carefully washed with fresh water and dried to constant mass at 65°C. The total dry mass of all the four animals in each group was also determined, and egestion of faecal material per total weight of urchin dry mass in each of the four replicates calculated for the 24 hour period.

To measure seasonal change in organ mass and energy content ten animals were sampled each month from each site. After being returned to the laboratory individual urchins were placed in 500ml beakers filled with clean seawater and maintained at ambient environmental seawater temperature by partial immersion of the beaker in a flow through aquarium. After 60 hours the urchins had completely evacuated their guts (S. Brockington, pers obs). Horizontal test diameter was measured in triplicate using knife edged calipers and a mean value calculated. The dry mass of four separate body components (test, lantern, gut and gonad) was determined by dissection and subsequent drying to constant

mass at 65°C. Care was taken to ensure no residual gut content material was transferred along with the gut itself, and the total dry mass of gut contents (retrieved from the original holding beaker) was also determined. Initial dissections over a size range allowed the determination of the scaling exponent for the mass of the four components with test diameter (Table 3.1), and this exponent was used to correct for small differences in urchin size by adjusting all measured values from the monthly samples to a standard animal of 30mm test diameter. After determination of dry mass the samples for gut and gonad tissue were divided into two portions, one half being retained for elemental analysis upon return to the United Kingdom, and the second half used for determination of the percentage ash content of the tissue calculated from material remaining after ignition at 465°C for 22 hrs.

Table 3.1 Scaling relationships relating the mass of body components to test diameter from a sample collected during May 1997 after $\log_e$ transformation of both variables. b = slope (scaling exponent), SD is standard deviation around regression line, F tests significance of slope (0 hypothesis, $b = 0$). Animal diameters ranged from 17.9 to 41.6 mm.

Dependant variable	b	SE	Intercept	r^2	SD	F	n
Gonad	2.76	0.20	-9.98	0.823	0.289	186.08	40
Gut	2.61	0.14	-11.51	0.896	0.200	346.14	40
Lantern	2.41	0.09	-9.17	0.942	0.135	648.89	40
Test	2.84	0.06	-9.16	0.980	0.091	1967.49	40
Total	2.77	0.06	-8.42	0.983	0.081	2354.66	40

In U.K. the preserved subsamples of gut and gonad tissue were redried for 24 hours at 60°C immediately prior to analysis. Elemental (C, H, N) composition was then determined using a Fisons Instruments EA 1108 elemental analyser calibrated with acetanilide. Soft tissue energy content was estimated from the percentage carbon using the relationship derived by Gnaiger and Bitterlich (1984), and proximate composition (ratio of

protein, carbohydrate, lipid) was estimated from elemental proportions using the stoichiometric algorithm developed by Gnaiger and Bitterlich (1984).

AFDM of the body wall was measured as loss in mass after ignition at 465°C for 22 hours. Energetic content of this tissue was estimated using a conversion of 23 $kJ.g^{-1}$ AFDM (Brey et al. 1988) rather than by carbon content (as for the soft tissues) because of the high inorganic carbonate content of the test. Proximate composition estimated from elemental analysis was checked by direct analysis for one month (January 1999) at the end of the study period. Samples were taken in duplicate for both gut and gonad tissue from ten animals for lipid, protein, carbohydrate, ash and percentage water content of fresh tissue. Lipid was assayed gravimetrically after extraction of fresh tissue with 2:1 v/v methanol / chloroform (Bligh and Dyer 1959). Carbohydrate was measured following the method of Dubois et al. (1956) calibrated with a D(+) glucose standard, and protein was

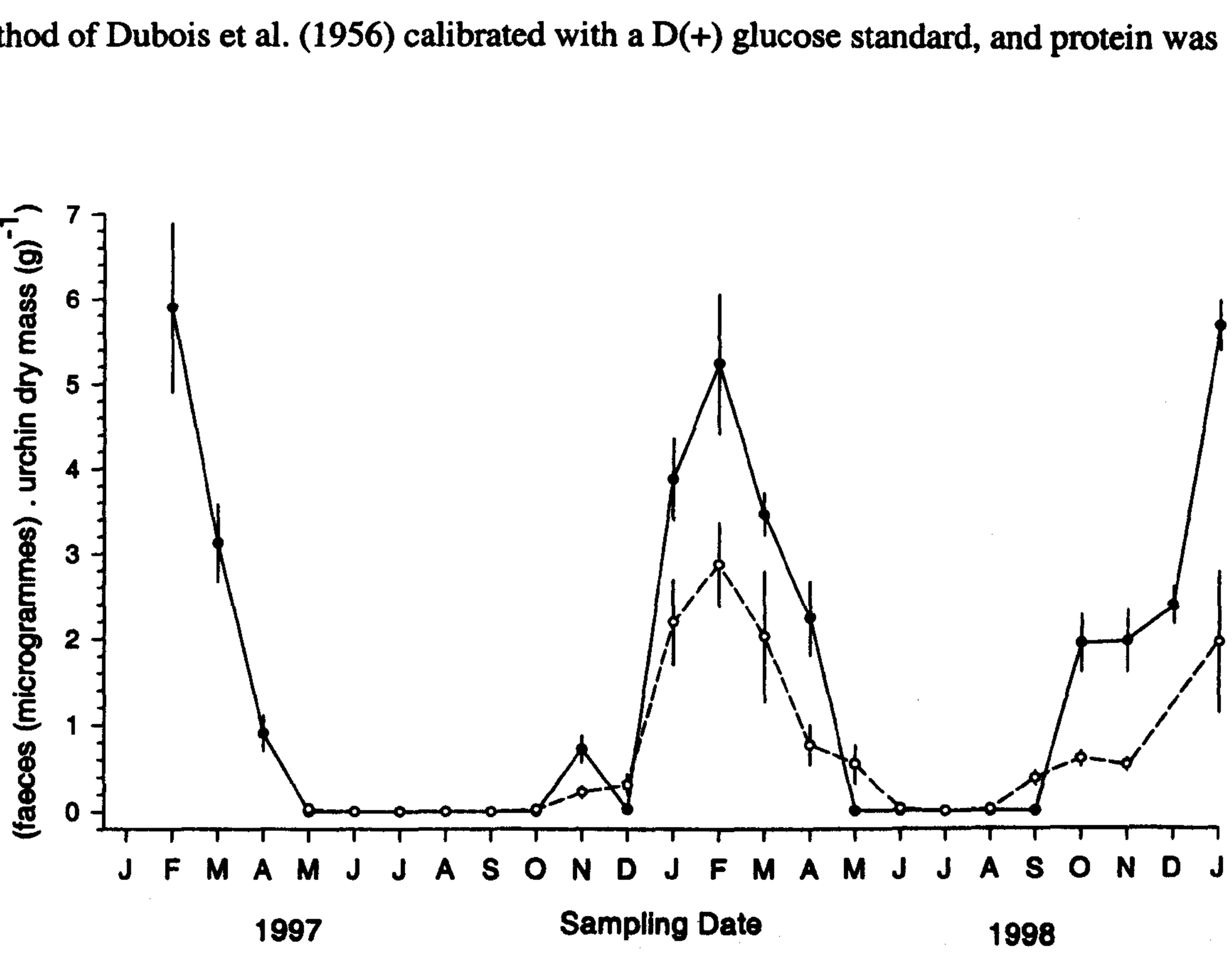


Figure 3.1a Seasonal variation in faecal egestion from both North (●) and South Cove (○). Each point represents a mean of four replicate determinations ± standard error.

measured in accordance with Lowry et al. (1951) after homogenisation of tissue in 0.2M perchloric acid, and subsequent solublisation of the protein pellet in NaOH. The protein assay was calibrated with bovine serum albumin (supplied by Sigma-Aldrich). In all cases 100 mg (wet mass) samples were used, and percentage water of fresh tissue was established by drying 20 x 100 mg replicates of both gut and gonad tissue to constant mass at 65°C. Ash was measured after ignition at 465°C for 22 hrs.

3.3 Results

Rates of faecal egestion can be used to estimate food intake and consequently energy assimilated through the gut wall if organic contents of food and faeces are known and a correction is applied for assimilation efficiency. Rate of faecal egestion varied enormously throughout the year (Fig 3.1a) and was closely tied to food availability as

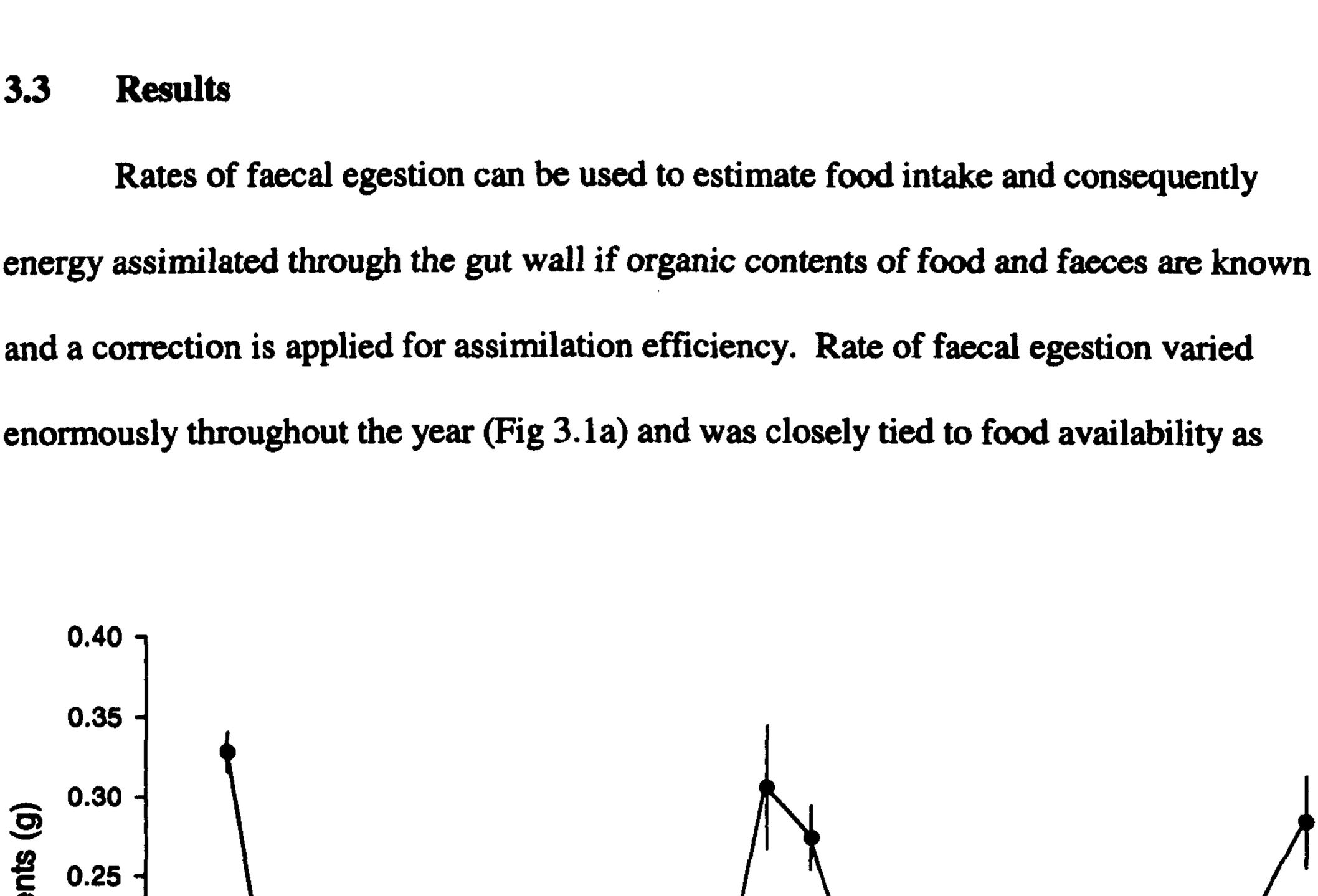


Figure 3.1b Total mass of gut contents determined by dissection. Each data point represents mean of 10 values ± standard error for North (●) and South (○) Cove. Data have been corrected allometrically to a standard animal of 30mm test diameter.

inferred from sediment chlorophyll biomass (Fig 2.2). For North Cove echinoids, feeding rate was zero for a six month period during the winter of 1997, and again for a four month period in winter 1998. During the middle summer season of this study (austral summer 1997 / 98) 84 % of the total material egested for the entire year occurred between January through March, leaving almost three quarters of 1997 essentially devoid of feeding activity. The diet of animals from North Cove was observed to consist entirely of sediment (and consequently also benthic diatoms), whereas at South Cove a considerably more varied diet was taken, including mainly sediment and macroalgae, but also occasional bryozoan fragments, crustaceans and seal faeces. Whilst *S. neumayeri* from both sites showed similarities in feeding activity, and also the degree of interannual variation in the length of period of winter starvation, sea urchins from South Cove passed considerably less material through their guts than those from North Cove. The mass of gut content material from the

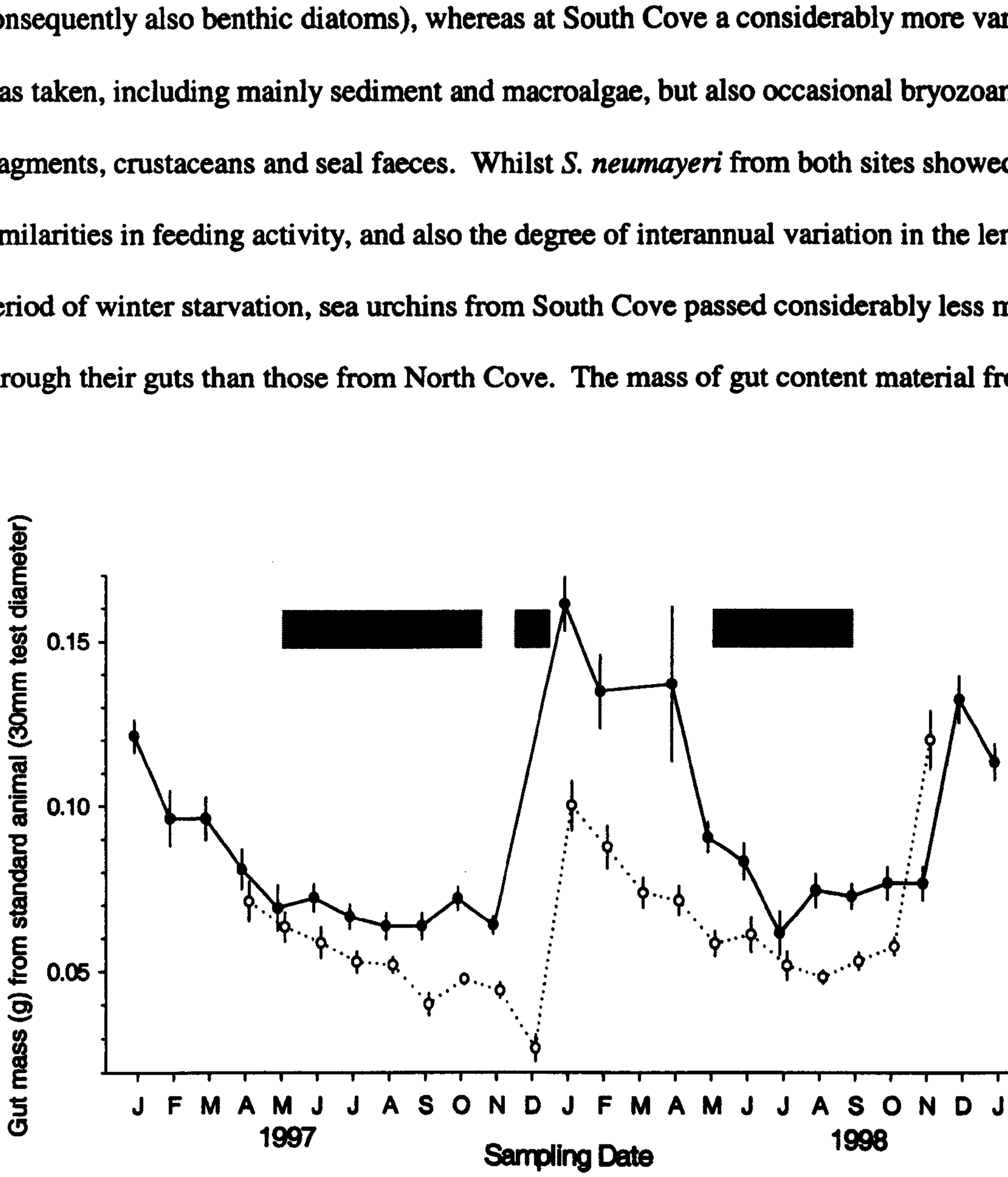


Figure 3.2 The annual cycle of gut mass for urchins at North Cove (●) and South Cove (○). Data are standardised to an animal of 30mm test diameter ± SE, $n = 10.\text{month}^{-1}$. Horizontal bars indicate absence of feeding.

dissected animals (Fig. 3.1b) indicated that the gut was completely empty during the periods of zero faecal egestion. This observation confirmed that *S. neumayeri* ceased feeding in winter (as opposed to simply passing material through the gut at an extremely slow rate).

Observations by divers indicated that throughout the starvation periods the sea urchins aggregated into dense groups on the seafloor where they appeared motionless. These groups, often centered on occasional rocks or other solid substratum within the sediment at the North Cove site appeared unchanged for the entire winter. Aggregative grouping also took place at South Cove around rocky outcrops and ledges (Plate 1.2).

Previous work has shown that echinoids may utilise the gut as a short term energy store, and the gonad and body wall as longer term energy sources (Lares and Pomory

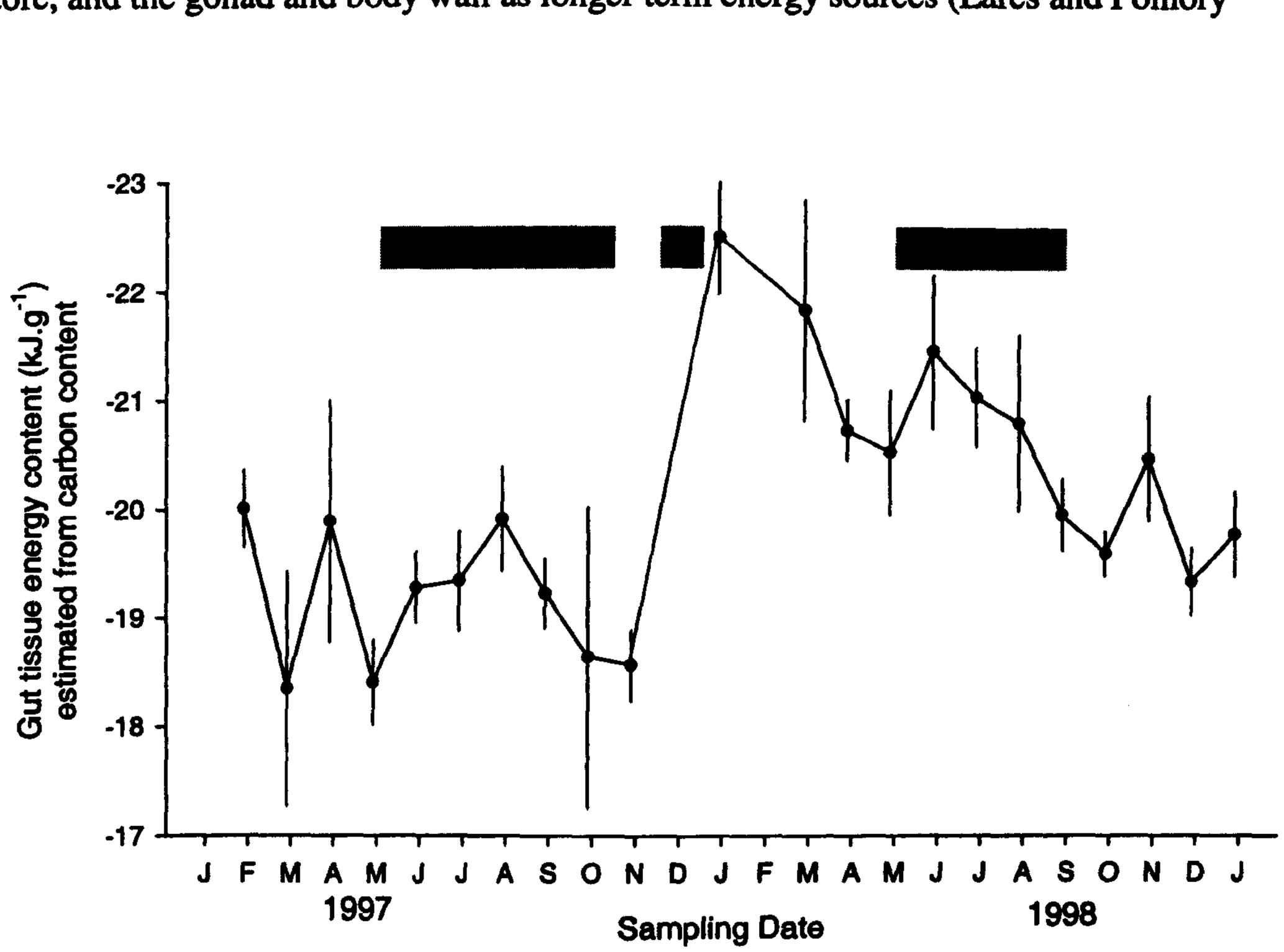


Figure 3.3 Gut energy levels estimated from carbon content (North Cove urchins only) using stoichiometric algorithm developed by Gnaiger and Bitterlich (1984). $n = 5.\text{month}^{-1}$, data are means ± SE. Horizontal bars indicate absence of feeding.

1998). These tissues might therefore be expected to show changes in either or both of mass and energy content in response to seasonal changes in feeding. There were pronounced within year changes in gut tissue mass of a standard animal (Fig. 3.2). For all but one month of the sampling period the South Cove population displayed a reduced gut mass compared to North Cove, although urchins from both sites showed the same broad seasonal changes. This was characterised by a steady decrease in gut mass during the first half of the austral winter followed by a subsequent stabilisation until the onset of feeding in November. The start of feeding correlated with an abrupt increase in gut tissue mass between November 1997 and January 1998. At South Cove gut tissue mass did not stabilise and the reduction continued throughout the 1997 winter, although the subsequent rise at the onset of feeding was as pronounced as at North Cove. At North Cove gut mass

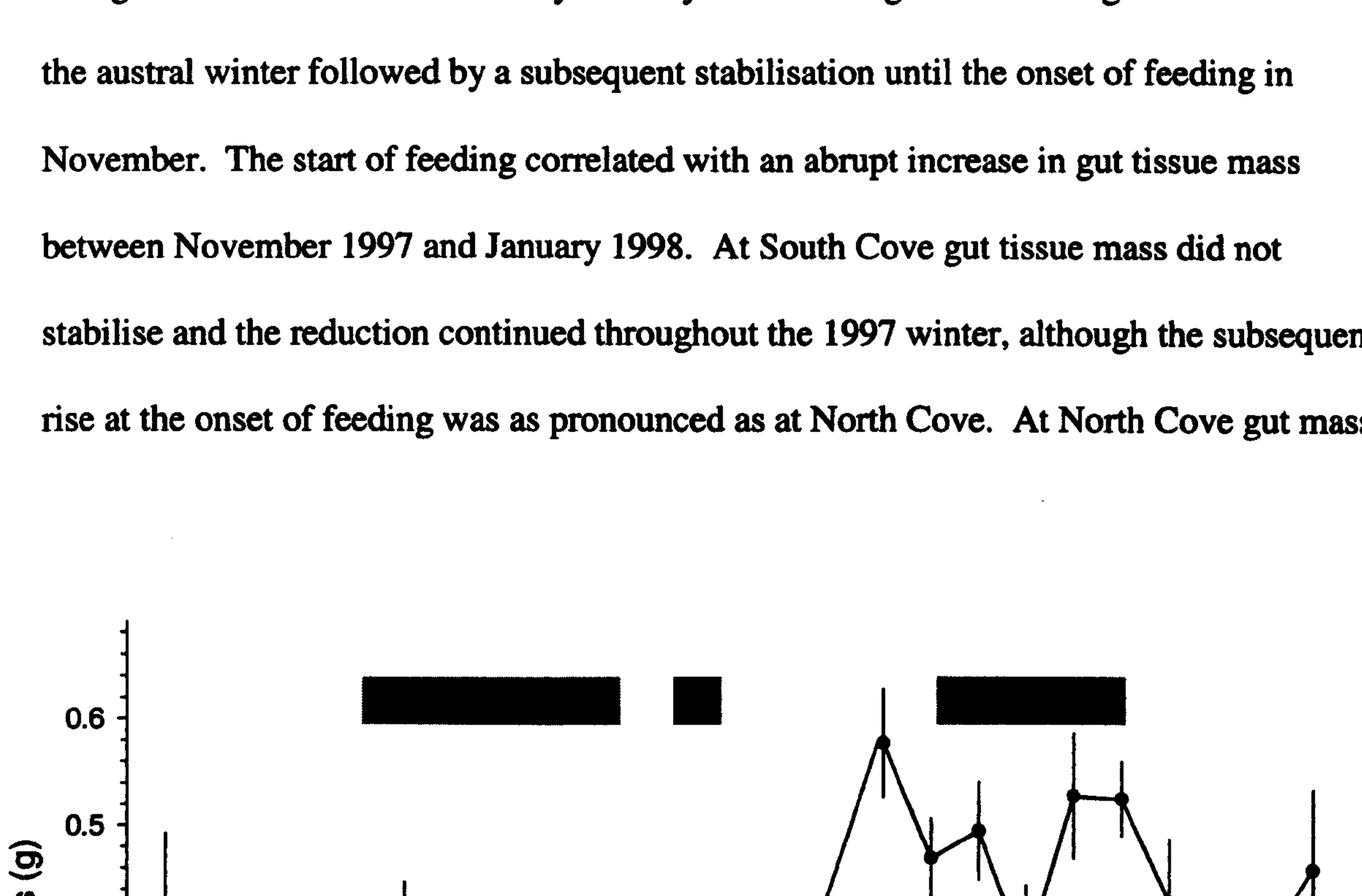


Figure 3.4 The annual cycle of gonad dry mass for North Cove (●) and South Cove (○). Data are standardised to an animal of 30mm test diameter and presented as mean ± SE, n = 10.month^{-1}. Horizontal bars indicate absence of feeding.

remained high throughout the four month 1997/98 summer feeding period before showing a similar trend of loss in mass during winter as displayed in the previous year. In the second winter South Cove urchins showed an identical pattern of gradual loss to a stable minimal midwinter value before a rapid summer rise.

There was no discernable change in gut tissue energy content throughout the 1997 austral winter (Fig 3.3), although a sharp rise did occur at the start of the 1997/98 summer. From a maximum value in December 1997 a regular decline took place throughout the following winter, and gut energy content did not recover by the end of the study. The variation over the whole study period was from 18 - 22 $kJ.g^{-1}$, a change over the yearly cycle of 18% of the maximum figure. This compares with a change of 63% in gut tissue mass, indicating that although changes in gut tissue energy concentration do occur, it was gut mass rather than gut tissue composition which had greater influence in determining whole organism energy status.

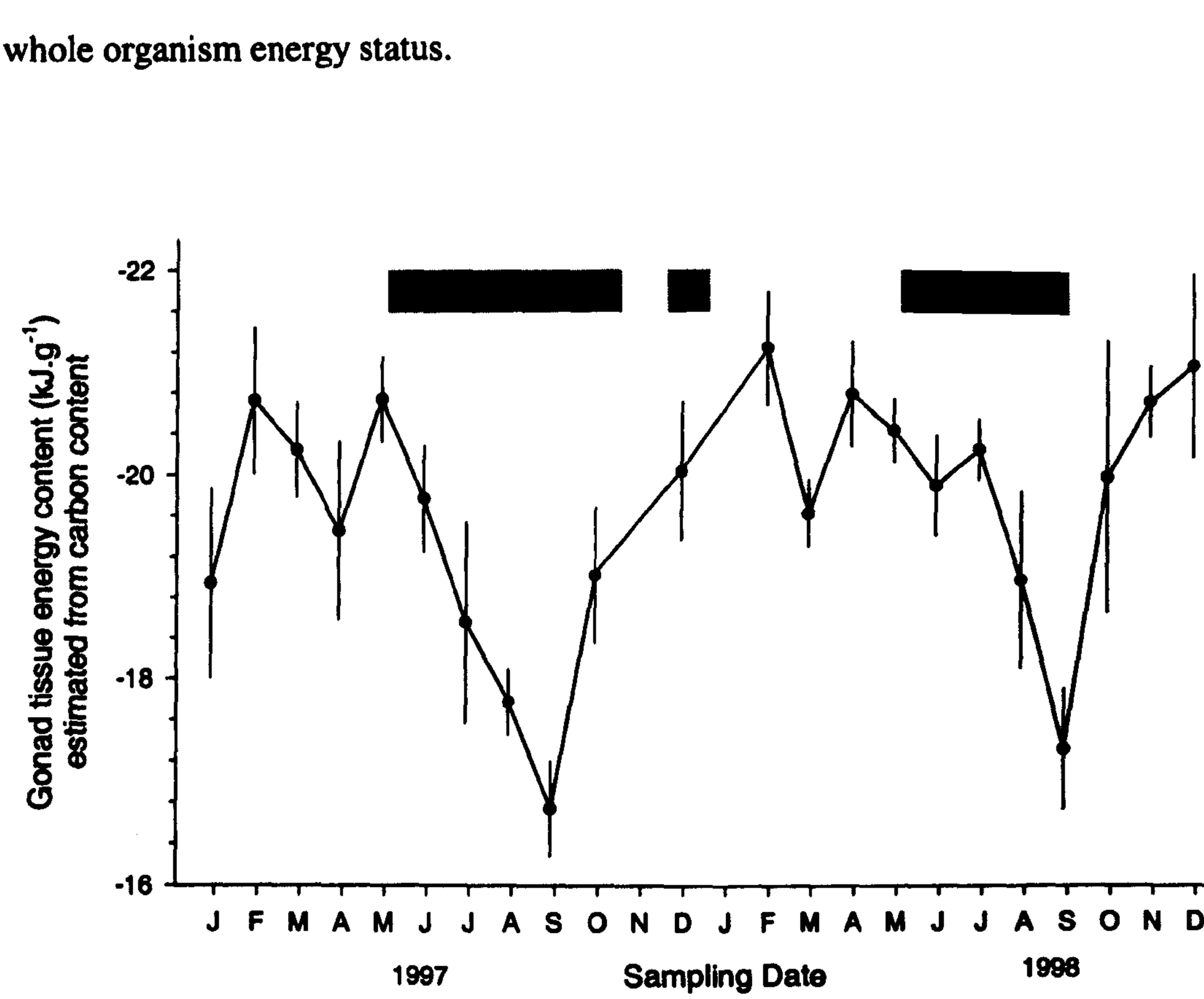


Figure 3.5 Gonad energy levels estimated from carbon content of North Cove samples. $n = 5$ per month, data are means ± standard error. Horizontal bars indicate absence of feeding.

Figure 3.4 illustrates gonad dry mass for a standard animal (30mm test diameter) rather than the more traditional gonad index (gonad mass as a function of total organism mass). This is because test diameter shows only slight variation over the year, whereas gonad index could be influenced by simultaneous changes in the mass of other tissues. The most striking aspect of these data are the large differences in the mass of gonad between the two sites. Throughout the entire duration of the study the gonads of the South Cove population were 2 to 5 times smaller than those from North Cove, and no strong seasonal mass cycle was apparent from either site. There was, however, an increase in gonad mass in the North Cove urchins between October 1997 and March 1998 (the austral spring and summer feeding period). The North Cove animals had a slightly higher mass of gonad tissue during the second winter when compared to the first, and a distinct rise was apparent at South Cove in November 1998 coincident with commencement of feeding.

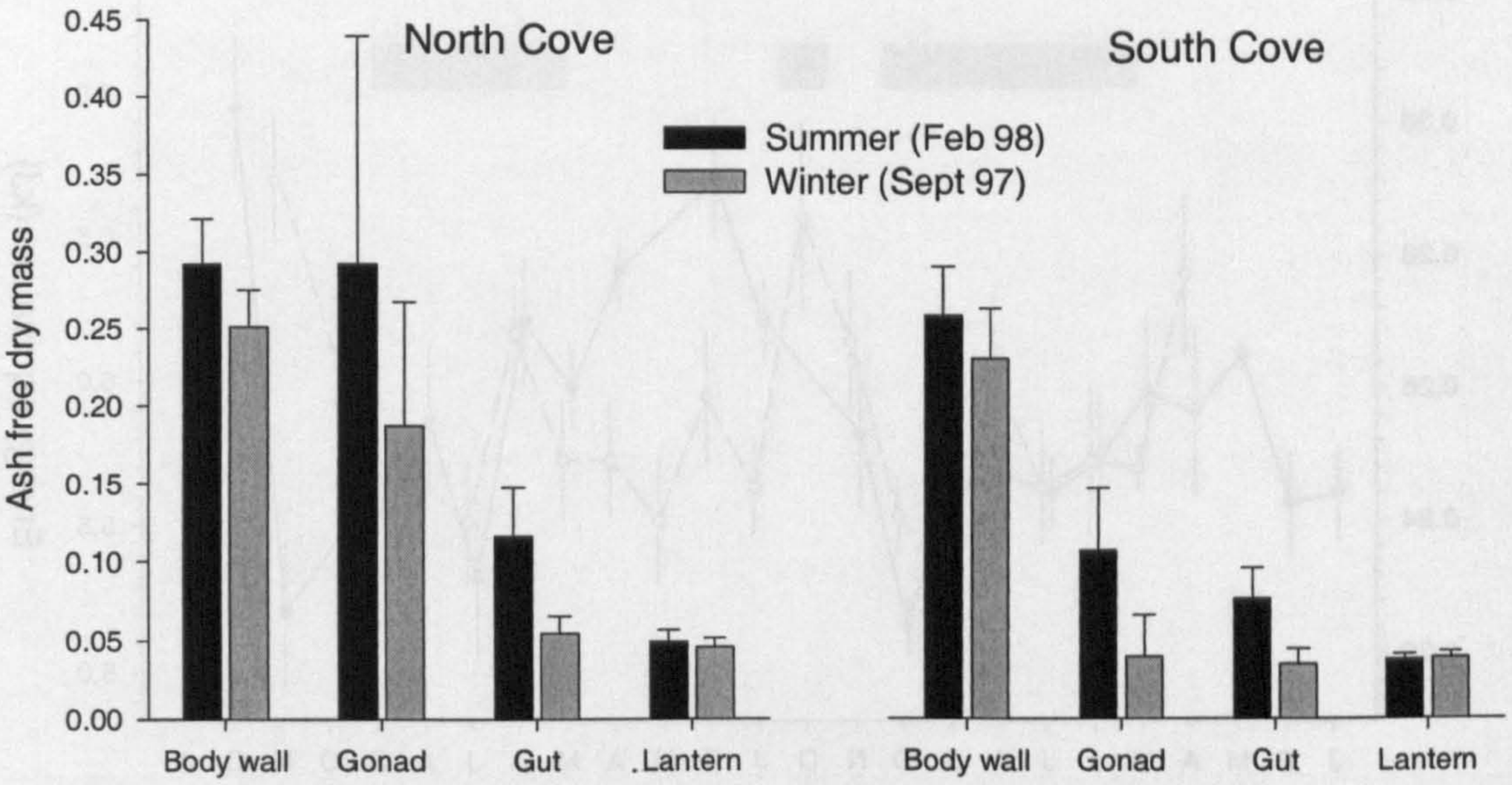


Figure 3.6 Relative contributions of each of the four major body components to total organic mass of the echinoid. Representative summer and winter samples (n=10, ±SE) are contrasted from both North and South Cove sites.

In contrast to the somewhat aseasonal pattern of gonad mass, gonad energy content (Fig. 3.5) declined markedly during the second half of both winters. In 1997 the mean value dropped continuously from 20.8kJ.g^{-1} in May to 16.8kJ.g^{-1} in September, and there was a similar fall from 20.4kJ.g^{-1} in July 1998 to 17.2kJ.g^{-1} in September 1998. The recovery to summer levels at the end of the winter took two to three months and was not as rapid as for either gut mass or energy content. In contrast to gut tissue, the energy content of the gonad was the main variable during the seasonal cycle, although considerably larger interannual and intersite variations in gonad mass affected total animal energy content.

Despite being formed predominantly (~80%) of skeletal carbonate, the echinoid body wall contains a large percentage of the total organic mass of the urchin (Fig. 3.6); variations in body wall biochemical composition and energy content will therefore exert a strong influence on that of the whole organism. The mass of organic tissue associated with

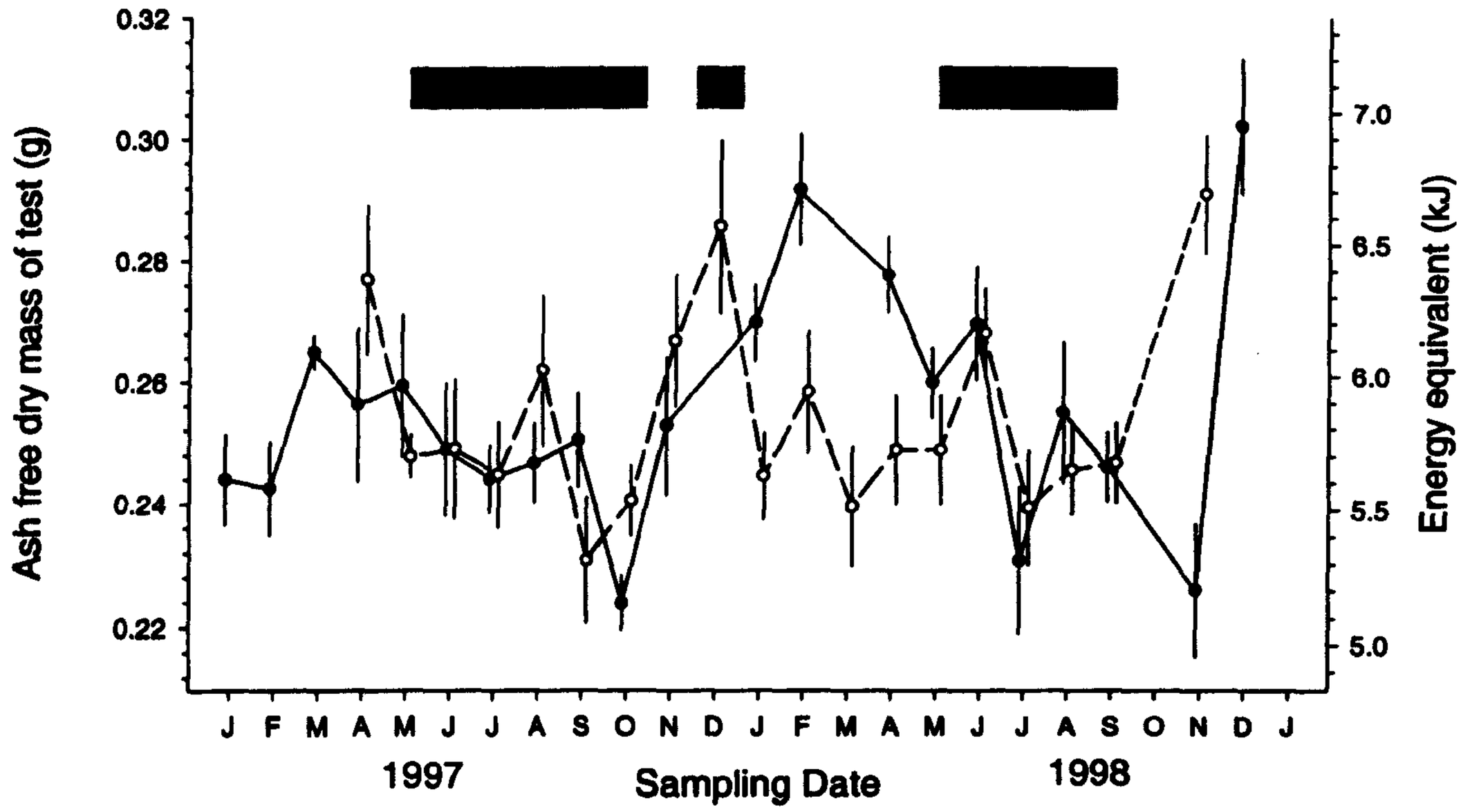


Figure 3.7 Mass of organic tissue (ash free dry mass) associated with the standard 30mm test diameter body wall for North and South Cove during the study period. n = 10.month^{-1} and are means ± SE. Horizontal bars indicate absence of feeding. ● = North Cove, ○ = South Cove.

the body wall far outweighed that associated with the gut, and was also considerably larger than gonad AFDM in urchins from South Cove. North Cove urchins had a higher body wall AFDM throughout the first half of 1998 (Fig. 3.7), although there were no pronounced intersite differences for the remainder of the study period. Both sites showed evidence of a reduction in test organic mass over the latter part of the winter period in both years, coupled with a sharp increase in November and December.

Organic content (AFDM) provides a valuable first order estimate of the relative investment in different tissues, but a much greater insight is provided by proximate or elemental composition. This is particularly so in respect of distinguishing mainly structural tissue (typically high in protein and so with a low C:N ratio). This study has used elemental analysis to estimate broad patterns of proximate composition, but comparison was also made with proximate composition measured directly for two tissues in January 1999 (Table 3.2).

Direct measures of proximate composition for marine invertebrates typically explain around 90% of the AFDM. Some organisms or tissues can however contain significant amounts of material which eludes direct proximate analyses, usually insoluble proteins, chitin and amino-polysaccharides (Clarke et al. 1992; 1994). Once allowance has been made for these, the remaining unexplained fraction comprises nucleic acids and small molecular weight metabolites.

Estimation of proximate composition from elemental analysis is based on stoichiometry - i.e. the quantitative relationship between elemental ratio present in dry

tissue and ratio of protein : carbohydrate : lipid (Gnaiger and Bitterlich 1984). It does, however, require knowledge of three variables: ash content, the extent of residual water retained in the oven dried tissue, and the fraction of non protein nitrogen in the total nitrogen pool. Ash can be measured directly, as can some components of non-protein nitrogen (for example chitin and nucleic acids). For this study ash content was measured directly, and as chitin is not present in echinoderms non protein nitrogen was taken to be 5%. Residual water was assumed to be 6% - the default value proposed by Gnaigler and Bitterlich (1984). The stoichiometric algorithm was then run iteratively with small deviations either side of the default values until the most consistent proximate composition was obtained (see Clarke et al. 1992; 1994 for examples of this process with both straightforward and difficult marine invertebrate tissue).

The comparison of proximate composition measured directly and estimated from elemental composition (Table 3.2) generally showed excellent agreement and explained effectively all of the observed dry mass. The significant exception was the gonad where direct measurement significantly underestimated protein in comparison with the stoichiometric assay. This may have been caused by interference in the assay by lipid, or a nitrogen rich component in the gonad not detected by the Lowry assay (as was found previously in squid tissue: Clarke et al. 1994). Notable results were the relatively high lipid content of the gut tissue (17%), suggesting a possible role in energy storage, and the high content of carbohydrate (16%) in the reproductive tissue in January 1999 (Table 3.2). There was very little variation in proximate composition with season for either gut (Fig 3.8) or gonad tissue (Fig 3.9). The carbohydrate content of the gut was low at all times, but appeared to show a minor peak around midwinter 1997. Lipid in gut tissue also decreased

at this time, but these patterns were not repeated in 1998. Protein also showed little variation over the study period, with the exception of a pronounced reduction in the early austral summer of 1997-98 which recovered to normal winter levels over the remainder of the summer. A more minor, but comparable drop in gut protein concentration was observed between November and December 1998. These trends were not reflected by the gonad tissue, where proximate composition was almost invariant with season.

Table 3.2 Proximate composition of gut and gonad tissue during January 1999. Protocols for direct analysis are outlined in the text, and proximate composition was also estimated stoichiometrically from elemental (C:H:N) composition according to Gnaiger and Bitterlich (1984). Mineral ash data were measured directly, whereas data for residual water and non-protein nitrogen (NPN) were set default values. Mean percentage water in fresh tissue found to be 80.1% (SD = 1.52) for gonad and 79.2% (SD = 2.07) for gut (n = 20 for both tissues). Data are presented as means, with standard deviation in brackets.

	PROTEIN (%)	LIPID (%)	CARBO-HYDRATE (%)	NPN (default estimate)	ASH (%)	WATER (%) (default estimate)	Total
GUT							
Direct	42.7 (3.0)	21.7 (2.8)	3.9 (0.8)	5	14.0 (1.9)	6	93.3
C:H:N	54.7 (2.8)	17.0 (3.9)	4.6 (5.0)	5	14.0 (1.9)	6	101
t	-7.65	2.42	-0.33				
p	0.0001***	0.052	0.76				
df	8	6	4				
GONAD							
Direct	19.6 (2.9)	16.7 (3.7)	19.0 (6.6)	5	10.4 (4.2)	6	76.7
C:H:N	40.9 (6.2)	24.3 (10.3)	15.8 (5.7)	5	10.4 (4.2)	6	100
t	-7.24	-1.59	0.97				
p	0.0019**	0.19	0.36				
df	4	4	9				

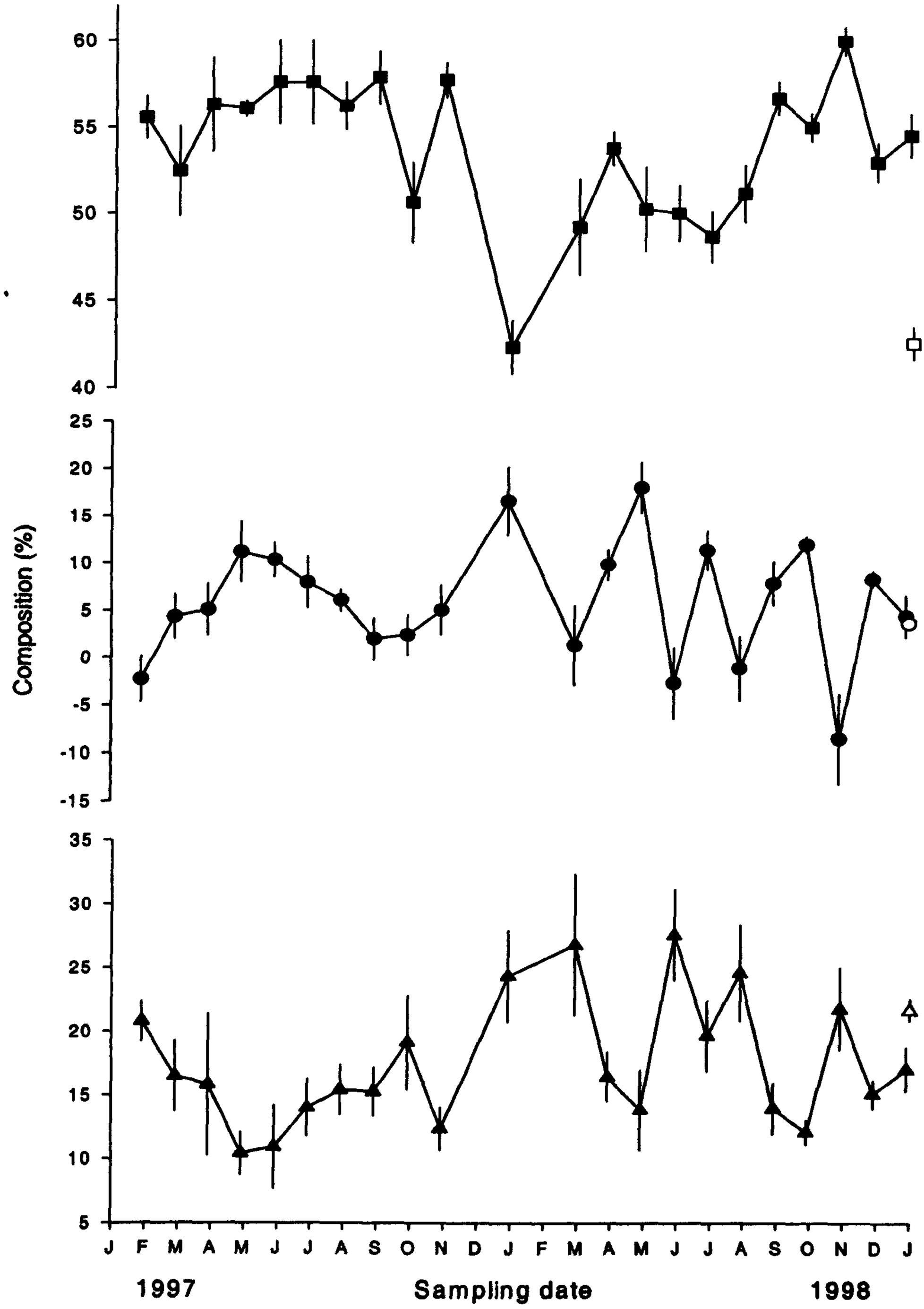


Figure 3.8 Monthly changes in proximate composition of *S. neumayeri* dry gut tissue, from both stoichiometric assay (filled symbols, n=5 per month) and comparative direct assay (hollow symbols, *n*=10). ■ = protein, ● = carbohydrate, ▲ = lipid. Data are means ± standard error. Note different scales.

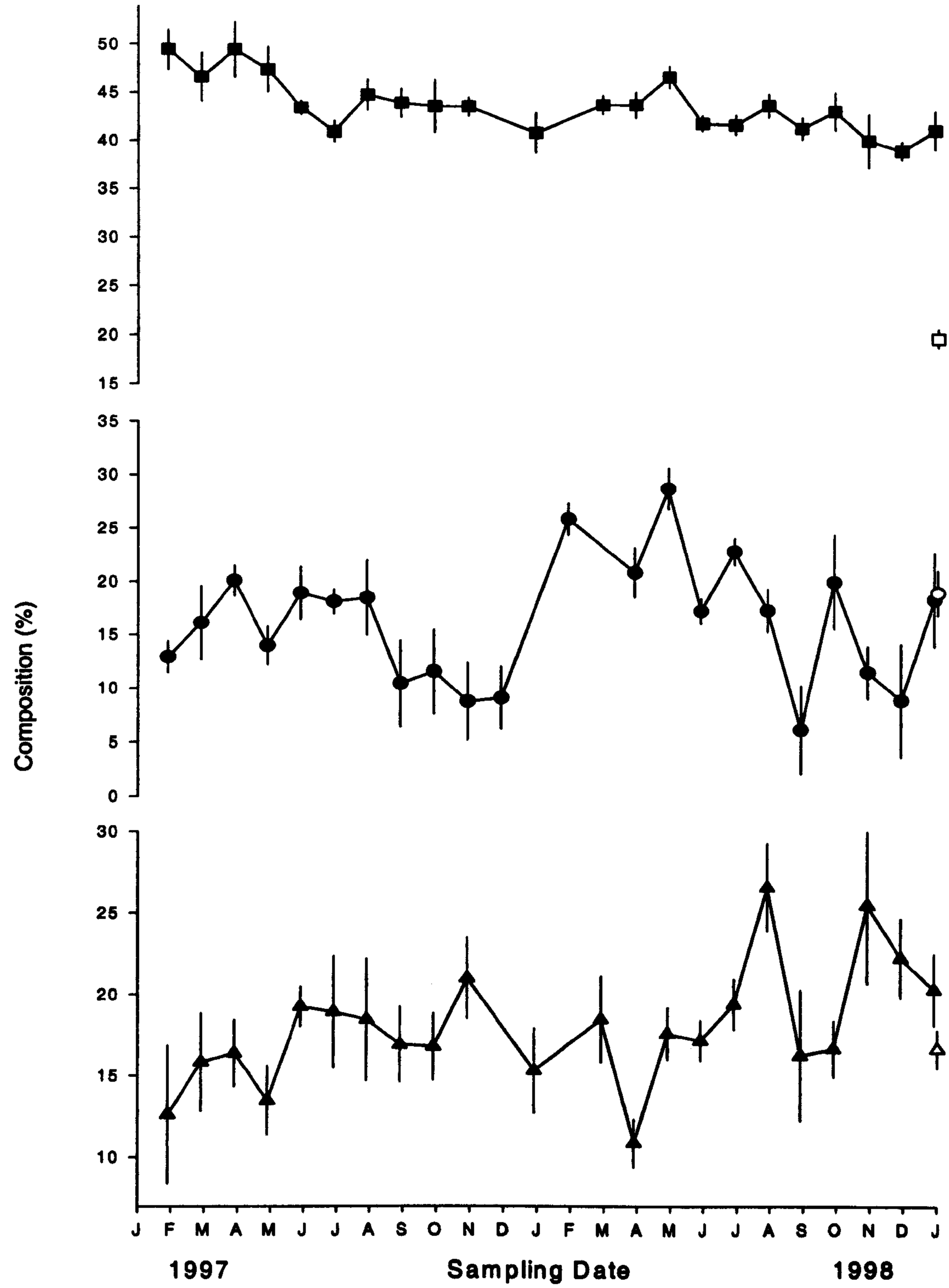


Figure 3.9 Monthly changes in proximate composition of *S. neumayeri* dry gonad tissue, from both stoichiometric assay (filled symbols, n=5 per month) and comparative direct assay (hollow symbols, n=10). ■ = protein, ● = carbohydrate, ▲ = lipid. Data are means ± standard error. Note different scales.

The Aristotle's lantern contributed only a small percentage to the total organic mass of the urchin (Fig. 3.6). The dry mass of this component showed a small degree of erratic variation over the annual cycle. Summation of all standard lantern dry masses for the entire period showed highly significant differences between the populations in North and South Cove (Student's *t*-test, $t = 6.95$, $p<0.0001$). The mean size corrected lantern dry mass of urchins from North Cove was 0.42g (SD ±0.049) and for South Cove was 0.39g (SD ±0.047).

3.4 Discussion

A comprehensive review of feeding in echinoids (De Riddler and Lawrence 1982) indicated that feeding rates vary with several factors including size, reproductive state, type of food and population density. Seasonal changes in feeding rates were also recorded but these were generally attributed to the effects of temperature rather than food availability. For *S. neumayeri* at North Cove, feeding activity was closely tied to sediment chlorophyll levels (Fig 2.2). Feeding activity was greatest during the summer months, and absent in winter when sediment chlorophyll concentrations were generally <20mg.m^{-2}. Feeding restarted earlier in the 1998 winter, and this appeared to be the result of a greater build up of microalgal production linked to the reduced sea ice cover relative to the 1997 winter. A strong sedimentation of ice associated autotrophic biomass in November 1997 caused minor feeding activity, which subsequently diminished until the breakout of the fast ice two months later. Although seasonal increase in light levels act to increase rates of autotrophic production, it is unlikely that light acts as a cue for increase sea urchin activity because light levels reaching the benthic environment are strongly controlled by sea ice extent. Also given the extremely small annual fluctuation in seawater temperature (~3°C) recorded

at the Rothera Station it is further unlikely that the strong seasonal variation in feeding activity of *S. neumayeri* was caused by temperature, and was far more likely to be related to changes in food quality and availability (see also Chapter 7). Some sea-urchins show faster rates of feeding when fed on low quality diets (Lares and McClintock 1991), and this may explain the higher rates of faecal egestion observed for the North Cove population which was taking a poor quality sediment diet. Ingestion of sediment either occasionally or as a matter of course has been widely reported for a number of species of regular echinoids, especially those from the deep sea (De Riddler and Lawrence 1982), and also for the Antarctic ophiuroid *Ophionotus victoriae* from various localities (Fratt and Dearborn 1984).

Variations in gut tissue mass under conditions of nutritive stress are well known, and have been recorded from field populations of *Stronglyocentrotus purpuratus* (Lawrence et al. 1965; 1966), *Mellita quinquiesperforata* (Moss and Lawrence 1972) and also from laboratory studies on *Lytechinus variegatus* (Klinger et al. 1988; Lares and Pomory 1998). In these studies gut tissue mass decreased rapidly to a minimal value and then remained constant as other body stores (gonad) reduced in mass. Bishop and Watts (1992) were able to show that the stomach constitutes the main storage organ of the gut, and both changes in cell number and cell size have been identified as being important during overall changes in mass. On this evidence the gut has been ascribed a role in short term nutrient storage (< four weeks of starvation). The patterns observed for *S. neumayeri* are in general agreement with this hypothesis, although the decline in gut mass took place as feeding intensity reduced, and continued for two months after feeding ceased. Reduction in mass of gut tissue at the start of the winter may be energetically

advantageous, since this will reduce the basal energy demand of the gut tissue for the remaining starvation period.

Although the primary function of gonad tissue in echinoderms is clearly reproductive, it may also play a role in nutrient storage (Lawrence and Lane 1982). Reproductive output in echinoids is closely linked to food availability (Chapter 5), and the variation in *S. neumayeri* gonad size between sites suggested a degree of small-scale spatial heterogeneity in food availability. For *S. neumayeri* the combination of a relatively minor spawning event coupled with gonad mass increase from the laying down of energetic reserves for the forthcoming winter caused mass reductions related to spawning to be masked.

Table 3.3 Comparison of energy reduction during the non feeding period of the austral winter for 1997 and 1998 (30mm test diameter sea urchin). Energy values (kJ) have been calculated as mass of tissue multiplied by energy concentration as determined by either carbon concentration (for gut and gonad) or by ash free dry mass using the energetic conversion of Brey et al 1988 for test and lantern.

	Energy Content of Tissues (kJ)			
	March 1997	November 1997	February 1998	October 1998
Gut	1.93	1.19	3.04	1.50
Gonad	6.28	4.73	6.77	7.43
Test	6.10	5.82	6.70	5.45
Lantern	1.16	1.22	1.13	1.13
Total body energy	15.47	12.96	17.64	15.51
	Austral winter 1997		Austral winter 1998	
Total energy used	2.51		2.13	
Total energy as a percentage of pre-winter value	16 %		12 %	

Although the test contains a greater absolute mass of organic material than the gut or in some cases the gonad (Fig. 3.6), its potential for nutrient storage in echinoids has been generally regarded as limited. Lawrence and Guille (1982) reported low levels of carbohydrate and lipid in a wide range of echinoderm body walls, and the absence of specialized nutrient storage cells would suggest that this tissue is not likely to play a significant role in energy storage. Nevertheless the seasonal pattern of test AFDM (Fig. 3.7) does indicate a role for this tissue in the provision of winter energy supply in *S. neumayeri*.

These data indicate that three of the four body components of *S. neumayeri* play a role in storage of overwintering energy reserves (Table 3.3). The total overwintering energy demand supplied by stored reserves was 2.5 kJ and 2.1 kJ in 1997 and 1998 respectively, which equated to 16% and 12% of total body energy value. The reduction in energetic status under starvation took place at a considerably slower rate than for species from lower latitudes; after eight weeks of starvation *L. variegatus* retained only 20% of its initial mass of gonad (Lares and Pomory 1998) whereas *S. neumayeri* showed no appreciable drop in gonad mass over the whole of the winter period. These differences reflect, at least in part, lower metabolic demands over winter in *S. neumayeri* (Chapter 4a) in comparison with echinoids from lower latitudes.

Proximate composition data have previously been reported for *S. neumayeri* (at McMurdo Sound) by Pearse and Giese (1967), and their estimates for lipid and carbohydrate agree well with those presented here. This proximate composition ratio also falls in broad agreement with the ranges of gut and gonad tissue of Antarctic echinoderms

reported by McClintock and Pearse (1987), although these authors comment that the ash content of the gut of two of the echinoid species presented by their study are unusually high (reflecting inefficient separation of gut contents from the gut wall before the assay) and this biased the percentage ratios of the organic components. *S. neumayeri* gonad carbohydrate is higher than reported for other Antarctic species, or echinoids in general (Giese 1966), though similarly high values have been recorded for *Mellita quinquiesperforata* (Moss and Lawrence 1972) and *Echinus esculentus* (Stott 1931). The cause of these occasional high values is not clear though they may be related to food quality. Comparison of tropical, temperate and polar echinoderm tissues led Lawrence and Guille (1982) to conclude that there is no evidence for latitudinal variation in biochemical composition within the Echinodermata, and the data reported here for *S. neumayeri* fit this general pattern.

Moss and Lawrence (1972) showed that although soft tissue lipid and protein underwent minor changes over the annual cycle for *Mellita quinquiesperforata*, carbohydrate in both gut and gonad tissue fluctuated markedly during the year and increased to annual maxima during the winter period. No such variability could be distinguished for *S. neumayeri*, and this suggests that stored reserves were being used in the same ratio as they are present in the tissues for the vast majority of the austral winter. However respiration and excretion data for this species (Chapter 4a) indicated an accelerated use of protein at the end of winter, and the sudden drop in gut protein concentration at this time suggests that this tissue provides metabolic reserves which are important in breaking the winter dormancy. Rapid recovery of the energetic concentrations of both gut and gonad tissue are apparent once feeding resumes. Fast regeneration of gonad tissue has also been recorded in *L variegatus* (Klinger et al. 1988; Lares and Pomory

1998), and may be of significance for the efficient utilisation of the brief summer peak in autotrophic production.

Differences in the absolute size of the Aristotle's lantern between field populations within a species have been recorded for *Diadema setosum* (Ebert 1980), *Echinometra mathaei* (Black et al. 1984) and *Sterechinus neumayeri* at McMurdo Sound (Brey *et al.* 1995). These studies have demonstrated that echinoids growing in areas of restricted food availability develop larger lanterns than do individuals from comparable populations in areas of more abundant food. The differences are assumed to be related to the more efficient grazing action associated with larger jaws. In this study *S. neumayeri* from North Cove had larger jaws when compared to animals of South Cove, and this may be related to the reduced quality of the sediment only diet at North Cove. Levitan (1991) suggested that within an individual, starvation increases the relative size of the lantern because test diameter shows a small decrease. Lewis et al. (1990) reported that deposition of labeled calcium to jaws was as great under conditions of starvation as in their control animals thereby confirming that it is relative rather than absolute lantern size which varies under starvation. Relative lantern size showed no seasonal variation in *S. neumayeri* despite variations in all other body components. This observation, coupled with only minor changes in gonad tissue and slow rate of depletion of gut tissue relative to tropical species undergoing starvation suggest that the austral winter is not a physiologically harsh period for this species. This is in contrast to some original anthropomorphic expectations that the long winter presented survival problems for polar species.

3.5 Conclusion

Seasonality of solar radiation increases with latitude, whereas both intensity of incident radiation and mean annual sea surface temperature decrease. These conditions form a natural gradient, and study at the endpoint of this gradient (the polar regions) helps provide strong tests of hypotheses concerning adaptation to these fundamental ecological factors. This study has shown that a common Antarctic echinoderm is able to survive long periods of starvation without either undue depletion of reproductive mass or body energy level. Although the prolonged cessation of feeding presents little problem for annual survival, the extended period of dormancy will undoubtedly have implications for polar life history strategies. In particular although the switch from summer to winter conditions is predictable there are annual variations in the timing, intensity and duration of summer food availability (Chapter 2, Clarke and Leakey 1996). These interannual variations require a flexible life-history strategy and reproductive ecology, whereby a poor season in one year can be offset by good seasons at other times.

Chapter 4a Seasonality of oxygen consumption and ammonia excretion.

Data also presented in: Brockington S & Peck LS (in press). Seasonality of respiration and ammonium excretion in the Antarctic echinoid *Sterechinus neumayeri*. Mar. Ecol. Prog. Ser.

4.1 Introduction

Antarctic marine ectotherms live at low but fairly stable temperatures (annual variation typically less than 3°C). Several investigations of individual taxa have shown that they exhibit low resting metabolic rates (White 1975; Peck et al. 1987; Davenport, 1988, Peck 1989; Chapelle & Peck 1995, Peck 1996; Ahn & Shim 1998). Comparative studies indicate a positive relationship between water temperature and resting metabolic rate (Luxmoore 1984; Clarke 1991; Peck & Conway 2000). Clarke & Johnston (1999) showed that a typical tropical fish at 30°C requires approximately six times more oxygen for resting metabolism as does a polar fish at 0°C (Q_{10}=1.83). The lowered metabolic rate of Antarctic ectotherms has been suggested to be a consequence of reduced basal costs, of which protein turnover appears to be a major component (Clarke 1998). Such reduced costs could influence relative growth efficiencies of polar species (Clarke 1987) and may also be significant for the survival of polar benthos during the energy limited austral winter (Chapter 2).

A consequence of reduced resting metabolic rates may, however, be a diminished capacity for power generation (aerobic scope) in polar species. The maximum level of activity attained by an organism is linked factorially to its resting metabolic rate, and for sessile or slow moving ectotherms is usually in the range ×2 to ×4 (Peck 1998). Because

of the underlying effect of temperature on resting metabolic rate, the same factorial increase will result in a greatly reduced absolute metabolic rise for a cold water species compared with a similar species from warmer water. In this context seasonal variations in metabolism of polar marine invertebrate populations are of considerable interest.

The aim of this study was to document seasonal variation in oxygen consumption (MO_2 μmol.h^{-1}) for the common Antarctic sea-urchin *Sterechinus neumayeri* over a two year period. This enabled comparison with echinoid oxygen consumption rates from temperate and tropical latitudes, and also provided a field-derived factorial and seasonal aerobic scope. Oxygen consumption was compared with loss of standard animal mass during the period of winter starvation via an oxycalorific coefficient. Rates of ammonium excretion (NH_4^+.μmol.h^{-1}) were also determined over the same period, and comparisons of atomic oxygen:nitrogen (O:N) ratios were used to give an indication of metabolic substrate utilised.

4.2 Methods

After collection specimens were returned quickly to the Bonner Laboratory, where they were carefully sorted and any adherent material removed. They were held in gently running seawater overnight in a flow through aquarium to allow for recovery from handling before metabolic measures were taken. Oxygen consumption and ammonium excretion rates were measured simultaneously on the same individuals to allow calculation of O:N ratios. The non feeding period for *S. neumayeri* each austral winter was between May and December 1997 and between May and September 1998 (Chapter 3). Specimens returned to the laboratory during this time showed very little locomotor activity or spine movement.

Measurements of both oxygen consumption (MO_2) and ammonium excretion were conducted monthly, using 24 individuals from North Cove and 8 from South Cove. A wide size range of sea urchins was assessed each month and overall the study size range was 3.8 - 59.9mm. After respiration and ammonium excretion measurements were completed, the test diameter of each individual was measured in three horizontal directions and a mean value calculated. Dry mass was then determined for whole animals using the entire body including gut contents after drying to constant mass at 60°C, and ash-free dry mass (AFDM) obtained by difference following ignition of the entire body (and gut contents) at 465°C for 22hrs. The biomass to diameter relationship (using AFDM for biomass due to the large percentage of inorganic material present in echinoids) was then calculated for each site for each month.

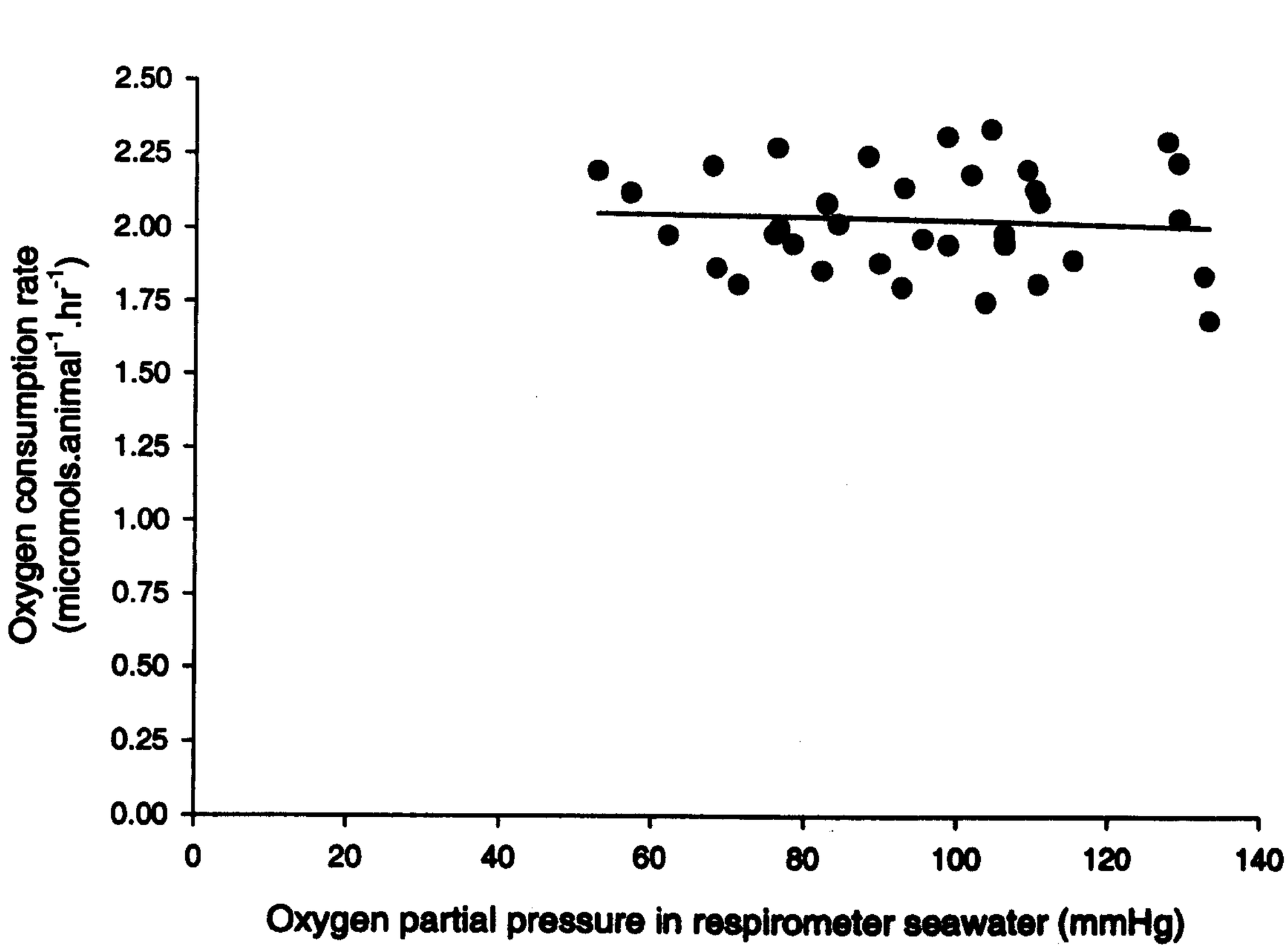


Figure 4.1 Oxyregulating behaviour of *S. neumayeri* between 140 and 50mmHg. The regression slope is not significantly different from zero ($F = 0.13$, $p = 0.723$).

MO_2 values were assessed using closed chamber respirometry techniques similar to those described by Chapelle & Peck (1995), and individual oxygen consumption was obtained by comparisons with control chambers (no animal). Three sizes of respirometer (60, 80 or 180ml) were used, and incubation times were adjusted to allow for variations in MO_2 due to body size and time of year. Two replicate measures of oxygen tension were made from each respirometer during the main study, and oxygen consumption was calculated after a correction of the respirometer volume for water displaced by the urchin. Chambers were inverted carefully three times at the end of each trial to ensure adequate mixing, and replicate 25 μl samples of water were withdrawn using a gas tight syringe through a septum and injected into a couloximeter (Peck & Uglow 1990) to measure oxygen content. A couloximeter is a fuel cell based system for measuring small quantities of oxygen in seawater, and this small sample method has an advantage over traditional polarographic systems which require animals to be held in relatively large volumes of seawater. This has important implications for the measurement of the very low MO_2 rates often exhibited by polar benthic invertebrates.

The relationship between oxygen consumption rate of *S. neumayeri* and environmental oxygen tension was initially investigated by placing five similarly sized sea urchins in respirometer chambers, and measuring rate of oxygen consumption at roughly hourly intervals. This initial experiment revealed that the oxygen consumption rate of *S. neumayeri* is independent of environmental oxygen tension down to 50mmHg, indicating that closed chamber respirometry methods are fully appropriate for this species (Fig 4.1). Consequently the oxygen tension in respirometer chambers was not allowed to fall below 100mmHg the main part of the study.

Immediately after oxygen measurements were completed the respirometer lid was removed and 10ml aliquots of water were transferred to duplicate teflon tubes (i.e. two tubes each containing 10ml) for ammonium analysis. Ammonium levels in the seawater were determined spectrophotometrically using the phenol hypochlorite method of Solorzano (1969) as modified by Liddicoat et al. (1975) and Clarke et al. (1994). The incubation stage of the assay was also modified according to Catalano (1987) by using teflon tubes and tungsten illumination. The assay was calibrated by spiking seawater with ammonium sulphate, and background ammonium concentration in seawater controls were subtracted from the experimental values. As for MO_2, ammonium excretion rates were calculated after correction of chamber volume for the displacement of the animal.

Data were analysed using multiple regression and ANOVA, performed using Statistical Analysis Software (SAS) Version 6.12 for Windows.

4.3 Results

A total of 723 measurements of echinoid ash free dry mass (AFDM) were made during the 25 month study period, over a total size range of 3.8 to 59.9 mm test diameter (mean = 24.4 mm, SD = 8.6 mm). Data were expressed for a standard animal of 24.4 mm test diameter which was the mean size of sea urchins used. Standard animal values were obtained following double $\log_e$ transformed regressions of AFDM vs test diameter (Fig 4.2).

Multiple regression and ANOVA provided a good fit to both the North and South Cove data (overall fits: North Cove $F = 2653$, $p < 0.0001$; South Cove $F = 1468$,

p<0.0001) and indicated that echinoid AFDM was higher in 1998 than 1997 at both sites (North Cove F = 4.086, p = 0.044, South Cove F = 4.194, p = 0.042). The same technique also indicated that the North Cove sea urchins had a significantly greater AFDM in both years than the South Cove sea urchins (F=26.89, p<0.0001)

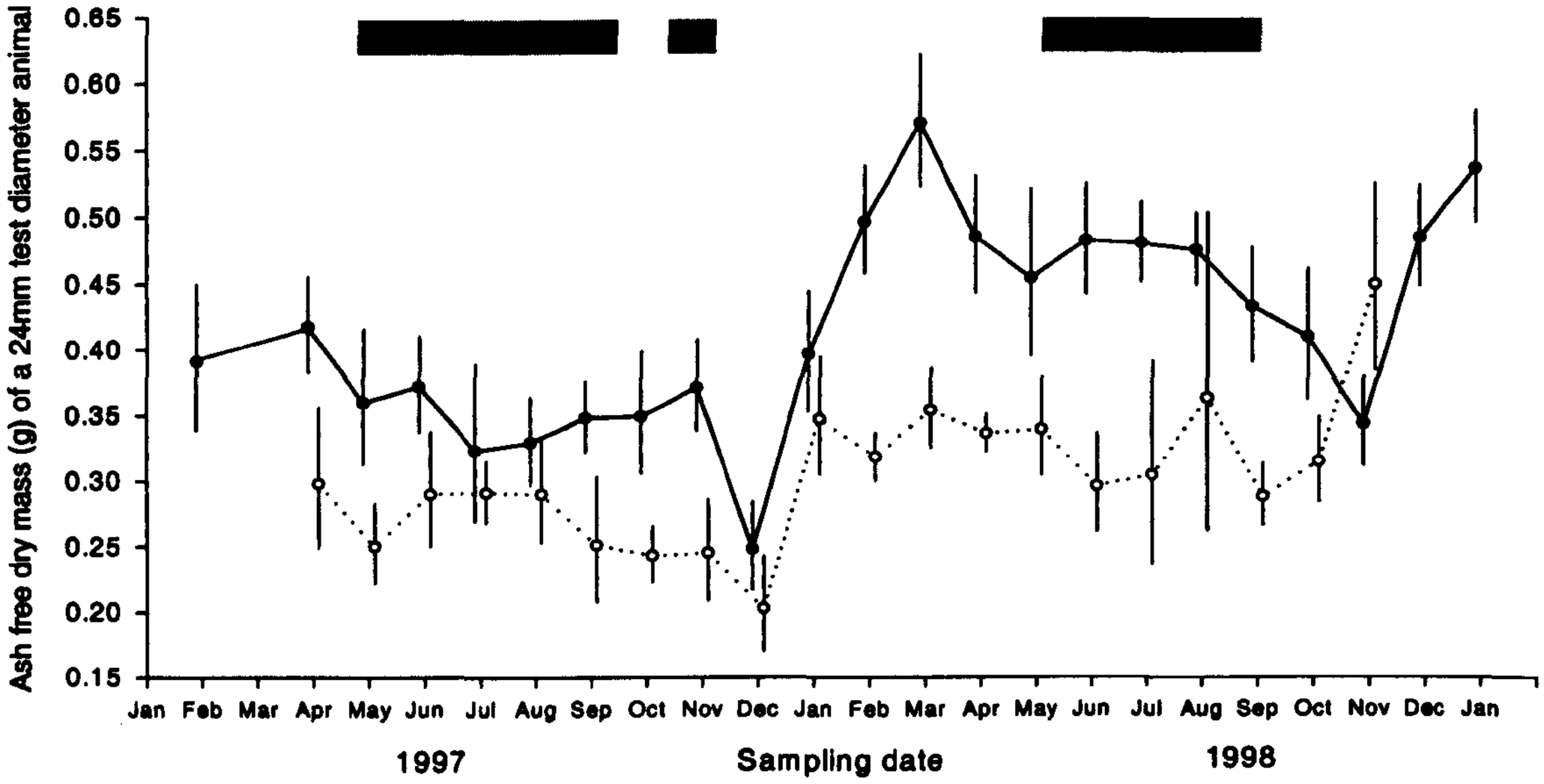


Figure 4.2 Organic mass (AFDM) of a standard 24mm test diameter urchin from North Cove (●) and South Cove (○). Plotted results are from solved regressions of $\log_e$ transformed data for mass against test diameter (n = 24.month^{-1} North cove, n = 8.month^{-1} South Cove). Error bars represent 95% confidence intervals of the regressions. Shading represents non feeding period.

Changes in echinoid AFDM during the non feeding period were estimated from the multiple regression and ANOVA model. In May 1997 the AFDM of a standard 24.4 mm test diameter animal at North Cove was 0.362 g, which had reduced to 0.345 g by October (a drop of 0.003 g.month^{-1} over the winter non feeding period). For 1998 (North Cove) the reduction between May and September was from 0.508 g to 0.433 g for the same test diameter animal (0.019 g.month^{-1}). For South Cove, the reduction in mass over the same periods was 0.006 g.month^{-1} and 0.011 g.month^{-1} for 1997 and 1998 respectively (Table 4.1).

Table 4.1 Comparison of energy available from loss of standard (24mm test diameter) sea urchin ash free dry mass during non feeding period of austral winter with metabolic energy use (calculated via an oxycalorific coefficient from winter respiration rate).

Winter	Site	Non feeding period * months	Reduction in AFDM g.month $^{-1}$	Energy liberated J.month $^{-1}$	Mean molar oxygen consumption (Winter) μmols.month $^{-1}$	Heat dissipated J.month $^{-1}$
1997	North	6	0.003	64	336	145
	South	6	0.006	138	236	102
1998	North	4	0.019	432	471	204
	South	4	0.011	242	418	181

* Data from Chapter 3

Standard animal oxygen consumption rates rose rapidly at the end of the austral winter period and coincided exactly with the onset of the phytoplankton bloom. It then remained high during the feeding period described in chapter 2 in both years (Fig 4.3).

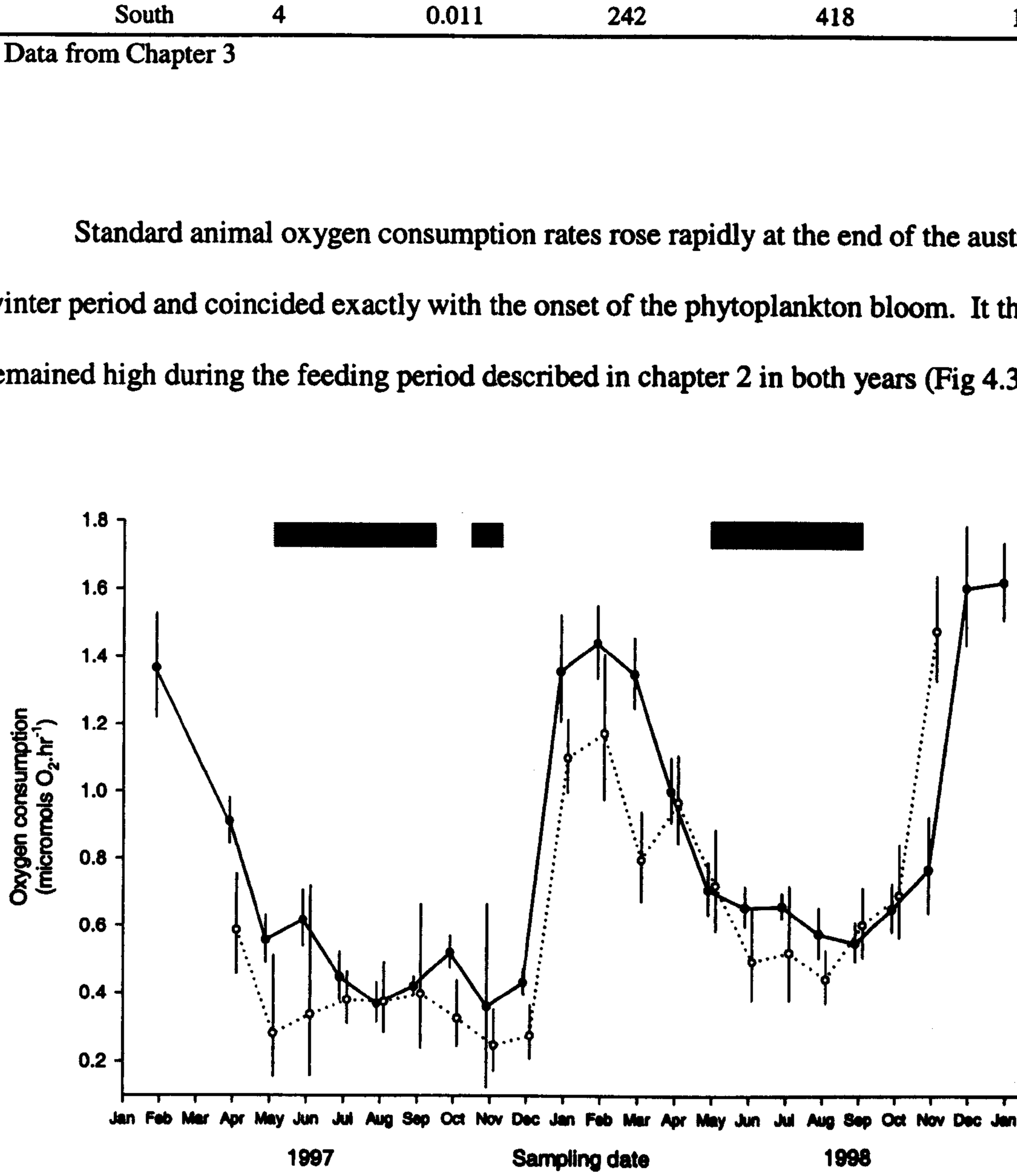


Figure 4.3 Oxygen consumption rate of a 24mm test diameter sea urchin from North Cove (●) and South Cove (○). Data are from solved regressions of $\log_e$ transformed data for oxygen consumption rate against test diameter (n = 24.month^{-1} at North Cove and 8.month^{-1} at South Cove). Error bars represent 95% confidence intervals of the regressions. Shading represents non feeding period.

Rates of oxygen consumption decreased after the summer peak as feeding activity and phytoplankton standing stock also reduced, and minimum winter MO_2 values (coincident with non feeding state) were recorded for seven months in the 1997 winter and six months in 1998 between May and November. As for echinoid biomass, oxygen consumption was adequately described by a model using multiple linear regression and ANOVA on $\log_e$ transformed data ($F = 737.2$, $p < 0.0001$). Overall oxygen consumption was significantly lower at South Cove than at North Cove ($F = 42.41$, $p < 0.0001$). For North Cove, the highest recorded standard animal values were 1.44 μmols $O_2.hr^{-1}$ in February 1998, and 1.62 μmols $O_2.hr^{-1}$ in February 1999. Mean winter MO_2 values were estimated by calculating the mean of the standard animal values for the months between May and December in 1997 and May to November in 1998: North Cove = 0.46 μmols $O_2.hr^{-1}$ in winter 1997, and 0.65 μmols $O_2.hr^{-1}$ in winter 1998. Thus the factorial increase in

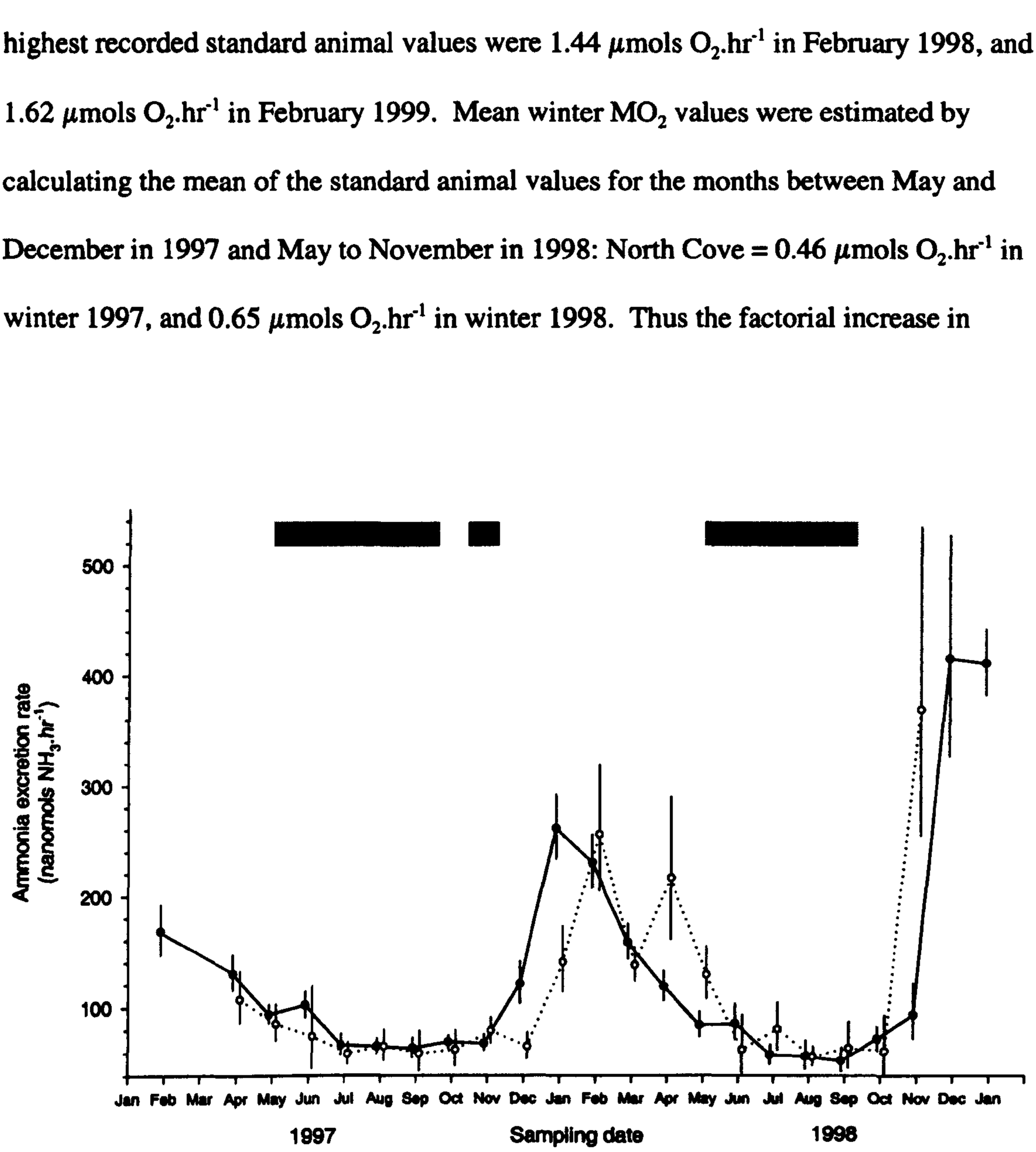


Figure 4.4 Ammonia excretion rate of a standard 24mm test diameter sea urchin from North Cove (●) and South Cove (○). Data presented as for Fig 4.3. Shading represents non feeding period.

metabolic rate between winter and summer was ×3.1 for 1997, and ×2.5 for 1998 at North Cove. Factorial increases for South Cove calculated in the same way are of similar magnitude, at ×3.6 for 1997 and ×2.6 for 1998.

Strong seasonal and interannual variation also occurred in ammonium excretion (Fig 4.4). The multiple regression and ANOVA model again provided a good fit to the data ($F= 280.6$, $p<0.0001$) but indicated no significant differences between sites ($F=0.328$, $p=0.567$). Large between year differences were evident in the magnitudes of the summer maxima recorded (262 nmols NH_4^+.standard animal^{-1}.hr^{-1} in the 97/98 summer, and 415 nmols NH_4^+.standard animal^{-1}.hr^{-1} in the 98/99 summer). Winter excretion levels were very similar for both years (mean = 76.6 nmols NH_4^+.standard animal^{-1}.hr^{-1}).

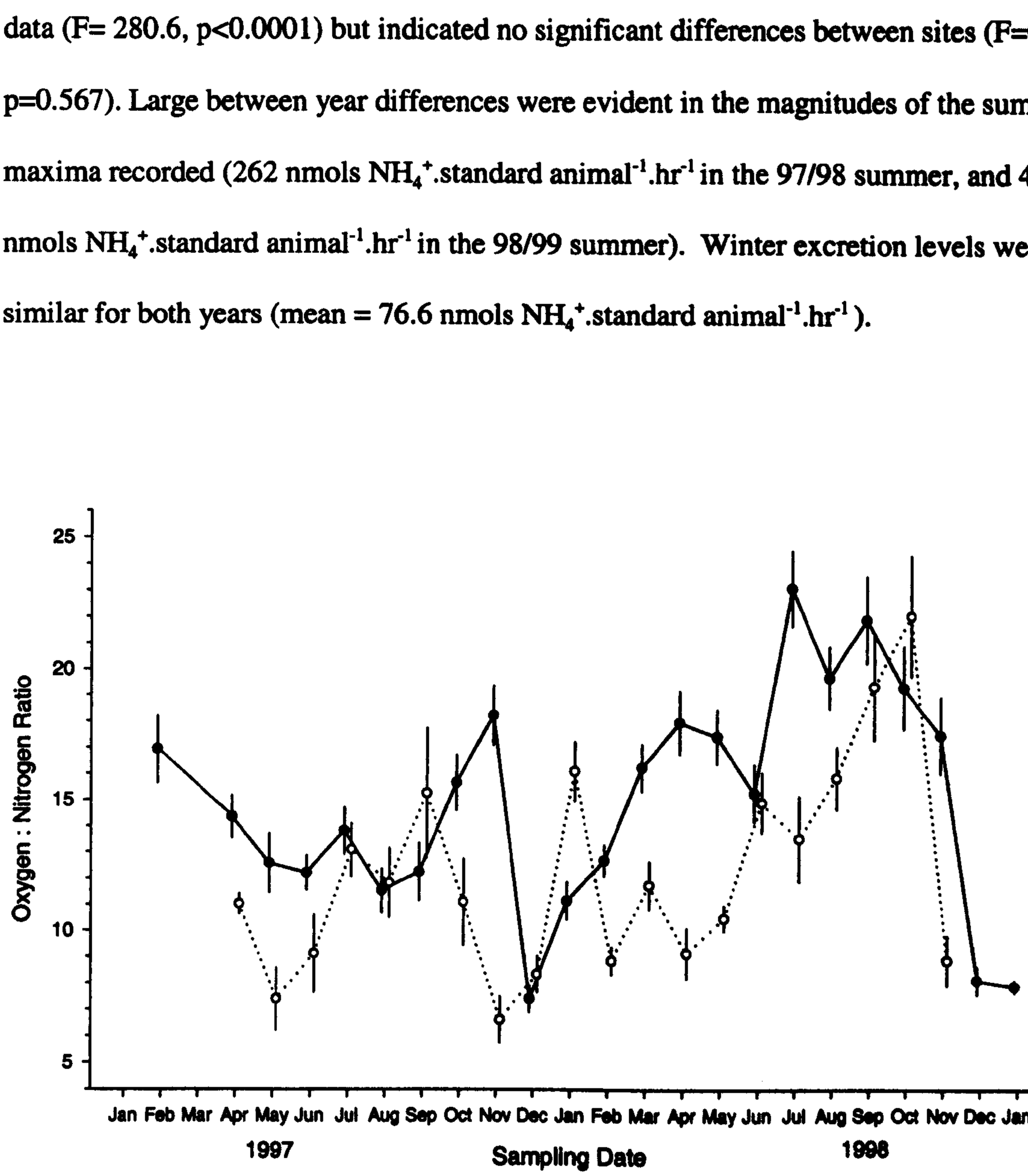


Figure 4.5. O:N atomic ratios calculated for North Cove (●) and South Cove (○). O:N ratio was calculated as nanogram-atoms of oxygen consumed per individual per hour divided by nanomols of ammonium excreted per hour. To convert from µmols oxygen to ng-atoms multiply by 2000.

Regressions of O:N ratio against echinoid diameter were not significant for any of the months investigated. The O:N ratio was always in the range 5 to 26 (Fig 4.5). The peak in ammonium excretion at the end of the austral winter period took place before the peak in MO_2 and hence the lowest ratios (7 - 8) were recorded early in the austral summer (December) at the end of both 1997 and 1998. These lows were followed by small rises during the remaining summer and winter periods. This pattern was clearer at both sites during the 1998 winter than in 1997.

4.4 Discussion

The oxygen consumption rate of *S. neumayeri* displayed prominent summer peaks and prolonged winter minima, which closely reflected phytoplankton abundance in both form and timing (Figs 2.1 & 2.2). *S. neumayeri* is a generalistic feeder (McClintock 1994), and such a strategy has been suggested to increase fitness by allowing feeding over a much longer period (Dell 1972). Echinoids from the North Cove study site were observed to feed only on sediment, whereas those from South Cove had a more varied diet including sediment, macroalgae, bryozoan fragments, crustaceans and also seal faeces. Despite these differences in diet urchins at both sites fed only during the period of the pelagic bloom, and indicated that *S. neumayeri* was reliant on downward flux and advection of settled phytoplankton for a substantial part of its energy intake (Chapter 3). The strong annual variability in oxygen consumption was shown by echinoids from both populations, despite the broader diet of the South Cove population. This indicated that a greater breadth of diet did not support higher metabolic rate beyond the duration of the summer phytoplankton bloom.

Feeding ceased in *S. neumayeri* for a six month period in winter 1997, and for four months in winter 1998 (Chapter 2). During these times metabolism must have been fueled by body reserves. Data presented here indicate that mass was lost by a standard 24.4 mm urchin in the non feeding winter period at a rate of 2.8 - 18.8 mg.month^{-1}. The strong contrast in standard animal AFDM between sites was related to differences in reproductive condition of the two populations: individuals from the North Cove site exhibited a higher gonad index (~ ×3) than those from South Cove despite the narrower diet (Chapter 6). Release of gametes took place at the start of the austral summer, indicating that over wintering reduction in biomass resulted from metabolic demands alone. The energy content of macrobenthic aquatic invertebrate tissue has been summarised by Brey et al. (1988), who suggest that a conversion factor of 23 kJ.g^{-1} AFDM may be used to convert mass data to energy. Using this conversion, the amount of energy liberated by the reduction of standard animal biomass in the winter non-feeding period was between 64.4 and 432 J.month^{-1} (Table 4.1).

The power generation during the non feeding period of the austral winter may also be calculated from MO_2 via the use of an oxycalorific coefficient. Brafield & Llewellyn (1982) provide data for oxycalorific coefficients for different metabolic substrates. An appropriate conversion is 0.434 J.μmol O_2 based on the substrate utilisation indicated by mean winter O:N ratio. These data are compiled for periods when feeding is entirely absent. The general agreement of these two energy use estimates (mass loss and MO_2: Table 4.1) suggest that it is unlikely that the echinoids derived any significant nutritional benefit from other sources (e.g. dissolved organic compounds) during the austral winter. Rate of loss of AFDM by *S. neumayeri* during the non-feeding periods is low. Lares &

Pomory (1998) demonstrated that *Lytechinus variegatus* lost 30% of initial total organic content during ten weeks of starvation, and that the maximum time *L. variegatus* can survive without food is ~90 days. By contrast, *S. neumayeri* from the North Cove site lost 4.6% and 14.8% of initial AFDM during the 1997 and 1998 winters.

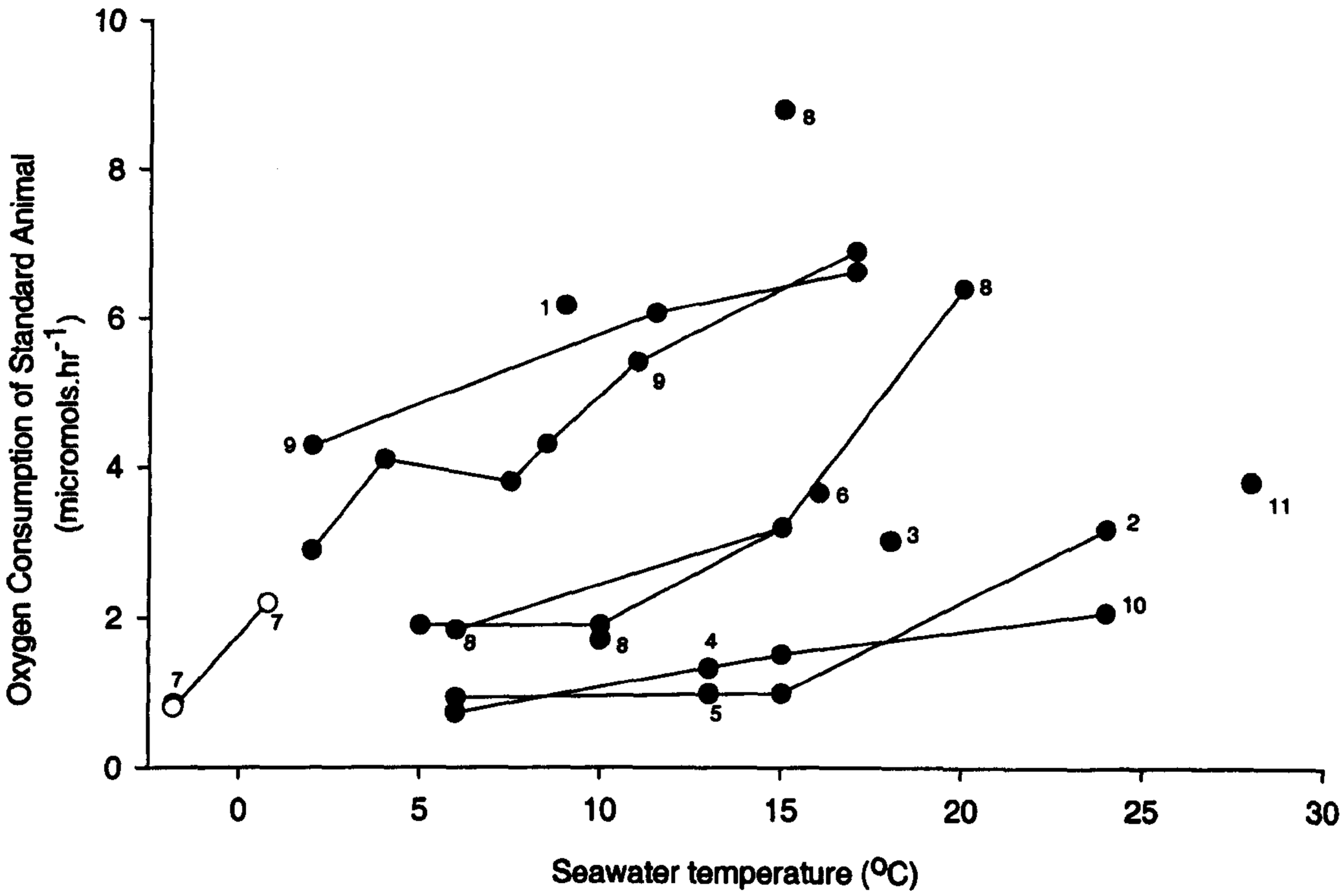


Figure 4.6 Plot of respiratory rate of echinoids against environmental temperature. Data compiled from Féral & Magniez (1988) and from summary in Lawrence & Lane (1982). 1) *Abatus cordatus*; 2) *Allocentrotus fragilis*; 3) *Arbacia lixula*; 4) *Evechinus chloroticus*; 5) *Goniocidaris umbraculum*; 6) *Lytechinus anamesus*; 7) *Sterechinus neumayeri*; 8) *Stronglyocentrotus purpuratus*; 9) *Stronglyocentrotus droebachiensis*; 10) *Stronglyocentrotus francisanus*; 11) *Tripneustes ventricosus*. Data converted to μmols oxygen.hr $^{-1}$ (where necessary) and corrected to a standard animal of test diameter 30mm, wet mass 5.5g, dry mass 2.3g or AFDM 0.7g. Single data points indicate individual determinations for a species at ambient temperature, points linked by lines indicate studies for a single species over a range of temperatures. Data for *S. neumayeri* from this study shown in unfilled symbols (○).

Antarctic teleost fish (Clarke & Johnston 1999) and bivalve molluscs (Peck & Conway 2000) have lower metabolic rates than similar species from temperate or tropical environments. The metabolic rates found here for *S. neumayeri* are at similar levels to those recorded by Belman & Giese (1974) working on the same species, although their use of wet mass precludes accurate comparison with the data presented by this study. Although the scatter in the data is wide, the respiratory rate of echinoids generally increases with temperature (Fig 4.6) and *S. neumayeri* has one of the lowest rates reported for any sea-urchin. This finding agrees with that of Féral & Magniez (1988) who also showed a decrease in metabolic rate for echinoids with decreasing environmental mean seawater temperature. Such findings contrast with predictions that the mitochondrial proliferation observed in polar species (Johnston et al. 1994, 1998, Guderley 1998) should lead to elevated resting metabolic rates due to increased costs of mitochondrial maintenance in low temperature species (Pörtner et al. 1998). The reduced whole organism metabolic rate in comparison with warmer water species helps to explain the considerably reduced rate of consumption of body reserves under polar conditions.

Aerobic factorial scope is the maximum increase in aerobic metabolic rate over resting displayed by a species. Basal metabolic rates are reduced under polar conditions, and consequently a fixed factorial scope will result in a greatly reduced absolute power generation for cold water species when compared to similar warm water forms. The specific dynamic action of feeding (SDA) response may be indicative of maximum metabolic (aerobic) capacity for sessile or sluggish marine ectotherms (Peck 1998). The rise in metabolic rate following consumption of a meal is associated with the costs of processing the meal and consequent growth, and in echinoids may include an anaerobic

component because of a poor internal circulatory system (Bookbinder & Shick 1986). SDA factorial responses are independent of habitat temperature, although under polar conditions reduced resting metabolic rates cause a decrease in absolute power generated at the height of the SDA. As a consequence the duration of the response is extended and this allows the total size of the SDA to remain constant across the latitudinal temperature range. Peak factorial scopes determined from investigation of the SDA response are in the range 1.6 to 2.5 times pre-feeding levels for sedentary polar species (Peck 1998). These estimates correlate well with the ×2.4 to ×3.8 seasonal rise in metabolism for *S. neumayeri* provided by this study.

Despite their inherent stenothermy members of the Antarctic benthos show normal responses to temperature (measured as Q_{10}) over the range they normally encounter (White 1975, Ralph & Maxwell 1977, Ikeda & Hing Fay 1981, Peck 1989, Pörtner et al. 1999). These Q_{10} values are typically in the range 2 - 3, and may be applied to the winter resting metabolic rates derived by this study to estimate the increase in basal rate which may be expected simply as a result of the warmer seawater temperatures of the austral summer. Assuming a Q_{10} of 2.5, the change in temperature from -1.8° C to +1.2°C recorded at Rothera during this study would result in a summer rise over winter metabolic rates of ×1.3; compared with observed rise in field rates of ×2.4 and ×3.8 in 1997 and 1998 respectively at North Cove. Accurate calculation of the summer basal metabolic rate is confounded by concomitant changes in mass of the standard sized animal in the transition to the summer period. Without correcting for mass, the rise of ×1.3 caused by temperature accounts for 13% of the summer respiratory rise in 1997/8 (compared to 1997 winter) and 21% in 1998/9 (compared to 1998 winter) at North Cove. The data therefore support the

conclusion of Clarke (1993) and also data presented in Chapter 7 in that the slight seasonal elevations in temperature encountered at polar latitudes have only a minor influence upon the seasonal oxygen demand.

Although many echinoderms excrete some nitrogen as urea, echinoids are predominantly ammonotelic (Jangoux 1982, Stickle 1988). The major route for ammonium formation is through catabolism of both ingested and cellular protein. A seasonally changing pattern of ammonium excretion in relation to food availability was recorded for the ophiuroid *Ophiothrix fragilis* by Davoult et al. (1991), who found a ×2.7 increase in rate of ammonium excretion in summer compared to winter. Nitrogen excretion has been more intensively studied for bivalve molluscs, where again the major nitrogen product is ammonium. Significant seasonal changes in ammonium excretion were recorded for the temperate bivalve *Mytilus edulis* by Bayne & Scullard (1977). These were interpreted as responses to the seasonal gametogenic cycle and of the metabolic effects of changes in ration size and quality. For *S. neumayeri* the very strong seasonal cycle of excretion provides a second method for evaluating the effects of intense seasonal food limitation under polar conditions. As for respiration, excretion of ammonium by *S. neumayeri* increases during the summer, although the timings of the changes in ammonium excretion do not exactly coincide with changes in oxygen consumption, giving rise to a seasonal variation in O:N ratio.

Theoretical O:N ratios were calculated by Ikeda (1977) as 415 to oxidise pure lipid, falling to 6.8 for pure protein; intermediate values around 25 indicate 50% protein utilisation. Values less than 7 were found by Peck et al. (1987) and Peck (1996) for

Antarctic brachiopods. Values down to 3 may be explained by differences in the C:N ratio of the particular protein (Mayzaud & Conover 1988). Some further additional variation will exist if nitrogen excretion and oxygen consumption for a given activity or process do not occur simultaneously.

The strikingly low O:N ratios encountered in both years in the early part of the austral summer are indicative of a metabolic substrate derived almost solely from protein. During the winter period the O:N ratio rose slightly suggesting that although protein was still providing the major metabolic substrate lipid or carbohydrate began to play an increasing role as winter progressed. A similar profile of gradually rising O:N ratio under prolonged starvation has also been recorded for other polar invertebrates, e.g. the limpet *Nacella concinna* (Clarke et al. 1994). Proximate composition estimated stoichiometrically from tissue elemental ratios (C:H:N) showed that both gut and gonad tissue were dominated by protein (54.7% and 40.9% respectively: Chapter 3). Gut tissue also showed notably high concentrations of lipid (17%), and this tissue also underwent the greatest loss in mass during winter relative to its initial pre-winter value. Gonad contained 24% lipid and15.8% carbohydrate at the start of winter, and it is likely that both gut and gonad tissue supplied energy to fuel metabolism during the non feeding period.

4.5 Conclusion

The metabolic rate of *S. neumayeri* shows a strong seasonality and is correlated to the intense summer phytoplankton bloom development rather than seawater temperature. The respiratory rate is one of the lowest recorded for regular echinoids. This reduced rate may be of importance to allow efficient usage of body reserves during the extended non-

feeding period of the austral winter. Seasonal factorial aerobic scope is comparable with aerobic scopes recorded for other Antarctic marine invertebrates. The low basal aerobic rate confers a constraint on the total power that may be generated in comparison to species from lower latitudes. O:N ratio indicated that although protein is the main metabolic substrate lipid and carbohydrate became more important as winter progressed. A sharp decrease in O:N ratios to around 7 indicated a change to a 100% protein based metabolism at the onset of the summer season.

Chapter 4b The effects of temperature on the metabolism of winter conditioned sea urchins

Data also presented in: Brockington S and Peck LS (submitted) Temperature induced changes in oxygen consumption and supply in winter conditioned *Sterechinus neumayeri* (Echinoidea: Echinodermata) from the Antarctic. Polar Biology.

4.6 Introduction

The high Antarctic marine ecosystem exists in one of the most thermally stable environments on Earth - at 585m depth at McMurdo (78°S) the annual temperature variation is just ±0.07°C around a mean of -1.89°C (Picken 1984). In the seas around Antarctica the annual temperature range increases slowly with latitude, and at Rothera (67°S) is only 3°C . The Antarctic marine benthos have therefore evolved to live in an environment with an exceptionally high degree of thermal stability, and as a consequence many species are stenothermal. Pörtner et al (1999) studied temperature responses of the bivalve *Limopsis marionensis* from the Weddell Sea (normal temperature range -2.2°C to +0.5°C), and found that a temperature elevation to just +2°C caused a continued net ATP depletion and the onset of long term stress. Other Antarctic species also show a similar degree of stenothermy (e.g. the brachiopod *Liothyrella uva* (Peck 1989), but notable exceptions to this trend are found in littoral regions, e.g. the limpet *Nacella concinna* which is able to withstand a relatively much broader temperature regime. However, despite the inherent stenothermy Antarctic benthic species show metabolic responses to temperature changes within the normal environmental range which have Q_{10} values in the range 2 - 3 (White 1975, Ralph and Maxwell 1977, Ikeda and Hing Fay 1981, Peck 1989, Clarke 1993).

For marine invertebrates critical sub-lethal temperatures have been identified at both high and low extremes (Zielinski and Pörtner 1996; Sommer et al. 1997; Pörtner et al. 1998). These temperatures are characterised by the onset of anaerobic metabolism, and are caused by a failure of the oxygen ventilation mechanism. Above the upper critical temperature (Tc_{II}) mitochondrial oxygen demand becomes excessive, the mechanism of ventilation and circulation becomes insufficient to meet this demand and a transition to anaerobis is observed. At low critical temperatures there is insufficient aerobic capacity to meet energy requirements. Low critical temperatures have been observed for temperate species, although evolutionary pressures have depressed the lower critical temperature of polar species so far studied to below the freezing point of seawater (Pörtner et al. 1999). The critical temperatures have been observed to shift within species both as a result of latitude and also of seasonal acclimation to temperature, and such shifts are associated with concomitant changes in mitochondrial density.

This experiment has set out to expose winter conditioned (i.e. non feeding) *S. neumayeri* to stepwise rises in temperature both within and above its normal environmental range to measure thermally induced changes in metabolic rate. Perivisceral coelomic oxygen partial pressures have also been determined in relation to the induced rises in metabolic rate so as to observe the efficiency of the oxygen supply mechanism to internal tissues and thereby provide an initial estimate of the upper critical temperature (Tc_{II}).

4.7 Methods

Ninety *S. neumayeri* were collected by SCUBA divers from North Cove in May 1998 (the beginning of the austral winter) and returned to the Station's aquarium facility.

At this time seawater temperature was -1.7°C. The echinoids were placed in clean seawater in a jacketed water bath of 40 litres internal volume initially cooled to -1.5°C using a thermocirculator. Twenty percent of the tank water was removed every two days throughout the entire experimental duration and replaced with clean seawater of the required temperature, and gentle aeration of the water in the bath was maintained at all times. The temperature of the bath was raised by 2.5°C at approximately weekly intervals, and on each occasion the bath took about eight hours to rise to the new temperature. The bath temperature was checked daily and never varied from the set value by more than ±0.2°C. The end point of the experiment (i.e. the upper lethal temperature) was decided when mortality (first indication of spine dropping) of the unused echinoids remaining in the bath reached 50%.

The sea urchins were allowed to recover from initial collection and handling shock for a period of four days at -1.5°C before the first oxygen consumption measure was taken. Molar oxygen consumption rates (MO_2) were assessed using closed chamber respirometry techniques identical to those described in Chapter 4a. MO_2 measurements were recorded individually for eight sea urchins at approximately daily intervals throughout the study period. Oxygen measurements were made in 180ml volume chambers for roughly eight hours initially, reducing eventually to three hours to compensate for the higher respiratory rates encountered at the higher temperatures. The same group of eight urchins was used for consecutive daily measurements at each incremental temperature after which they were removed from the main experiment to minimise error due to handling stress. A different group was used for each temperature step. A total of 56 animals were therefore used for MO_2 measures (seven temperature steps and eight animals per step). Dry masses of

experimental echinoids were obtained by drying to constant weight at 60°C

The increase in metabolic rate with mass for *S. neumayeri* in May 1998 was measured in Chapter 4a. Logarithmic transformation of this data allowed a least-squares linear regression to be fitted:

$\ln MO_2 = 0.730 \,.\, \ln dry\ mass - 0.756$ $(r^2 = 0.89; F = 178.06; p = <0.001; n = 24;)$ - (1)

The sea urchins used in the temperature experiment were all of roughly similar size (mean = 1.37g, standard deviation = 0.3g, n = 56). Differences in oxygen consumption rates resulting from small differences in sea urchin mass were corrected by arithmetically transforming data to a standard mass by application of the scaling coefficient presented in equation one. A standard animal mass of 1.37 g was chosen to coincide with the mean mass of the sea urchins used in the main experiment.

Oxygen content of the perivisceral coelomic fluid was measured at each of the temperature increments from sea urchins not used in respirometry trials. Five echinoids were sampled at each temperature increment and after sampling the individuals were no longer used in the main experiment. A 25 μl aliquot of coelomic fluid was withdrawn from the coelom of the echinoid by insertion of a microsyringe needle through the periproct. The sample was then injected directly into the couloximeter. Either two or three samples were taken from each individual and a mean value calculated; oxygen partial pressures were then calculated from the measured concentrations.

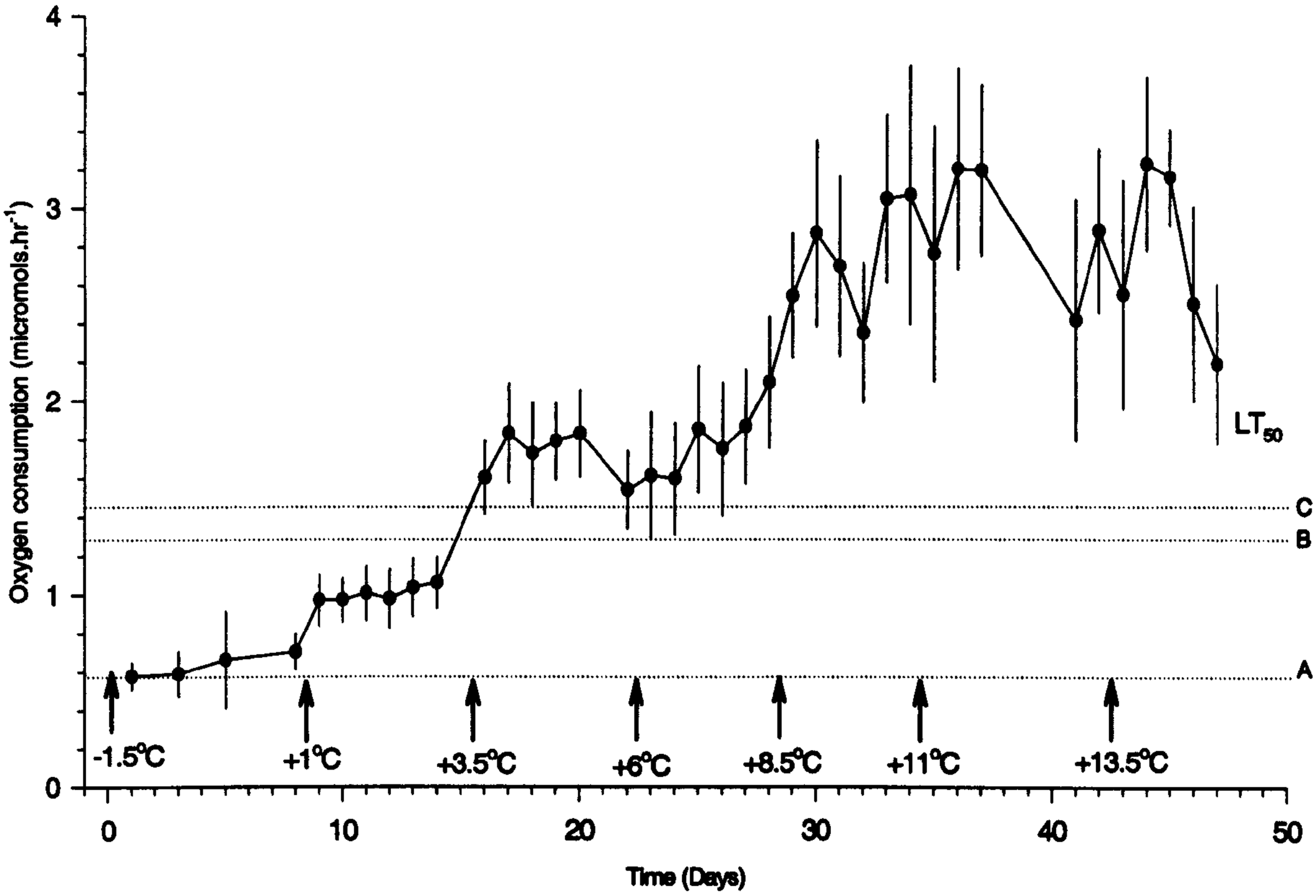


Figure 4.7 Response of *S. neumayeri* molar oxygen consumption rate to incremental rises in temperature. Values shown are corrected arithmetically to a standard animal of 1.37g dry mass, and data are presented as means (n=8) ± standard error. Timings of each incremental rise shown by arrows. Dotted line A indicates the size corrected oxygen consumption rate of the field population at the time of collection, B and C indicate maximum summer rate in January 1998 and 1999 respectively (data from Chapter 4a).

4.8 Results

The mean standard animal oxygen consumption recorded for *S. neumayeri* at the start of the experiment at -1.5°C was 0.644μmols.hr^{-1}, which compared well to the rate recorded in Chapter 4a for the wild population at Rothera Point at the same time of year (Fig 4.7). Stepwise increases in temperature to both 1°C and 3.5°C caused an immediate rise in the rate of oxygen consumption. There was no evidence of a return or acclimation of oxygen consumption (*sensu* Precht 1958) to original levels in the days following a temperature rise. Q_{10} values calculated for incremental temperature rises within the normal environmental range of *S. neumayeri* were very high: a value of 7.2 was found for changes

both from -1.5°C to 1°C and from 1°C to 3.5°C. No change in rate of oxygen consumption was recorded when temperature was raised from 3.5°C to 6°C (Students t = -1.22; p = 0.22). Further elevation in temperature beyond the normal environmental range of *S. neumayeri* resulted in progressively smaller rises in MO_2, until the maximum recorded value of 3.24 μmols.hr^{-1} on day 43 of the experiment at 13.5°C. From day 43 onwards metabolic rate decreased sharply until the LT_{50} was reached on day 47 and the experiment was terminated.

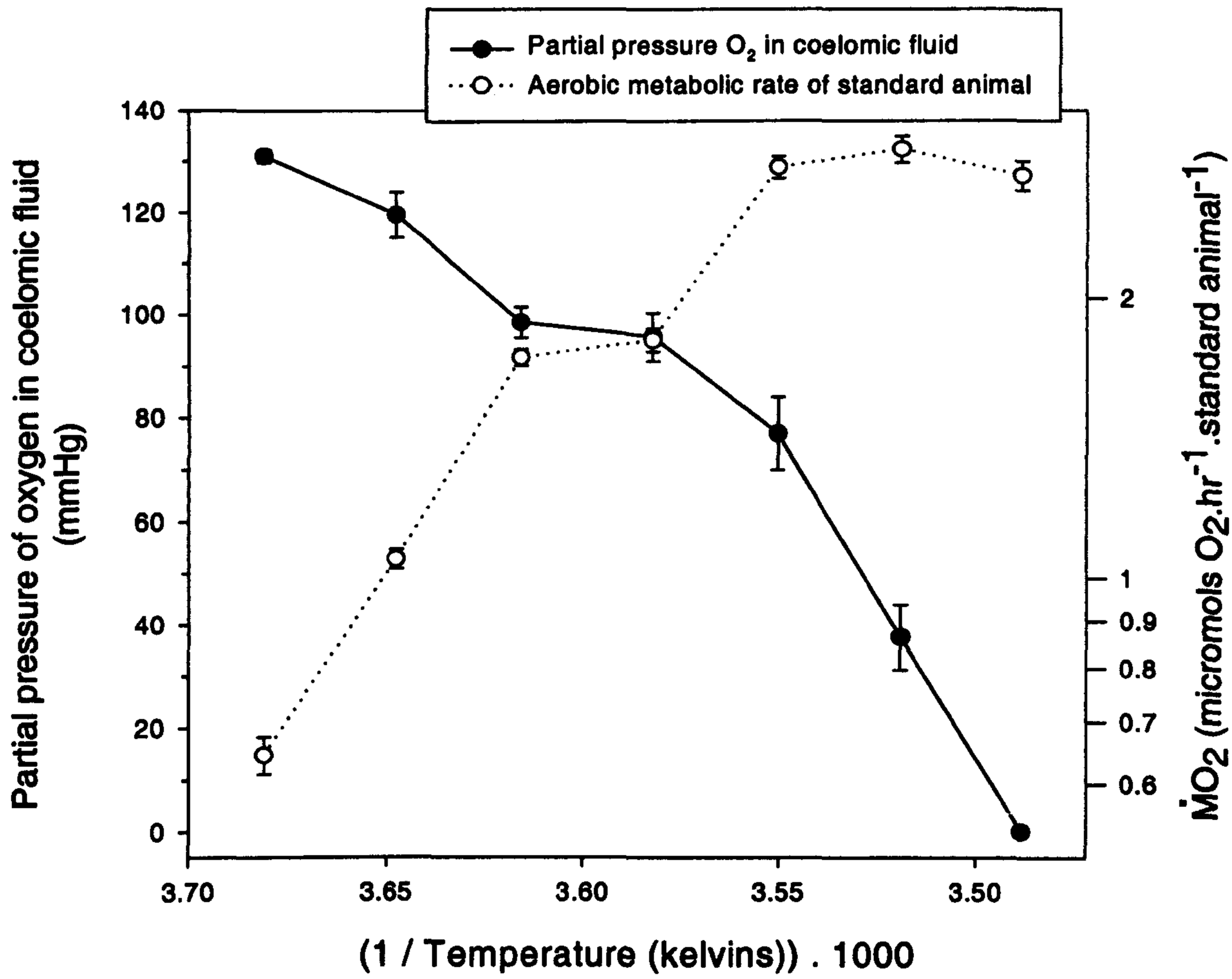


Figure 4.8 Arrhenius plot of mean aerobic metabolic rate ± standard error plotted alongside reduction in perivisceral coelomic fluid oxygen content; (n = 5 ± standard error).

Coelomic oxygen partial pressure initially decreased steadily from 131mmHg at -1.5°C to 98.4 mmHg at 3.5°C (Fig 4.8). A very small decrease was observed when the temperature was raised to 6°C, consistent with the plateau in sea urchin respiratory rate at this step. Above 6°C coelomic oxygen partial pressure continued to fall with increasing severity at each incremental temperature rise. A linear relationship between oxygen consumption rate and coelomic oxygen partial pressure was observed between -1.5°C and 8.5°C (Fig 4.9). Above 8.5°C internal oxygen partial pressure showed a sharp decline, despite an absence of increase in oxygen consumption rate.

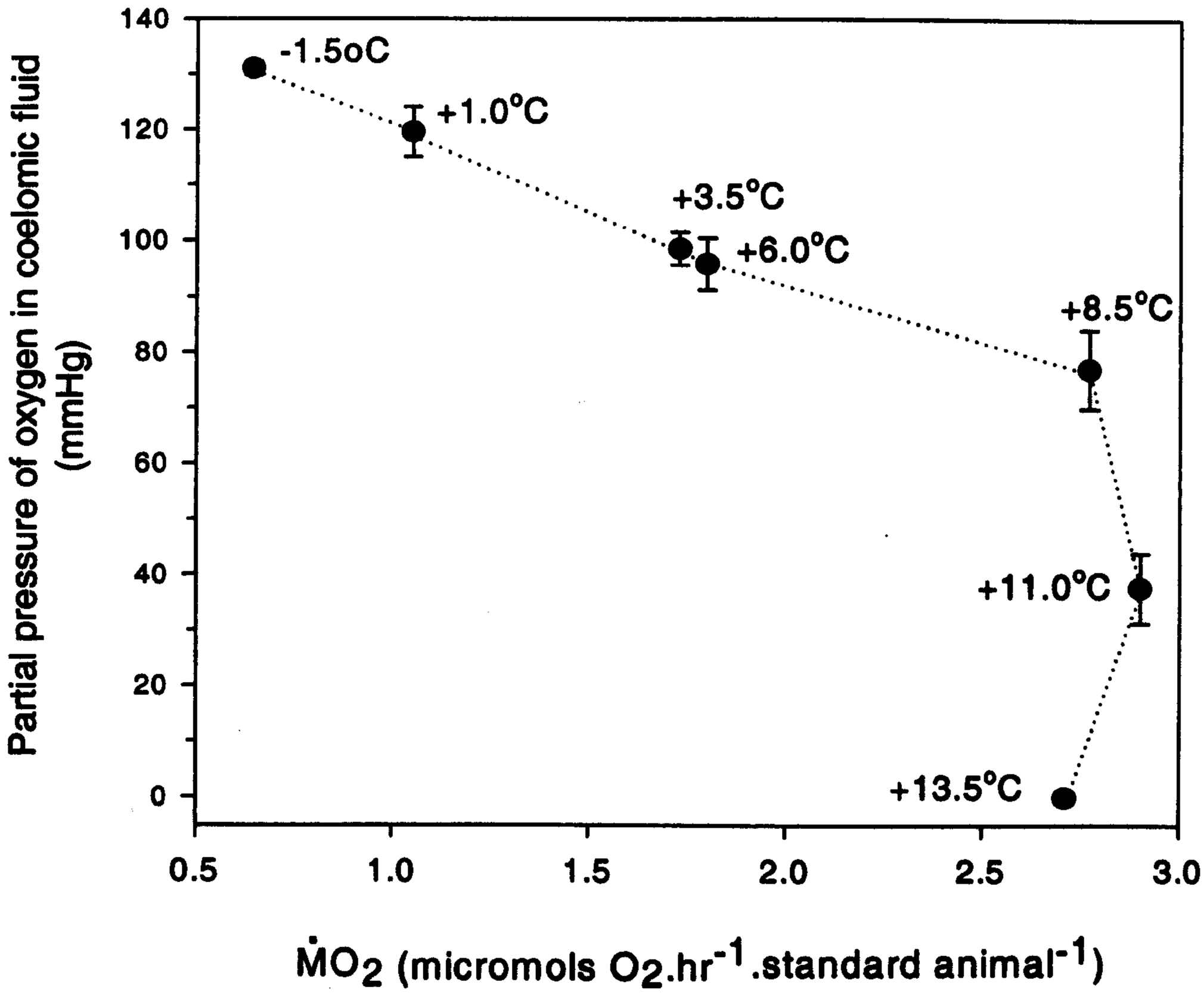


Figure 4.9 Percentage oxygen saturation of perivisceral coelomic fluid as a function of aerobic metabolic rate. Data are means ± standard error. Catastrophic failure of the respiratory ventilation mechanism was apparent above +8.5°C. Coelomic oxygen content was non detectable above 11°C.

4.9 Discussion

The oxygen consumption rates recorded for the lowest temperature in this study agreed well with observed rates from winter conditioned sea urchins. The initial temperature elevations however prompted substantial rises in oxygen consumption, and the Q_{10} values for the temperature effect on oxygen consumption displayed by winter conditioned *S. neumayeri* are extremely high. Recent investigations into cold adaptation suggest that an increased temperature sensitivity may result from raised mitochondrial respiration under polar conditions (Pörtner et al in press). Mitochondria do not show any evolutionary compensation to temperature (Johnston et al. 1994), and hence cold water species so far investigated maintain oxidative capacity by increasing tissue mitochondrial proliferation (Johnston et al. 1998). Enhanced temperature sensitivity is not, however, seen at the whole animal level. A Q_{10} of between 2.5 and 2.9 was calculated for *S. neumayeri* exposed to seasonal rise in temperature in the absence of food (Chapter 7), and White (1975), Ralph and Maxwell (1977), Ikeda and Hing Fay (1981), Peck (1989), Clarke (1993) and Pörtner et al. (1999) have all reported Q_{10}'s for polar ectotherms within the biologically expected range of 2-3.

Q_{10} values outside the normal biological range indicate a deviation from normal physiological function, and may represent either a breakdown of physiological systems such as are encountered at the limits of a species temperature range, or a change of physiological state, e.g. the onset of seasonal changes from winter torpor to summer activity (Clarke 1993). A change of physiological state may explain the acute Q_{10} values found for oxygen consumption in the Antarctic limpet *Nacella concinna* (20.1) when temperatures were raised to 3°C by (Peck 1989), and also the Q_{10} of 13.6 found for

development of embryos of *S. neumayeri* between -2°C and 0°C by Stanwell-Smith and Peck (1997), as development normally occurs during the summer months when temperatures are above 0°C at the site studied. The very high Q_{10} values for the rise in MO_2 over the two lowest temperature increments found in this study could either indicate high temperature sensitivity, or a change in physiology. The sea urchins in this study were winter acclimated at Rothera Station, where winter conditions are constantly at -1.8°C and food availability is low. 1°C is typical of high summer temperature in the environs of Rothera Station and may have induced a change in the sea urchins from winter to summer physiological condition (although no rise in mobility was observed). The following rises to 6°C and above would take *S. neumayeri* beyond its normal environmental temperature range and produced acute responses similar to those encountered by Peck (1989). Previous studies on the effect of temperature on metabolism in Antarctic marine invertebrates which have found low Q_{10} values in the normally encountered temperature range (eg Davenport 1988, Peck 1989, Pörtner et al 1999) were all on animals in summer condition. This is the first such investigation carried out in winter and this may explain the unusually high Q_{10} values obtained.

Critical temperatures have been defined for ectothermic species as the point of transition to anaerobic metabolism (Pörtner et al 1998). For polar species evolution has caused the depression of the lower critical temperature, Tc_I, to below the freezing point of seawater. However, the upper critical temperature, Tc_{II}, represents the long term survival limit for a species and occurs a few degrees below the experimental upper lethal limit; temperature elevation above Tc_{II} results in a time limited situation. Critical temperatures therefore reflect the thermal flexibility of the species and are entrained by the

environmental regime encountered over evolutionary time, although they may shift depending on latitude and seasonal temperature adaptation. The upper critical temperature for *S. neumayeri* may be indicated by a break in the coelomic oxygen tension above 8.5°C. This would suggest the long term upper temperature limit is well above the local maximum temperature and is above the highest value for the whole distributional range of the species (5.7°C). Whilst the stenothermal characteristics of the Antarctic benthos result primarily from evolutionary adaptation to a constant thermal environment, the precise degree of stenothermality varies amongst polar species in relation to their mode of life. (Pörtner et al. 1999) have shown that metabolism in the Antarctic limpet *Nacella concinna* is fully aerobic at 7°C, whereas the Antarctic bivalve *Limopsis marionensis* showed a much greater degree of stenothermality and was beyond tolerance limits at 4°C. Pörtner et al.(1998; 1999) suggested that the overall reduction of the upper critical temperature under polar conditions may be a combined consequence both of mitochondrial proliferation and reduction in motor activity.

4.10 Conclusion

The data presented by this study indicate that basal metabolic rate in winter conditioned *S. neumayeri* is strongly temperature dependant between -1.5°C and 3.5°C (Q_{10}=7). This result is in contrast to studies by Parry (1978; 1983) which suggest that seasonal biological responses (e.g. Chapters 3 & 4a) are caused by changes in food availability rather than direct effects of environmental temperature (see also Chapter 7). It is likely that the high Q_{10} values reported by this study are indicative of a change of physiological state (i.e. a break from winter torpor) rather than a direct effect of temperature. These data further indicate that the upper critical temperature (Tc_{II}) for *S.*

neumayeri as characterised by a failure of the oxygen supply mechanism lies between 8.5°C and 11°C. This temperature value lies just above the summer maximum at the northernmost extent of this species distributional range (5.7°C at South Georgia, 49°S), and indicates *S. neumayeri* to be a moderately stenothermal Antarctic species.

Chapter 5 Reproduction

Data also presented in: Brockington S, Peck LS & Tyler PA (submitted). Gametogenesis and gonad mass cycles in the Antarctic echinoid *Sterechinus neumayeri*. Mar Ecol Prog Ser.

5.1 Introduction

Gametogenesis within the Echinoidea has been well studied, and all species display an ordered sequence of events from initial gonial proliferation through spermatogenesis or vitellogenesis to final maturation of gametes. Different species show a broad range of reproductive periodicities, many of which are influenced by environmental factors, two of the most important being nutrition and temperature. Gonad size and gamete output is strongly related to food availability (Lawrence & Lane 1982), but there is little evidence that nutrition has much role in regulating the initiation of gametogenesis (Pearse & Cameron 1991). Temperature has often been regarded as a gametogenic regulator, but evidence is generally correlational except for species which inhabit areas with marked natural annual temperature regimes.

Polar latitudes provide a unique environment, with extremely low and fairly constant temperatures contrasting with highly seasonal food limitation (Clarke 1988, Chapter 2). Under these conditions the polar benthos have evolved to be generally stenothermal with very low metabolic rates, slow annual growth and consequently extended longevity (Arntz et al. 1994). Whilst the low temperature has facilitated the overall reduction in metabolic rates, the highly seasonal cycle of autotrophic production has led to strongly restricted feeding opportunities for many species during the polar winter (Barnes & Clarke 1994; Barnes & Clarke 1995). Metabolically active processes, such as

somatic growth are therefore often confined to summer periods of autotrophic production. As a consequence, somatic growth is slow or absent during winter for many Antarctic species, and extended longevity results (Dayton et al. 1974; Sagar 1980; Clarke 1988).

Polar conditions may also have implications for reproductive periodicities since vitellogenesis is extended at low temperature locations (Pearse & Cameron 1991). At higher latitudes the metabolic cost of gametogenesis may therefore have to be met during the polar winter, when both metabolic rate and feeding are minimal. Furthermore, it has been suggested that the effects of seasonal energy limitation, combined with extended longevity may balance a reduction in annual reproductive output (i.e. mass of gametes released) so that lifetime reproductive output is similar to that of a comparable non-polar species (Clarke 1987; Brey et al. 1995). Although somatic growth has been moderately well studied for benthic polar species, both by direct observation and via indirect methods (e.g. growth band counts), reproductive growth has been less well evaluated, mainly because of sampling difficulties.

This investigation recorded gonad mass monthly for three populations of *S. neumayeri* over a 25 month period. Spawning events were observed both directly by SCUBA divers, and time course of vitellogenesis as well as reproductive output was determined from histological investigation.

5.2 Methods

Sterechinus neumayeri were obtained at monthly intervals by SCUBA divers from three locations (North Cove, South Cove and Lagoon Island) near the British Antarctic

Survey Research Station at Rothera Point (Fig 1.1). Gonad mass and competence to spawn was assessed at all three sites, and histological investigation of reproductive periodicity was also carried out on North Cove specimens.

Sea urchins were returned to the laboratory after collection and sorted to remove adherent debris. Test diameter was measured in three horizontal directions using knife edged calipers, and a mean value calculated. Dry gonad mass was obtained by placing eviscerated tissue into pre-weighed crucibles and drying to constant mass at 60°C. Initially gonad mass was regressed on test diameter after logarithmic transformation of both variables from a large sample:

$$\ln(\text{gonad}) = 2.76 \times \ln(\text{test diameter}) - 9.98 \qquad (r^2 = 0.823;\ F = 186.08;\ n = 40) \qquad (1)$$

Subsequently dry gonad mass was determined from ten sea urchins at each sampling occasion, using sea urchins with test diameters as close to 30mm as possible. Gonad index was not calculated since the index may be altered through the year by simultaneous changes in the mass of other tissues (e.g. gut mass: Lawrence et al. 1965). Instead the scaling exponent from equation (1) was used to arithmetically correct for small differences in test size by adjusting gonad mass to that of a standard 30mm test diameter sea urchin.

Competence to spawn was assessed at each site on each sampling occasion by injecting 0.5 to 1.0 ml of 0.5 *M* KCl in seawater into the coelom of ten urchins (Bosch et al. 1987). After injection urchins were held individually in clean seawater in 500ml

beakers and maintained at environmental seawater temperature. Any gamete discharge was recorded after 12 hours.

Reproductive condition was assessed histologically for 5 male and 5 female sea urchins at roughly monthly intervals for the North Cove site only. Specimens were collected simultaneously with the gonad mass study, and fixed by injection and immersion into 4% formal saline. Samples were stored at +5°C, and the preservative changed, where necessary, after 12 months. Specimens were returned to the United Kingdom, dissected, and gonad samples dehydrated in an alcohol series followed by immersion in histoclear before embedding in wax. Sections were cut at 7μm on a hand microtome, and stained using Haematoxylin 'Z' and Putts Eosin (Cellpath, U.K.).

Reproductive condition was assessed for female urchins as oocyte feret (i.e. mean) diameter. Sections were examined at ×100 and feret diameters of 100 oocytes per animal were calculated using Sigmascan image analysis software (Jandel Scientific). Thus 500 oocyte diameters were recorded each month over a 21 month period. For male urchins testis development was classified into five maturity stages modified from the methods of Byrne (1990) and Fuji (1960):

Stage One	Spent. Gonad lumen empty or resorption of relict gametes in progress. Nutritive phagocytic tissue highly eosinophilic and of variable thickness. No evidence of new spermatogenesis.
Stage Two	Initial development. Nutritive tissue clearly eosinophilic and of medium thickness. Blue primary spermatocytes visible on germinal epithelium.

First traces of spermatozoa in lumen.

Stage Three Developing. Nutritive tissue stains more readily with haematoxylin (i.e. more blue). Blue primary spermatocytes visible on germinal epithelium and forming spermatid towers into lumen. Moderate density of spermatozoa in lumen.

Stage Four Mature. Lumen full of densely packed mature spermatozoa in swirls. Lumen intense blue. Spermatid production may still be evident. Nutritive tissue generally highly reduced.

Stage Five Spawning. Reduced density of mature spermatozoa. No spermatid production evident. Thickness of nutritive tissue highly variable, and becoming eosinophilic.

Reproductive output, defined as percentage loss of mass of reproductive tissue during a spawning event, is difficult to measure for *S. neumayeri* where the spawning period coincides with rapid gonad mass increase through intense feeding and storage of non reproductive reserves (Chapter 3). Male reproductive output was therefore assessed using the histological preparations described above. Sections of testis from pre-spawning mature sea urchins in October and early November 1997 and 1998 displayed a sharp boundary between mature spermatozoa and remaining nutritive phagocytic tissue lying against the wall of the testicular tubule. Percentage area of sperm was calculated in relation to area of remaining tissue using image analysis for ten sections from each individual male. Mass of potential reproductive output was calculated from this ratio and mean dry gonad mass for that month. Female reproductive output could not be assessed by this method.

5.3 Results

KCl injection showed that urchins from all sites had synchronised reproductive cycles, and all sites exhibited highest spawning competence in October and November of both years (Fig 5.1). This coincided with SCUBA divers observations of spawning in the

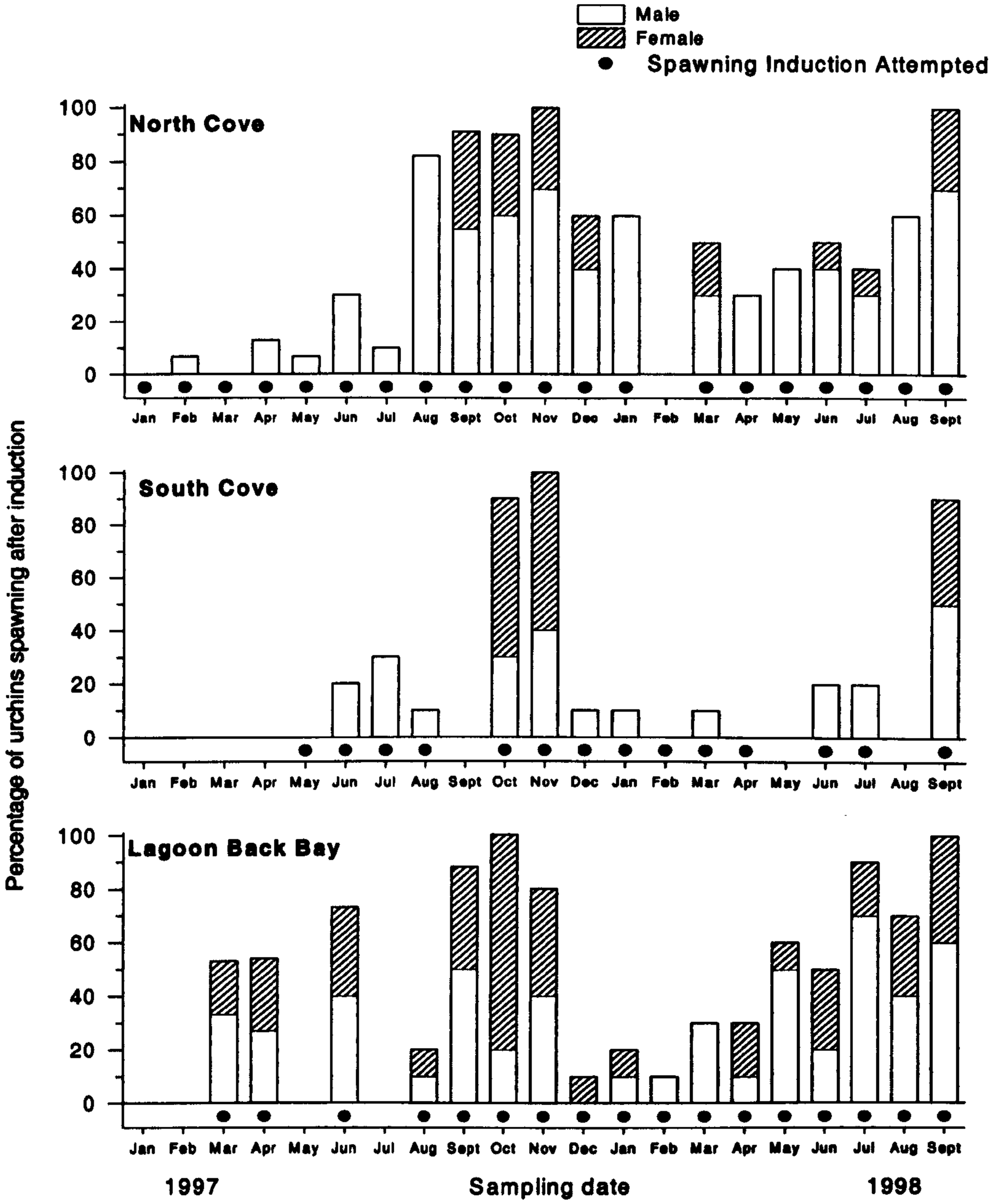


Figure 5.1 Seasonal cycle in competence to spawn for *S. neumayeri* at three sites around Rothera Point. Filled symbols (●) indicate dates when 10 individuals were injected with 0.5ml of KCl; bars represent the percentage of urchins spawning.

field. At both North Cove and Lagoon Back Bay spawning competence increased gradually in the austral winter, although males always spawned more readily than females. Spawning competence was usually lower at South Cove than for the other two sites. The smallest sea urchins observed spawning were ~20 mm test diameter, implying that sexual maturity is reached before this size.

Gonad mass showed large differences between both sites and subsequent years (Fig 5.2). Paradoxically, gonad mass often increased at the start of the austral summer, despite spawning being observed at all sites between November and January. Sea urchins from South Cove site had the lowest gonad mass in the study (0.05 to 0.32 g dry mass per standard animal), and no change in mass occurred either at spawning, or between years with the exception of a rise at the start of the 1998/99 austral summer. Gonad mass of

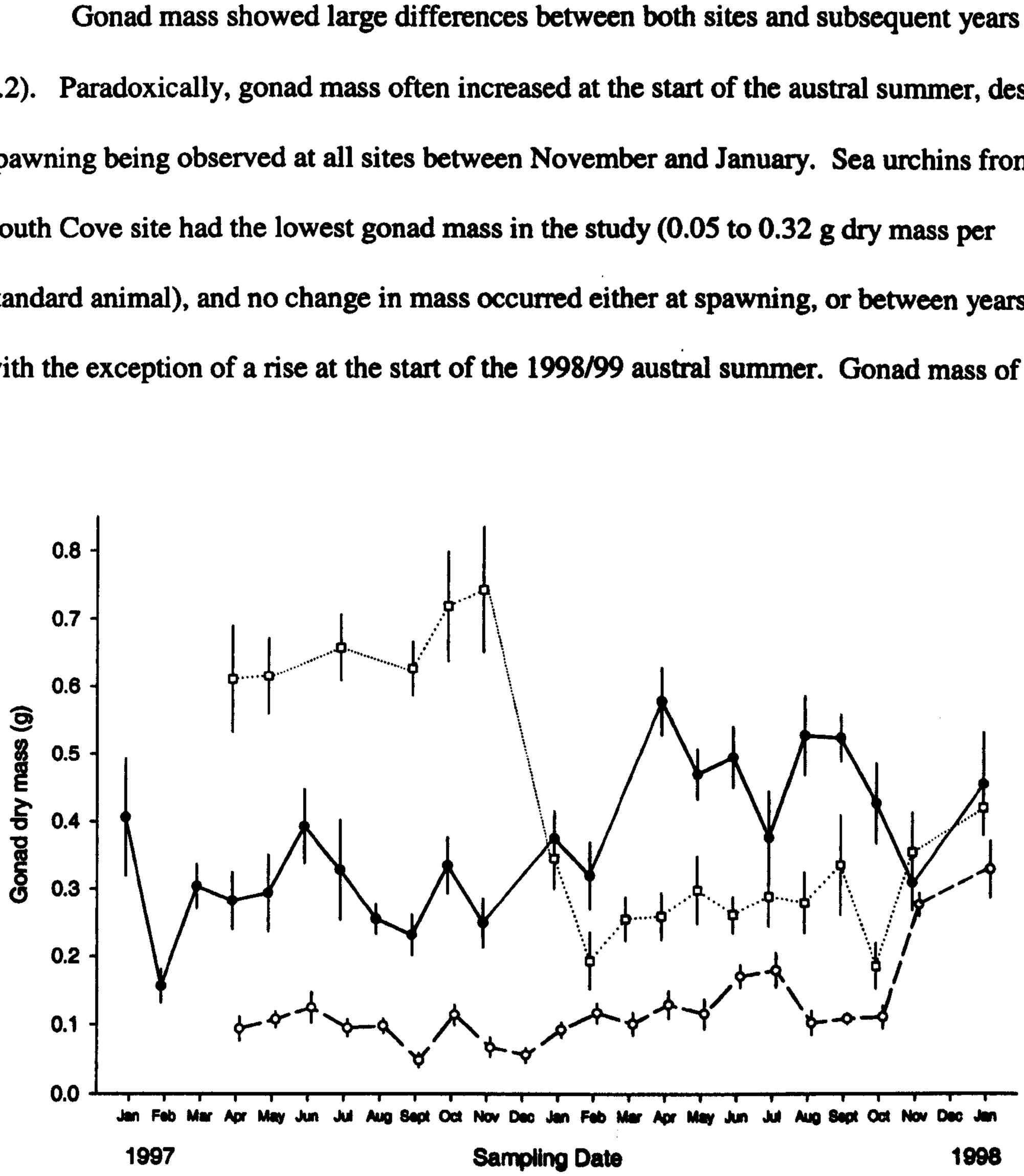


Figure 5.2 Seasonal variation in gonad mass for *S. neumayeri* at North Cove (●), South Cove (○) and Lagoon Islet (□). Data are standardised arithmetically to an animal of 30mm test diameter and presented as means ($n = 10$) ± standard error.

North Cove urchins increased during the 97/98 austral summer, and remained higher throughout the 1998 winter compared to 1997. The coeloms of urchins from Lagoon Island during winter 1997 were completely filled with reproductive tissue, and gonad masses at this time from this site were the highest recorded (0.62 to 0.75 g dry mass per standard animal). The mass reduced to much lower values during a major spawning event in the 97/98 austral summer. Gonad mass then remained at intermediate levels throughout the 1998 winter before rising again at the onset of the following austral summer.

A female gametogenic cycle lasting 18-24 months with spawning events in November 1997 and 1998 was clearly present in oocyte size frequency distributions (Fig 5.3). In August of both years there were only two cohorts of oocytes, one nearly full grown at roughly 120μm diameter, and a second of 40-60μm diameter. By September of both years the full grown cohort was unchanged in diameter, although maturation of secondary oocytes to form ova was commencing prior to spawning in November. However the mean size of the smaller cohort had abruptly decreased in September 97, and divided into two in September 1998 as the new population of pre-vitellogenic oocytes proliferated in the ovaries. The two smaller cohorts of oocytes were then observed through the timecourse of the experimental period, with the middle cohort (40-60μm in September and October 97) maturing to spawn in November 98, and the smaller cohort (20μm in September 97) maturing to spawn in November 99. Despite the prolonged overall developmental period, 93% of the increase in volume of the developing oocytes (equivalent to an increase in diameter from 50 to 120μm) occurred during the last 12 months of the cycle (November to November), with the initial increase over 6-12 months from 20 to 50μm accounting for only 7% of final volume.

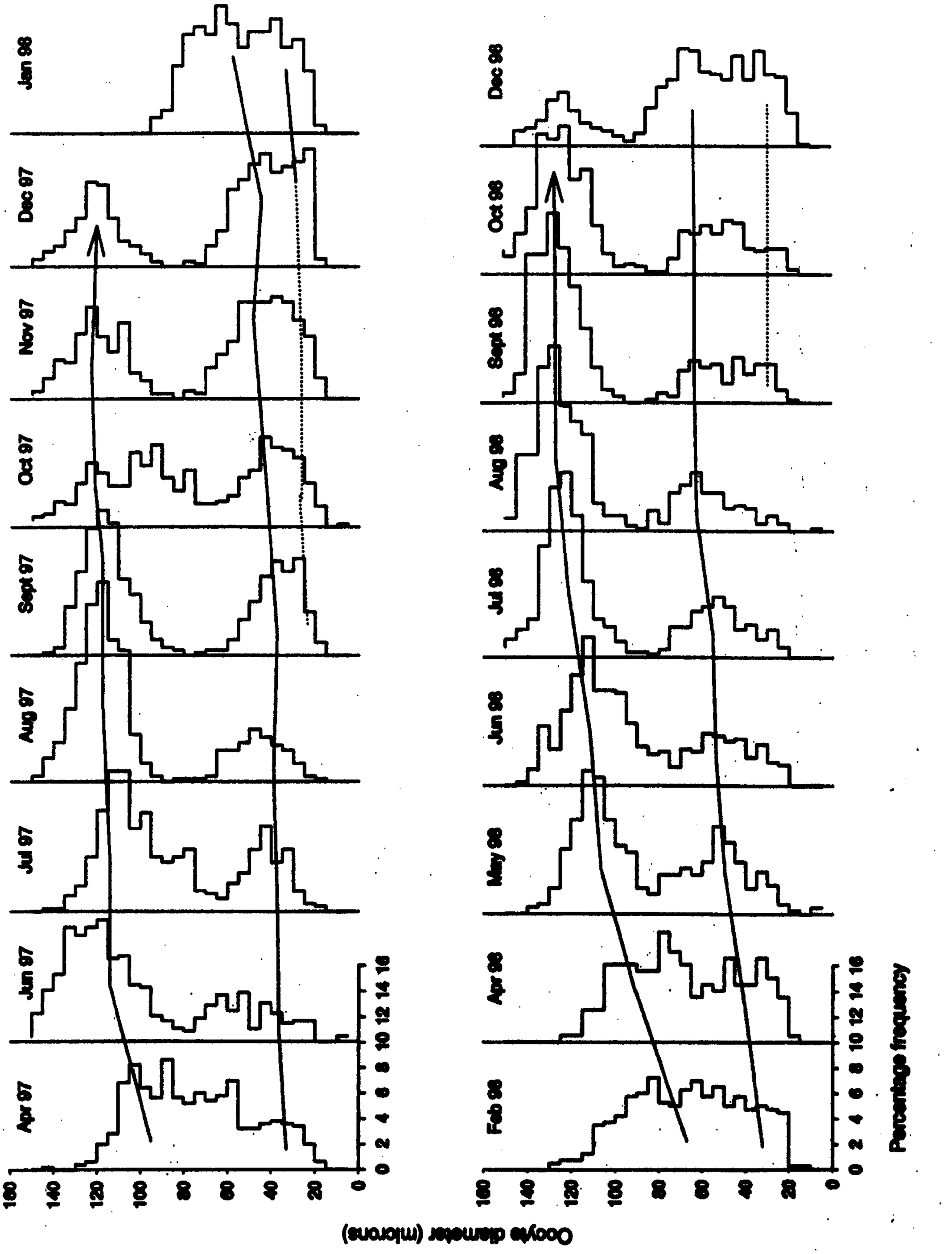


Figure 5.3 View from side. Oocyte feret diameter frequency histograms, in 5μm size classes. *n* = 500 per month (5 females, 100 oocytes each), except February 98 (*n* = 400), April 98 (*n* = 200) and September 98 (*n* = 400): total *n* = 8,500. Solid line represents increase in modal peak size, dotted line indicates recruitment of pre-vitellogenic oocytes.

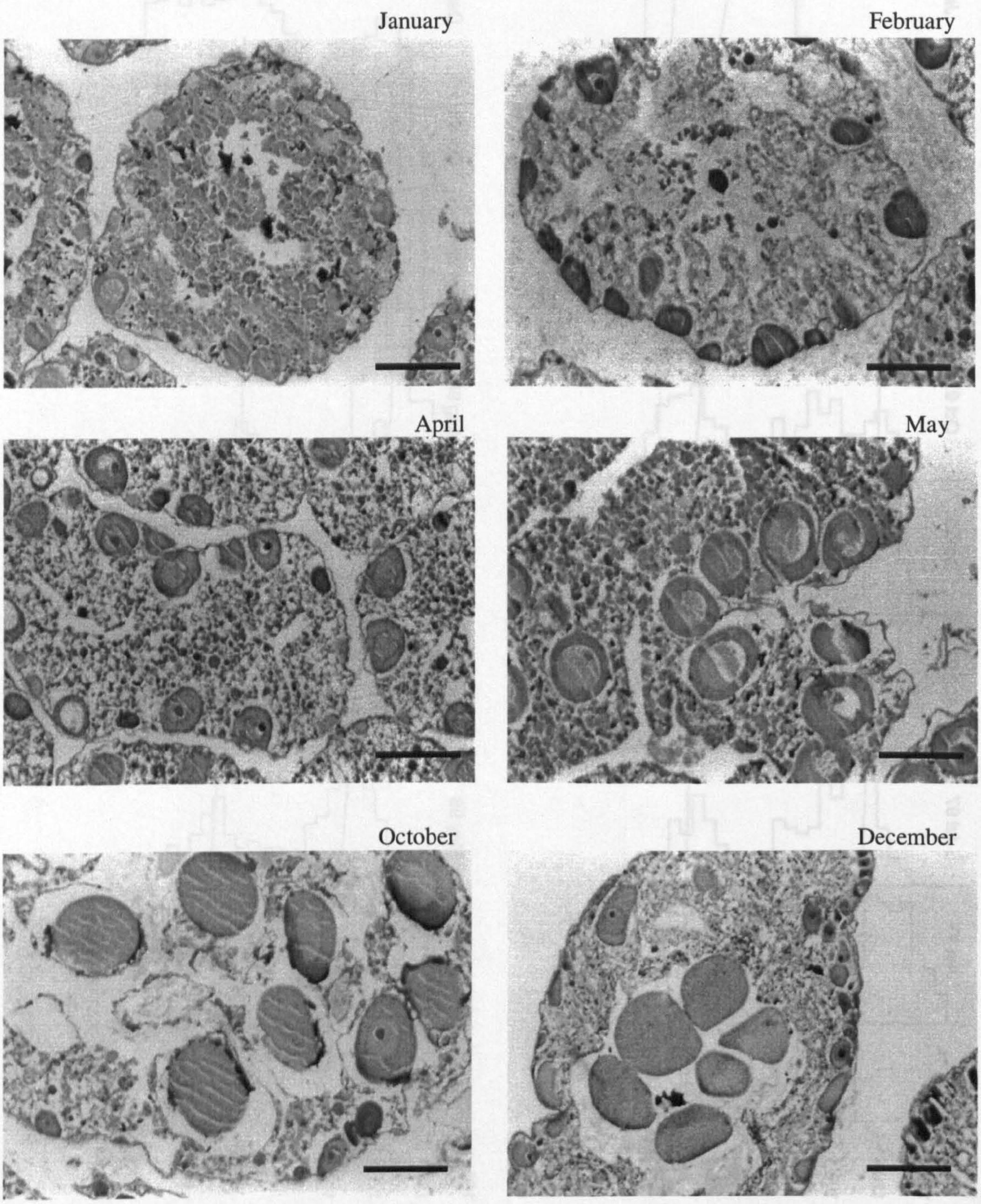


Figure 5.4 Histological sections of representative ovaries. Scale bar equals 120μm.

The final major vitellogenic phase started in January of both years (Fig 5.4) and was more clearly represented by re-plotting data to emphasize height of peaks in oocyte size frequency distributions (Fig 5.5). This phase of volume increase proceeded at a steady rate throughout the austral winter until August / September, when maximum oocyte diameter (120 µm) was attained. From September onwards post vitellogenic secondary oocytes matured to become ova without further increase in size. Mature ova were released as part of a synchronous spawning event over a two month period from November onwards.

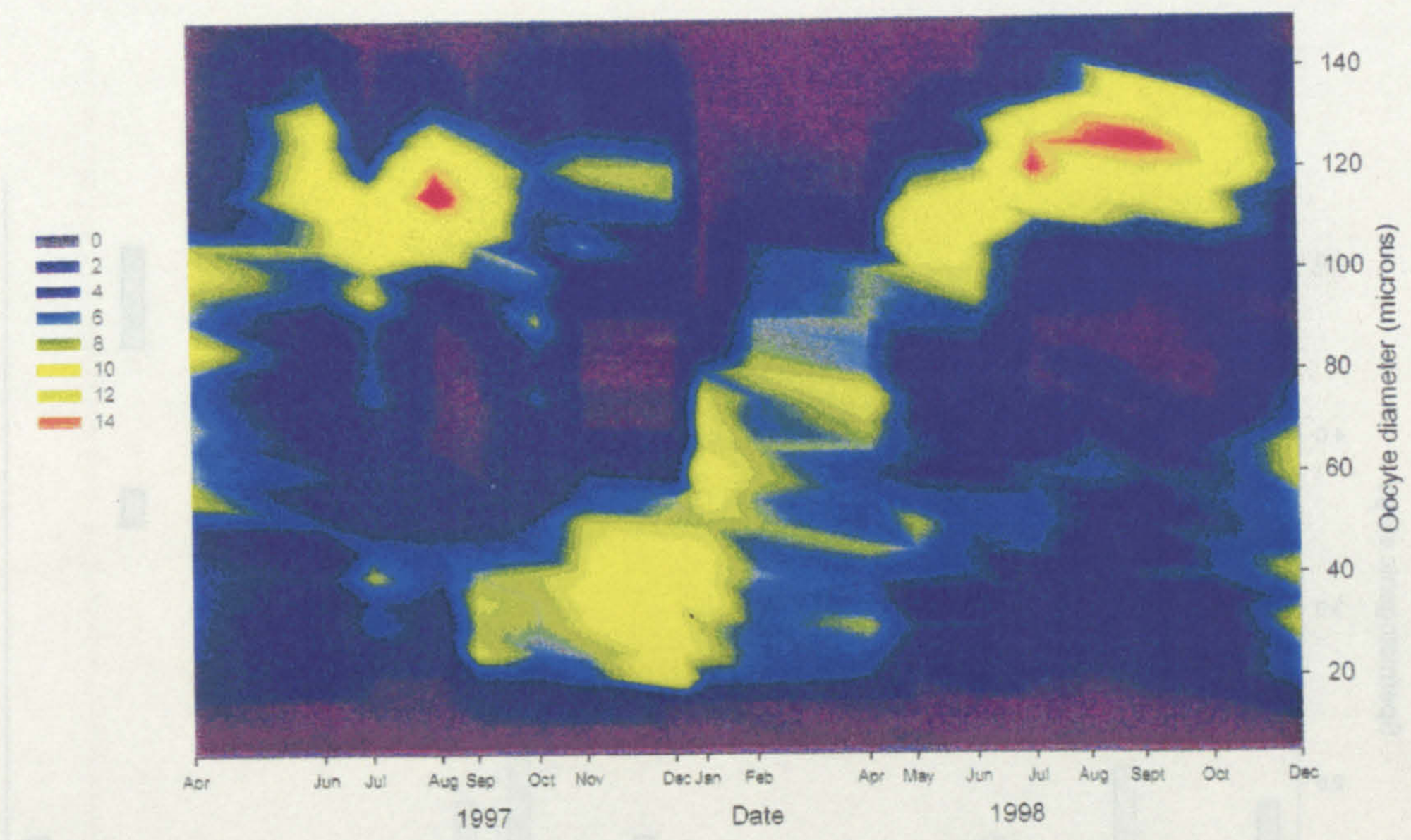


Figure 5.5 Oocyte feret diameter frequency histograms, in 5µm size classes. Figure compiled from same data as Fig 5.3, although colour intensity shows clear domination of the second year vitellogenic oocytes in the size frequency distributions, and the regular progression of vitellogenesis and maturation during the austral winter.

Spermatogenesis showed a 12 month cycle (Fig 5.6). Fully spent testes were observed mainly between November and February following the annual spawning event, although spent urchins were encountered sporadically at other times. In late summer (March and April) the thickness of eosinophilic nutritive phagocytic tissue increased to fill

the testicular lumen (Fig 5.7). Primary spermatocytes became visible on the germinal epithelium in April and May, and these started to form towers of spermatids by mid-winter. Spermatid development and reduction in thickness of nutritive phagocytic tissue progressed steadily throughout the austral winter. Mature sperm were observed in dense concentrations in the testis lumen by October (Fig 5.7), and spawning took place from November onwards.

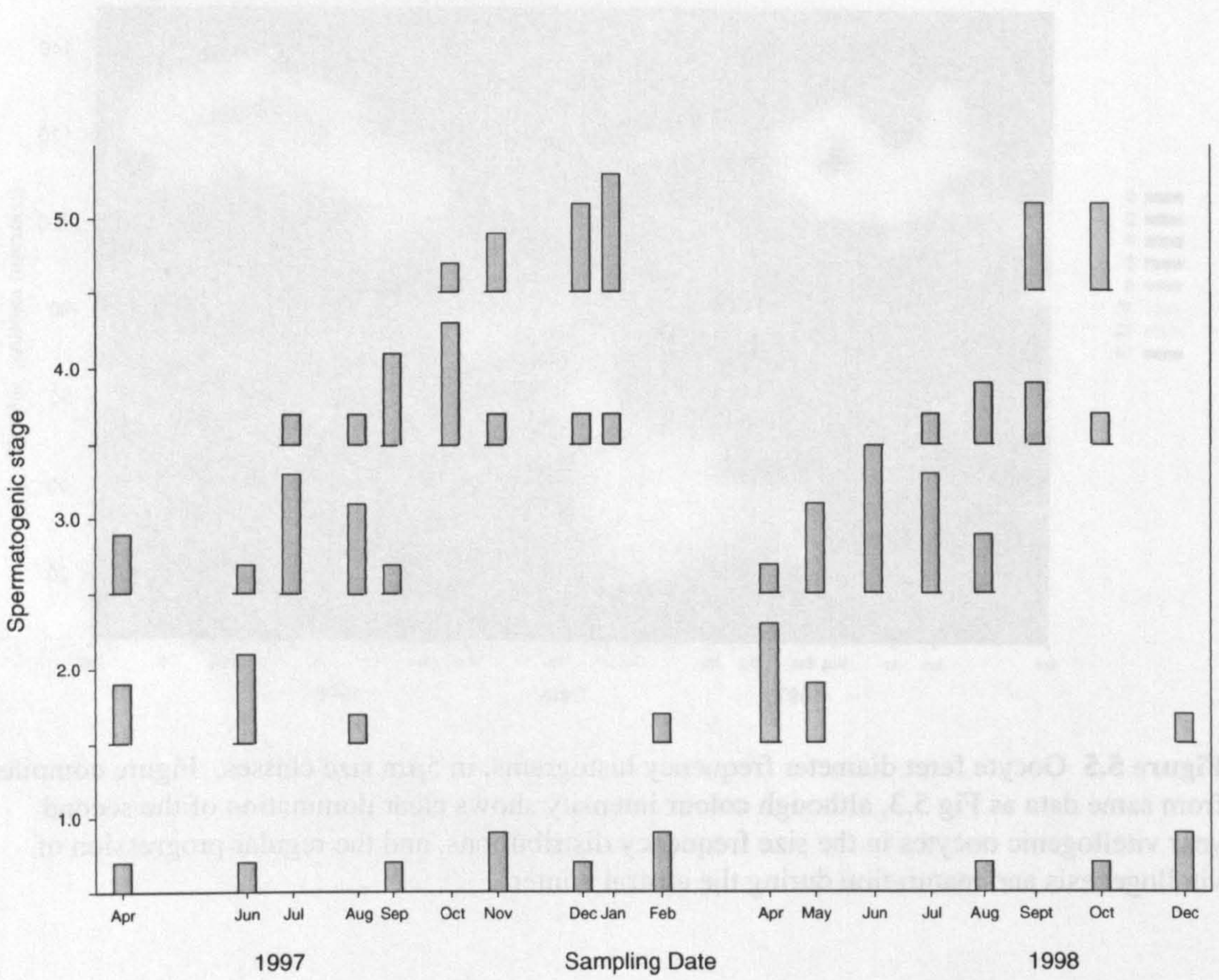


Figure 5.6 Maturation stage of testis of *S. neumayeri* (n = 5 individuals per month).

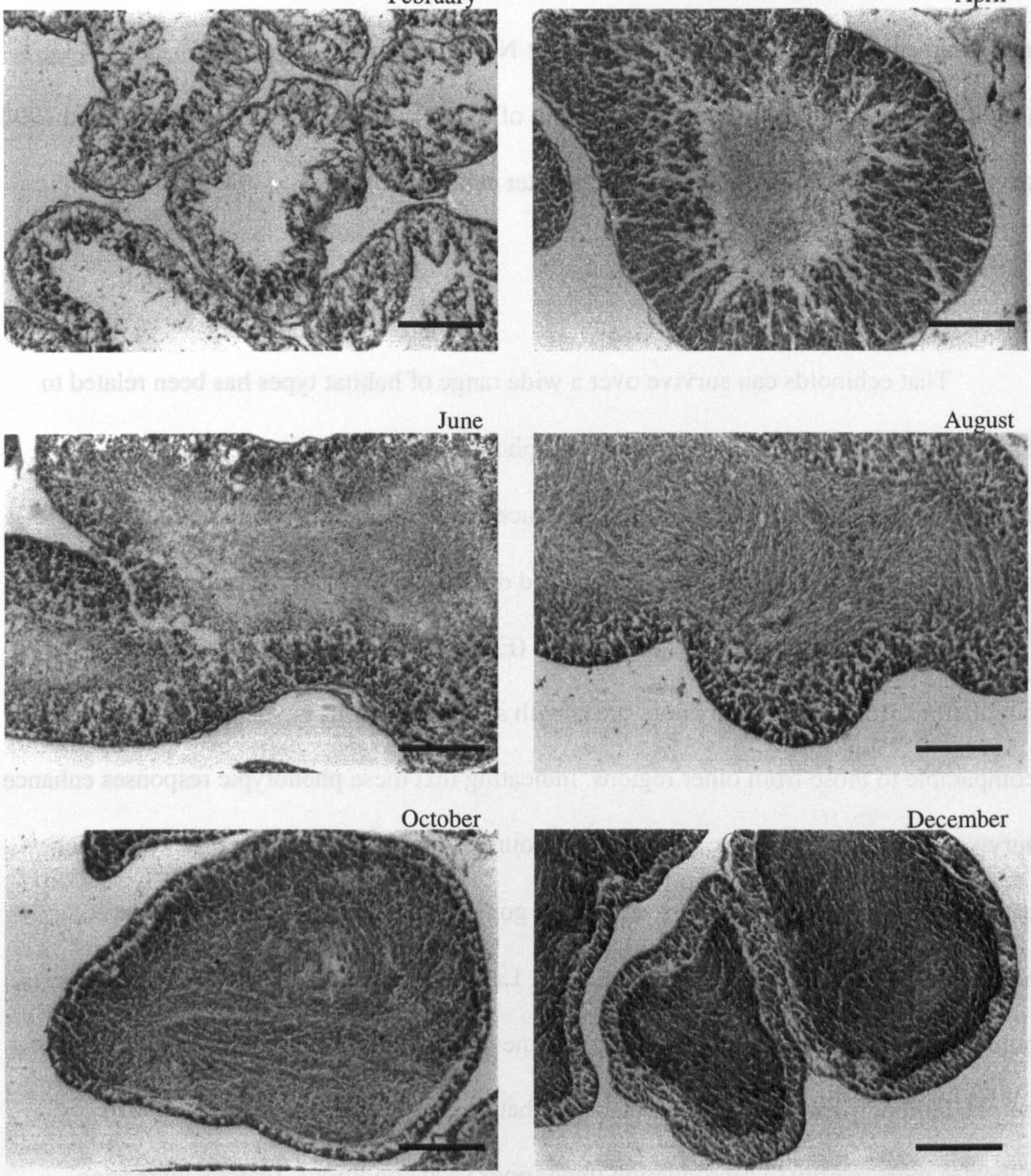


Figure 5.7 Histological sections of testis from *Sterechinus neumayeri* from North Cove. Nutritive tissue is at maximum thickness late in the austral summer (April) and shows a steady decline as winter progresses. Spermatids increase in concentration in the lumen of the testis prior to spawning in November and December. Scale bar = 120 μm.

Male potential reproductive output, assessed as area of sperm in testis histological sections from the North Cove population in November was 38.6% in 1997 (n = 9; standard

deviation = 3.95) and 41.9% in 1998 (n = 6; standard deviation = 0.76). Mean gonad dry mass immediately pre-spawning (October) at North Cove was 0.33 g (1997), and 0.43 g (1998). This translates to a spawning output of 0.13g and 0.18g dry mass in 1997 and 1998 respectively for a standard 30mm test diameter echinoid.

5.4 Discussion

That echinoids can survive over a wide range of habitat types has been related to the ability to display varying degrees of morphological plasticity under differing environmental regimes (Marcus 1983). Reduced food availability causes a relative increase in mass of the Aristotle's lantern and decrease in mass of gut under laboratory conditions in *Strongylocentrotus purpuratus* (Ebert 1996). In the field, sea urchin populations from nutritionally poor areas with such adaptations have survival rates comparable to those from other regions, indicating that these phenotypic responses enhance survival (Ebert 1996). One of the most pronounced morphological responses to reduction in food availability, however, is reduction in gonad size (Keats et al. 1984; Minor & Scheibling 1997; Andrew 1986; Lawrence & Lane 1982; Nichols et al. 1985). For populations from areas of reduced food this inevitably leads to reduced reproductive output, although Thompson (1984) showed that *Strongylocentrotus droebachiensis* allocates more energy to reproduction under poor food conditions, thereby increasing reproductive effort, although reproductive output was ultimately lower.

The pronounced differences in gonad mass recorded for the three *S. neumayeri* populations around Rothera point are likely to be due to inter-site variation in food availability. Sea urchins from the soft bottom population at North Cove ingested only

sediment, whereas *S. neumayeri* from both South Cove and Lagoon (with the lowest and highest gonad masses respectively) consumed a similar diet, comprising a large proportion of sediment but also crustaceans, macroalgae fragments, polychaetes, bryozoans and occasional seal faeces. Rates of feeding (estimated as rate of faecal egestion) were highest at North Cove and very similar at Lagoon (S. Brockington unpublished data) and South Cove (Chapter 3).

Differences in animal size did not contribute to the inter-site differences in gonad mass, since similar sized urchins were used throughout, and small differences in mass were corrected arithmetically using a scaling coefficient. One possible further explanation for the inter-site mass differences is that reproduction in *S. neumayeri* varies over several cycles of differing length. There is a seasonal build up of gonad mass with an annual spawning event but this may be overlaid on a much longer cycle of gonad mass over a period of several years, culminating a substantial spawning event such as that seen in the Lagoon population in 1997/1998 austral summer. A circaseptennial rhythm (seven year cycle) has been tentatively suggested for *Strongylocentrotus purpuratus* based on 114 monthly collections of data over a ten year period (Halberg et al. 1987). Our data would not rule out a cycle of this duration, however many years of investigation are needed to validate the exact period of such a cycle.

Spawning was observed each year at each site, but was not generally associated with a reduction in gonad mass. The annual feeding period for *S. neumayeri* at Rothera is restricted to the summer months (Chapter 3), and a reduction in mass on spawning was not observed because of rapid feeding activity and laying down of nutritional reserves in the

gonad. Histological examination of testis estimated that reproductive output was 38.6% in 1997/98, and 41.9% in 1998/99 at the North Cove population. An independent estimate of reproductive output in 1998/99 was made at the same site by collecting sea urchins at the end of the winter non feeding period (October 1998) and holding them without food in the seawater aquarium at Rothera Station for four months (Chapter 7). Gonad mass of the experimental group declined both through spawning and through the need to use reserves to fuel metabolism. Loss of mass to fuel metabolism was corrected by measuring rate of oxygen consumption. This controlled laboratory based study indicated a reproductive output of 43% of the pre-spawning biomass, which compares closely with the 41.9% estimated here from histological data. These estimates are comparable with reproductive outputs recorded from warmer water species (Fig 6.9). In some years however reproductive output for *S. neumayeri* will be considerably greater than 42%, e.g. Lagoon Island in 1997/98 where over 60% of gonad mass was lost (Fig 5.2).

Many Antarctic benthic species show prolonged longevity combined with regular annual reproduction (Arntz et al. 1994). It has been suggested that this may balance reduced annual reproductive output in Antarctic benthos such that lifetime reproductive effort is comparable to that of shorter lived temperate species (Clarke 1987). The data presented here suggest that this is not the case for this species, since annual reproductive output is not markedly lower than temperate species, even in years when little clear reduction in gonad mass was observed. Somatic growth is however reduced in comparison to warmer water species (Fig 6.7). Chapter 6 provides a calculation of reproductive production to total production for *S. neumayeri*, and comparisons with other species.

Gametogenesis in *S. neumayeri* was reported as taking 18-24 months for both sexes by Pearse & Giese (1967) and Pearse & Cameron (1991) at McMurdo Sound, but only 12 months by Yakovlev (1983) at Hasswell Island (Davis Sea). The latter study, however, did not include a quantitative examination of seasonal oocyte size frequencies, and may have failed to record the presence of smaller pre-vitellogenic oocytes amongst the larger maturing ova.

The time-course of vitellogenesis varies considerably among different echinoid species. *Strongylocentrotus purpuratus* has an oogenic cycle of 6-9 months in California where mean annual seawater temperature is 20°C (mean annual seawater temperatures derived from LEVITUS94 Ocean Climatology: http://ingrid.Ideo.columbia.edu). Oocyte proliferation occurs in summer (May - August), ova accumulate in winter (December - March), and are spawned in late winter or early spring (J. Pearse, pers comm). This timing is very similar to *Echinometra mathaei* in the Gulf of Suez (26°C) (Pearse 1969), and is about half the time required by *S. neumayeri*. Holland (1967) reported a 9 to 12 month cycle for *Stylocidaris affinis* from Naples (25°C), and *Centrostephanus coronatus* in southern California (24°C) has a one month lunar cycle, similar to that of *Diadema* spp in the tropics (27°C) (Pearse, pers comm). Although these results suggest that low water temperature does slow gametogenesis, other environmental factors (photoperiod, food availability) affect both timing of the initiation of gametogenesis and its subsequent tempo.

Sterechinus neumayeri displays a highly seasonal metabolic rate (Chapter 4a). Long periods of minimal metabolic activity or 'hibernation' (Gruzov 1977) during the austral winter correspond to restricted feeding opportunities at that time. Overwintering

survival during this period of severe nutritional limitation has been attributed to the very low metabolic rates displayed by polar marine ectotherms, which allow highly efficient usage of stored reserves (Clarke 1988; Clarke & Peck 1991). The low overall winter metabolic rates have been associated with a suspension of metabolically expensive processes such as growth, coupled with a reduced basal metabolic requirement through reduced protein turnover (Clarke 1988). However the current study indicates that 93% of vitellogenesis occurs in winter, when feeding is suspended. Although nutritive tissue to fuel gametogenesis is layed down during summer, thermodynamic costs of vitellogenesis must be met by stored reserves. The continuance of metabolically active processes such as gametogenesis during the energy limited polar winter reinforces the hypothesis that survival over the polar winter poses no major physiological difficulties.

Chapter 6 Somatic growth

Data also presented in: Brockington S (submitted). Growth and production of the Antarctic echinoid *Sterechinus neumayeri* at Rothera Point, Adelaide Island. Journal of Animal Ecology.

6.1 Introduction

The energy assimilated by a sessile ectotherm is predominantly divided between somatic growth, reproductive growth and respiratory costs (the latter including both basal maintenance costs and energy expended in synthesis of somatic and reproductive tissue). The balance between these competing sinks is a major facet of life-history. Basal metabolic rates decrease with environmental water temperature in teleost fish (Clarke & Johnston 1999), bivalves (Peck & Conway 2000) and echinoids (Chapter 4a). Reduced respiratory demands under polar conditions have been described for other taxa including isopods (White 1975), amphipods (Chapelle & Peck 1995), and brachiopods (Peck *et al.* 1987). Annual rates of somatic growth are also generally reduced in polar species (Arntz *et al.* 1994). In contrast latitudinal gradients in reproductive output of the benthos have received little attention. These are nevertheless important because variation of both basal maintenance costs and growth rates with temperature suggest that overall growth efficiencies (Clarke 1987), or relative allocation of energy to somatic or reproductive production (Brey *et al.* 1995) may be different in cold water species.

Although annual somatic growth rates of polar invertebrates are low, trends in maximum body size with temperature appear to be taxa specific. Many groups show an increase in body size under reduced temperatures (Atkinson 1994), and Chapelle & Peck (1999) have demonstrated that the maximum size attainable by amphipods is limited by

water oxygen concentration. Bivalves however appear to show an overall decrease in size in the Antarctic, with over 60% of species measuring less than 10mm in length (Nicol 1970).

This aim of this study was to accurately record annual growth rates using a calcein mark / recapture experiment for adult urchins coupled with monthly cohort analysis for juvenile year classes. Growth data was then combined with measurements of population density, size structure (Chapter 1) and two independently determined measures of reproductive output (Chapter 5 & 7) to calculate both individual and population productivity, and also the balance between energy invested in somatic and reproductive production on a population basis. Growth rates, maximal sizes, and reproductive outputs of echinoids under polar conditions were then compared with published data to elucidate potential latitudinal trends.

6.2 Methods

Measurement of growth rate and production estimates were carried out at North and South Coves (Fig 1.1). A description of population density and structure is provided in Chapter 1. The scaling relationship between total urchin dry mass and test diameter was established for the North Cove population by drying a broad size range of urchins to constant mass at 60°C. Ash free dry mass was determined from the same urchins after ignition at 450°C for 24 hours.

The growth of juvenile sea urchins was assessed at South Cove from monthly measures of population size frequency distributions over a 1.5 year period. Sea urchins

were collected from 23m and were often associated with the dense macroalgal mat found at this depth. Collection of urchins was carried out by placing a 0.25 m^2 quadrat on the seafloor and collecting all material (mainly macroalgae) and placing this in a mesh bag. Once back at the Bonner Laboratory sea urchins were carefully sorted from the other material in the sample, and test diameters measured with calipers. For sea urchins < 3 mm test diameter measurements were carried out using a graduated steel rule and a binocular microscope at ×20. Shift of modal year class peaks to the right was treated as size increment data. This type of analysis is not possible for older age classes and growth of larger sea urchins was measured at both North Cove and South Cove using a mark recapture technique. Freshly collected sea urchins were immersed for three days in a bath of calcein (1g.l^{-1}, with pH corrected back to that of seawater by addition of solid NaOH). Calcein binds to sites of calcification, and produces a fluorescent mark visible under ultra-violet light (Wilson 1987). Urchins were returned to the site of collection in March 1998 and at North Cove were held in mesh cages. At South Cove urchins were released to a prominent rock face (free ranging), and also to two further cages, although these were destroyed by icebergs after only seven days. Sea urchins were recovered from the North Cove cages and the South Cove rock face in February 1999, (i.e. after 343 days or 0.94 yrs), and subsequently dried and returned to the United Kingdom for microscopy.

In the United Kingdom test diameters of the recaptured and dried sea urchins were measured, and the Aristotle's lantern removed. The lantern was then immersed in hot 5% NaOCl for 12 hours to allow separation of jaws from organic tissue, and these were then washed in 95% ethanol and dried. Two half jaws or demi-pyramids from each sea urchin were mounted in clear polyester resin, and examined using a stereo-microscope (×40)

under U-V light. Jaw height (i.e. size at t_1 after 0.94 yrs growth) was measured for each demi-pyramid from the tip to the upper inner edge and a mean value calculated. These values were used to establish the scaling relationship between jaw height and test diameter for both sites. Size at time zero (t_0) was assessed by measuring distance from tip of jaw to the fluorescent calcein mark. These size increment data were then used to establish a growth curve using the generalised von Bertlalanffy growth function fitted iteratively using Solver (Microsoft Excel):

$$Dia_t = S_\infty \times (1 - e^{-kt})^D$$

Where Dia_t = Diameter at age t; S_∞ = asymptotic size; k = growth constant and D is a scaling parameter. Annual somatic production was estimated for each year class from the growth function, and total production per square metre was calculated from the size frequency distributions and population density. Gonad mass at North Cove was 18.5% of dry mass or 51.7% of ash free dry mass (recalculated from data in Chapter 3), and spawning output at North Cove was 41.9% of initial gonad mass in 1998 (Chapter 5). This value was therefore used to calculate population reproductive output from the original biomass relationship and size frequency distribution for North Cove. Total annual production per m^2, the fraction of total annual population production invested in reproduction and annual P/B ratios were subsequently calculated from these data.

6.3 Results

Population density at North Cove was 80.2 $ind.m^{-2}$ (n = 13 quadrats, standard error 23.2). At both North and South Cove two year classes of juvenile sea urchins of modal test diameter approximately 2mm and 4mm were recorded in June 1997 (Fig 1.2). At North

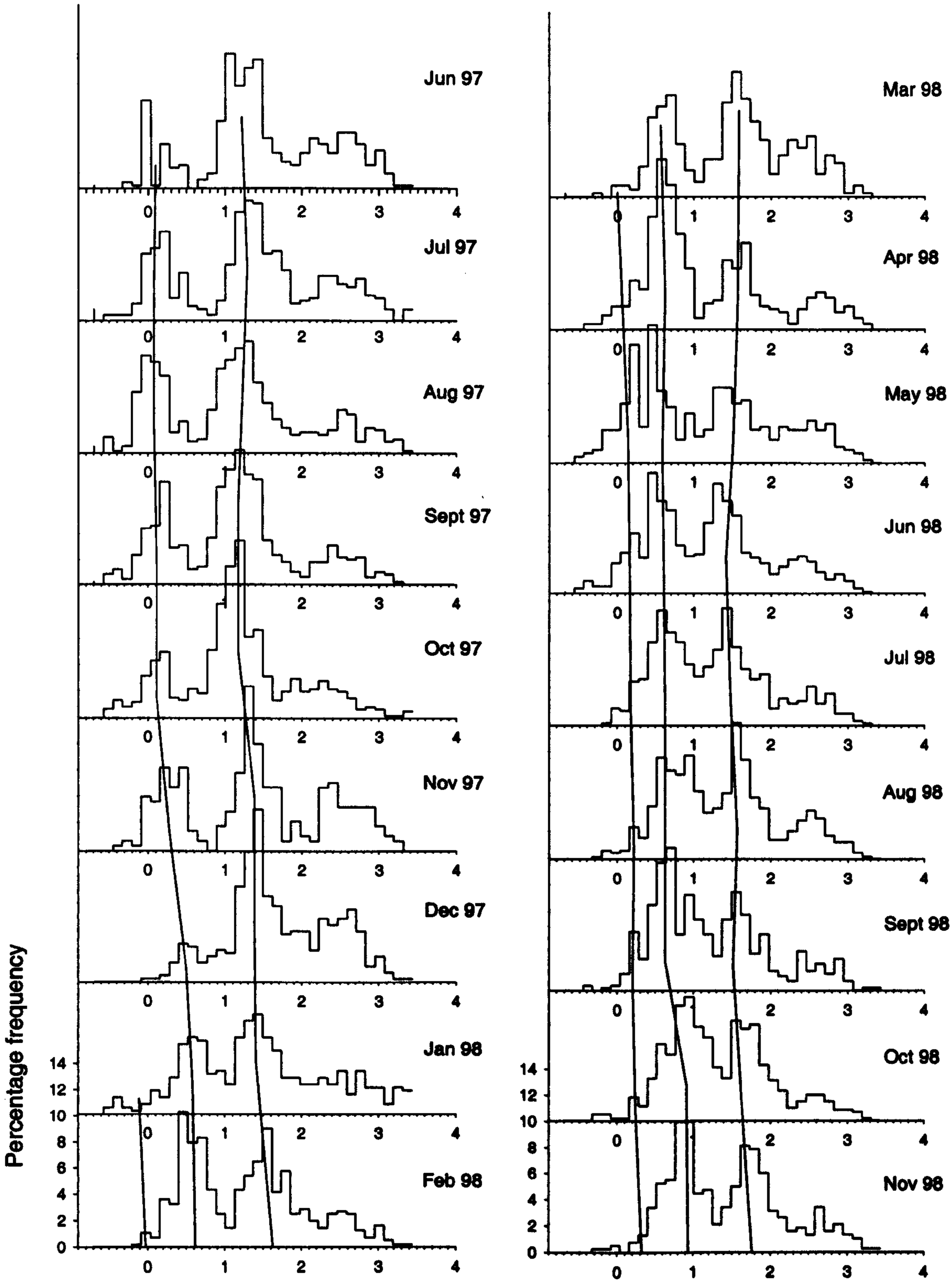


Figure 6.1 Sequential percentage population size frequency histograms indicating juvenile growth rate at South Cove. Note abscissa plotted as ln test diameter. Mean *n* per month = 496, total *n* = 8919. Lines indicate progression of modal year class peaks to the right.

Cove there were very few individuals in the range 8-14mm, although a large proportion of the population was greater than 20mm test diameter. This was in strong contrast to the South Cove population, where roughly 70% of individuals collected at 23 m were members of the two youngest year classes. Only a small proportion of the sea urchins were greater than 20mm diameter at this location.

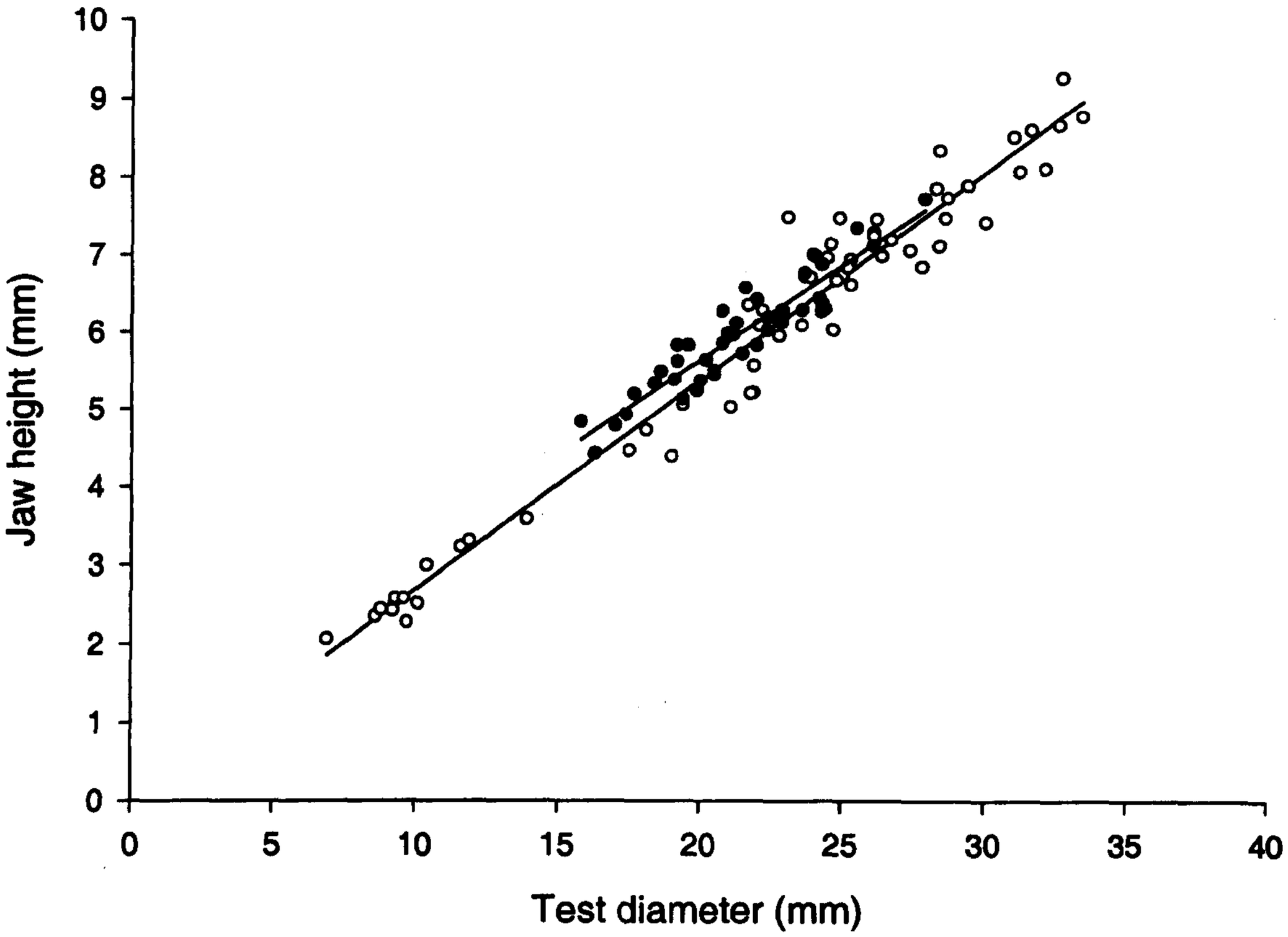


Figure 6.2 Least squares regressions of jaw height against test diameter for North (○) and South (●) Coves. For North Cove test diameter (mm) = 0.269.jaw ht + 5.39×10^{-4}; $r^2 = 0.96$, $n = 63$, intercept is not significantly different from zero. For South Cove test diameter (mm) = 0.245.jaw ht + 0.736; $r^2 = 0.87$, $n = 40$.

For the South Cove population an improved separation of the initial year classes was achieved by re-plotting size frequency histograms using the natural logarithm of test diameter for the abscissa (Fig 6.1). In June 97 peaks were visible at 0.1 and 1.2,

corresponding to test diameters of 1.1mm and 3.3mm respectively. The modal values of these peaks did not change between June and September 1997, and the first growth of the austral summer was observed in October 1997. By the end of the summer (March 1998) the peaks had progressed to 0.6 (= 1.8mm test diameter) and 1.6 (= 5.0mm). Growth then ceased during the 1998 winter, and recommenced in October 1998. By November 1998 the final peak diameters were 1.0 (= 2.7mm) and 1.8 (= 6.0mm). *Sterechinus neumayeri* is an annual spawner, and newly settled and metamorphosed urchins were first recorded in

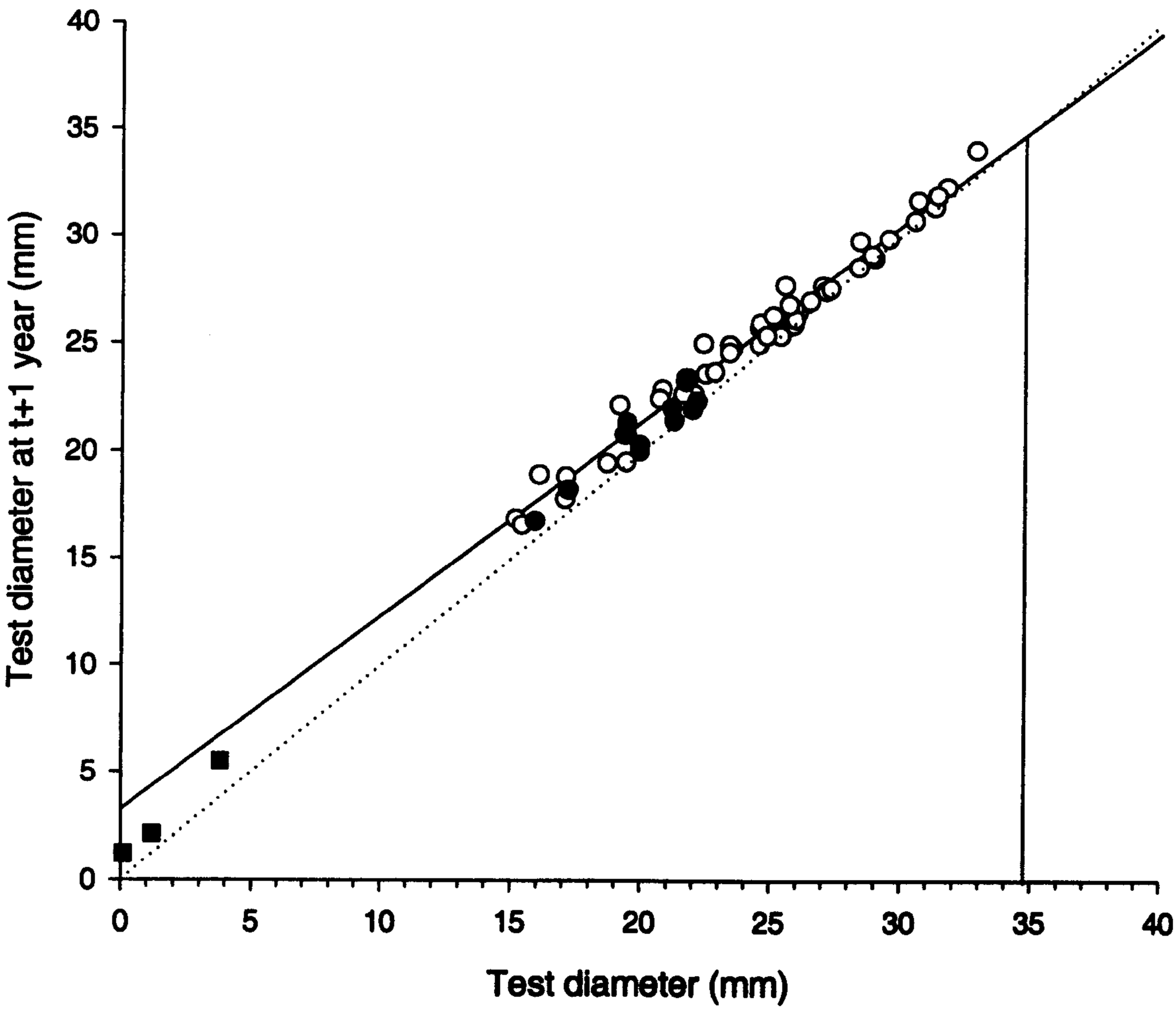


Figure 6.3 Walford plot indicating growth during 0.94 years as size increment data from North Cove (○) n = 50, South Cove (●) n = 11 and juvenile growth rates (■) estimated from Fig 6.1. Dotted line indicates zero growth. Solid line is a least squares regression fitted through the North Cove data points only; intercept of regression with line of zero growth (i.e. S_{∞}) = 34.5mm.

January and February 1998 (three months after spawning) with a modal peak diameter of -0.1 (= 0.9mm). The proportion of new recruits in the population continued to increase until May 1998, and in May and June three year classes could clearly be distinguished in the population distributions (Fig 6.1). Growth of the youngest cohort continued at a slow rate during winter, increasing from 0 (= 1.0mm) in March 1998 to 0.2 (= 1.2mm) in September 1998.

At North Cove 63 sea urchins recovered from cages were examined microscopically for the presence of a calcein label, and 50 displayed a prominent flourescent band. All 63 urchins examined were used to establish a jaw height vs test

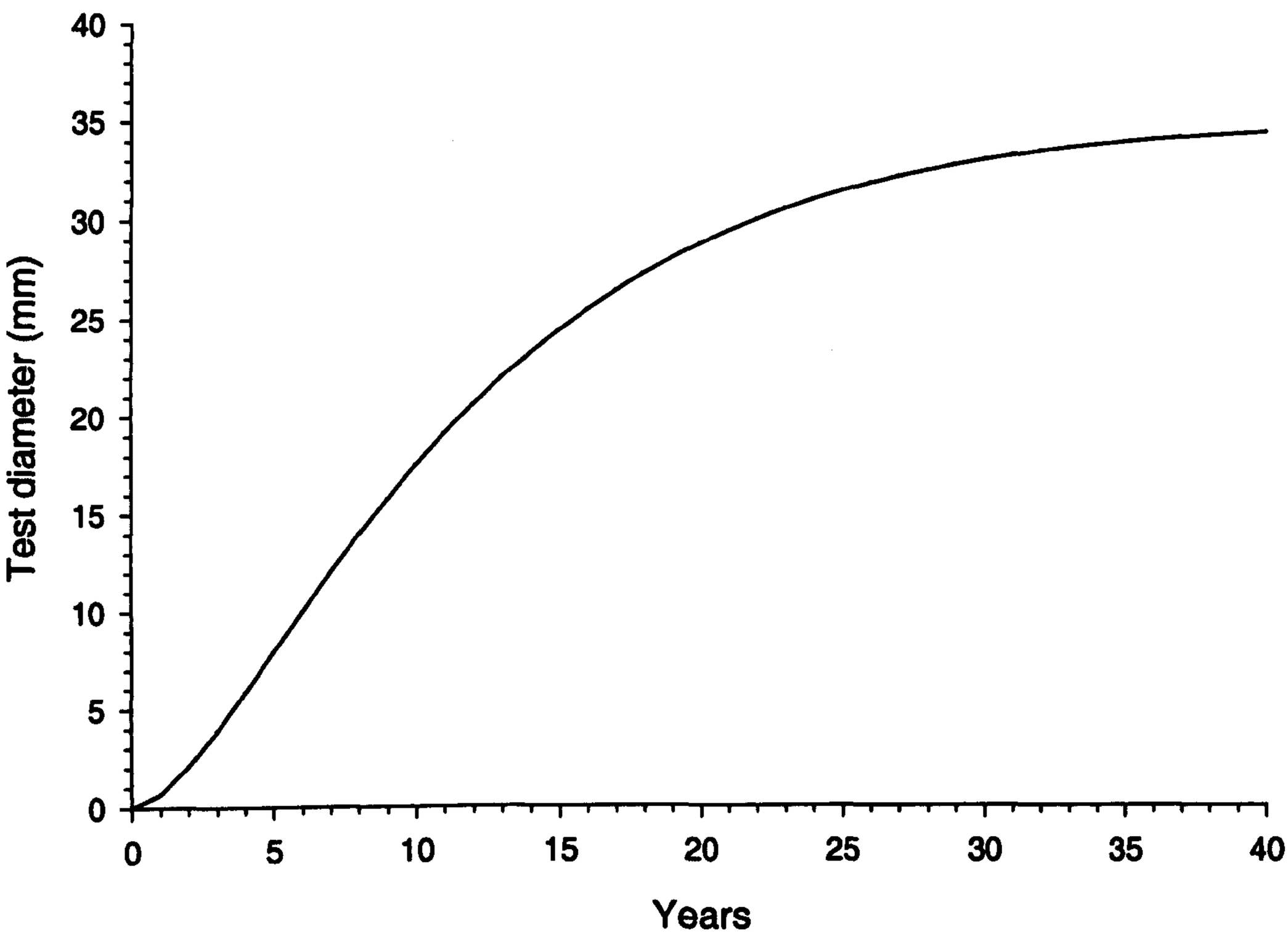


Figure 6.4 Generalised von Bertalanffy function fitted iteratively from North Cove size increment data and juvenile cohort rates. $S_\infty = 34.98$, $k = 0.133$, $D = 1.767$, $r^2 = 0.991$. Too few recaptures were recorded at South Cove to allow the fitting of a separate growth function.

diameter relationship (Fig 6.2). At South Cove 40 urchins were recovered, of which 11 were labeled. ANOVA indicated that the slopes of the regressions for the North and South Cove data sets were not significantly different ($F = 1.2$, $p = 0.276$), although the intercepts differed ($F = 11.29$, $p = 0.001$). Relative jaw size was therefore greater at South Cove.

The calcein marked urchins were used to generate pairs of size increment data. Juvenile growth rates (from population size frequency data) were converted to size increment data assuming that time zero (and therefore size zero) was at spawning in November. In the 12 months following November 1997 the three cohorts grew from 0.0mm, 1.2mm & 3.8mm to 1.2mm, 2.1mm and 5.5mm respectively. All growth data were collated onto a Walford plot (Fig 6.3). The S_∞ obtained from this plot ($S_{t+1} = S_t$) was at 34.5mm test diameter. Size increment data from North Cove mark recapture (adult growth) and South Cove size frequency distributions (juvenile growth) were pooled and a single generalised von Bertalanffy function was fitted iteratively to these data (Fig 6.4):

(1) $$Dia_t = 34.98 \times (1 - e^{-0.113t})^{1.717} \qquad r^2 = 0.991$$

The small number of replicates from the South Cove mark recapture experiment precluded the fitting of a separate growth function for this location. ANOVA however indicated that the slopes of regressions fitted for the North and South Cove data on the Walford plot (Fig 6.3) were not significantly different ($F = 0.02$, $p = 0.887$). However the intercept of the two regressions was significantly different (ANOVA: $F = 2032.9$, $p < 0.001$), indicating that growth was slower, and asymptotic size lower at South Cove.

The dry mass (DM) and ash free dry masses (AFDM) of sea urchins from North Cove were related to the test diameter (Dia) by the following relationships:

(2) Log_{10} DM(g) = -3.321 + (2.496.$\log_{10}$ Dia (mm)) $r^2 = 0.99$, n = 80

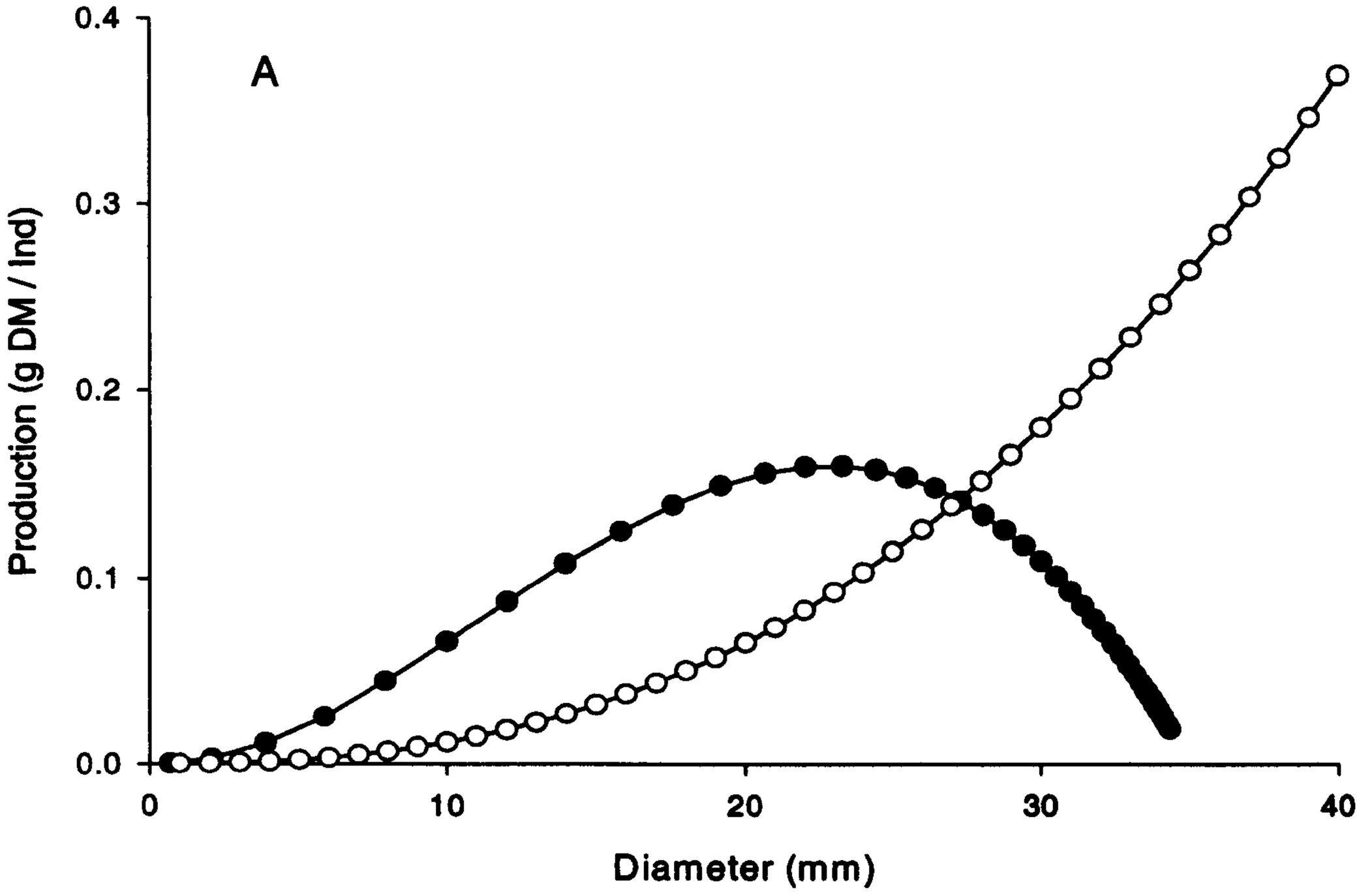
(3) Log_{10} AFDM(g) = -4.209 + (2.744.$\log_{10}$ Dia (mm)) $r^2 = 0.98$, n = 80

Somatic production, calculated for each year class using equations 1, 2 & 3 (Fig 6.5), peaked at 23mm test diameter (0.18 g DM.yr^{-1} or 0.05 g AFDM.yr^{-1}). Annual reproductive output was calculated using the estimate of 41.9% initial gonad mass (Chapter 5) and increased exponentially with test diameter. Reproductive production exceeded somatic production at 28 mm (DM) or 20 mm (AFDM).

The P:B ratio for *S. neumayeri* at North Cove was 0.21 (DM) or 0.35 (AFDM).yr^{-1} (Table 6.1). Using the population distribution described by Fig 1.2, total production invested in reproduction by the population was 62% (AFDM).

Table 6.1 Biomass and productivity at North Cove, calculated using population density of *S. neumayeri* = 80.m^{-2}. B biomass; P production; S somatic; G reproductive; T total; DM dry mass; AFDM ash free dry mass. Energy content of organic tissue calculated using the value of 23kJ.g^{-1} (Brey 1988), and skeletal (inorganic) enthalpy of precipitation using the Gibbs free energy equation cited in text (p149).

	DM	AFDM
B_T (g m^{-2})	80.89	23.26
P_S (g m^{-2} yr^{-1})	10.33	3.14
P_G (g m^{-2} yr^{-1})	6.28	5.04
P_T (g m^{-2} yr^{-1})	16.61	8.19
P_T / B_T (yr^{-1})	0.21	0.35
P_G / P_T	0.38	0.62



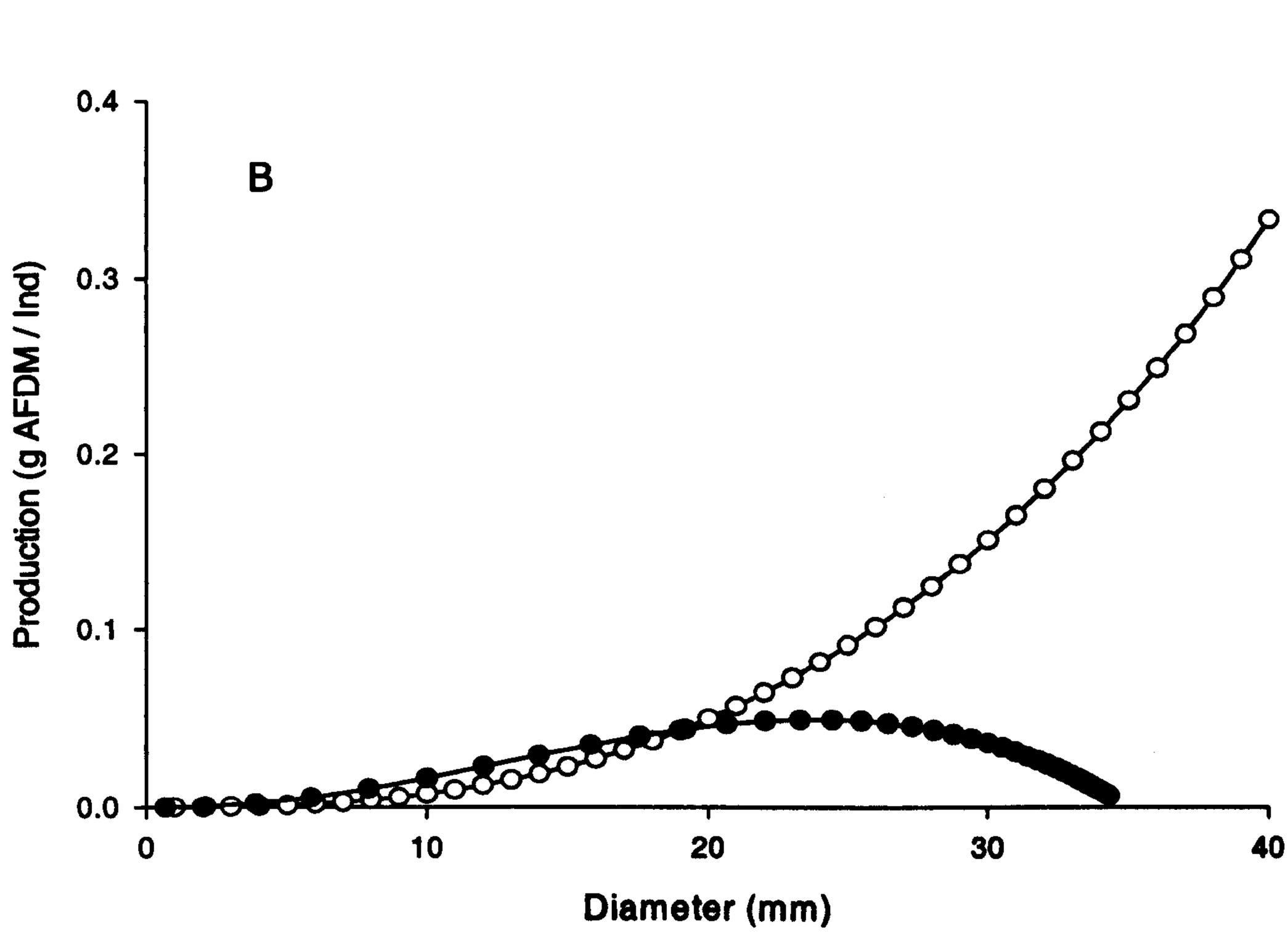


Figure 6.5 Individual somatic (●) and reproductive (○) production in relation to test diameter for S. neumayeri at North Cove. A) Dry mass, B) Ash free dry mass.

6.4 Discussion

An inverse relationship between relative jaw size and environmental food availability has previously been established for echinoids (Ebert 1980, Edwards & Ebert 1991 and Levitan 1991). Brey *et al.* (1995) demonstrated that *Sterechinus neumayeri* from sites in McMurdo Sound where food was abundant grew faster and had a lower relative jaw size than those from sites with a lower food availability. Although the data from South Cove in the present study were insufficient to allow the fitting of a separate growth function, ANOVA indicated that urchins from this location had significantly larger jaws, and a significantly slower growth rate. Furthermore, faecal egestion rate at this location was lower compared to North Cove (Chapter 3) and reproductive output was also markedly reduced (Chapter 5). Taken together these observations suggest that production by urchins at South Cove was restricted because of a comparatively reduced food availability.

The growth rate of different species may be compared using the von Bertalanffy growth coefficient, k, which generally falls in the range 0.01 to 1.30 for echinoids. If k is large growth is comparatively fast and asymptotic size is reached early; if k is small then growth proceeds slowly and the overall growth function approaches the asymptote later and as a gentle curve instead of a sharp inflexion. The value of k for *S. neumayeri* at McMurdo Sound was 0.031 when data from all sites investigated were pooled, but growth varied between site in relation to food availability (Brey *et al.* 1995). One other study on echinoids from polar regions also reported slow growth (k = 0.011) and extended longevity of *Stronglyocentrotus pallidus* in the northern Barents Sea at 80°N (Bluhm *et al.* 1998). Among other echinoderms McClintock *et al.* (1988) recorded extremely slow growth rates for the ubiquitous Antarctic sea star *Odontaster validus* at McMurdo.

Growth rates of echinoids have been widely studied and have been shown to be strongly correlated to food quantity and quality (Lawrence and Lane 1982). This variability has tended to mask any trend in growth with latitude (Ebert 1975, 1982). A detailed investigation of the growth of the red sea urchin *Strongylocentrotus franciscanus* at 18 localities over a latitudinal range of almost 30° found that growth differed amongst sites ($p < 0.001$), but failed to find a north - south correlation ($p > 0.80$). Annual survival rates were however correlated with latitude, being higher in the north (Ebert *et al.* 1999). A combination of all available data from the published literature suggests that for echinoids the upper limit of the von Bertalanffy growth coefficient is determined by mean annual seawater temperature, or by a factor which co-varies with temperature (e.g. environmental

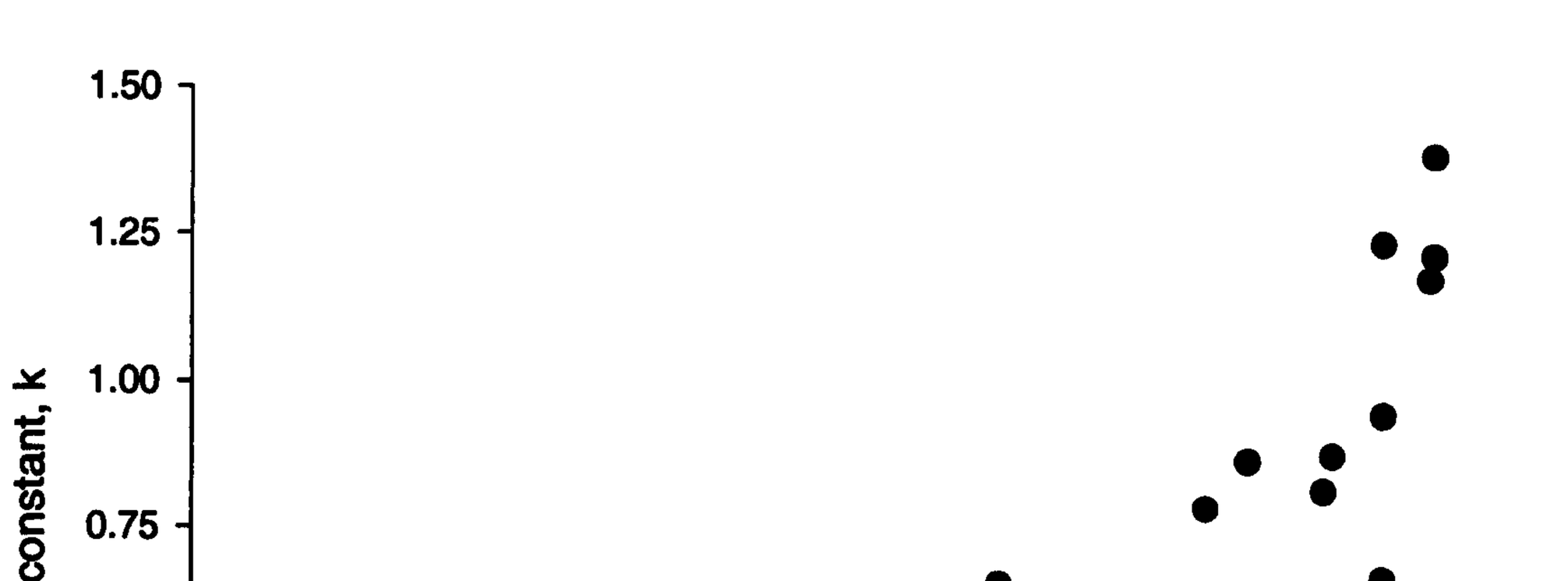


Figure 6.6 Growth constant, k, from von Bertalanffy equations for 34 echinoid species from 64 different investigations. Mean annual seawater temperatures at specific locations obtained directly from original publications or from the LEVITUS94 Ocean Climatology data set (http://ingrid.ldeo.columbia.edu) Original data sources listed in Appendix 2.

seasonality) (Fig 6.6). However, at any given location not all species achieve the maximum potential, and growth rates of some echinoids from warm water or tropical locations may be as slow as those from polar regions.

Seasonality in echinoid growth may be caused by variations in food supply, a decrease in feeding associated with spawning, or as a consequence of an inverse relationship between somatic and gonadal growth in some species (Lawrence and Lane 1982 and references therein). Primary production in polar environments is highly seasonal (Clarke 1988, Clarke & Leakey 1996), with the austral winter representing a period of low food availability. Feeding in *S. neumayeri* ceased entirely during the 1997 winter for 6 months, and for 4 months in the 1998 winter (Chapter 3). Nutritional reserves for gamete production were laid down during summer, although gametogenesis continued over an 18+ month cycle, (Chapter 5) indicating a decoupling of reproductive development (although not acquisition of energetic reserves to fuel gametogenesis) from feeding. Somatic growth in juvenile urchins at Rothera ceased for 4 months in winter 1997 and 7 months in winter 1998. This strong seasonality was caused by nutritional limitation associated with an absence of feeding, and implies that growth in adult urchins is also highly seasonal.

For many ectotherm species temperature plays an important role in determining differences in size between subsequent generations. Atkinson (1994) showed that in 91 out of 109 studies a decreased environmental temperature (within a non stressful range) led to an increased size at a given stage of development, although suggested explanations for this trend have remained contentious (Atkinson and Sibly 1997, and references therein). Increased body size under polar conditions has been reported for a few taxa (e.g.

nudibranchs: Wägele 1988), and a cline of increasing size with latitude within the Southern Ocean has been recorded for caridean shrimps (Arntz *et al.* 1994). Chapelle & Peck (1999) showed increasing size with latitude in amphipods and provided convincing evidence that oxygen concentration of seawater (as restricted both by environmental temperature and salinity) causes a restriction on maximal size attainable by amphipods over a global range. Other taxa may however show a decrease in maximum size attainable under polar conditions: Nicol (1970) commented that over 60% of Antarctic bivalves are less than 10mm in length.

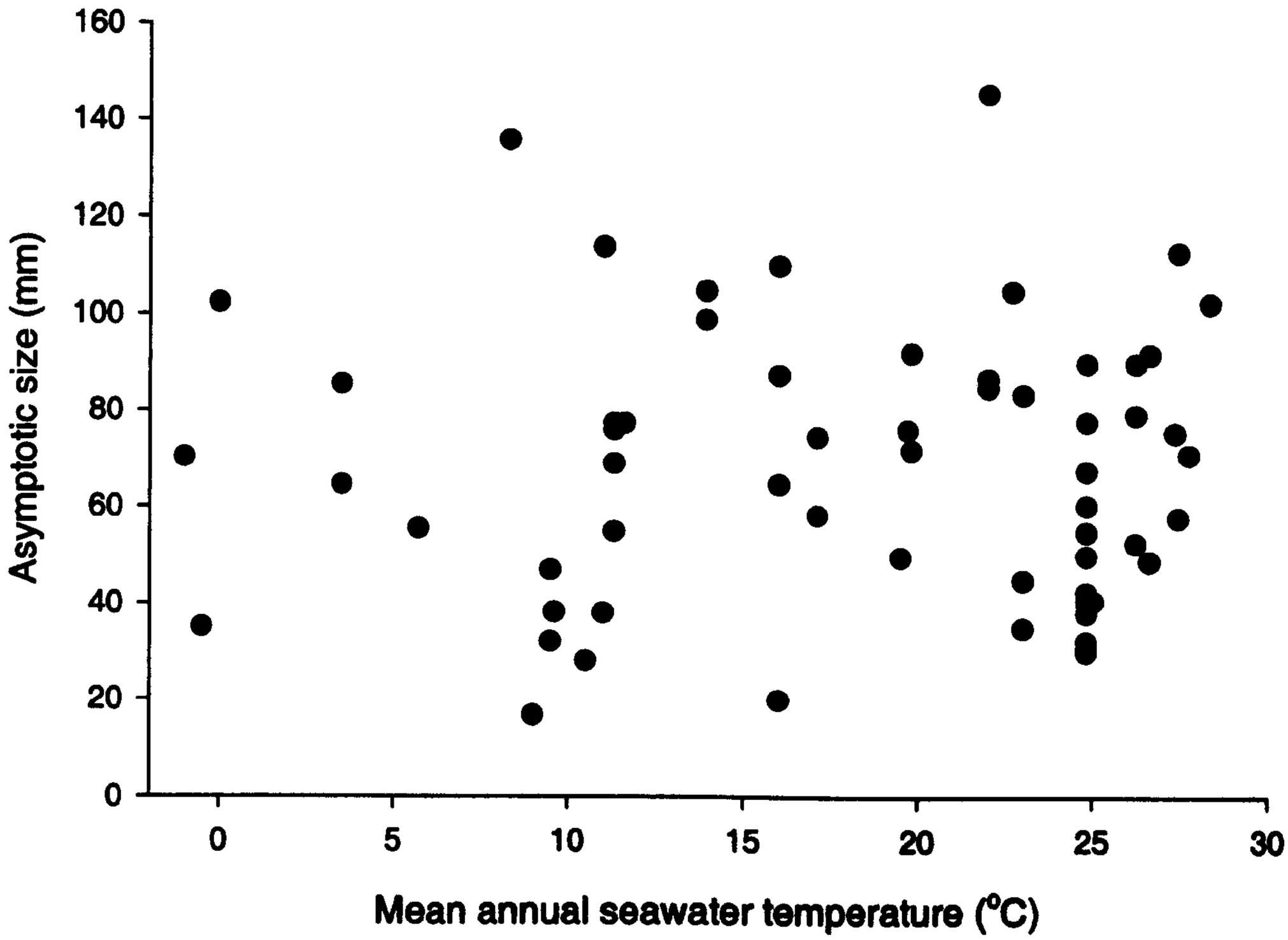


Figure 6.7 Asymptotic size of 34 echinoid species compiled from S∞ values of von Bertalanffy equations from 64 separate investigations. Data sources as for Fig 6.6.

The intraspecific variability in maximum body size of echinoids in the field is well established and generally ascribed to food availability (Lawrence and Lane 1982), although excessive mortality as a result of predation or disease may be important at some locations. Increased echinoid density (with the consequent increase in intraspecific competition for food) does not however always result in a decrease in mean population body size. Many combinations of density and size of individuals occur, suggesting multiple control of alternate states (Lawrence 1984). Maximum size of *S. neumayeri* at Rothera revealed by this study (35mm) is much smaller than for the populations at either McMurdo (S_∞ = 70.2mm, location = 77°S; Brey *et al.* 1995), or Signy Island (location = 60°S, maximum size of 59 randomly collected urchins = 54.6mm, mean = 41.6mm; S. Brockington unpubl. data). A collation of maximal (i.e. asymptotic) test diameter estimates for sea urchins from published literature where von Bertalanffy functions have been fitted indicates that S_∞ is not temperature dependent, and hence not latitude dependent(Fig 6.7). However the estimation of S_∞ for a population using growth functions is notoriously imprecise because outlying data has a large effect on the asymptotic value obtained, and hence Fig 6.7 may indicate a greater variability in echinoid maximal sizes than is actually present.

Overall growth performance may be assessed using the index ϕ where:

$$\phi = \log (K) + 0.667 \times \log S_\infty \qquad \text{(Munro and Pauly 1983).}$$

This index provides a composite measure of growth which encompasses both size and rate. ϕ was calculated for 28 Antarctic and 141 non-Antarctic marine invertebrate species by Arntz *et al.* (1994), and was found to be significantly smaller in Antarctic populations,

indicating that on average growth performance is reduced in polar regions, although there was a large degree of overlap between the populations. Calculation of ϕ for echinoids reveals that the majority of the values fall in a broad band which correlates positively with mean annual seawater temperature, although six values in the range 24 - 30°C were recorded below the general broad trend (Fig 6.8). Three of these latter values were species with massive body walls (*Heterocentrotus trigonarius*, *H. mammillatus* and *Colobocentrotus atratus*), developed as a response to an exposed physical environment (Ebert 1982). The remaining three species to show unusually low growth performance (*Echinothrix diadema*, *Diadema setosum* & *Stomopneustes variolaris*) may have been recorded from areas of excessively poor food availability, especially since one of these

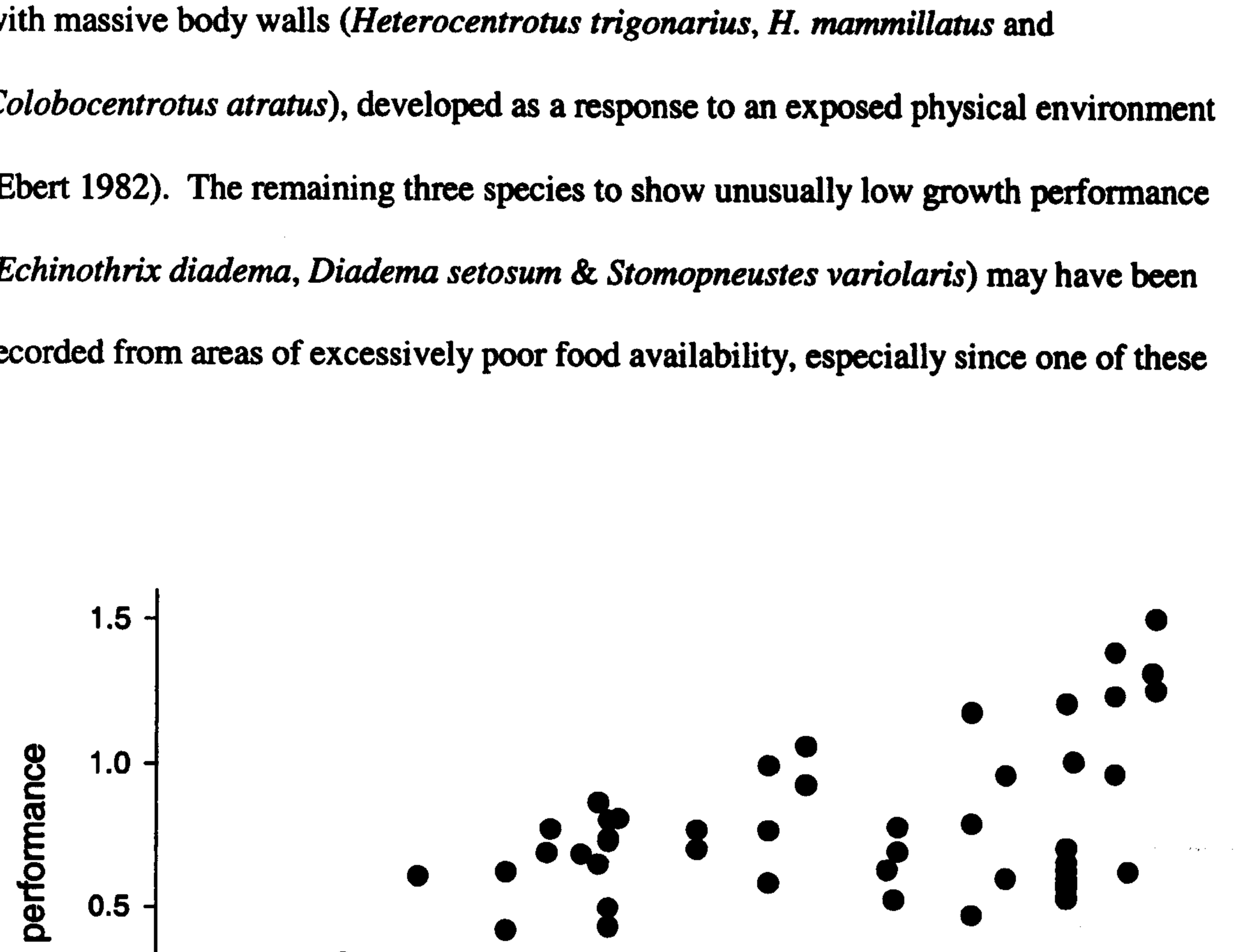


Figure 6.8 Growth performance, ϕ, = log (k) + 0.667 × $\log_{10}$ ($S\infty$) (Munro and Pauly 1983). Data sources as for Fig 6.6.

species (*E. diadema*) was recorded as growing at a considerably faster rate by Ebert (1975) at a different location (Hawaii). If these six values are removed, a least squares regression through the remaining points is described by $\phi = 0.032 \times \text{Temperature} + 0.132$. The overall relationship described by Figs 6.6 and 6.8 may represent either a fundamental constraint of temperature on growth, or is a reflection of an increase in seasonality towards polar regions (long periods of winter starvation reducing annual growth rate).

P:B ratios for Antarctic marine benthic populations range between 0.116 for *Sterechinus antarcticus* from the Weddell Sea and 0.87 for *Philine gibba* at South Georgia (Arntz *et al.* 1994). Brey & Clarke (1993) showed that although Antarctic populations have significantly lower P:B ratios than warmer water counterparts, the differences were removed by taking into account the effects of depth and mean seawater temperature (i.e. Antarctic populations do not display any special adaptations). The P:B ratio for *S. neumayeri* from the current study (0.21 DM or 0.31 AFDM) falls within the range of values already recorded for polar benthic invertebrates and indicates a low overall rate of production. Ratio of individual reproductive production to total production was reported as between 0.96 and 0.98 (AFDM) for *S. neumayeri* at McMurdo (Brey *et al.* 1995). This estimate is extremely high, but the ratio was calculated using an assumed reproductive output of 60% maximum gonad mass, which is the value estimated by Pearse & Giese (1967). The current study, using an accurately determined and site specific reproductive output indicates a ratio of reproductive production to total production of 0.62 (AFDM). This result is considerably lower than that for *S. neumayeri* at McMurdo, but still at least 50% greater than the values compiled by Brey *et al.* (1995) for *Mellita quinquiesperforata*: 0.34 AFDM (Lane 1977), *Moira atropes*: 0.30 AFDM (Moore and Lopez 1966),

Parechinus angulosus: 0.18 AFDM (Greenwood 1980) or *Strongylocentrotus droebachiensis*: 0.19 AFDM (Miller and Mann 1973).

The reproductive output of a variety of species of echinoids has been summarised by Lawrence and Lane (1982). As for somatic growth rate, reproductive output of a species is highly dependent upon the nutritional conditions at the study location. However, unlike somatic growth rate, maximum reproductive output is not influenced directly by mean annual seawater temperature, and echinoids at cold water locations may have

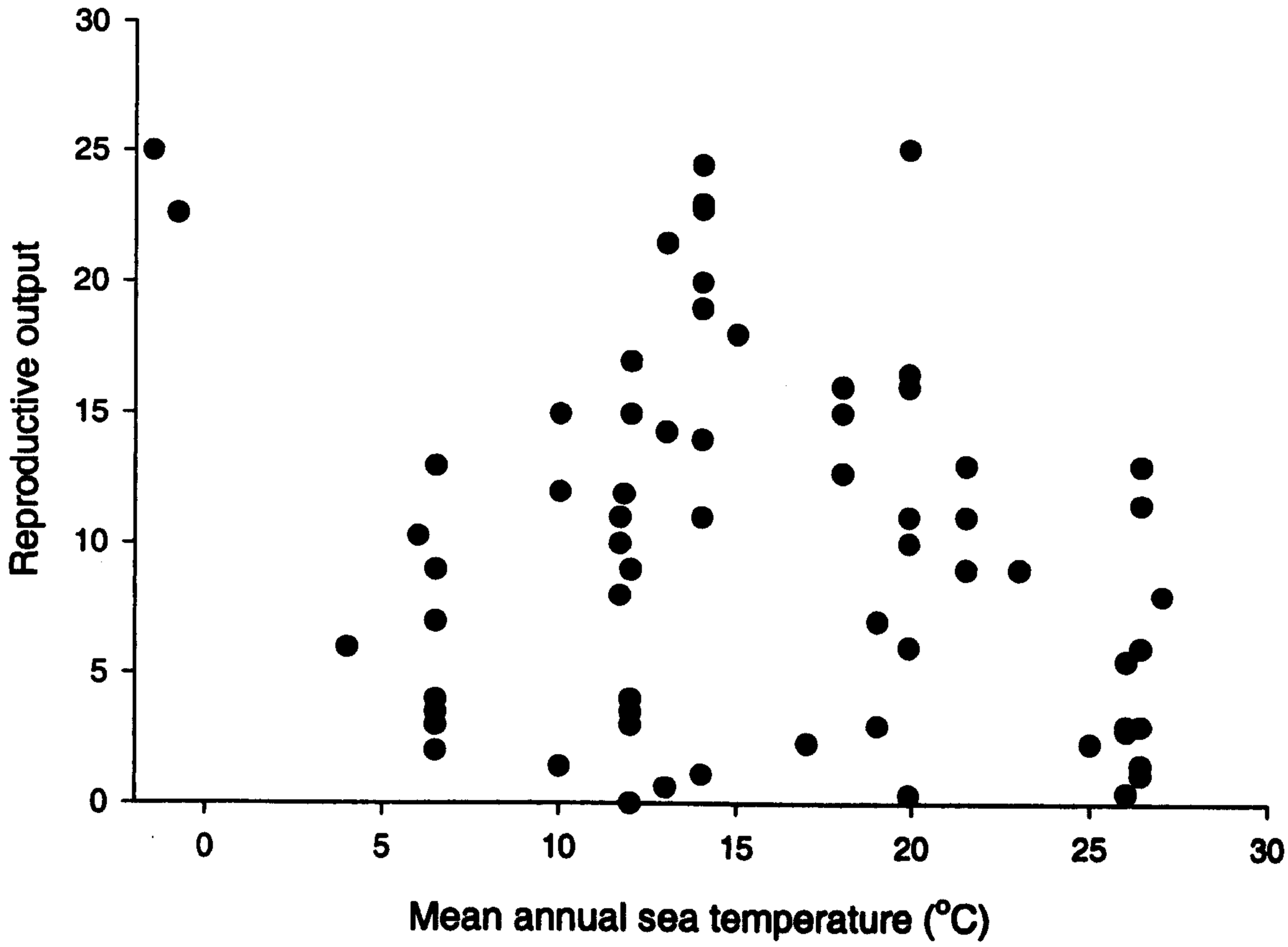


Figure 6.9 Reproductive output of 39 species of echinoid, compiled from 71 separate studies. Reproductive output was calculated as maximal (i.e. pre-spawning) annual gonad index minus minimal (immediately post spawning) gonad index. Only studies where gonad index was expressed as either wet mass (or volume, since density of gonad tissue is roughly equivalent to seawater) have been used so that data is directly comparable. Mean annual seawater temperatures from LEVITUS94 Ocean Climatology data set. Original references listed in Appendix 2.

reproductive outputs as high, if not higher than warmer water species (Fig 6.9). In the environs of Rothera Point *Sterechinus neumayeri* showed a broad range of reproductive outputs, from near maximal at Lagoon Island to minimal at South Cove (pers obs). Reproductive output of 13 polar asteroids from McMurdo (77°S) were also found to be similar to published values for temperate, tropical and subtropical species (McClintock 1989). The maintenance of reproductive output over the latitudinal range coupled with a decrease in metabolic rate and somatic growth rate indicates that polar echinoids devote a larger fraction of assimilated energy to reproduction than their warmer water counterparts. Hence in contrast to model predictions made by Clarke (1987) reproductive effort for echinoids is greater under polar conditions.

6.5 Conclusion

These data indicate that somatic growth of *Sterechinus neumayeri* is highly seasonal, and that overall annual growth rate is very low. Decreasing seawater temperature along a latitudinal gradient has had no effect on maximal individual size for echinoids. Combining growth rate with an accurately derived estimate of reproductive output showed that overall annual production (compared as P:B ratio) for *S. neumayeri* is low in common with other polar species. However, ratio of reproductive production to total production is much higher than would be expected by comparison with non polar ectotherms. This may reflect the need to maintain reproductive output to optimize recruitment. The low somatic growth rates may therefore be in part a consequence of severe seasonal energy limitation in polar environments, and in part due to a higher than normal fraction of assimilated energy being devoted to reproduction.

Chapter 7 The relative influence of temperature and food upon seasonal metabolism.

Data also presented in: Brockington S, Clarke A (in press). The relative influence of temperature and food on the metabolism of a marine invertebrate. J. Exp. Mar. Biol. Ecol.

7.1 Introduction

Throughout many of the continental shelves of the world marine invertebrates are subject to coincident seasonal variations in environmental factors including photoperiod, food availability and temperature. The biological response to this seasonal variability by benthic invertebrates can include elevated rates of growth, feeding and oxygen consumption in summer (Chapters 3, 4 and 6). It can, however, be difficult to attribute the rise in biological tempo to any specific environmental factor (for example food or temperature) because of their parallel variation. Many laboratory studies have shown a positive correlation between metabolic rate (oxygen consumption) and temperature, and as a result much of the seasonal variation in biological activity has traditionally been related to elevated environmental temperature. More sophisticated experimental designs (Parry 1978; 1983) have however suggested that food may be important, and this is now becoming more widely recognised. In polar regions the seasonal variation in seawater temperature is small or very small (Chapter 2), whereas that of food availability is very marked (Clarke 1988; Clarke and Leakey 1996). This natural separation of factors which co-vary over the annual cycle almost everywhere else in the world provides a natural laboratory for distinguishing the effects of food and temperature on marine invertebrate animals.

Antarctic benthic invertebrates typically exhibit low resting metabolic rates, slow growth, long development times and a highly seasonal biology (Arntz et al.1994). The generally reduced P:B ratios of the Antarctic benthos may be attributed either directly to low temperature or to seasonal food limitation. Pearse et al. (1991) suggested that the prolonged development times of lecithotrophic larvae (with adequate energy stores) under polar conditions indicate a direct developmental retardation resulting from low temperature, and both Bosch et al. (1987) and Stanwell-Smith and Peck (1997) have demonstrated a strong temperature dependance for developmental rates of polar echinoderm larvae. Clarke (1988) however has argued that the short and intense period of phytoplankton supply to the benthic community results in a reduced period of time during which biological secondary production can take place, causing slower mean annual rates of growth for the Antarctic benthos. This would lead to reduced annual growth irrespective of any temperature compensation. Indirect evidence for the energy limitation hypothesis is supplied by the relatively fast growth rates shown by some Antarctic benthic invertebrates at the height of the summer phytoplankton bloom (Dayton 1974; Sagar 1980; Clarke 1988); in these cases the biochemical capacity for faster growth exists, despite the low seawater temperatures.

There are two obvious tests for the hypothesis that seasonal energy limitation, rather than direct reduction by temperature is responsible for the polar benthic life history characteristics, namely:

1. To feed organisms in winter. In general this approach has not been successful, since benthic invertebrates and fish held in captivity typically refuse to take food

during the winter months, even when it is supplied. This indicates either that higher level hormonal controls may have switched off appetite and synthetic machinery (Clarke 1991) or alternatively that the gut and food processing apparatus has atrophied (e.g. Chapter 3).

2. To deprive organisms of food in summer. The summer rise in metabolism may be expected to be in part because of costs of feeding, production and other activities and partly because of a rise in basal metabolism over winter levels due to higher water temperature. Following the change in metabolic rate of starved animals held at summer temperatures may allow the influence of temperature and food to be distinguished. This approach is a crucial initial exploration of the relative influence of temperature and food, but it does assume that starvation induces no other metabolic changes.

Data is presented here on the metabolic responses and changes in organ mass of winter conditioned *S. neumayeri* held in the laboratory subject to a natural rise in seawater temperature over the austral summer period, and is compared with the results obtained from the wild population (Chapters 3 and 4a).

7.2 Methods

Sixty sea urchins of roughly 30mm test diameter were collected from North Cove (Fig 1.1) by SCUBA divers on 9 October 1998, a time when the wild population was still in a state of winter dormancy (i.e. not feeding: Chapter 3). Seawater temperature at this time was -1.7°C and water column chlorophyll standing stock <0.2 $\mu g.l^{-1}$. Sea urchins

were transferred to the Research Station aquarium facility, and held in a large flow through tank containing fresh running seawater. Photoperiod in the laboratory was controlled to approximate natural variations in day length, and the tank was cleaned regularly to remove any microalgal build up. Temperature in the aquarium was continually recorded by a logger.

Measurements of metabolic rate and organ mass were carried out on 16 randomly selected individuals from the aquarium tank on three occasions: October 1998 (shortly after collection), January 1999 and February 1999. Simultaneous assessments of oxygen consumption and ammonia excretion rate were performed using methods described in Chapter 4a. Organ masses and horizontal diameters of the sea-urchins used for the metabolic measures were then recorded using methods described in Chapter 3.

The sizes of sea urchins used in this experiment ranged from 24.9 to 39.6mm test diameter (mean 30.2mm). Rates of oxygen consumption, ammonia excretion and organ masses were corrected arithmetically for sea urchin size using the scaling exponents calculated in Chapters 3 & 4a. Values were corrected to a standard animal size of 30mm test diameter.

7.3 Results

Seawater temperatures in the flow through aquarium facility at Rothera Station never varied by more than 0.15°C from the sea temperatures recorded at 15m depth at the oceanographic time series station in Ryder Bay for the duration of this study. During the austral summer of 1998/1999 seawater temperature in the aquarium increased from -1.7°C

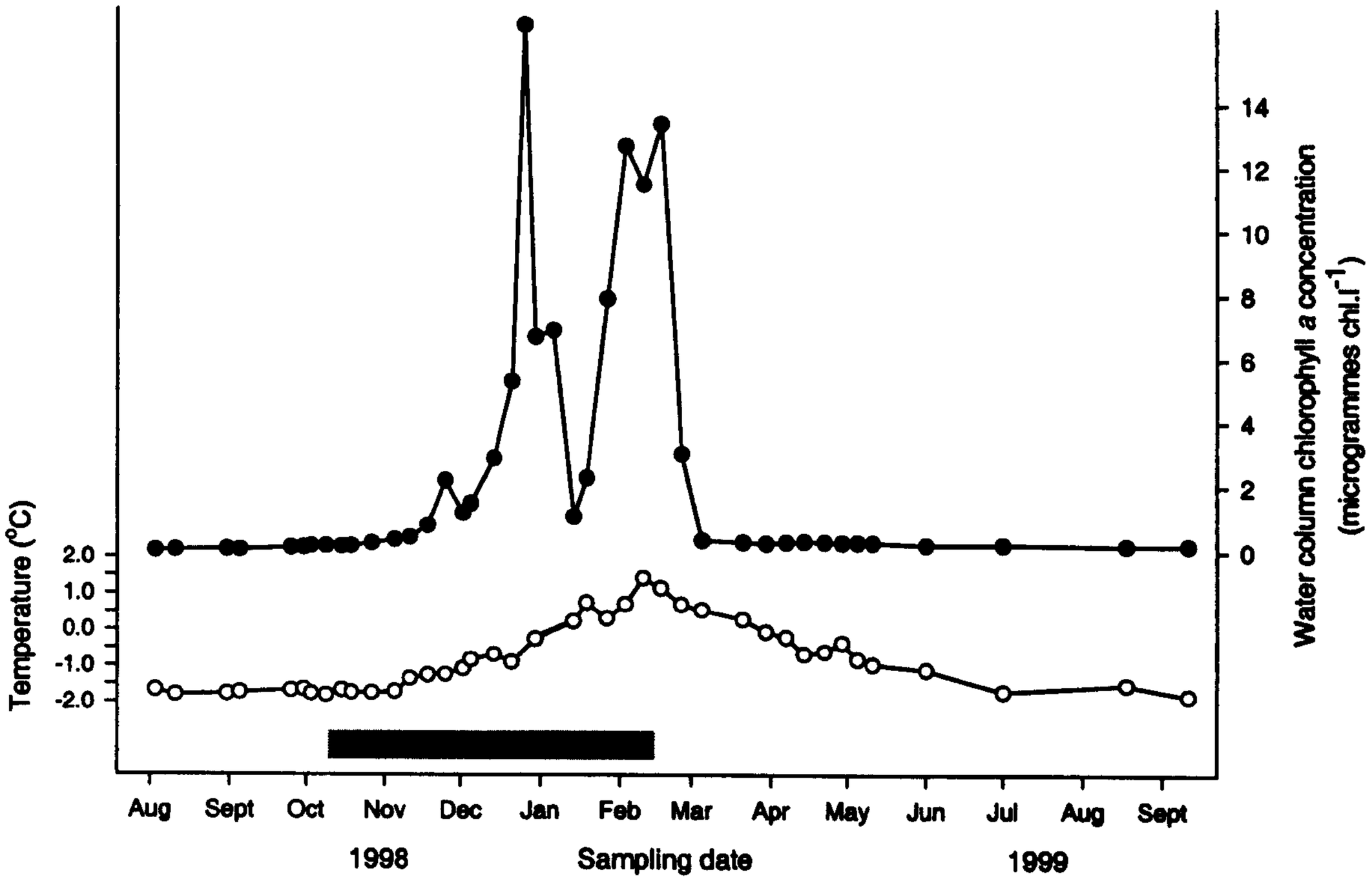


Figure 7.1 Water column chlorophyll standing stock and seawater temperature at Rothera Station. Data were collected from 10m depth as part of the Rothera Time Series (RaTS) oceanographic monitoring programme. The grey bar indicates the duration of this study - sea urchins were removed to the aquarium whilst chlorophyll concentration was still at winter levels, and metabolic responses to temperature were recorded in the absence of food.

in October to +0.6°C in January and +0.9°C in February. A strong phytoplankton bloom was recorded in the environs of the Rothera Station from November until February which peaked at 16.7 μg chl.l^{-1} (Chapman 1999). This profile of roughly 3°C seasonal variation in temperature and strong intense peak of primary production (Fig 7.1) is typical of maritime Antarctic nearshore locations (Clarke et al. 1988). Feeding rates of the wild population increased sharply from zero in October, to a maximum in January (Chapter 3). No feeding activity (i.e. faecal egestion) was observed in the starved sea urchins held in the aquarium. Mean oxygen consumption rates of the starved sea-urchins in October 1998 was 0.79 μmols.hr^{-1} and this compared well with the rate recorded for the wild population at

this time (Fig 7.2). By January and February the oxygen consumption of the starved sea urchins had increased slightly to 0.98 and 1.05 μmols.hr^{-1} respectively. If this small increase is assumed to be driven solely by the slight rise in temperature, then a Q_{10} value can be calculated. Although both the increase in metabolic rate and temperature were small, calculation reveals biologically realistic Q_{10} values of 2.54 (January) and 2.99 (February) over the winter October levels. The rise in respiratory rates of the starved sea urchins contrasted strongly with the much larger increase in oxygen consumption rates displayed by the wild population at the height of the austral summer (Fig 7.2). If it is assumed that the summer increase in metabolic rate in field sea urchins is driven by

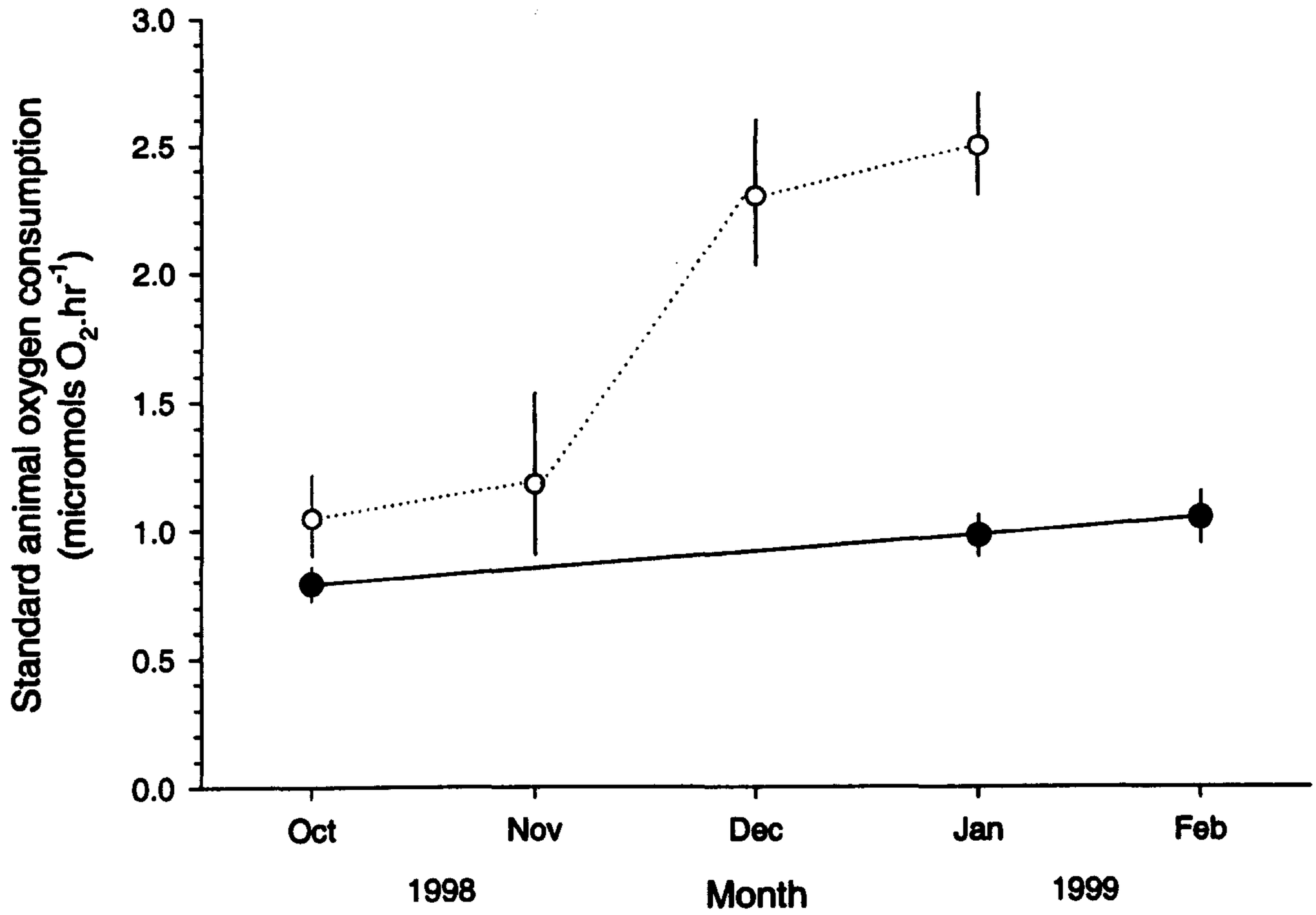


Figure 7.2 Oxygen consumption of starved *S. neumayeri* (●) compared with wild population (○) described in Chapter 4a. Data are presented as means for a 30mm test diameter sea urchin ± 95% confidence limits.

temperature alone, the Q_{10} value is ~30. This is a biologically unrealistic value, and shows clearly that more than temperature is involved in seasonal variations in the metabolic rate of field urchins.

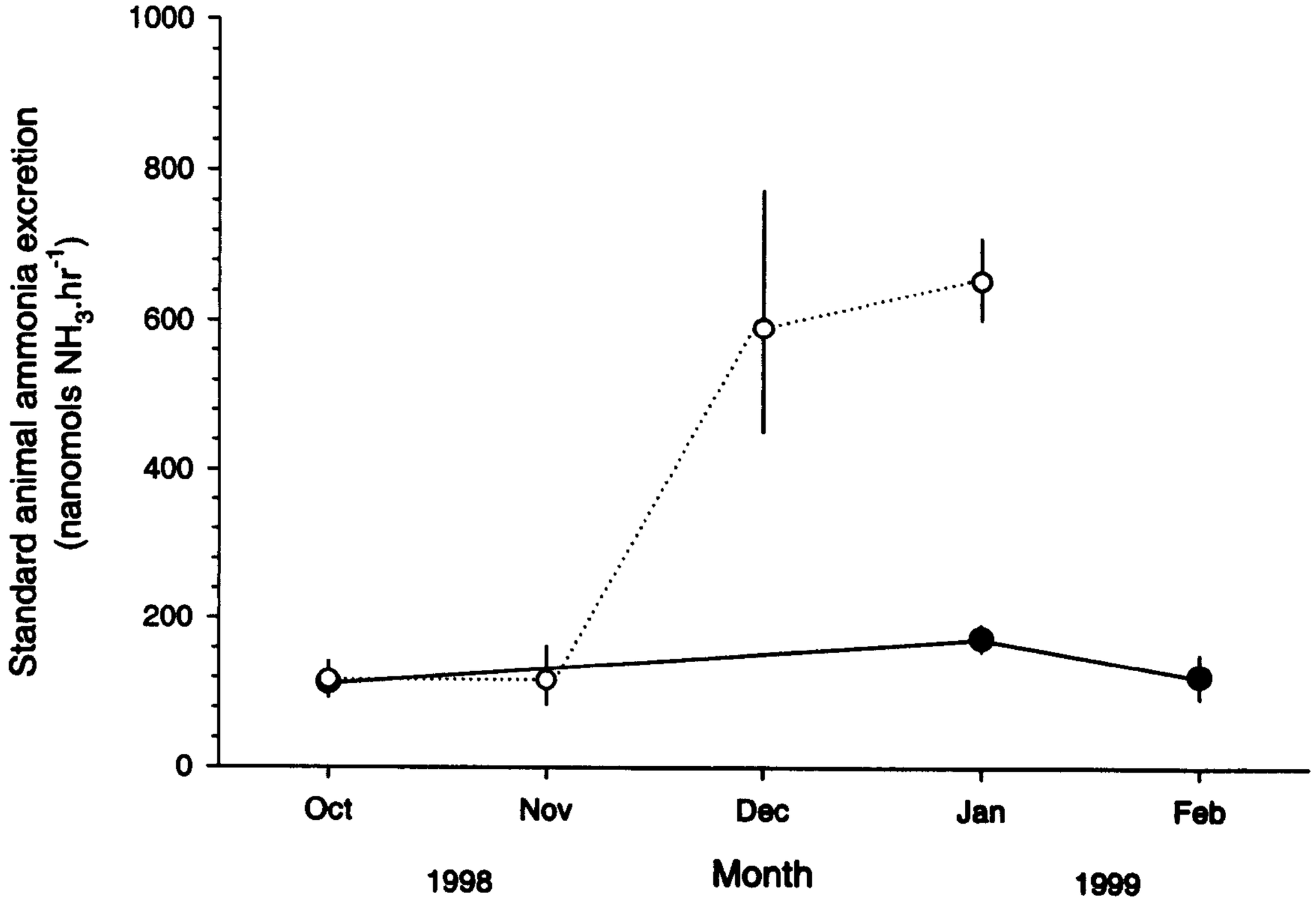


Figure 7.3 Ammonia excretion rate of starved *S. neumayeri* (●) compared with the wild population (○) described in Chapter 4a. Data are presented as means for a 30mm test diameter sea urchin ± 95% confidence limits.

Ammonia excretion rate also showed a marked difference between the starved sea urchins and the wild population (Fig 7.3). Values recorded for both groups in October 1998 were very similar at about 115 nmols.hr^{-1}. By January the excretion rates of the starved sea urchins had increased slightly to 174 nmols.hr^{-1}, whereas the wild population showed a considerably greater increase to 590 nmols.hr^{-1}. The atomic ratio of oxygen consumed to nitrogen excreted may be used to compare metabolic substrate being utilized,

and the reduction in the February excretion rate for the starved sea urchins resulted in an increase in the O:N ratio to a mean value of 21 for this month demonstrating an increased reliance on lipid and carbohydrate at this time (Fig 7.4). O:N values for the wild population were much lower during the summer feeding period indicating a heavier dependance on protein for metabolic fuel.

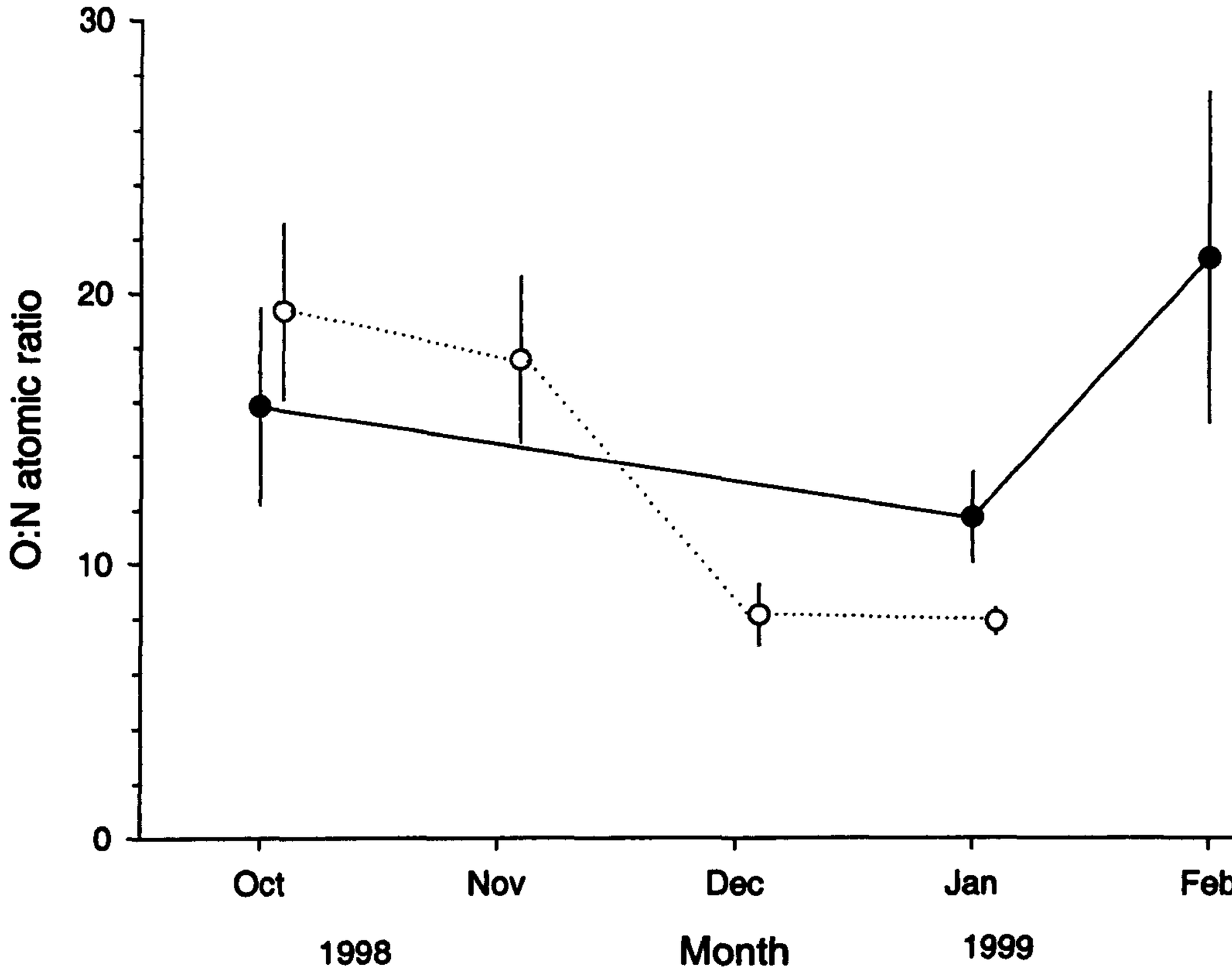


Figure 7.4 O:N atomic ratio of starved *S. neumayeri* (●) and the wild population reported in Chapter 4a (○). Data are presented as means ± 95% confidence limits.

Under conditions of starvation energetic demands must be met by stored reserves. For echinoids this energy is generally supplied from the soft tissues (gut, gonad) and also to a limited extent the test (Lares and Pomory 1998; Chapter 3). The gonad masses of starved animals in October compared well with those recorded from the wild population, but gonad

mass of the starved population had been reduced to 49% of the October value by January, primarily as a result of spawning but also due in part to metabolic demands (Fig 7.5). This drop was not recorded from the wild population, presumably because field urchins were feeding and laying down reserves. Between January and February a further minor loss in mean mass was recorded, from 0.21g to 0.20g, but this difference was not significant (Student's t = 0.29, $p = 0.77$).

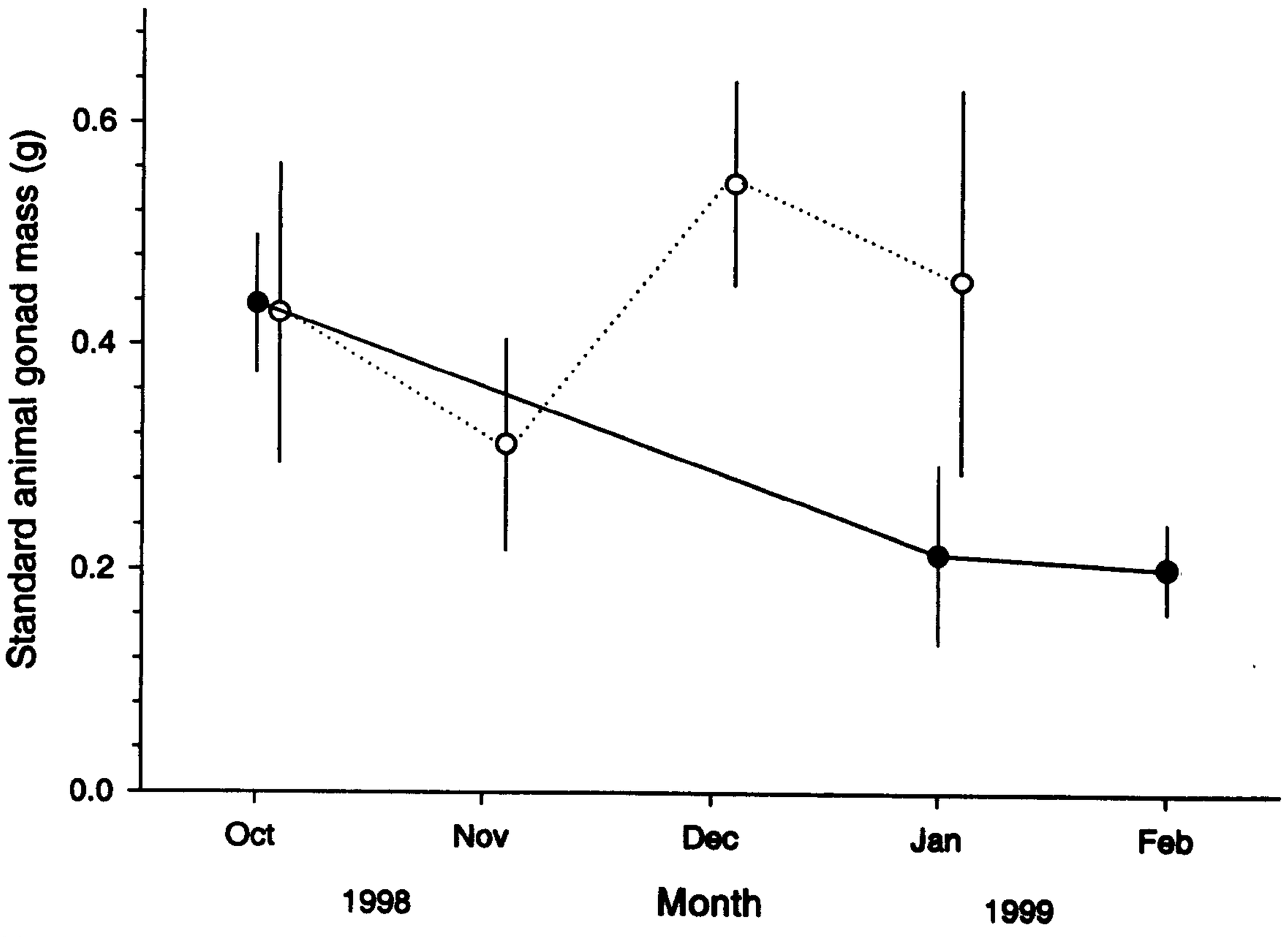


Figure 7.5 Gonad mass of starved *S. neumayeri* (●) contrasted with the wild population (○) presented in Chapter 3. Data are means ± 95% confidence limits.

Gut tissue mass of echinoids reduces to a stable level during periods of reduced food availability (Lawrence et al. 1965), and this was recorded for the wild population at North Cove (Chapter 3). The gut tissue mass recorded from the starved animals was

initially similar to that of the atrophied guts in the wild population (Fig 7.6). Gut masses of the starved sea urchins remained unchanged during the experimental period and did not show the abrupt increase caused by the onset of feeding displayed by the wild population. Standard animal test ash free dry mass also remained stable during the starvation period indicating that a proportion of the loss in mass of reproductive tissue was due to metabolic demands.

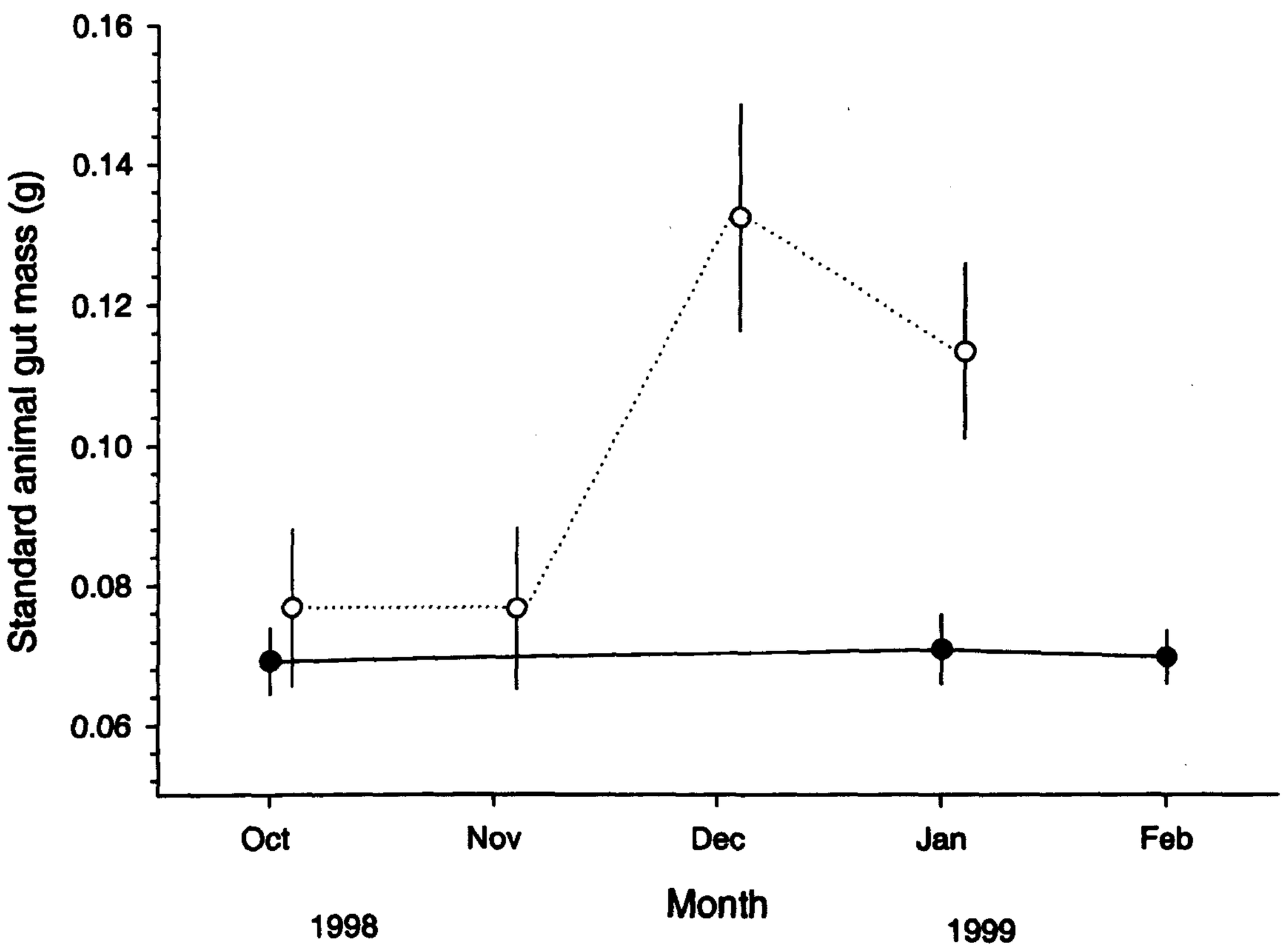


Figure 7.6 Gut mass of starved *S. neumayeri* (●) compared with data from the wild population presented in Chapter 3 (○). Data are means ± 95% confidence limits.

7.4 Discussion

An increase of temperature in an ectotherm system has two major effects at the molecular level: firstly it will result in an increase in the proportion of molecules possessing sufficient kinetic energy to achieve the activation level for any given reaction, and secondly it will influence the equilibrium constant of reactions, especially those involving weak chemical bonds (Hochachka 1991). Q_{10} values for individual biochemical functions can fall anywhere from one through to very high vales, although the Q_{10} for purely physical processes such as diffusion is typically around 1.5 or lower. Many studies however report Q_{10} values in the range 2-3 for whole animal physiological processes under normal conditions (Clarke 1983; Clarke and Johnston 1999). The maintenance of whole organism Q_{10} values within this range as a result of evolutionary adjustment allows for the integration of enzyme mediated biochemical reactions with physical processes which is essential to physiology (Hochachka 1991)

Many polar species however show an unusual degree of stenothermality, and evidence based primarily around larval developmental rates has been used to suggest that an increased sensitivity to temperature change may play a role in metabolic regulation (Bosch et al. 1987; Pearse et al. 1991; Hoegh-Guldberg and Pearse 1995). This concept has been extended to modelling bivalve culture in the Arctic, where high Q_{10} values in the range 2-6 have been used to relate mussel tissue growth to environmental variables at the time of onset of the spring bloom (Grant et al. 1993; Grant 1996). Stenothermal adaptation or 'fine tuning' to the Antarctic environment has been investigated for the bivalve *Limopsis marionensis* in relation to ambient temperature (Pörtner et al. 1999), and these authors were able to show a temperature dependence for this species described by a Q_{10} of 2.2.

Other temperature dependence studies on less stenothermal Antarctic ectotherms have also reported normal Q_{10} results (White 1975; Ralph and Maxwell 1977; Ikeda and Hing Fay 1981; Davenport 1988; Peck 1989). The present study on *Sterechinus neumayeri* however suggests that it is food, and not temperature, which is the dominant factor driving the summer rise in metabolic rate. The extra oxygen consumption induced by feeding will include the handling costs of food, and the metabolic cost of growth (which together comprise the heat increment of feeding or specific dynamic action, SDA). Also included will be factors such as the costs of restructuring the gut tissue and locomotor costs.

The data presented here for *S. neumayeri* show that the summer rise in metabolism of the starved population may reasonably be attributed to the rise in water temperature alone. The Q_{10} is within the normal biological range for temperature mediated change, and suggests that basal or maintenance processes in this species will respond to relatively small changes in temperature. In wild urchins this small rise will presumably also be present, but here it is masked by the much greater signal from the costs of growth, activity and other processes. The data suggest that in wild *S. neumayeri* only 10-15% of the summer rise in metabolism is caused directly by the temperature rise whereas 80-85% is related to the increased physiological activity associated with feeding, growth and spawning. The size of the absolute metabolic rise over basal rate as a result of summer feeding may however be constrained by the low basal rates of polar organisms (Peck 1998). It would be valuable to repeat the experiment undertaken here for temperate and tropical echinoids to determine the extent to which maximum aerobic scope may be related to absolute basal costs (Clarke 1991; Peck 1998).

For *S. neumayeri* at Rothera, synchronous spawning takes place between October and January (Chapter 5). The substantial drop in gonad mass of the starved population at this time is indicative of reproductive output (Fig 7.5). Whilst the primary function of gonad tissue is reproductive, in many echinoids this tissue also plays a role in energy storage and supply under conditions of starvation. The reduction in mass due to metabolic demands alone may be estimated from oxygen consumption by the use of an oxycalorific coefficient (Brafield and Llewellyn 1982). This indicates that a standard sized *S. neumayeri* utilised 830 J during the period October to January, and assuming typical marine invertebrate tissue contains $23kJ.g^{-1}$ (Brey et al. 1988) this equates to a mass loss of 0.036 g of gonad to fuel metabolic demands. The total mass drop between October and January was 0.222g. This suggests that 0.186g was therefore due to spawning. This figure represents a reproductive output of 43% of the pre-spawning gonad mass, although in wild urchins the spawning event was masked by feeding activity and accumulation of reserves in the gonad at the height of the phytoplankton bloom.

7.5 Conclusions

The data from this comparison of laboratory starved with field population indicate that the bulk of seasonal change of metabolism of *S. neumayeri* (and by inference other marine invertebrates) is related to increased feeding. Temperature has only minor influence, and may be estimated by the use of Q_{10} values within the normal biological range.

Chapter 8 Seasonality of the Antarctic bivalve *Laternula elliptica*.

Data also presented in: Brockington S (in press). The seasonal energetics of the Antarctic bivalve *Laternula elliptica* at Rothera Point, Adelaide Island. Polar Biol.

8.1 Introduction

The Antarctic benthic fauna characteristically exhibits slow growth, low P/B ratios (Brey and Clarke 1993), prolonged longevity and low activity (Arntz et al. 1994). These life history traits are a result of low and stable water temperatures and seasonal energy limitation. *L. elliptica* is, however, unusual in having a relatively fast growth rate compared to other Southern Ocean bivalves (Ralph and Maxwell 1977, Brey and Mackensen 1997), attaining a shell length of 90mm in 12-13 years. *L. elliptica* is also a relatively large bodied form: over 60% of Antarctic bivalve species are less than 10mm in length (Nicol 1970). This combination of fast growth rate and large final size indicate that in contrast to *S. neumayeri*, *L. elliptica* has a high capacity for somatic growth in an environment which exhibits an extreme seasonal energy limitation (Clarke 1988).

Many temperate bivalves show seasonal cycles of mass gain and loss related to variations in phytoplankton abundance (i.e. food availability) throughout the year (Ansell 1972; Ansell et al. 1980; Bayne and Widdows 1978; Bayne and Worrall 1980; review in Gabbot 1983; Newell and Bayne 1980). Although phytoplankton concentrations fall below maintenance requirements in temperate zones feeding does continue albeit at reduced rates. It may be expected that the winter period of nutritional stress will be more extreme under polar conditions, both due to the prolonged duration of the winter period and also because of the extreme reduction in phytoplankton standing stock at this time (Clarke 1988,

Chapter 2). For Antarctic benthos the ability to feed during the austral winter varies between taxa. Barnes and Clarke (1995) reported that holothurians at Signy Island (South Orkneys) ceased feeding during winter for between four and five months: polychaetes and hydroids also ceased feeding, but for less time.

Fuel for maintenance metabolism under non-feeding winter conditions is supplied from stored reserves. Many European bivalve species have periods of minimal reproductive activity in winter (Gabbot 1983), and under these conditions energy is lost from tissues through respiration and nitrogen excretion. The rate of utilisation of reserves is therefore dependent on metabolic rate and Bayne and Newell (1983) showed that during starvation bivalves reduced their metabolism to maintenance levels to conserve body reserves. Clarke and Johnston (1999) and Peck and Conway (2000) have further shown that for Antarctic fish and bivalves respectively resting metabolic rates are low compared to tropical and temperate counterparts, and this reduced rate may be important for efficient resource utilisation in winter. A consequence of reduced maintenance costs under polar conditions may however be a diminished capacity for power generation. Peck (1998) records that sessile marine ectotherms typically display aerobic scopes of between ×2 and ×4 over resting metabolic rate. Because the relationship is factorial and cold water species have a reduced minimal metabolic rate, the maximal absolute power generation is restricted when compared to warmer water counterparts with a faster resting metabolism. Such a reduction in capability for maximal absolute power generation may potentially set limits for production in Antarctic species.

The aims of this study were 1) to assess seasonal changes in the metabolic rate of

L. elliptica in relation to chlorophyll standing stock and feeding activity; 2) to measure changes in *L. elliptica* body condition during the Antarctic winter from decline in major organ masses and energy concentrations; 3) compare tissue energy loss with total winter metabolic power generation.

8.2 Methods

Fieldwork was carried out at the British Antarctic Survey Rothera Research Station on Adelaide Island (67°34'S, 68°07'W) between September 1998 and August 1999. *L. elliptica* feeding activity was observed in the field at roughly fortnightly intervals by direct observation. SCUBA divers recorded either presence or absence of *L. elliptica* siphons at the sediment surface.

The field site at North Bay has a sediment substratum and *L. elliptica* occur in large numbers from the shallow sublittoral to beyond 30m (parallel with the northern most extension of the runway: Fig 1.1). Specimens were carefully collected by SCUBA divers from 15 metres depth on the western side of the runway, and transported underwater in buckets to the aquarium in the Bonner Laboratory. Freshly collected *L. elliptica* were sorted to remove any specimens damaged during collection. They were then gently cleaned of any adherent sediment using a toothbrush before being left in aquarium tanks to recover from collection for four days.

Oxygen consumption rates (MO_2) and ammonium excretion rates were determined simultaneously to allow calculation of individual O:N ratios. Methods used were identical to those described in Chapter 4a, although respirometer chamber volumes varied from 80

to 1550ml. Measurements were repeated five times during the year (Sept 98, Nov 98, Jan 99, Mar 99 and Aug 99), and on each occasion 32 animals were measured (except Jan 1999; n=24). Bivalves are predominantly ammonotelic, but urea excretion was also measured in September 98 to evaluate its contribution to overall nitrogen excretion. As large a size range of animals as possible was used on each sampling occasion (shell length 13.2 to 80.2mm for the whole study, mean = 48.8mm). For each date rates of oxygen consumption, ammonium excretion and whole body energy content were calculated for a standard sized animal of shell length 50mm (chosen to coincide with the mean size of animal used in the study) from covariance analysis of logarithmically transformed data for each variable as a function of shell size.

In September 1998, after respirometer seawater was taken for the ammonium assay a further 2 x 10 mls were removed and placed into replicate stoppered test tubes. Urea was measured using a the method of Clarke et al (1994a) modified from Grasshoff et al (1983). NaCl was added as a solution (3.6mls of 300g.l^{-1} NaCl solution to each reaction vessel) rather than a solid, and the assay was calibrated by spiking seawater with urea. Background urea levels were assessed using controls (fresh seawater), and urea excretion was calculated after correction for the volume of the bivalve.

Thirty *L. elliptica* were dissected in both September 1998 and March 1999 to contrast end of winter and end of summer tissue masses when body condition was expected to be respectively lowest and highest. Size ranged from 23.8 mm to 86.5 mm shell length; bivalves smaller than this could not be dissected reliably. Body components were separated into five groups: ctenidia (including palps), shell, digestive tissue (including gut,

digestive gland and viscera), gonad and musculature (siphons, adductor muscles and remaining mantle tissue). Although the digestive and gonad tissues are closely associated, a reliable separation of the two was possible after practice. Dissected tissues were placed into pre-weighed crucibles, and dried at 60°C to constant mass. Sub samples from digestive tissues, gonad, musculature and ctenidia were then taken from 12 animals on each of the sampling occasions, and saved in airtight vials for C:H:N analysis in the United Kingdom (see Chapter 3 for elemental analysis methods). Ash content of the remaining tissues was determined by ignition in a muffle furnace at 465°C for 24 hours.

8.3 Results

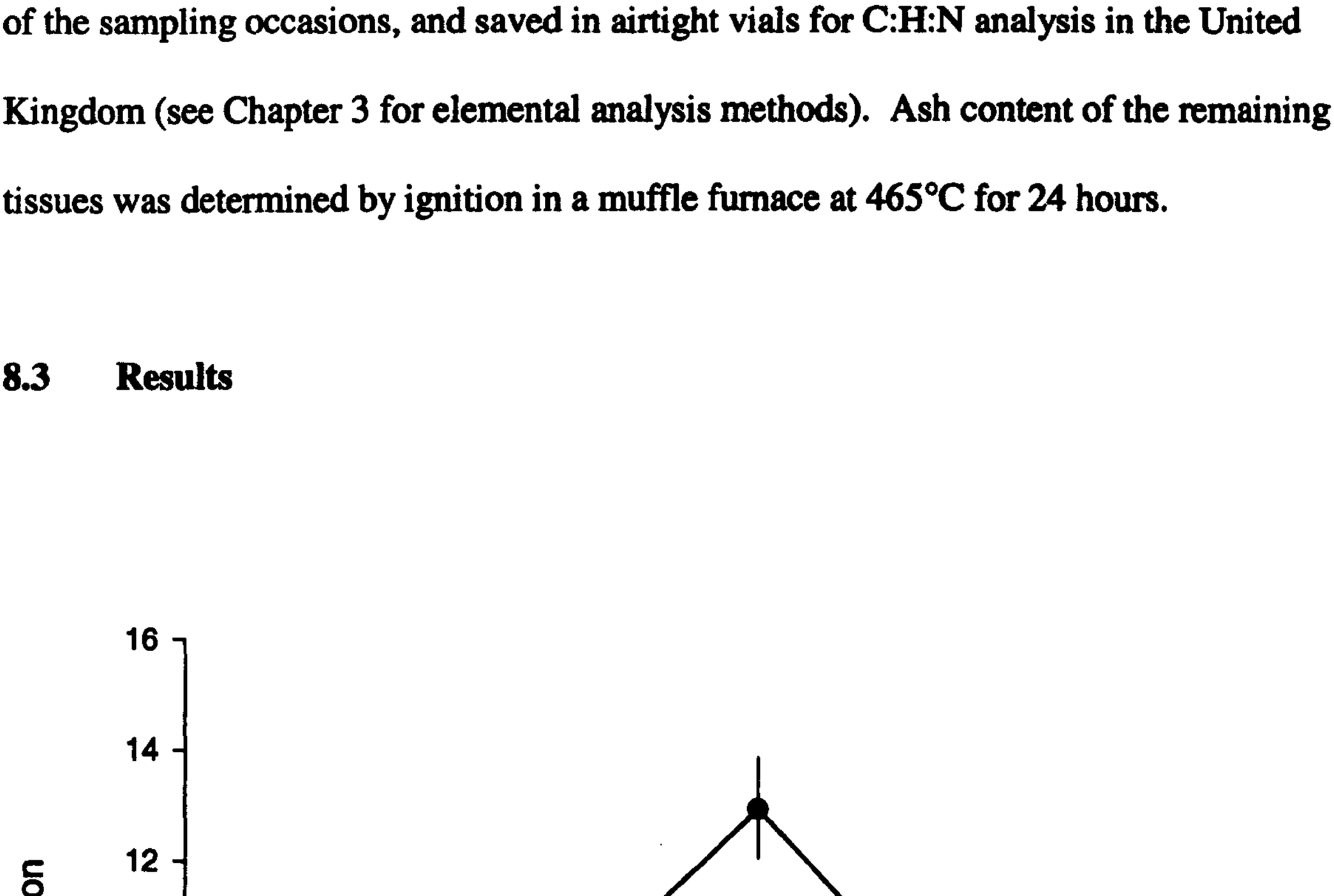


Figure 8.1 Seasonal variation in molar oxygen consumption (MO_2) of a 50mm shell length *L. elliptica*. Data are solved from regressions of $\log_e$ transformed data for oxygen consumption vs shell length (n = 32.month^{-1}, except January n = 24). Error bars represent 95% confidence intervals.

Water column phytoplankton standing stock exhibited the sharp seasonality characteristic of polar latitudes (Fig. 7.1). Minimum chlorophyll concentrations occurred in August 1998 (0.053 mg.m^{-3}), and the rise in phytoplankton concentration began relatively early in the summer (November) due to the paucity of sea-ice in 1998. The bloom consisted of two peaks, the first reaching a maximum of 6.99 mg chl.m^{-3} in mid December, and the second rising to 13.42 mg chl.m^{-3} by early February. Chlorophyll concentrations then declined steeply, and had returned to winter levels by March 1999. *L. elliptica* at Rothera Point had ceased feeding (i.e. siphons retracted below the sediment surface) by 26th May 1999, and re-emerged and commenced feeding at the end of September indicating a non-feeding period of at least four months. Divers made

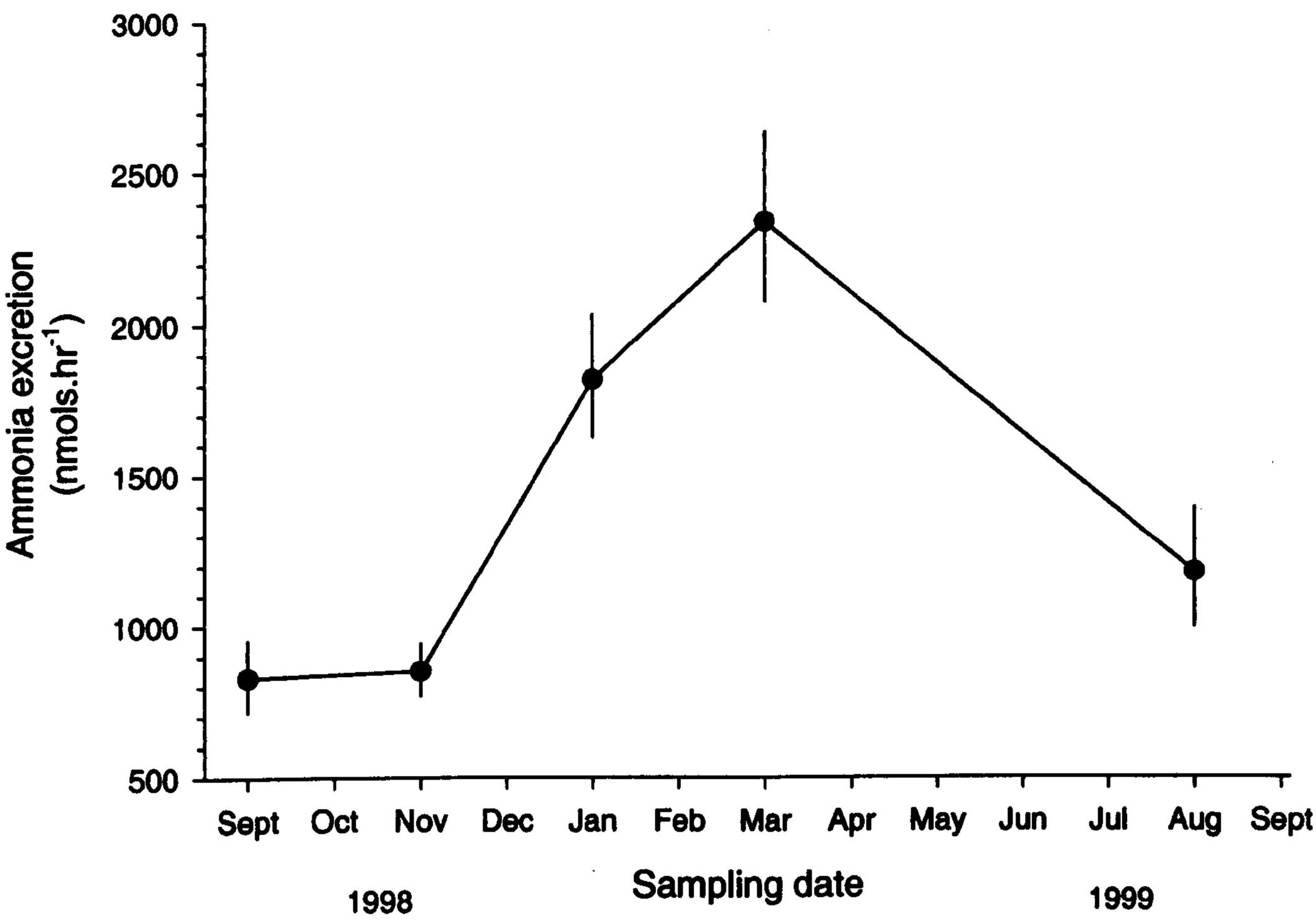


Figure 8.2 Ammonium excretion rates for a standard 50mm shell length *L. elliptica*. Data shown were obtained from regressions after log$_e$ transformation of both variables and are presented as for Fig 8.1.

observations of the *Laternula elliptica* bed on 20 occasions between these dates and no siphons were visible at any time.

Oxygen consumption in *L. elliptica* varied strongly with season. Minimum winter standard animal respiratory rates of 5.1 and 4.3 μmols.hr^{-1} were recorded in September 1998 and August 1999 (Fig 8.1). Rates started to rise in November coincident with the onset of the phytoplankton build up, and peaked in March at 12.9 μmols.hr^{-1}. This indicated a seasonal factorial rise of ×3 over lowest winter metabolic rates. Ammonium excretion (NH_3) also varied with season and minimal rates (0.82 µmols.hr^{-1}) were recorded

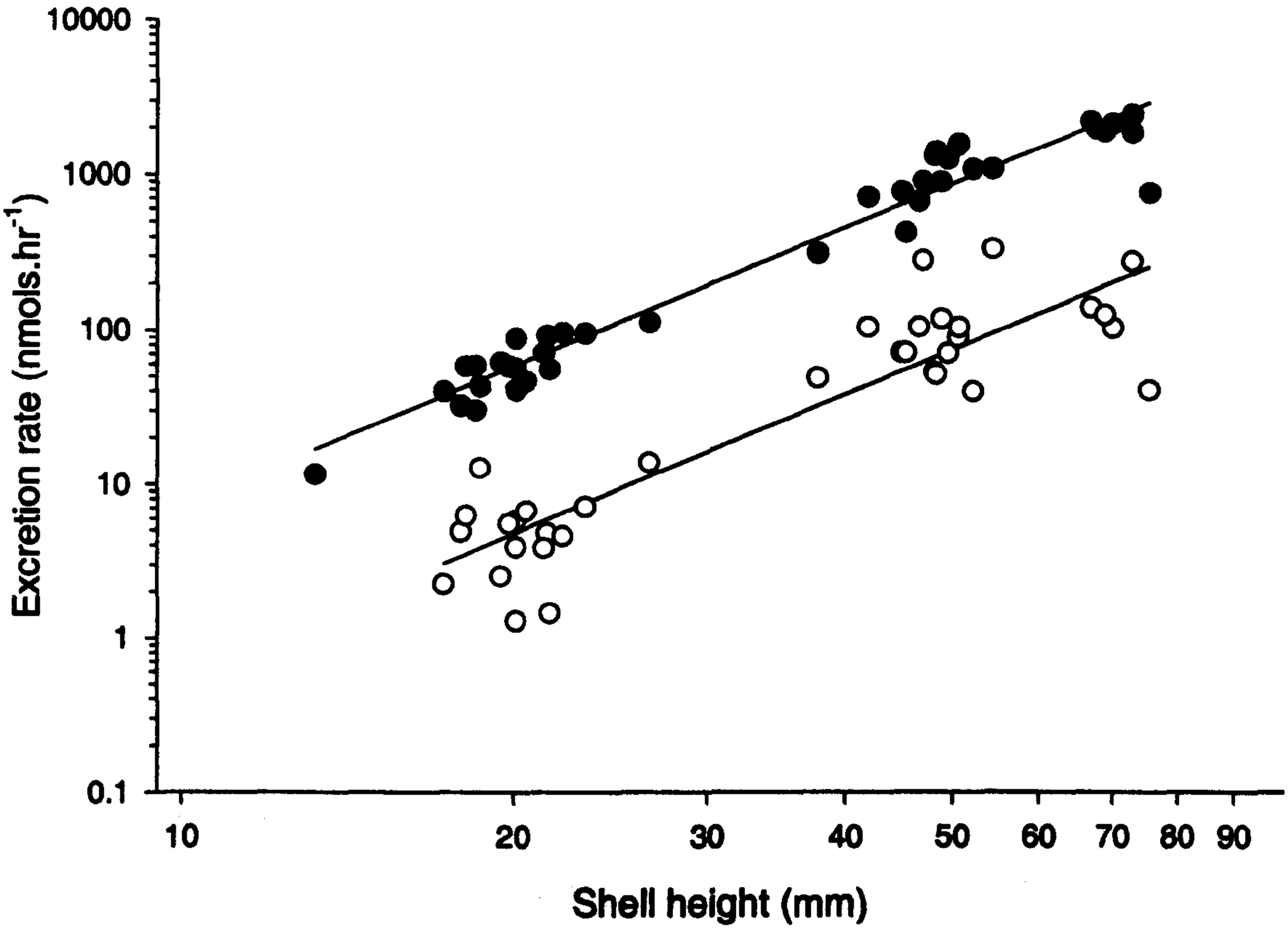


Figure 8.3 Rate of urea excretion (○) and rate of ammonium excretion (●) for September 1998. Note logarithmic scales. *L. elliptica* is predominantly ammonotelic; a 50mm shell length individual excreted 90% of N as ammonium, and 10% as urea.

in late winter 1998 (Fig 8.2). Maximum NH_3 excretion (2.3 µmols.hr^{-1}) coincided with the peak of oxygen consumption (March 1999). By August 1999 NH_3 excretion was similar to the minimal values recorded the previous winter.

In the urea experiment a standard 50mm shell length animal excreted 824 nmol NH_3.hr^{-1} and 47 nmol Urea.hr^{-1} (Fig 8.3). Since each molecule of urea contains two N atoms, this indicates that *L. elliptica* was excreting 10% of nitrogen as urea and 90% as ammonium. For the remainder of the study ammonium only was measured. O:N ratios calculated from ammonium only (Fig 8.4) and varied from 3.4 to 32.9 over the year.

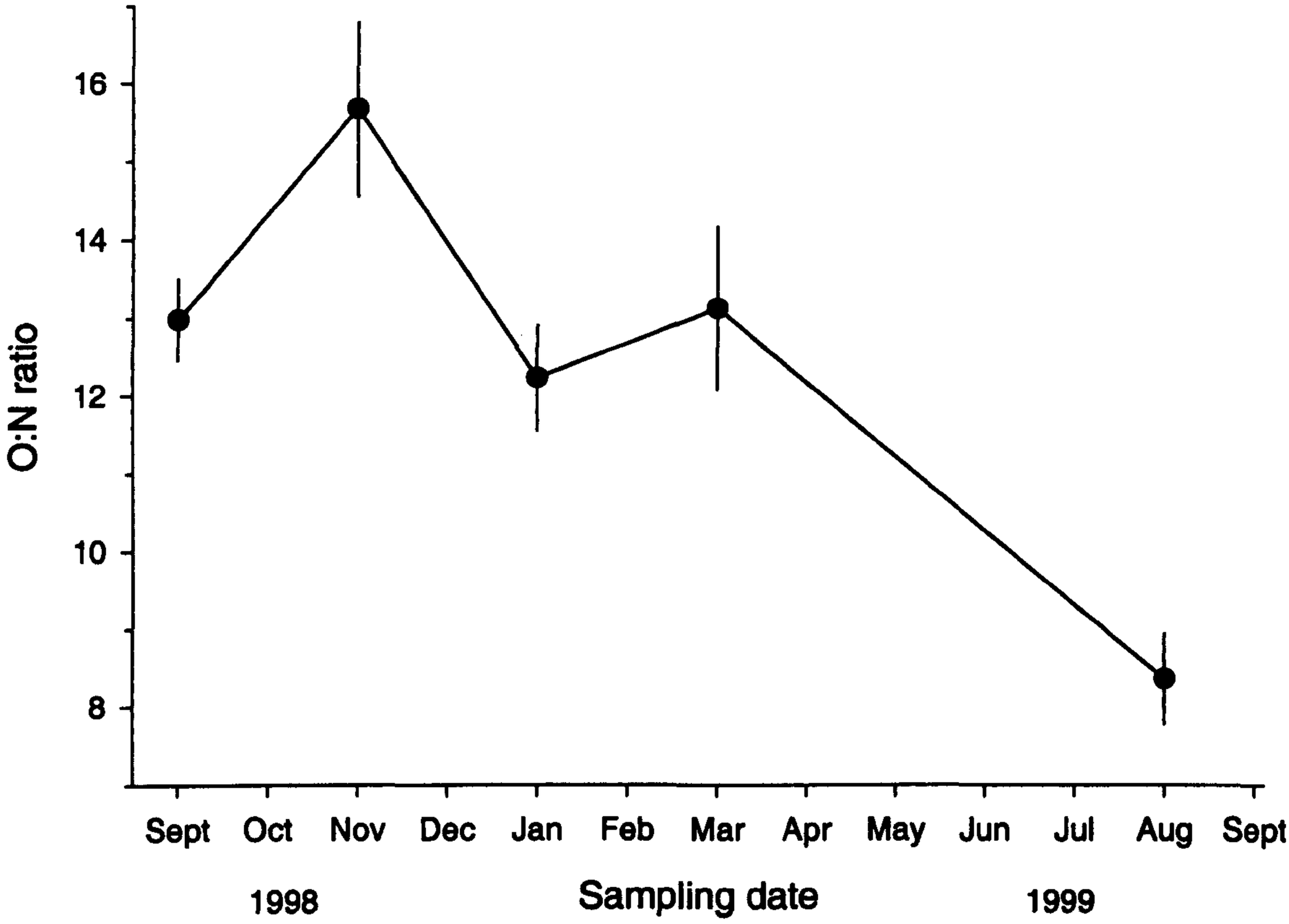


Figure 8.4 O:N ratios (ng atoms of oxygen consumed per individual per hour divided by nmols of ammonium excreted per hour). To convert from µmols oxygen to ng-atoms multiply by 2000. Data are expressed as mean ± standard error for a standard 50mm shell length individual and calculated from solved regressions of O:N ratio against shell length.

Regressions of O:N ratio against shell length were not significant for winter samples (September 1998 $p = 0.303$, August 1999 $p = 0.163$), but were significant in summer (Nov 1998 $p = 0.017$, Jan and March 1999 $p = 0.000$), showing lower ratios in larger bivalves during the feeding period. The lowest values were recorded in Aug 1999 (range 3-16).

Dry mass of body components in September 1998 and March 1999 were regressed against shell length after $\log_e$ transformation of both variables (Table 8.1). ANCOVA showed no change with season in either the slopes or the intercepts of the regressions for musculature (slope $F = 0.02$, $p = 0.897$; intercept $F = 0.247$, $p = 0.247$), ctenidia (slope $F = 0.03$, $p = 0.869$; intercept $F = 0.53$, $p = 0.471$), or digestive tissues (slope $F = 0.00$, $p = 0.989$; intercept $F = 2.07$, $p = 0.155$). ANCOVA also showed that the regression slope for gonad mass against shell length was greater in late summer than in late winter (slope $F = 7.37$, $p = 0.009$), and gonad mass was greater in all animals in later summer.

Table 8.1 Scaling exponents relating body tissue mass to shell length from samples in late winter (September 1998) and late summer (March 1999). Regression models were fitted after $\log_e$ transformations of both variables. b = slope, SD = standard deviation around regression line, F tests significance of slope (null hypothesis, $b = 0$). Bivalve shell lengths ranged from 23.8mm to 86.5mm.

Dependant variable	b	Intercept	r^2	SD	F	n
Muscle	3.21	-11.7	0.887	0.309	457.5	60
Ctenidia	2.83	-12.73	0.941	0.191	903.1	59
Digestive gland	3.04	-12.91	0.917	0.246	632.4	59
Gonad (summer)	4.99	-20.44	0.833	0.509	139.39	30
Gonad (winter)	3.87	-16.43	0.827	0.478	129.26	29

Energy concentration of all body tissues varied significantly with season (Fig 8.5). Muscle and digestive tissue energy content showed the greatest decrease from summer to

winter (2.09 and 1.78 $kJ.g^{-1}$ respectively), although gonad and ctenidia also declined significantly, by 0.97 and 1.09 $kJ.g^{-1}$ respectively. Combining energy content changes with tissue mass variation indicated that the greatest proportion of the loss of whole body energy content during winter was accounted for by the mantle musculature (4.97kJ). Digestive tissues contributed 0.66kJ and ctenidia contributed 0.20kJ (Fig 8.5). Gonad energy content was 0.98kJ higher in summer than winter.

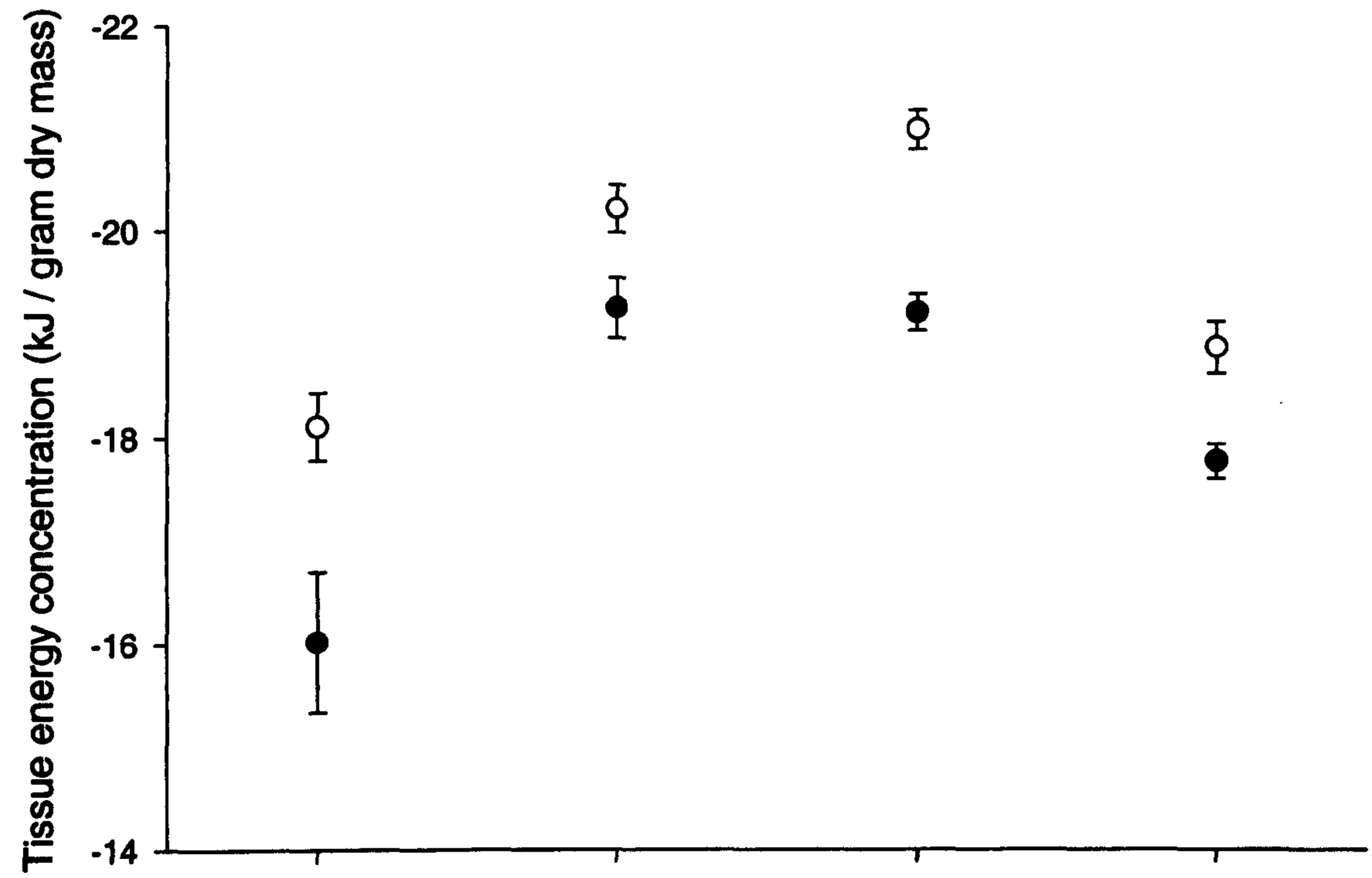


Figure 8.5 Tissue energy content for late austral summer (March 1999: ○) and late austral winter (September 1998: ●) samples. Data are means ± standard error, n = 12 for each tissue and season and are calculated from tissue carbon content. Student's T indicates seasonal differences are all significant: muscle T = 2.76, $p = 0.011$; gonad T = 2.59, $p = 0.017$; digestive tissues T = 6.82, $p < 0.001$; centidia T = 3.65, $p = 0.001$; degrees of freedom = 22 for all comparisons

The energetic cost of surviving the polar winter may also be estimated from metabolism data. The mean standard animal MO_2 was 4.7 μmols $O_2.hr^{-1}$, and O:N ratios indicated a predominantly protein based metabolic substrate. Oxidation of protein to ammonium liberates 0.427 J.μmol^{-1} oxygen (Brafield and Llewellyn 1982), which indicates a power utilisation of 48.17 J.day^{-1} for *L. elliptica* in winter. This equates to a total power use of 5.78kJ for the four month non feeding period indicated by siphon retraction. The reduction in total body energy content (excluding reproductive tissue) recorded between the height of summer feeding period (March 1999) and the end of winter (September 1998) was 5.84kJ. 85% of this energy was liberated from muscle and mantle tissue (Fig 8.6). Proximate composition analysis of tissues (Table 8.2) showed that muscle tissue displayed the largest reduction in protein content.

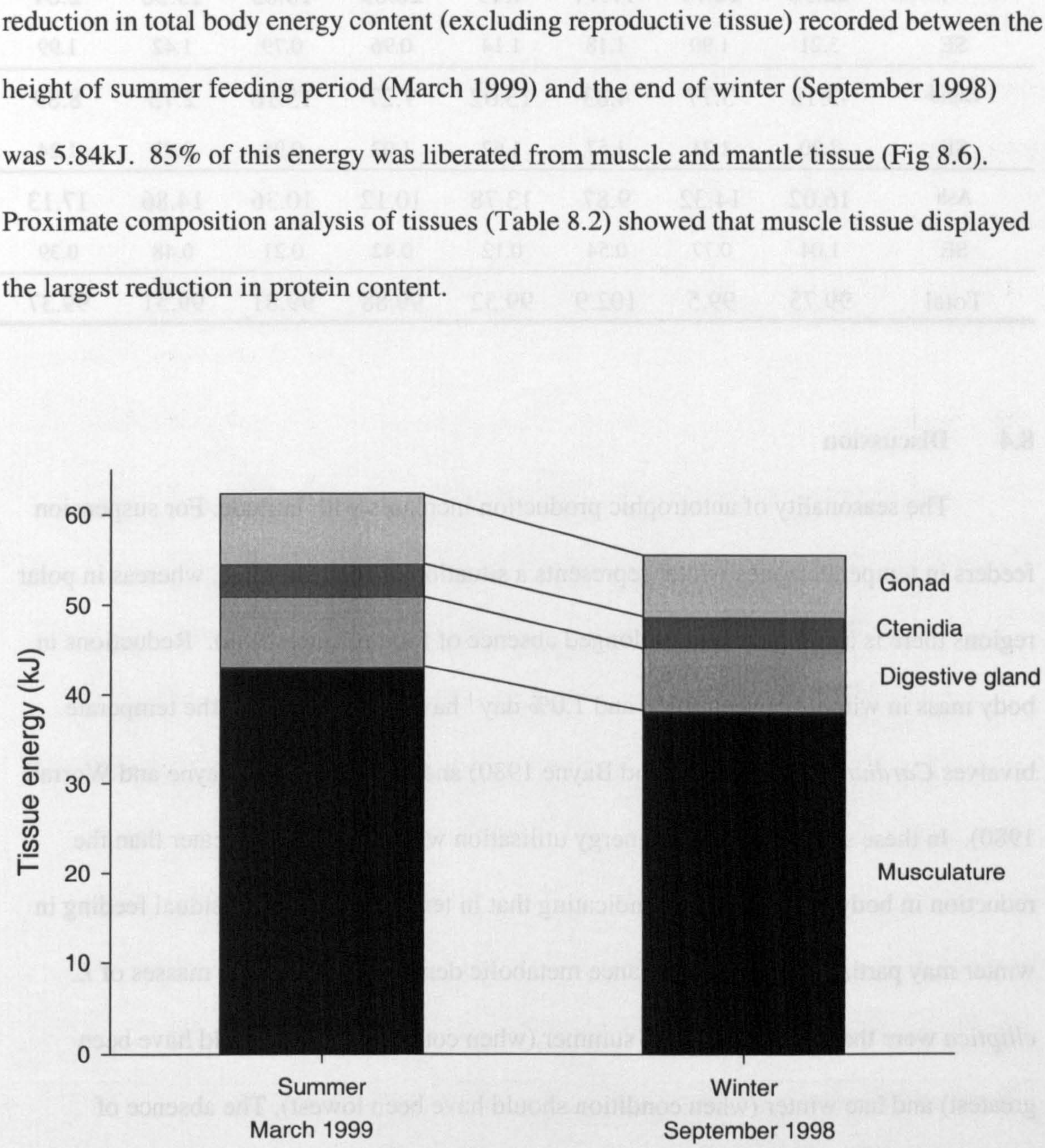


Figure 8.6 Seasonal differences in whole body energy content for a standard 50mm shell length *L. ellipitca*. Total reduction in body energy content was 5.84kJ.

Table 8.2 Proximate composition of *L. elliptica* body tissues in summer and winter (% dry mass). Data presented are means ± standard errors (n = 12), and were estimated stoichiometrically from C:H:N ratio. Ash was measured directly, and data for residual water (6%) and non protein nitrogen (5%) were default values suggested by Gnaiger and Bitterlich (1984). Totals calculated include residual water and non protein nitrogen.

	Muscle		Gonad		Digestive		Ctenidia	
	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer
Protein	53.55	57.64	59.71	57.23	51.4	52.66	57.56	59.71
SE	1.49	0.76	0.74	2.30	0.56	0.75	0.65	0.50
Carbohydrate	22.36	12.77	17.44	4.49	20.09	10.63	13.36	2.64
SE	3.21	1.90	1.18	1.14	0.96	0.79	1.42	1.99
Lipid	-3.18	3.77	4.83	13.02	7.27	15.16	2.73	8.89
SE	2.20	1.21	1.57	1.62	1.02	0.95	0.82	1.24
Ash	16.02	14.32	9.87	13.78	10.12	10.36	14.86	17.13
SE	1.04	0.77	0.54	0.12	0.42	0.21	0.48	0.39
Total	99.75	99.5	102.9	99.52	99.88	99.81	99.51	99.37

8.4 Discussion

The seasonality of autotrophic production increases with latitude. For suspension feeders in temperate zones winter represents a situation of food shortage, whereas in polar regions there is a complete and prolonged absence of food (Clarke 1988). Reductions in body mass in winter between 0.5% and 1.0% day^{-1} have been shown for the temperate bivalves *Cardium edule* (Newell and Bayne 1980) and *Mytilus edulis* (Bayne and Worrall 1980). In these studies metabolic energy utilisation was significantly greater than the reduction in body energy content, indicating that in temperate regions residual feeding in winter may partially offset maintenance metabolic demands. The tissue masses of *L. elliptica* were the same in both late summer (when condition index should have been greatest) and late winter (when condition should have been lowest). The absence of detectable body mass reduction despite the protracted non feeding period of the polar

winter suggests that overwintering physiological costs (i.e. supported through consumption of reserves) may be harsher in temperate than in higher latitudes. This may be caused by elevated winter metabolism at higher temperatures in temperate regions and is supported by the data of Honkoop and Beukema (1997) who showed that higher than normal overwintering temperatures caused an accelerated reduction in body mass index for adult *Macoma baltica* in the Dutch Wadden Sea.

Mass loss during starvation is dependent on resting metabolic rate, and this is generally low for polar benthic marine ectotherms (Clarke and Johnston 1999, Peck and Conway 2000). Ahn and Shim (1998) reported summer MO_2 for *L. elliptica* of 12.9 μmol.hr^{-1} for a 60 mm shell length individual, which is close to the rate observed for a 50mm shell length standard animal in late summer at Rothera. These peak summer metabolic rates are, however, substantially lower than for temperate suspension feeding bivalves (Ahn and Shim 1998; Peck and Conway 2000). The seasonal cycle in oxygen consumption reflects the metabolic costs of suspension feeding and somatic growth which takes place during the austral summer (Brey and Mackensen 1997). Vitellogenic transformation of primary oocytes to mature ova takes place at a constant rate over an 18 month cycle in *L. elliptica* (D. Powell, pers comm). Consequently it may be expected that the respiratory costs of reproductive growth are more or less constant throughout the year.

Ammonium excretion rates displayed a similar seasonal cycle with increased overall protein catabolism in the austral summer as a result of substantially increased feeding and somatic production. The atomic O:N ratio is an indicator of the fraction of protein used to fuel metabolism. The lowest ratios possible are around three, indicating the

sole use of protein as a metabolic substrate (Mayzaud and Conover 1988). Ratios between 20 and 25 are obtained when protein fuels around 50% of the metabolic substrate (Ikeda and Hing Fay 1981) and progressively higher ratios indicate lower proportions of protein utilisation. Utilisation of protein as the main metabolic substrate was observed throughout the study, although the lowest ratios were encountered in winter 1999. Relative increases in ammonium excretion during starvation have been commonly observed in temperate bivalves as glycogen stores are used up and protein is catabolised to meet maintenance metabolism requirements (Bayne and Newell 1983). Reduced O:N ratios as a result of seasonal nutritive stress have also been observed both in temperate bivalves (*Mytilus edulis*: Bayne 1973) and other Antarctic species including the bivalve *Limopsis marionensis* (Pörtner et al 1999), the brachiopod *Liothyrella uva* (Peck et al. 1986). The higher ratios encountered in late winter 1998 are comparable with summer values, and indicate a reduced protein dependence at this time. This may be attributed either to an early break from winter dormancy fueled by lipid catabolism, or more favourable nutritional conditions in the preceding summer.

The body energy content of Antarctic benthic invertebrates is similar to temperate and tropical species in that energetic reserves are not laid down before the austral winter (Lawrence and Guille 1982; Clarke and Peck 1991). A low metabolic rate is, therefore, crucial in allowing economic utilisation of body tissue between feeding periods. The reduction in whole body energy content measured between late summer and late winter was consistent with the predicted energy loss estimated from MO_2 during the four month winter starvation. Most of the energy to fuel metabolism at this time was supplied from *L. elliptica* muscle, and this tissue also had the greatest reduction in protein content between

summer and winter which corresponds well to calculated winter O:N ratios. Summer tissue lipid contents (Table 8.2) are low compared to temperate and other polar species, but show close agreement with estimates for *L. elliptica* (gills 14.9%, gonad 10.9%, digestive gland 9.9% and remaining tissues 8.2%) reported for King George Island by Ahn (2000) and Ahn et al. (2000). Lipid levels decrease after spawning in temperate bivalves (Gabbot 1983), and the reduction in lipid levels observed in late winter may be attributed to the early winter spawning. It is likely that lipid levels will increase with feeding at the start of summer.

Although a low overall metabolic rate is necessary for economic utlization of resources, the potential for metabolic elevation when food becomes available is important to optimise capacity for work. Metabolic scope in sessile benthos has been estimated from the rise in oxygen consumption following a meal and is referred to as the specific dynamic action of feeding or SDA (Peck 1998). The SDA response is composed of the physical costs of processing food along with both anabolic and catabolic processes associated with the feeding event. In marine ectotherms, SDA peak metabolic rates are typically ×2 to ×4 higher than pre-feeding standard or basal metabolic rates. SDA derived metabolic scopes for Antarctic ectotherms are usually in the range ×1.6 to ×2.5 (Peck 1998). The range of values reported from Antarctic species is therefore slightly lower than the normal for temperate species, although the relative contribution of environmental and phylogenetic factors to this remains to be quantified. The seasonally energy limited Antarctic environment has, however, favoured species with low energy lifestyles, and the low range of SDA scopes reported for Antarctic benthos may be due to evolutionary pressure rather than a direct limitation of aerobic scope by temperature.

Metabolic scope may also be estimated in sessile benthos by exposing unfed animals to incremental rises in temperature. As temperature rises metabolic rate increases to a maximum, and Peck et al. (in press) used this method to estimate the aerobic scope for *L. elliptica* to be ×3.3, which is close to the seasonal scope of ×3.0 in the current study. Although a scope of between ×3.0 and ×3.3 falls well within the range normally expected for temperate and tropical marine sedentary marine invertebrates, it is somewhat higher than the range of ×1.6 to ×2.5 reported from SDA work for most Antarctic species so far investigated. One facet of the narrow range of polar values reported may however be because of the lack of polar studies in comparison to investigation from warmer water regions. Nevertheless the relatively broad range of metabolic capability shown by *L. elliptica* may reflect a stronger than usual ability to depress metabolism in winter, and these bivalves may enter a physiological state akin to dormancy when siphons are retracted below the sediment surface in winter. Further data to clarify the degree of anaerobic metabolism sustained by *L. elliptica* during winter when siphons are retracted, and also to provide a link between seawater pumping rates in field and laboratory specimens in winter would however help quantify the degree of dormancy.

8.5 Conclusion

Annual production under polar conditions is affected by the three way relationship between the length of winter non-feeding period, minimal metabolic rate and metabolic scope (i.e. capacity for metabolic work under conditions of adequate food supply). The data presented here indicate that for *L. elliptica* the austral winter is not a period of harsh physiological stress despite the prolonged starvation. The strongly reduced rate of mass loss during winter compared to temperate bivalves was facilitated by the highly depressed

minimal metabolic rates characteristic of polar ectotherms, and this avoided the need to lay down high energy (lipid) reserves before winter. The field derived metabolic scope is amongst the highest yet reported for sessile polar benthic species, and this increased capacity for metabolic work in summer months, combined with a dormant phase in winter may explain the enhanced productivity and relatively fast growth displayed by *L. elliptica* compared to other polar suspension feeders.

Chapter 9 Overview: A seasonal energy budget.

Data also presented in: Brockington S, Peck LS, Clarke A (in prep). Latitude and life history modification: a seasonal power budget for the Antarctic echinoid *Sterechinus neumayeri*.

9.1 Introduction

The Antarctic benthic marine fauna is characterized by low rates of metabolism, activity, slow growth, prolonged longevity and low productivity to biomass ratios (Arntz et al. 1994). These specific life history traits are a consequence of the polar environment, i.e. primarily low and relatively constant seawater temperature coupled to a highly seasonal cycle of food availability (i.e. phytoplankton production). Since both mean sea surface temperature and the seasonality of phytoplankton production vary with latitude, then it may be expected that life history characteristics of benthic communities may also show change with latitude. The energy assimilated by a predominantly sedentary marine ectotherm is divided between respiration, losses through excretion of dissolved organic substances, somatic growth and reproductive growth. Studies from polar regions have shown that the separate effects of temperature and highly seasonal food availability have affected each of the different energy budget sinks in different ways.

Seasonal food availability to polar communities has resulted in many taxa suspending feeding for a variable period in the austral winter (Chapter 3). Consequently metabolically expensive processes such as somatic growth are confined to the austral summer for many species. Low temperature has resulted in a lowered rate of basal and consequently resting metabolism, probably as a consequence of a lowered requirement for protein turnover or reduced membrane transport costs. Clarke (1987) argued that the

reduced basal metabolic requirements of polar ectotherms will lead to an increased growth efficiency, since for a given amount of food consumed by a polar invertebrate a relatively greater proportion can be directed to production (either somatic or reproductive), with less being diverted to maintenance. Whilst this may be true during periods when feeding is taking place, the effect of the prolonged annual period without feeding must be considered. During this time reserves are used to fuel maintenance, leading to a negative growth efficiency over that period.

Polar conditions have also induced changes in the relative size of the other energy budget sinks. Evidence from P:B ratios from a variety of taxa suggest that total annual energy assimilated by polar taxa is lower than for warmer water counterparts. Both somatic growth rates and metabolic losses through respiration are lower for most taxa studied. However, mass specific reproductive output does not change with latitude in such a consistent pattern. This chapter compiles results documented in other sections of this thesis with a view to 1) summarising the energetic cost of enduring the polar winter and 2) estimating whether growth efficiencies are changed under polar conditions as a result of changes in relative sizes of energy budget sinks compared to warmer water species. Results have been recalculated from earlier chapters and are presented as energy terms.

9.2 Methods

Methods for the measurement of dietary assimilation efficiencies are based on the principle of comparing the digestion of organic material relative to an unabsorbed tracer substance. In this study the Conover ratio approach was attempted, where the natural ash content of the food was used as an inert tracer. This indirect procedure has provided

reliable results for determining the assimilation efficiency of suspension feeders as long as the inorganic content of the food is not too low (Navarro and Thompson 1994). Ash contents of the food were derived from the North Cove sediment core sampling (Chapter 1), and dry masses and ash free dry masses of faeces were determined from samples collected for the faecal egestion study (Chapter 3).

Data on somatic growth, reproductive output, respiration and ammonia excretion were converted to a power utilisation (using conversion factors detailed in the results section) and the relative magnitudes of each of the energy sinks was compared on a seasonal basis for both 1997 and 1998. The sampling period ran for 695 days (i.e. 35 days less than two complete years), so power totals for 1997 were calculated for one year from the start of the data run in mid February (Julian day 41). Power totals for 1998 were calculated for one year ending on the last day of the data run (day 695 in January 1999). Consequently the same 35 day period (January 1998) was counted in the annual estimates for both years. Data on seasonal energy utilisation are expressed for both a 24mm and a 30mm test diameter urchin from North Cove. 24mm represents the size at which somatic growth is maximal for *S. neumayeri*, and also coincides with the mean size of urchin used for monthly monitoring of oxygen consumption and ammonia excretion rates. 30mm represents the largest size class commonly occurring in the *S. neumayeri* population at North Cove.

9.3 Results

Feeding was highly seasonal (Chapter 2), and the organic content of *S. neumayeri* faeces was invariably higher than the sediment on which they were feeding due to the

production of a faecal envelope comprised largely of mucous. Since organic composition of faeces was higher than the food, assimilation efficiencies could not be calculated using Conover ratios (Fig 9.1). Total energy assimilated was therefore calculated from summation of energy devoted to somatic growth, reproductive output, respiration and ammonia excretion.

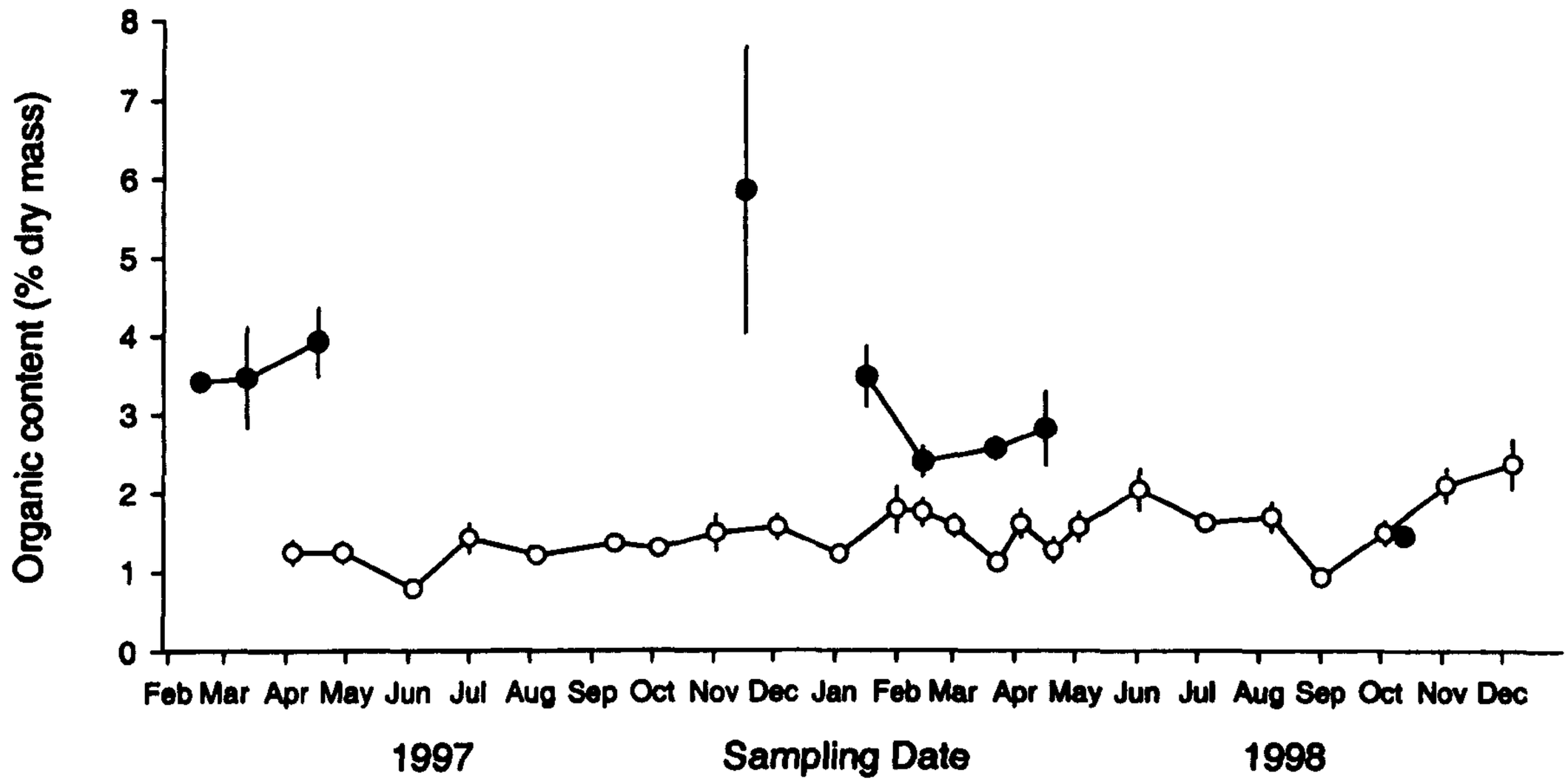
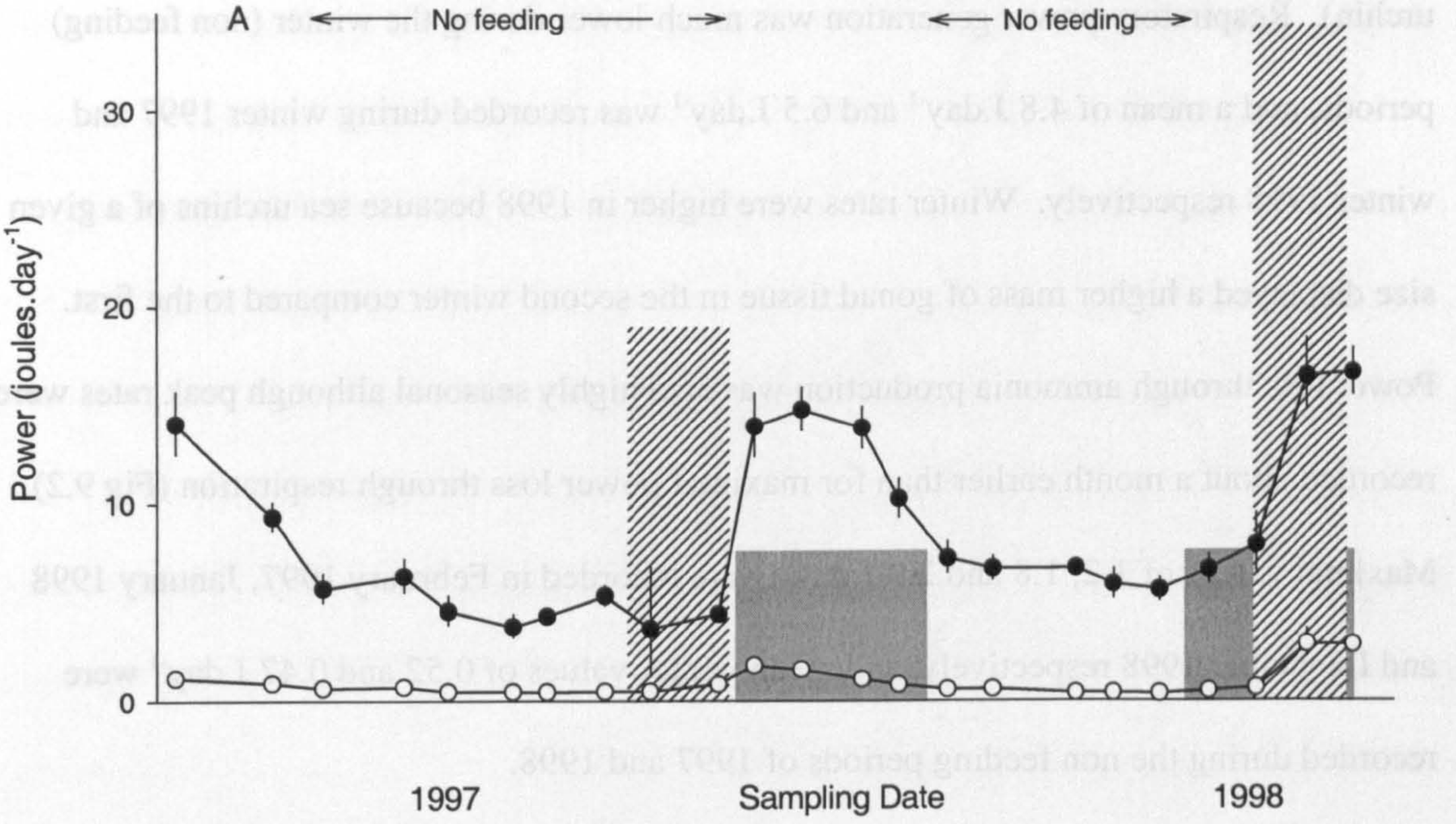


Figure 9.1 Seasonal changes in sediment organic content at North Cove (○): data are redrawn from Chapter 2 and represent means of five replicate sediment cores and standard errors. (●) organic content of faeces of *S. neumayeri* from North Cove; data are means of 16 observations with standard error.

Rate of power generation attributable to respiration showed strong seasonal variation (Fig 9.2 and Chapter 4a). O:N ratios varied between 7 and 26 during the two year study period, and indicated a predominantly protein based metabolic substrate. A power conversion of 0.427 J.μmolO_2 (Brafield and Llewellyn 1982) was therefore used to calculate energy dissipated as heat, and maximal respiratory power output of 14, 14.7 and 16.6 J.day^{-1} were recorded in February 1997, February 1998 and January 1999 respectively



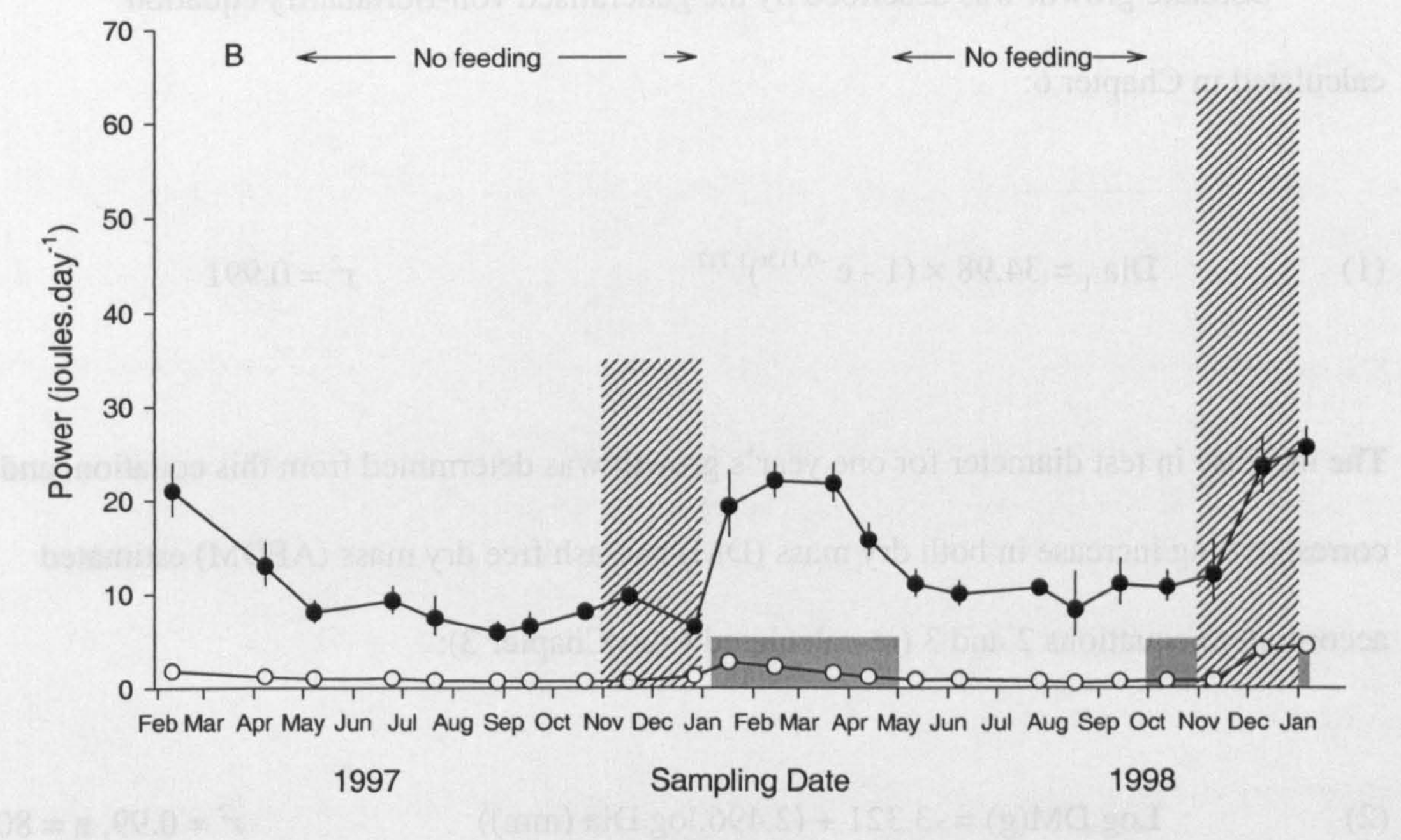


Figure 9.2 Seasonal energy utilisation for *S. neumayeri*: A) 24mm test diameter and B) 30mm test diameter. (●) Daily metabolic heat equivalent measured from rate of oxygen consumption: data recalculated from Chapter 4a. (○) Daily power loss from ammonia excretion: data recalculated from Chapter 4a. Grey shading represents daily energy utilised for somatic growth recalculated from Chapter 6. Hatched shading represents daily reproductive output assuming a two month spawning period, recalculated from Chapter 5.

for a 24mm diameter urchin (values were approximately 50% higher for a 30mm diameter urchin). Respiratory power generation was much lower during the winter (non feeding) periods, and a mean of 4.8 $J.day^{-1}$ and 6.5 $J.day^{-1}$ was recorded during winter 1997 and winter 1998 respectively. Winter rates were higher in 1998 because sea urchins of a given size displayed a higher mass of gonad tissue in the second winter compared to the first. Power loss through ammonia production was also highly seasonal although peak rates were recorded about a month earlier than for maximal power loss through respiration (Fig 9.2). Maximal values of 1.2, 1.8 and 2.9 $J.day^{-1}$ were recorded in February 1997, January 1998 and December 1998 respectively, and mean winter values of 0.52 and 0.47 $J.day^{-1}$ were recorded during the non feeding periods of 1997 and 1998.

Somatic growth was described by the generalised von-Bertalanffy equation calculated in Chapter 6:

(1) $$Dia_t = 34.98 \times (1 - e^{-0.113t})^{1.717} \qquad r^2 = 0.991$$

The increase in test diameter for one year's growth was determined from this equation, and corresponding increase in both dry mass (DM) and ash free dry mass (AFDM) estimated according to equations 2 and 3 (re-calculated from Chapter 3):

(2) $$\text{Log DM(g)} = -3.321 + (2.496.\text{log Dia (mm)}) \qquad r^2 = 0.99,\ n = 80$$

(3) $$\text{Log AFDM(g)} = -4.209 + (2.744.\text{log Dia (mm)}) \qquad r^2 = 0.98,\ n = 80$$

Annual increase in organic matter was taken to correspond to annual increase in AFDM,

and energetic growth was calculated using the conversion factor of 23 kJ.gram AFDM for marine invertebrate tissue (Brey et al. 1988). Annual increase in skeletal material was estimated as increase in DM subtracted from increase in AFDM. Energy required to precipitate calcite from solution was estimated from the apparent solubility product (K') as follows:

(4) $K'_{calc} = (0.1614 + 0.02892\ C - 0.0063\ t) \times 10\text{-}6$ (Clarke 1983)

where C = chlorinity and t = temperature (°C). The change in Gibbs free energy ($\Delta G°$) required to precipitate one mole of $CaCO_3$ from solution is a function of the solubility product:

(5) $\Delta G° = R\ T \ln K'_{calc}$

where R is the universal gas constant and T the absolute temperature. Consequently $\Delta G°$ for calcite at 0°C and salinity of 34ppt (assuming chlorinity = salinity / 1.80655) = 32.167 $kJ.mol^{-1}$. Equations one to five were used to calculate energetic investment in somatic growth (both organic and skeletal) for 1998.

Somatic growth of juvenile sea urchins was highly seasonal, and took place only during feeding periods (Chapter 6). The generalised von-Bertalanffy function (Chapter 6, equation 1) indicated that a 24mm test diameter urchin (14.6 years old) grew to 25.1mm in one year. This represented an increase in dry mass (DM) of 0.155 g and an increase in ash-free dry mass (AFDM) of 0.048g. AFDM (taken to be organic tissue) represented 1111.4 J, and the remaining growth (0.107g) was assumed to be calcite ($CaCO_3$). The energy to

precipitate this quantity of calcite was estimated from ΔG° to be 34.4 J, making the total annual energy invested in growth 1146 J. This can be converted to a daily power generation (Fig 9.2) of 7.53 $J.day^{-1}$ assuming a constant growth rate throughout the feeding period for 1998, although in reality growth was likely to be faster at mid summer than in either spring or autumn because of differences in food availability. Although skeletal growth (precipitation of calcite) accounted for almost 70% of the increase in total dry mass, it represented only 3.0% of the total annual energy dedicated to somatic growth. Over the same period the generalised von Bertalanffy equation (Chapter 6, equation 1) indicated that a 30mm diameter urchin (22 years old) grew to 30.52 mm, which represented an increase in DM of 0.101g and AFDM of 0.033g. This growth indicated a total (organic + calcite) energetic investment of 781 J or 5.14 $J.day^{-1}$ (Fig 9.2).

S. neumayeri spawning commenced at the start of the annual feeding period, and any reductions in gonad mass resulting from spawning were masked by gonad mass increase related to increased feeding. No reduction in gonad dry mass was observed during the preceding winter, so pre-spawning gonad mass was estimated from the mean mass for the six non feeding months in 1997 and the four non feeding months in 1998. The relationship between $\log_{10}$ (gonad mass) and $\log_{10}$ (test diameter) was described by a scaling exponent of 2.77 (recalculated from Chapter 3), and this was used to calculate gonad masses for both a 24 mm and a 30mm test diameter urchin. Mean gonad mass during the winter non feeding periods of 1997 and 1998 was 0.165g and 0.258g respectively for a 24mm diameter urchin. Spawning output was measured for both years using histological methods and was 38.6% in 1997 and 41.9% in 1998 (Chapter 5). This spawning output was independently corroborated by experimentation described in Chapter

7, which indicated a figure of 43% for 1998 by holding mature pre-spawning urchins in the laboratory without food during the spawning period, and correcting observed mass loss for respiratory power generation. The energy content of gonad immediately pre-spawning (September) was 17.7 $kJ.g^{-1}$ and 19.0 $kJ.g^{-1}$ in 1997 and 1998 respectively (Chapter 3). Summation of values for mean overwintering gonad mass, percentage reproductive output and gonad energy concentration indicated a total energetic reproductive output (24mm diameter) of 1.1 kJ and 2.1 kJ in 1997 and 1998 respectively (Table 9.1). The values for the second year are almost double those for 1997 mainly because of the higher mass of gonad tissue in overwintering urchins in the second year, but also because of the higher percentage reproductive output. The total reproductive output of a 30mm diameter urchin was 2.1kJ and 3.8kJ in 1997 and 1998 (Table 9.1). These values were converted to a daily power expenditure by division over a sixty day spawning period in November and December (Fig 9.2).

Total energy assimilated (calculated by summation of energy values for respiration, ammonia excretion, somatic and reproductive growth) could not be calculated for 1997 because somatic growth was not measured over this period. For 1998 total energy assimilated was 7.1 kJ and 10.6 kJ for a 24mm and 30mm diameter urchin respectively. Respiration accounted for 50% of this total and ammonia excretion for 5% of the total in both 24 and 30mm diameter urchins. Somatic growth accounted for 16% and 7% of total annual energy in 24mm and 30mm diameter urchins respectively, and reproduction accounted for 29% and 36% respectively (Table 9.1). Overwintering (non feeding) energy costs amounted to 18% and 16% of total annual energy for 24 and 30 mm diameter urchins in 1998 (Table 9.1). The dominant component of winter energy lost was respiration. The

proportion of total annual respiration expended during the winter was higher in 1997 (48% for a 24 mm urchin) than in 1998 (28%) due to the longer period of starvation in 1997.

Growth efficiencies of marine invertebrates have previously been defined using either somatic production alone or somatic production + reproductive production to represent growth. Measurements using somatic production alone are valuable in studies of food web dynamics since these can be used to estimate the efficiency of upward transmission of available energy from prey to predator. However, for studies attempting to compare the consequences of environmental constraints on life history strategies of invertebrates (where a large proportion of their total annual energy is diverted to production of free spawned gametes) a measure of growth efficiency including both somatic and reproductive production is more appropriate:

(6) Gross growth efficiency, $(K_1) = (P_G + P_S) / C$ (Crisp 1984)

Where P_G is reproductive (gonad) production, P_S = somatic production and C = food energy consumed. This may be modified to calculate a net growth efficiency, (K_2) as follows:

(7) Net growth efficiency, $(K_2) = (P_G + P_S) / A$

Where A = energy assimilated. Because we cannot measure energy consumption or assimilation independently in *S. neumayeri*, total energy assimilated must be estimated from the sum of energy demands from respiration (R), ammonia excretion (U), somatic growth (P_S) and reproductive growth (P_G). For the current study, the net growth efficiency

(K_2) was therefore estimated from:

(8) $$K_2 = (P_G + P_S) / (R + U + P_G + P_S)$$

It was not possible to calculate K_1 because C was not obtained, but net growth efficiency was 0.45 for a 24mm diameter urchin and showed little change with animal size (Table 9.1).

Table 9.1 Relative and seasonal energy sinks for *S. neumayeri* from North Cove.

Energy term		24mm diameter (Joules)	30mm diameter (Joules)
Respiration	Summer 1997	1316	1902
	Winter 1997	1202	1975
	Total 1997	2518	3877
	Summer 1998	2566	3854
	Winter 1998	998	1575
	Total 1998	3564	5429
Ammonia	Summer 1997	137	203
	Winter 1997	139	216
	Total 1997	276	419
	Summer 1998	300	444
	Winter 1998	74	121
	Total 1998	374	565
Somatic Growth	1998	1145	781
Reproductive output	1997	1131	2085
	1998	2053	3812
Summation			
Total winter energy	1998	1072	1696
Total summer energy	1998	6064	8891
Total energy assimilated	1998	7136	10587
Winter energy as % of total	1998	17.7	16.0
Net growth efficiency (%)	1998	45	43

9.4 Discussion

The data presented here for each of the energy budget terms was collected simultaneously and from the same field population over a one year period (or two years for respiration, ammonia excretion and reproduction). This method of data collection overcomes many of the criticisms of previous energy budgets, where data have been compiled from sub-annual periods, or extrapolated from laboratory observations to field populations (Davies and Hatcher 1998). Only one previous energy budget has been constructed for an Antarctic marine benthic invertebrate, for the isopod *Serolis polita* (Luxmoore 1985). That study did not however measure seasonal changes in metabolism, and instead relied upon an experimentally determined relationship between water temperature and oxygen consumption. Such methodology would have failed to take into account rises in metabolism as a consequence of either growth or feeding, and these are major components of the annual respiratory power term for *S. neumayeri* (Chapter 7).

Very low assimilation efficiencies (1-10%) have previously been reported for other detrital feeding marine invertebrates. The protodetritus ingested by these species is often highly refractory by the time it reaches the seafloor, and rather than assimilating the inert detrital material itself it is the bacteria, fungi and protists which colonise the detritus that instead form the digestible fraction of the food (Barnes and Hughes 1988). From the data presented here is it impossible either to derive an overall assimilation efficiency, or to make an assessment as to whether *S. neumayeri* is digesting freshly settled and non-refractory phytodetritus or the microbial colonisers of protodetritus. However, the data do provide for a minimum estimate of total net energy assimilated to be calculated by the addition of annual and seasonal power devoted to somatic growth, reproductive growth, respiration and

ammonia excretion. This summation implies that net energy assimilated is equal to the energy contained in organic matter consumed minus the energy in organic matter which is egested. In reality, however, the secretion of the mucous envelope to the faecal pellets means that some energy is added to the faeces during their passage through the gut.

The data indicate strong seasonal trends which have been discussed in earlier chapters. Feeding and other metabolically expensive processes are confined to the austral summer as a result of changes in food availability, leading to a reduced winter metabolic rate. Although vitellogenesis continues during the winter, it is fueled by metabolic reserves stored in summer. Previous studies have recorded that the biochemical composition and organ masses of Antarctic echinoderms are no different from warmer water species (McClintock and Pearse 1987), which implied that high energy reserves are not laid down for the austral winter (Clarke and Peck 1991). The current study moderates this view: reserves are indeed stored, but they are small in relation to the overall mass and therefore energy content of the animal. Monthly changes in winter whole body energy content (Chapter 3) are in broad agreement with the rate of heat dissipation calculated from oxygen consumption (Table 9.2). This confirms that the slow rates of mass loss described in Table 3.3 are representative, and that they are attributable to a low overwintering resting metabolic rate.

Some marine organisms, notably shallow water gastropods, show a decrease in calcification (calculated as mass of shell divided by some measure of animal size) at low seawater temperatures. Clarke (1983) argued that this is likely to be related to the increased apparent solubility products of calcite (or aragonite) in cold water. However, the data

presented by this study for *S. neumayeri* indicate that the costs of calcification represent only 3% of the energy diverted to somatic growth, which equates to 0.48% of the total annual energy for 1998. Furthermore, an increase in temperature from 0°C to 40°C would represent a change of only 20% in the value of ΔG° for calcite. Such a change would have only the most minor influence on the annual energy budget, and this data does not therefore support the hypothesis that increased thermodynamic cost of precipitating calcite from seawater at low temperatures is the reason for thin shelled marine organisms at high latitudes.

Table 9.2 Comparison of energy lost as heat (calculated from oxygen consumption) and energy liberated from mass loss during overwintering period for a 24mm diameter *S. neumayeri*. Energy lost as heat calculated in Table 4.1. Energy liberated from mass loss recalculated from Table 3.3 for a 24mm diameter urchin.

Winter	J.month^{-1} dissipated as heat, calculated from rate of oxygen consumption. (Chapter 4a)	J.month^{-1} lost in mass during overwintering period. (Chapter 3)
1997	145	182
1998	204	178

Previously published energy budgets for marine organisms indicate that loss as nitrogenous waste represents only a small fraction of total energy assimilated (Davies and Hatcher 1998), and the data presented here for *S. neumayeri* (Table 9.1) agree with this statement. Respiration however accounts for a substantial proportion of previously published energy budgets for echinoids. Hawkins and Lewis (1982) have shown that respiration accounts for 72% of the monthly energy budget of *Diadema antillarum*, and Miller and Mann (1973) found that *Strongylocentrotus droebachiensis* devoted 78% of assimilated energy (where total assimilated energy is calculated by summation of individual components for somatic and reproductive growth and respiration). The equivalent figure

for *S. neumayeri* is lower (50% for 1998), indicating a higher P:R ratio, and therefore a higher overall net growth efficiency. Comparison of net growth efficiencies for echinoids (Table 9.3) shows that the value for *S. neumayeri* is amongst the highest reported. Of the three major energetic sinks, two, i.e. respiration (Fig 4.6) and somatic growth rate (Fig 6.7) both decrease with latitude. However reproductive output (Fig 6.9) does not reduce with latitude, and hence growth efficiency is high for *S. neumayeri*. This conclusion agrees with one of the hypotheses proposed by Clarke (1987), that if annual weight specific gonad output is held constant, then true reproductive effort must increase at high latitudes. Growth efficiency also increases, despite an overall reduction in total annual energy assimilated by species at high latitudes.

An absence of latitudinal variation in reproductive output, as evidenced by comparison of polar species with warmer water species has important consequences for our understanding of life history adaptations of cold water ectotherms. However, this trend may be specific to echinoderms. Brey (1995) demonstrated that for Mollusca (54 species compared, $p = 0.005$) and Crustacea (14 species, $p = 0.032$) annual reproductive output did decrease with decreasing temperature (i.e. increasing latitude), although the same study did not find any correlation between latitude and reproductive output for Echinodermata (20 species, $p = 0.148$), and additionally (McClintock 1989) studied polar asteroids and showed no change in gonad mass of asteroids with latitude (see Chapter 6). Sainte-Marie (1991) demonstrated a significant (Kruskal-Wallis; $p < 0.01$) reduction in reproductive potential (number of embryos per female per year) between cold water and warm water populations of gammaridean amphipods. However no significant difference was found for lifetime fecundity (i.e. brood size multiplied by maximum number of broods in a life-span) between

the warm and cold water groups, indicating that although annual reproductive effort was decreased for cold water populations, the extended life-span of cold water amphipods made lifetime reproductive output comparable with warmer water species.

Table 9.3 Comparison of echinoid net growth efficiencies.

Species	Temperature (°C)	Size test diameter (mm) or dry mass (g)	Net Growth efficiency (%)	Reference
Diadema antillarum	27.3	15mm	8.0	(Hawkins and Lewis 1982)
		30mm	9.8	
		45mm	16.3	
Eucidaris tribuloides	26.2		17 - 44	(McPherson 1968)
Mellita quinquiesperforata	26	62mm	7.1	(Lane 1977)
		89mm	12.4	
		100mm	16.8	
Sterechinus neumayeri	-0.5	24mm	45	Current study.
		30mm	40 - 43	
Strongylocentrotus droebachiensis	9	15mm	7.2 - 7.8	(Miller and Mann 1973)
		30mm	6.7	
		40mm	5.8	
Strongylocentrotus droebachiensis	5	0.35g DM	19	(Propp 1977)
		17.4g DM	28	
Strongylocentrotus intermedius	11.3	1.75g	28.1	(Fuji 1967)
		49.9g	12.4	

9.5 Conclusion

This thesis has, for the first time, quantified the energetic cost of enduring the polar winter for a marine invertebrate. The energy expended by *S. neumayeri* and *L. elliptica* during this prolonged non feeding period is minor in relation to whole body energy content. This thesis also represents the first complete calculation of growth efficiency for a benthic marine invertebrate at polar latitudes. The increased growth efficiency, and increased ratio of reproductive production to total production represents an important alteration to the life history pattern of this free spawning species, and one which may be true for other polar echinoderms. Evidence suggests however that this trend is not likely to extend to bivalves or some crustacean taxa, where reproductive output diminishes with increasing latitude.

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Appendix One Estimation of proximate biochemical composition from elemental CHN analysis

The stoichiometric method of estimating tissue proximate composition from CHN elemental ratios (Gnaiger and Bitterlich 1984) centres upon the different mass fractions of each element in standard carbohydrate, lipid and protein (Table A1.1):

Table A1.1 Elemental CHN composition of standard carbohydrate, lipid and protein (redrawn from Table 1 in Gnaiger and Bitterlich 1984)

	Carbon	Hydrogen	Nitrogen
Carbohydrate	0.444	0.062	0.000
Lipid	0.776	0.114	0.000
Protein	0.529	0.070	0.173

Proximate composition ratios presented in this thesis were calculated using a MINITAB macro written by Andrew Clarke ("CHNprox.mtb" :Version 2, May 1996). The main stages of the calculation are as follows:

1) Calculate mass of organic carbon in sample by subtracting mass fraction (%) of residual water and ash from sample mass.

2) Calculate the amount of protein (allowing for non-protein nitrogen) from the nitrogen in sample.

3) Calculate mass fraction of carbon and hydrogen associated with protein, and hence mass of protein in sample. Consequently calculate mass of organic matter remaining.

4) Lipid and carbohydrate are calculated from remaining organic matter using the mass fractions of carbon defined in Table A1.1. When carbohydrate alone is present in the sample, the mass fraction of carbon is 0.444. When lipid is the sole constituent, the carbon mass fraction is 0.776. Non-protein carbon fractions which are intermediate between 0.444 and 0.776 indicate the mass fractions of carbohydrate and lipid in the mixture according to a linear relationship.

5) Negative values of lipid occur when carbon explained by protein has been overestimated. Negative values of carbohydrate occur when carbon explained by protein has been underestimated.

Appendix Two Data sources for Figures

A2.1 Data sources for Figs 6.6, 6.7 & 6.8

Species	Location	Citation	Temp	S∞	K	ϕ
Sterechinus neumayeri	McMurdo, Antarctica	(Brey et al. 1995)	-1	70.23	0.031	-0.278
Strongylocentrotus pallidus	Barents Sea, 80N	(Bluhm et al. 1998)	0	102.3	0.011	-0.618
Sterechinus neumayeri	Rothera	This study	-0.5	34.98	0.113	0.082
Echinus esculentus	Dunstaffanage, shallow water	(Gage 1992)	11	114	0.317	0.872
Echinus acutus var. norvegicus	220 -1075 m, Scotland	(Gage et al. 1986)	3.5	64.7	0.126	0.308
Echinus elegans	220 - 1075 m, Scotland	(Gage et al. 1986)	3.5	85.56	0.096	0.271
Heterocentrotus trigonarius	Enewetak Atoll	(Ebert 1982)	28.3	102.4	0.032	-0.155
Heterocentrotus mammillatus	Honaunau, Hawaii	(Ebert 1982)	24.8	67.6	0.032	-0.275
Colobocentrotus atratus	Ulupau, Hawaii	(Ebert 1982)	24.8	60.5	0.032	-0.307
Echinometra mathaei	Hawaii, Kapapa Island	(Ebert 1982)	24.8	54.9	0.322	0.668
Echinometra mathaei	Hawaii, Makua	(Ebert 1982)	24.8	42.5	0.322	0.594
Echinometra mathaei	Rottnest Island, W. Australia	(Ebert 1982)	19.5	49.7	0.322	0.639
Echinometra mathaei	Ras Iwatine, Kenya	(Ebert 1982)	26.6	48.9	0.322	0.634
Echinometra mathaei	Eilat, Israel	(Ebert 1982)	23	44.9	0.322	0.609
Echinometra mathaei	Hawaii	(Ebert 1982)	24.8	40.95	0.292	0.540
Echinometra oblongata	Hawaii, Kapapa Island	(Ebert 1982)	24.8	40.4	0.322	0.579
Echinometra oblongata	Hawaii, Lani Overlook	(Ebert 1982)	24.8	30.8	0.358	0.546
Heliocidaris erythrogramma	Port Jackson, New South Wales	(Ebert 1982)	19.8	71.9	0.356	0.789
Heliocidaris erythrogramma	Cape Peron, W. Australia	(Ebert 1982)	19.7	76.1	0.191	0.535
Echinothrix diadema	Hawaii, Kapapa Island	(Ebert 1982)	24.8	77.8	0.05	-0.040
Centrostephanus rodgersi	Port Jackson, New South Wales	(Ebert 1982)	19.8	92.1	0.247	0.702
Diadema setosum	Zanzibar	(Ebert 1982)	26.6	91.9	0.019	-0.412
Diadema setosum	Eilat, Israel n=7	(Ebert 1982)	23	83.5	0.008	
Stomopneustes variolaris	Negombo, Sri Lanka	(Ebert 1982)	27.7	71.1	0.013	-0.651
Salmacis belli	Morton Bay, Queensland, Aust.	(Ebert 1982)	22.7	104.7	0.077	0.233
Sphaerechinus granularis	Southern Brittany	(Jordana et al. 1997)	13.9	99	0.28	0.778

Sphaerechinus granularis	Southern Brittany	(Jordana et al. 1997)	13.9	105	0.23	0.709
Loxechinus albus	Mehuin, Southern Chile	(Gebauer and Moreno 1995)	8.3	136	0.16	0.627
Loxechinus albus	Mehuin, Southern Chile	(Gebauer and Moreno 1995)	8.3	136	0.10	0.422
Tripneustes gratilla elatensis	Red Sea, Israel	(Dafni 1992)	23	35	0.87	0.969
Strongylocentrotus franciscannus	San Nicholas Island, California	(Ebert and Russell 1992)	16.0	87.5	0.063	0.094
Eucidaris tribuloides	Virginia Key, Florida	(McPherson 1968)	26.2	52.7	0.67	0.974
Diadema antillarum	Virgin Islands & Florida Keys	(Randall et al. 1964)	27.3	75.6	1.18	1.324
Diadema antillarum	Barbados, B.W.I.	(Lewis 1966)	27.4	58.0	1.22	1.262
Echinothrix diadema	Kapapa Island, Oahu, Hawaii	(Ebert 1975)	24.8	90.0	0.82	1.217
Psammechinus miliaris	Loch Creran, W. Scotland	(Gage 1991)	10.5	28.1	0.53	0.690
Lytechinus anamesus	La Jolla, California	(North 1965)	16	20.0	0.53	0.591
Lytechinus variegatus	Miami, Florida	(Moore et al. 1963)	26.2	79.3	0.95	1.244
Tripneustes ventricosus	Barbados, B.W.I.	(Lewis 1958)	27.4	112.8	1.39	1.511
Tripneustes ventricosus	Virginia Key, Florida	(McPherson 1965)	26.2	90.0	1.24	1.396
Echinus esculentus	Port Erin, Isle of Man	(Moore 1935)	11.6	77.5	0.36	0.816
Psammechinus miliaris	Raunefjorden, Norway	(Jensen 1969)	9.0	16.8	0.28	0.264
Psammechinus miliaris	Raunefjorden, Norway	(Jensen 1969)	9.0	16.8	0.13	-0.069
Psammechinus miliaris	Aquarium, Northumberland	(Bull 1939)	9.6	38.3	0.53	0.780
Allocentrotus fragilis	Newport, Oregon, USA	(Ebert 1975)	11.3	77.5	0.30	0.737
Allocentrotus fragilis	Newport, Oregon, USA	(Ebert 1975)	11.3	77.5	0.15	0.436
Strongylocentrotus drobachiensis	Friday harbour, Washington	(Swan 1961)	17.1	74.6	0.66	1.068
Strongylocentrotus drobachiensis	St Margarets Bay, Canada (W)	(Miller and Mann 1973)	0-18	55.6	0.28	0.611
Strongylocentrotus echinoides	Friday harbour, Washington	(Swan 1961)	17.1	58.4	0.57	0.934
Strongylocentrotus franciscannus	San Diego, California	(Baker 1973)	16	110.0	0.26	0.776
Strongylocentrotus intermedius	Ishiya & Sumiyoshi, Japan	(Fuji 1967)	11.3	69.2	0.33	0.745
Strongylocentrotus nudus	Urakawa, Japan	(Kawamura 1966)	11.3	76.2	0.36	0.811
Strongylocentrotus pulcherrimus	Hatsuse, Japan	(Fuji 1963)	11	38.1	0.4	0.656
Strongylocentrotus purpuratus	Sunset Bay, Oregon	(Ebert 1968)	11.3	55.0	0.22	0.503
Strongylocentrotus purpuratus	Aquarium, La Jolla, California	(Leighton 1968)	16	64.8	0.62	1.000

Colobocentrotus atratus	Oahu, Hawaii	(Ebert 1975)	24.8	30.0	0.45	0.638
Echinometra mathaei	Oahu, Hawaii	(Ebert 1975)	24.8	38.1	0.46	0.717
Echinometra mathaei	Oahu, Hawaii	(Kelso 1970)	24.8	55.0		
Echinometra oblongata	Oahu, Hawaii	(Ebert 1975)	24.8	32.2	0.40	0.607
Echinometra oblongata	Oahu, Hawaii	(Kelso 1970)	24.8	50.0		
Echinocardium cordatum	Northumberland, 10m	(Buchanan 1966)	9.5	32.1	0.11	0.046
Echinocardium cordatum	Northumberland, Intertidal	(Buchanan 1966)	9.5	47.0	0.38	0.695
Moira atrops	Biscayne Bay, Florida	(Moore and Lopez 1966)	25	40.7	0.88	1.018

A2.2 Data sources for Figure 6.9

Species	Location	Reference	Temp.	Max GI	Min GI	Max-Min
Allocentrotus fragilis	Deep Sea	(Giese 1961)	4	7	1	6
Anthocidaris crassispina	Seto, Japan. 35N 137E	(Kobayashi 1969)	19.9	18	8	10
Anthocidaris crassispina	Tateyama, Japan 34N 139 E	(Masuda and Dan 1977)	19.9	12	1	11
Arbacia lixula	Villefranche-sur-Mer France 43N 7E	(Fenaux 1968)	18	17	2	15
Arbacia lixula	Villefranche-sur-Mer France 43N 7E	(Fenaux et al. 1977)	18	9.3	2.2	12.7
Arbacia punctulata	Cape Cod. 42N 70W	(Boolootian and Turner 1965)	11.8	13.4	1.5	11.9
Centrostephanus rodgersii	Solitary Islands, Australia. 30S, 153E	(O'Connor et al. 1976)	21.5	13	2	11
Dendraster excentricus	Los Angeles, USA 34N118W	(Timko 1975)	16	6.5		
Dendraster excentricus	San Diego, USA 32N 117 W	(Niesen 1977)	17	3.8	1.5	2.3
Diadema setosum	Seto, Japan. 35N 137E	(Kobayashi and Nakamura 1967)	19.9	35	9.9	25.1
Diadema setosum	Gulf of Suez	(Pearse 1970)	26	17		
Echinarachinus parma	Orono, Maine, USA 45N, 68W	(Cocanour and Allen 1967)	11.7	13	5	8
Echinarachinus parma	Orono, Maine, USA 45N, 68W	(Cocanour 1969)	11.7	14	4	10
Echinocardium cordatum	Port Erin, Isle of Man	(Moore 1936)	10	0.085		
Echinometra lucunter	Florida, 25S 80W	(McPherson 1969)	26.4	15	2	13
Echinometra viridis	Florida, 25S 80W	(McPherson 1969)	26.4	8	2	6
Echinometra mathaei	Gulf of Suez	(Pearse 1969)	26	5	2.2	2.8
Echinometra mathaei	Seto, Japan. 35N 137E	(Kobayashi 1969)	19.9	14	8	6
Echinostrephus aciculatus	Seto, Japan. 35N 137E	(Kobayashi 1969)	19.9	18	8	10
Echinostrephus aciculatus	Seto, Japan. 35N 137E	(Kobayashi and Tokioka 1976)	19.9	21	4.5	16.5
Echinus esculentus	50N, 5W, Plymouth., 1981 8-10m	(Nichols et al. 1985)	12	24	7	17
Echinus esculentus	50N, 5W, Plymouth., 1982	(Nichols, Bishop et al. 1985)	12	20	5	15
Echinus esculentus	50N, 5W, Plymouth., 1983	(Nichols, Bishop et al. 1985)	12	14	10	4
Echinus esculentus	50N, 5W, Plymouth.,1981 20-22m	(Nichols, Bishop et al. 1985)	12	10	1	9

Echinus esculentus	50N, 5W, Plymouth.,1982	(Nichols, Bishop et al. 1985)	12	4	1	3
Echinus esculentus	50N, 5W, Plymouth., 1983	(Nichols, Bishop et al. 1985)	12	2	2	0
Echinus esculentus	Port Erin, Isle of Man	(Moore 1934)	10	1.75	0.35	1.4
Eucidaris tribuloides	South East Florida 25N 80W	(McPherson 1968)	26.4	5	2	3
Eucidaris tribuloides	South East Florida 25N 80W	(McPherson 1968)	26.4	15	3.5	11.5
Evechinus chloroticus	Kaikoura + Kaiteriteris, South Island, NZ 42S, 173E	(Dix 1970)	14	1.9	0.8	1.1
Heliocidaris erythrogramma	S.E. Tasmania	(Dix 1977)	13	19.19		
Heliocidaris erythrogramma	S.E. Tasmania	(Dix 1977)	13	1	0.4	0.6
Heliocidaris tuberculata	Solitary Islands, Australia. 30S, 153E	(O'Connor, Riley et al. 1976)	21.5	14	5	9
Hemicentrotus pulcherrius	Tateyama, Japan 34N 139 E	(Masuda and Dan 1977)	19.9	24	8	16
Lexicons albus	53S, 71W, Chile, Cockburn Channel	(Oyarzun *et al.* 1999)	6.5	16	9	7
Lexicons albus	Dawson Island	(Oyarzun, Marin et al. 1999)	6.5	14	11	3
Lovenia elongata	Gulf of Suez	(Pearse 1969)	26	4	1	3
Lovenia elongata	Gulf of Eilat	(Ferber and Lawrence 1976)	26	6	0.5	5.5
Lytechinus variegatus	Key Largo, S.E. Florida	(Moore et al. 1963)	26.4	1.75	0.3	1.45
Lytechinus variegatus	Miami	(Moore and McPherson 1965)	26.4	1.7	0.6	1.1
Lytechinus variegatus	Miami	(Moore and Lopez 1972)	26.4	2	0.5	1.5
Mellita quinquiesperforata	27N, 82W	(Moss and Lawrence 1972)	25	3.3	1	2.3
Meoma ventricosa	Florida Keys	(Chesher 1969)	26	0.55		
Mespilia globulus	Seto, Japan	(Kobayashi 1967)	19.9	0.5	0.19	0.31
Moira atrops	Biscayne Bay, Florida.	(Moore and Lopez 1966)	26	0.5	0.1	0.4
Paracentrotus lividus	Villefranche-sur-Mer France 43N 7E	(Fenaux 1968)	18	17	2	15
Parechinus angulosus	South Africa, 33S, 18E.	(Greenwood 1980)	19	5	2	3
Phyllacnathus parvispinus	Solitary Islands, Australia. 30S, 153E	(O'Connor, Riley et al. 1976)	21.5	3.7		
Prionocidaris baculosa	Gulf of Suez	(Pearse 1969)	26	5	2	3
Psammechinus microtuberculatus	Villefranche-sur-Mer France 43N 7E	(Fenaux 1968)	18	21	5	16

Sterechinus neumayeri	McMurdo, Antarctica	(Pearse and Giese 1967)	-1.5	30	5	25
Sterechinus neumayeri	Rothera Environs, Antarctica (Recalculated)	(Brockington et al.)	-0.8	34	11.4	22.6
Stomopneustes variolaris	Madras Harbour 44N, 121W	(Giese et al. 1964)	15	23	5	18
Strongylocentrotus droebachiensis	Orono, Maine, USA 45N, 68W	(Cocanour and Allen 1967)	11.7	17	6	11
Strongylocentrotus droebachiensis	52W, 47N Newfoundland 1981 0-2m	(Keats et al. 1984)	6.5	13	6	7
Strongylocentrotus droebachiensis	52W, 47N Newfoundland 1982	(Keats, Steele et al. 1984)	6.5	17	4	13
Strongylocentrotus droebachiensis	52W, 47N Newfoundland 1983	(Keats, Steele et al. 1984)	6.5	12	3	9
Strongylocentrotus droebachiensis	52W, 47N Newfoundland 198112-18m	(Keats, Steele et al. 1984)	6.5	3	1	2
Strongylocentrotus droebachiensis	52W, 47N Newfoundland 1982	(Keats, Steele et al. 1984)	6.5	5	1	4
Strongylocentrotus droebachiensis	52W, 47N Newfoundland 1983	(Keats, Steele et al. 1984)	6.5	4.5	1	3.5
Strongylocentrotus droebachiensis	44N, 64W E. Canada Kelp Bed	(Meidel and Scheibling 1998)	10	20	5	15
Strongylocentrotus droebachiensis	44N, 64W E. Canada Barren Grounds	(Meidel and Scheibling 1998)	10	14	2	12
Strongylocentrotus droebachiensis	St. Johns, Newfoundland	(Percy 1971)	6	14.02	3.72	10.3
Strongylocentrotus nudus	Southern Hokkaido, Japan.	(Fuji 1960)	14	30	10	20
Strongylocentrotus nudus	Vostock Bay, Sea of Japan	(Yakolev 1976)	14	14	3	11
Strongylocentrotus nudus	Peter the Great Bay, Sea of Japan.	(Yakolev et al. 1976)	12	4.1	0.6	3.5
Strongylocentrotus intermedius	Hokkaido, Japan.	(Fuji 1960)	14	26.7	3.9	22.8
Strongylocentrotus intermedius	Southern Hokkaido, Japan.	(Fuji 1960)	14	30	5.5	24.5
Strongylocentrotus intermedius	Vostock Bay, Sea of Japan	(Yakolev 1976)	14	20	1	19
Strongylocentrotus purpuratus	Western seaboard, USA	(Bennett and Giese 1955)	13	23.5	2	21.5
Strongylocentrotus purpuratus	Western seaboard, USA	(Giese et al. 1958)	13	17	2.7	14.3
Strongylocentrotus purpuratus	Pacific Groove, California	(Holland and Giese 1965)	14	15	1	14
Strongylocentrotus purpuratus	Pacific Groove, California	(Lawrence 1966)	14	24	1	23

Tripneustes gratilla	Seto, Japan	(Kobayashi 1969)	19	12	5	7
Tripneustes gratilla	Solitary Islands, Australia. 30S, 153E	(O'Connor, Riley et al. 1976)	21.5	15	2	13
Tripneustes ventricosus	Bermuda	(Moore et al. 1963)	23	12	3	9
Tripneustes ventricosus	Miami.	(Moore, Jutare et al. 1963)	27	11	3	8