Condition indicators for Antarctic krill, *Euphausia superba*

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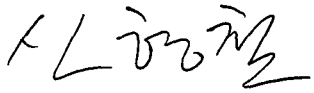

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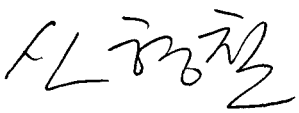

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Abstract

Antarctic krill use a variety of strategies to cope with, and thrive in the highly variable Southern Ocean environment. Despite much detailed information on its basic biology produced so far, the linkages between krill populations and the environment are yet to be systematically investigated. There is a practical need to have standardised indicators to assess the 'condition' of krill in relation to seasonal cycles and shifts in physical regimes and this study has aimed to develop such indicators.

The level of nucleic acids in abdominal tissue was determined as an estimator of growth rates of individual krill that could otherwise only be obtained by on-board experiments. The dynamics of the major digestive organ, the digestive gland, in its size, protein and lipid content and enzyme activities, were examined in relation to changing food regimes. The potential of using eye diameter as a long-term starvation indicator was also examined.

The amount of RNA and RNA:DNA ratio in krill muscle exhibited a significant relationship with individual growth rates, although the predictability was only modest. This was the case with both field-caught specimens and experimental juveniles. RNA-based indices were clearly different between well-fed, high-growth krill and underfed low-growth krill, and the RNA content levelled off when the growth rates became negative. The moult cycle had no significant effect on nucleic acid content. Overall, the content of nucleic acids varied considerably between individuals. Starved krill also tended to have higher DNA per unit biomass, which implies shrinkage of cells rather than loss of cells. The experimental krill showed a rapid response to the food conditions in their growth rates, either in a positive or negative direction, well within a single moult cycle.

The digestive enzyme activities in the digestive gland of field-caught adults decreased considerably during one week of starvation. The size of the gland decreased substantially both in length and weight, accompanied by a loss of lipid and protein, with the former being more readily utilised. In a laboratory experiment where juvenile krill were alternately fed and starved, the digestive enzyme activities changed in response to the food regime. These changes largely mirrored the mass gain and loss of the digestive glands. The gland size-specific activities of digestive enzymes showed no consistent trends even after a long period of starvation. When the food supply was resumed, the gland regained its mass and enzyme activities. The digestive gland appears to serve as a reserve, which can provide against a few days' starvation and be rebuilt relatively quickly. Its size showed a prompt and steady response to short-term changes in feeding regime, proving a reliable indicator of recent feeding activities.

By tracking individuals over time and examining specimens sampled as groups, it was demonstrated that fed and starved krill are distinguishable by the relationship between the eye diameter and body length. The eye diameter of starved krill did not decrease even when the animals were shrinking in overall body length. The eye diameter of well-fed krill continued to increase as overall length increased. This created a distinction between fed and starved krill while no simultaneous separation was detected in terms of the body length to weight relationship. It would take approximately 2 moult cycles of shrinkage or more at modest rates for the eye diameter to body length relationship to significantly change. Whether this feature is manifested in the wild would be best seen at the end of winter, after the most likely period of extended food limitation.

Nucleic acid content has only limited predictive power as an estimator of growth rates. Growth rates measured by the 'instantaneous growth rate' technique are still the best representation of *in situ* growth, which is determined by the condition during the period since the last moult. The size of the digestive gland of krill, a crucial short-term storage organ, was more responsive to food condition than enzyme activities. The gland size is a result of feeding activities over the past few weeks and will not be affected by immediate past events such as cod-end feeding. The digestive

gland size should, at least, be a simple measure of whether krill have recently undergone severe, sustained food shortage. Long-term, seasonal starvation and the shrinkage it caused over a few moult cycles can be seen in the body length to eye diameter relationship more obviously than the traditional body length to weight relationship. This suite of measurements will provide a matrix of methods to determine the 'condition' of krill, in time scales from a week to a few months. These techniques are now ready for repeated measurements in the field over wider temporal and spatial extent to examine their applicability and to contribute to unravelling the outstanding questions in krill biology.

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Chapter 1. General introduction

Antarctic krill (*Euphausia superba*, hereafter called krill), with an estimated biomass of approximately 500 million tonnes and with its ecological role as a major trophic link between primary production and vertebrate predators, is unquestionably the keystone species in the Antarctic marine ecosystem. The huge biomass together with the key position it occupies in the food web also means that krill plays a significant role in biogeochemical cycles (Nicol 1994). Further, Antarctic krill is the target of one of the world's largest crustacean fisheries (Nicol and Endo 1997).

These features of krill have justified a long research history, which has led to answers to many basic biological questions. Early investigations including the 'Discovery' Expedition provided much knowledge about the distribution of krill throughout its life cycle and in relation to its physical environment (Marr 1962, Mackintosh 1972, 1973). It became known early on that krill forms swarms and that its distribution is highly patchy. Its distribution and spawning grounds were identified and the seasonal association of krill with sea-ice was also noted (Mackintosh 1972, 1973). These early studies resulted in a life history model which saw krill as a short-lived, strictly herbivorous, pelagic species (Fraser 1936, Bargman 1945, Mackintosh 1972). However, subsequent field surveys and laboratory experiments have revealed the exceptional capabilities of krill to adapt to its highly seasonal and annually fluctuating environment (see review by Quetin and Ross 1991, Quetin et al. 1994). Its life span is now believed to be longer than 5 years (Ikeda and Thomas 1987b, Siegel 1987). It has been demonstrated that krill can use a range of habitats and a variety of food sources from microalgae to copepods (Kawaguchi et al. 1986, Price et al. 1988, Stretch et al. 1988) and is capable of high energy throughput under favourable conditions (Quetin et al. 1994).

Krill life history; a summary of key aspects

Longevity and growth

The depiction of krill as a short-lived species came from the simple application of length frequency analysis (Bargmann 1945, Mackintosh 1972). It has been

recognised that this approach is subject to possible errors because it is difficult to sample the same population over time (Mauchline 1980). It has also been shown that krill can shrink during periods of food scarcity (Ikeda and Dixon 1982), and this may seriously affect body length-based ageing methods (Ettershank 1983, 1984). Ikeda and Dixon's (1982) starvation experiment and their demonstration of shrinkage not only suggested a new overwintering strategy but changed the perception of krill from a short-lived species with a life cycle of only 2-3 years to a species with a longevity of several years. Later re-analysis and interpretation of length data, taking into account the seaonality of growth, presented a 5 to 6 year life span (Rosenberg et al. 1986, Siegel 1987). Yet there is still no means of reliably determining the age of krill. There have been attempts to develop ageing techniques for krill based on fluorescent age pigments that do not rely on length frequency analysis (Ettershank 1983, 1984, 1985, Nicol et al. 1991). Although Ettershank (1983, 1985) estimated a 5 year life span, similar to the results by more recent length frequency analysis, many technical and fundamental difficulties have been encountered, which have precluded its systematic use (Nicol 1987). Considerable progress has been made in the development of age pigment techniques for other crustacean species (Sheehy et al. 1995, 1996, Ju et al. 1999), however, these are yet to be applied to krill.

Documenting the growth of krill is of importance to understanding both its energetics and population dynamics. The marked seasonality of growth rates is probably the most difficult issue, and determination of this depends on the accurate measurement of growth itself. Growth rates of krill have often been calculated from length frequency analysis. Although this approach can generate reasonable values, a lingering potential problem is that there is no guarantee the same population is being sampled over time. Rearing krill under controlled laboratory conditions has provided valuable information (Ikeda and Thomas 1987b), however it is difficult to achieve natural conditions in the laboratory and to maintain them for a long period of time. An alternative approach, called the 'instantaneous growth rate' (IGR) technique, to determine individual growth rates from fresh krill in the field has been developed and representative values of growth rates are now in the literature (Quetin and Ross 1991, Nicol et al. 1992a, 2000, Ross et al. 2000). Using the IGR technique, krill can be observed growing during summer, often at rates above 5% increase in length per

moult (Nicol et al. 1992a) and these rates appear to be in reasonable agreement with the values obtained by other methods. However, there is little agreement on winter growth of krill. Daly (1990) reported that during winter, juvenile krill can grow at rates comparable to those in summer, at least when supported by sea-ice biota. Huntley et al. (1994) also reported apparent growth in winter. Quetin and Ross (1991), on the other hand, observed negative growth of adult krill from waters west of the Antarctic Peninsula along with negligible ingestion and faeces production. But positive growth of larval krill was observed under the ice (Ross and Quetin 1991). There is no reason why these results cannot all be correct, and this points to the need to have a data set of growth rates collected over a wide area and throughout the year.

Feeding

The image of krill as a strict pelagic herbivore has also changed. The complex feeding basket of krill allows them to ingest a wide size range of particles (Suh and Nemoto 1987) and its powerful enzyme system enables krill to digest food from various origins, including other animals (Mayzaud et al. 1985, McConville et al. 1986). Krill also exhibit a variety of feeding behaviours from filtration to raptorial feeding and are capable of scraping ice-algae off from the bottom of sea-ice (Hamner et al. 1983, Price et al. 1988, Stretch et al. 1988). It has now been demonstrated, from laboratory experiments and field studies, that krill can, and do utilise a variety of food sources, detritus, sea-ice biota and copepods, apart from usually favoured large-sized diatoms (Kawaguchi et al. 1986, Marchant and Nash 1986, Price et al. 1988, Stretch et al. 1988, Huntley et al. 1994, Atkinson and Snÿder 1997). Recently, it has been shown, by comparing the gut pigment level corrected for degradation, with faeces production or total organic carbon from the gut, that there is likely to be a substantial contribution from heterotrophic carbon to meet the energetic demand of krill (Pakhomov et al. 1997, Perissinotto et al. 2000). Additionally, filtration rates that were estimated to be too low to meet the carbon requirement of krill (Ikeda and Bruce 1986) have been updated from more refined experiments (Price et al. 1988) and from field egestion rates (Clarke et al. 1988). Hence it is accepted that krill can filter in the order of litres per hour, not millilitres per hour as previously thought. Although krill is essentially a herbivore, it is now well established that krill not only

has the capability of assimilating a variety of food sources but exercise that ability, with a high capacity, depending on its need and the circumstances.

Reproduction and recruitment

The reproductive strategies of the krill population have also been subject to revision. There have been arguments that recruitment of krill is not necessarily sourced from only one spawning in its peak but can come from multiple events during summer (Ross and Quetin 1983, 1986, Cuzin-Roudy 1987). The timing of onset of spawning may determine whether and how frequently multiple spawnings can occur and this is related to food conditions. However, Nicol et al. (1995) estimated the energy loss of females in spawning and calculated that multiple spawnings would only be possible, if there was an exceptionally high food concentration and at the upper end of the large filtration rates suggested. Recruitment of krill is determined not only by spawning success but also by larval survival during winter, which appears enhanced by well-developed winter sea-ice (Kawaguchi and Satake 1994, Loeb et al. 1997). The link between the sea-ice extent and krill recruitment is thought to be as a result of better feeding, particularly on sea-ice biota, although this link is yet to be directly shown.

Over-winter biology of krill

The habitat of krill extends from the most inward border of ice cover during summer to the maximum outward extent of ice cover during winter; an exception is the northern population in the South Atlantic which lies outside this zone. The apparent association of krill distribution with seasonal sea-ice, although described from early observations, was not necessarily thought of as a close connection until the 1980s, when the advent of scientific ice-breakers led to numerous publications which reported krill living and feeding under sea-ice (O'Brien 1987, Marschall 1988, Hamner et al. 1989). Quetin et al. (1996) showed habitat segregation in adult (water column) and larval (under-ice) krill in the winter with a combination of diving and net observations. The differentiation between the adults and the larvae is important and often glossed over. Nevertheless, the results from studies under-ice during summer, spring and even winter indicated that krill of varying sizes have the ability to make use of the sea-ice habitat (Stretch et al. 1988, Daly 1990, 1998, Lancraft et

al. 1991). It has been suggested that sea-ice provides food and also protection from predators (Daly 1990, Daly and Macaulay 1991). Smetaceck et al. (1990) provided a conceptual framework which indicated that winter sea-ice is an essential habitat for krill and provides an important nursery and protection ground for krill during the food-scarce period. Siegel and Loeb (1995) and Loeb et al. (1997) further maintained that the timing and extent of sea-ice cover during winter determine the recruitment and abundance of krill for the following seasons.

Although there are still limited observations of krill during winter, the following avenues for winter survival of krill have been proposed; combustion of its own body mass leading to shrinkage, use of stored lipid reserve, lowered metabolism and reliance on a season-specific diet of ice algae, copepods and detritus (see review by Quetin and Ross 1991). However, there have been different views regarding the general well-being of the species during the most unfavourable period of the year. Quetin and Ross (1991) reported shrinking krill in winter and assessed the relative importance of each proposed mechanism. They concluded from their data that the three-fold decrease in metabolic rates is probably most important, with some contribution from lipid utilisation and shrinkage and minor gain from carnivory. Conversely, Huntley et al. (1994) reported that krill were metabolically at a normal level, deriving the energy from feeding on copepods. Both of these studies are from direct observations and it is thus apparent that krill can utilise a variety of options to overwinter. It seems clear that krill are equipped with various means to cope with food scarcity during autumn and winter. Krill are likely to exploit any type of food resource in an opportunistic manner as it becomes available. While shrinkage is certainly one of the options krill can utilise, how often it occurs in the wild has yet to be determined. There is now direct evidence of all of these strategies occurring at different places and times. To examine which strategy is followed under what situations is the next step in understanding the winter biology of krill.

The question of the whereabouts of krill during winter also remains largely unanswered. Whether winter is particularly stressful or not, it is still a critical season when krill of all life cycle stages have to deal with a number of severe challenges but it also remains the least known season. Understanding the overwinter biology of krill

will assist us in learning about winter distribution of the species, together with development of the quantitative methods to record under-ice abundance of krill.

Outstanding topics in krill biology; the linkages between the changing environment and the species

The key biological features of krill during summer when they are probably most active and also best studied, are now fairly well known. Its capacities in major functions, including filtration rates, fecundity, respiration and excretion rates have been investigated and revised. Nevertheless, how these features vary as seasons progress and environments change, and how these change interannually are poorly known. Understanding the linkage between the species and the environment remains as a major task in krill biology.

There remains a list of critical questions to be answered. What key factors affect the reproduction of krill between seasons? What are the geographical variations in krill distribution in relation to the between- and within- season variability in the physical environment? What effects do the extent and nature of winter ice have on the overwinter survival of krill and their recruitment to adult populations and how does this mechanism operate? Do the seasonal and geographical differences in food availability lead to different overwintering strategies?

Direct and routine measurements of many of the physical variables of the krill habitat are now possible. Together with monitoring the change in physical regimes, the response of krill populations to the shifts in physical environment needs to be studied. The data representative of key activities of krill, particularly during non-summer seasons will need to be collected and analysed in relation to the major physical features. There will need to be standardised indicators to describe the activities and the resultant 'condition' of krill.

Many of the hypotheses put forward regarding overwinter strategy or sea-ice use by krill, can only be tested by examining appropriate specimens from the field. To fill the gap in our knowledge of krill biology in various settings, more data needs to be

collected over wider spatial and temporal extent. To have a data set of krill's physiological condition and growth against a gradient of environmental conditions, geographic locations and seasons is of paramount importance.

Studies into condition of krill, to date

There have been surprisingly few attempts to apply condition indicators for the purpose of regional and seasonal comparison of krill populations. Classic biometric indices such as body length to weight relationship have been used a number of times. These should be able to indicate whether krill at a particular stage are either over- or under-weight for the size. Kawaguchi et al. (1986) reported a change in the relationship between the carapace length and dry weight in overwintering krill. Siegel (1989) indicated there was a marked seasonal trend for the exponent as well as for the absolute weight; the weight of both sexes was lowest in June. Quetin and Ross (1991) also reported the decrease in proportional volume accompanying the shrinkage in body length during winter. Huntley et al. (1994), however, cited an apparently similar body length to weight relationship between two seasons to support their concept of copepod-feeding, well-growing winter krill. Nicol et al. (2000) also reported changes in weight per unit length with the progress of the summer season. More complex multiple morphometrics have also been used occasionally but have not been generally adopted (Farber-Lorda 1990, 1991, 1994)

The *in situ* growth rate of individuals could be the best reflection of the condition of the animals, particularly for non-reproductive animals. Buchholz (1991) described a protocol for this type of measurement - using differences in size between moults and recently moulted krill, with some data from laboratory experiment. Quetin and Ross (1991) provided field data obtained from fresh animals of different seasons and called this the 'instantaneous growth rate' (IGR) technique. Nicol et al. (1992a) further suggested that instantaneous growth rates coupled with time to onset of shrinkage give some indication of the nutritional condition of the krill at any one time.

Ikeda (1989) measured RNA concentrations of krill and attempted to derive natural growth rates of krill by applying equations obtained from other zooplankton species. However, there was no calibration conducted with the animals of known growth rates. Although not intended as condition indicators, other biochemical indices have also been used. Torres et al. (1994) compared proximate composition of krill between different seasons and inferred the overwinter strategies from there. Virtue et al. (1993) investigated the changes in lipid content and composition of the digestive gland of starved krill and reported a substantial loss of lipid and a change of lipid profiles with starvation.

Feeding related indicators such as stomach content and fullness, and digestive gland colour have also been used. Antezana and Ray (1984) described differing food conditions between the areas, based on stomach colour and fullness. Endo and Kadoya (1991) showed that the vividness and darkness of the colour of the digestive gland declined in starving krill.

Ross and Quetin (1991) used a group of 3 measurements to describe the nutritional history of larval krill; instantaneous growth rates, condition factor in the form of carbon amount per volume, and lipid content. Their measurements were used to examine the condition of larval krill between two winters with different ice cover. Such suites of measurements may provide a composite condition measure over a wide temporal scale.

Considerations for the present study

There has been a relatively small number of attempts to apply condition indicators to krill, in spite of the clear need to make use of them. In this study, growth and feeding were considered as variables reflected by condition indices. Growth is an ultimate expression of the animal's condition as it is dependent on the residual energy once other key metabolic requirements have been met. Growth can, therefore, be an indication of how much energy a krill has gained beyond its basic needs. So far, the only reliable way to obtain field growth data at an individual level is the IGR technique, which involves laborious experiments requiring a large number of

animals. If an alternative estimator of growth rate can be established which dispenses with on-board incubations but requires collection of specimens which can be processed later in the laboratory, a more extensive data set of growth rates can be built. This can, then, be examined against a number of environmental factors. Feeding is the process that gains energy for maintenance, growth and reproduction and therefore will essentially determine the physiological condition of an individual. Although the need for such indices to indicate how actively energy has been gathered is obvious, no standard means have been in wide use for krill. How actively energy has been gained and how much is left beyond basic needs are the appropriate processes to observe in order to understand the effect of the environmental changes on the species.

It is also considered desirable to build up a database of 'condition' drawn from a large number of net catches. Experimental approaches under different conditions can provide valuable data but it is often difficult to repeat these experiments with the frequency necessary because they are usually highly labour-intensive. Development of techniques suitable for on-board use was another consideration in the experiments reported here. For instance, ingestion rate measurements, when properly performed, may be a measure of *in situ* feeding. But one set of measurements may involve a series of separate analyses and hence it will be difficult to routinely apply to a large number of net samples. A protocol which does not involve delicate experiments on live animals and which can extract data from net-collected samples or from later analysis of preserved specimens was the aim in this series of studies.

As an alternative estimator of individual growth rates, RNA levels of krill tissue were determined against the independently measured growth rates. The use of RNA:DNA ratio as an estimator of growth rates rests on the premise that the ratio of RNA, the molecule for protein synthesis to DNA, a measure of cell mass, should represent the intensity of cell growth and this index has been applied with some success, particularly for larval fish (Buckley 1984, Bulow 1987).

The role of the digestive gland as a major nutrition organ has been shown in a number of crustacean species and the digestive gland of krill is likely to respond

sensitively to food regimes. Digestive enzyme activities have been examined as an indicator of feeding and sometimes the make-up of diets in some copepod species (Hassett and Landry 1983, 1986, 1990a, b, Head et al. 1984, Harris et al. 1986). Changes in the size, lipid and protein content, and enzyme activities in the digestive gland were investigated in changing feeding regimes in a quest for indicators of recent feeding activity and possibly the type of food ingested.

Sustained food shortage during winter may force krill to adopt the strategy of shrinkage for survival, however, the extent of its occurrence has not been demonstrated from field samples. Although Sun (1997) offered a method utilising the relationship between the eye diameter and body size, its applicability is yet to be established. To develop an indicator to determine to what extent long-term, sustained starvation and subsequent shrinkage occur in the wild, the eye diameter to body length relationship was examined with a laboratory population of krill kept for an extended period in changing food regimes.

The lack of opportunities to study krill from various locations and throughout the year has been largely responsible for the gap in our current understanding of the species. Major opportunities for wider seasonal and areal coverage are likely to be forthcoming from research voyages (Anon 1999) and from fisheries. Standardised condition indicators, which can be applied on board ship or on samples brought to the laboratory, are required and these would allow regional and seasonal comparison of the 'condition' of krill. This study focused on developing a suite of measurements to assess the condition of krill with regard to nutritional condition, growth rates or growth potential, and to detect shrinkage caused by long-term sustained starvation. A series of experiments was conducted on board ship and in the laboratory with selected methods that have been applied to other species and which can also be applied with relative ease to determine the 'condition' of krill.

Chapter 2. Nucleic acid content as a potential growth rate estimator of Antarctic krill; results from field-caught krill from the Indian Sector of the Southern Ocean

2.1 Abstract

Nucleic acid contents of tissue were determined from field-caught Antarctic krill to determine whether they could be used as an alternative estimator of individual growth rates which can currently only be obtained by labour intensive on-board incubations. Krill from contrasting growth regimes from early and late summer exhibited differences in RNA-based indices. There was a significant correlation between the independently measured individual growth rates and the RNA:DNA ratio and also the RNA concentration of krill tissue, although the strength of the relationship was only modest. DNA concentration, on average, was relatively constant, irrespective of the growth rates. The moult stage did not appear to have a significant effect on the nucleic acid contents of tissue. Overall, the amount of both nucleic acids varied considerably between individuals. Nucleic acid-based indicators may provide information concerning the recent growth and nutritional status of krill and further experimentation under controlled conditions is warranted. They are, however, reasonably costly and time-consuming measurements.

2.2 Introduction

Antarctic krill is the central species in the Antarctic marine ecosystem and serves as a key link transferring energy from lower trophic levels to many vertebrate predators. Growth of krill is a key parameter for understanding its biology and for managing the krill fishery. Growth rates are also a response of the species to the prevailing environmental conditions. Although a considerable amount of data about krill growth has been gathered both from field studies and laboratory experiments, its variation along geographical and seasonal gradients remains to be systematically studied in relation to environmental changes (Quetin et al. 1994). Growth rates measured from field-collected animals in varying conditions are required to answer many of the outstanding questions of krill biology.

The traditional method of determining growth rates is to observe the size changes in sequential samplings of a single population. However, krill is not a discrete population enclosed in a limited area and there are always uncertainties as to whether the samples have been taken from the same population over time. In addition, shrinkage, a possible strategy of krill to cope with food shortage, can further confuse size frequency distributions (Ikeda and Dixon 1982, Ettershank 1983, 1984). In an alternative method, the 'instantaneous growth rate'(IGR) technique, size increment per moult in terms of uropod length is observed from live animals and individual growth rates can be reliably measured (Quetin and Ross 1991). This protocol of comparing the length of uropods from consecutive moults had been used for growth rate measurement of krill maintained for longer than one moult cycle in the laboratory (Ikeda and Dixon 1982, Poleck and Denys 1982, Buchholz 1985, 1991). The IGR approach, however, is novel in that the field-caught animals are kept for a short period of time and the measurements of the krill that moult for the first few days are obtained by sacrificing them. Despite the significant contributions brought by this method (Quetin and Ross 1991, Ross and Quetin 1991, Nicol et al. 1992a, Nicol et al. 2000, Ross et al. 2000), the on-board incubations are laborious and also require a large number of live animals to obtain a reasonable sample size. This is because, at 0 °C, only one animal in 20 to 30 will moult per day, and because only the first few days of the experiment are used to obtain growth rates.

Measurement of growth rates and assessment of nutritional condition are of critical importance to most ecologists in all realms. If growth rates can be reliably estimated from certain characteristics of field-caught specimens without conducting incubations, it will result in a more extensive assessment of recent growth against various biotic and abiotic factors. To this end, RNA:DNA ratio and the RNA concentration of somatic tissue have often been pursued as growth rate estimators or condition indicators for marine organisms, notably for larval fish (Buckley 1984, Clemmesen 1994, Folkvord et al. 1996). This approach rests on the following premise. The amount of RNA, i.e. the molecule primarily responsible for the synthesis of protein, should reflect, and vary with the rate of protein synthesis, which is closely linked to growth. On the other hand, the amount of DNA, the carrier of genetic information, is thought to be nearly constant in somatic tissues and thus its concentration should reflect cell numbers or mass. Hence the ratio of RNA to DNA should serve as an index of the cell or the individual's rate of growth. Numerous attempts have been made to use this relationship as a growth rate estimator in a range of organisms from bacteria to mammals. Some of these applications toward marine organisms have produced encouraging results (Clemmesen 1994, Moss 1994a, b, Saiz et al. 1998). However, a number of factors have also been noted which confound the relationship between these indices and the target variables. These include the effect of temperature, developmental stages and age, the species-specific nature of this relationship and diel rhythms (Dagg and Littlepage 1972, Ota and Landry 1984, Chicaro et al. 1998). As krill is a large, conspicuous species which is easily separable from net samples, not only is an approach at a species level simple but the control of the effect of size and sex of the animals is also feasible. More importantly, the individual growth rates of the krill can be obtained, through an independent means, the IGR technique, from the same animals subject to the nucleic acid measurement. Thus it is possible to determine how precisely these indices predict the growth rates at an individual level.

Ikeda (1989) sought to use the amount of RNA in krill tissue to estimate the growth rates. However, there was no calibration using animals of known growth rates and an equation obtained from other species was applied to derive the growth rates of krill.

There have been no further published attempts to use nucleic acid-based indicators to estimate growth rates of krill. Krill being a crustacean, has some inherent attributes other than growth rates, such as the continuous moult cycle, that may have a significant effect on the tissue contents of nucleic acids. Moulting involves considerable tissue build-up and resorption that may not be linked to overall somatic growth. This has the potential to disturb the relationship between nucleic acid levels and growth, making the two appear decoupled. To examine the applicability of nucleic acid-based indicators as estimators of growth rates, this study aimed to determine if the moult stage had a significant effect on the nucleic acid contents and whether there is a significant relationship between growth rates and nucleic acid contents. Measurements were made on nucleic acid levels from field-collected krill which had been moult-staged and also animals with known growth rates from high growth regimes and low growth regimes from early and late summer, respectively.

2.3 Materials and methods

Experimental animals

Moult-staged krill

The animals were collected at 2 stations in East Antarctica (66°00'02" S, 69°00'40" E, 66°30'69" S, 69°30'39" E) on 11 and 13 February 1991 with an RMT 8 net. Adult males were selected and sorted by their moult stages, D_{3-4}, A, B-C, D_0, D_1, D_2, according to Nicol and Stolp's (1990) scheme. These animals were then examined for nucleic acid content.

Krill with known growth rates

A large number of krill were collected from January to March 1996 at a number of stations between 80 °E and 150 °E in East Antarctica with an RMT 8 net. Healthy looking krill were chosen and randomly placed in 250 ml clear plastic jars continually receiving fresh seawater. The jars were maintained at 0 °C in the dark and inspected once a day. When the krill had moulted, the animal was sacrificed and the exuviae were collected. The lengths of uropods of the animal and those of the moult were compared. Increments in length of uropods between moults were used to determine 'instantaneous growth rates' (IGRs) of individuals. These krill were then examined for nucleic acid content.

Measurements of nucleic acid contents

The analysis protocol for the measurement of nucleic acid contents was modified from Canino and Caldarone (1995). Approximately 20 mg of krill abdominal muscle was excised and weighed, and then placed into a 1.5 ml microcentrifuge tube. Forty µl of 1% sarcosine TNE buffer (Tris 10 mM, EDTA 1 mM, NaCl 100 mM) was added to the tube and the tissue was ground with a plastic pestle for 1 min. Three hundred and sixty µl of 1% sarcosine TNE buffer was then added and the tubes were left for 2 h on a shaker. The samples were diluted by adding 600 µl of TNE buffer and centrifuged for 10 min at 10 000 g. Five hundred µl of the supernatant was taken and further diluted by adding 500 µl of TNE buffer. The tubes were centrifuged

again for 10 min. Hence the original extraction mixture was diluted 5 times, resulting in a sarcosine concentration of 0.2 %. One hundred µl of the sample was mixed with 2.9 ml of ethidium bromide (EB) solution (100 µg ml^{-1}) for determination of total nucleic acids. Another 100 µl of the sample was mixed with 2.9 ml of DNA specific dye, BisBenzimide (BB) solution (10 ng ml^{-1}). Fluorescence was read at 525 nm excitation and 600 nm emission for EB and at 356 nm excitation and 458 nm emission for BB. Concentrations of nucleic acids were determined by running standard curves with known concentrations of calf thymus DNA and yeast RNA.

2.4 Results

The effect of moult stages on nucleic acid levels

Between 3 to 6 animals were chosen from each moult stage group. The body size of the experimental animals, as measured by carapace length (standard measurement 4 as in Mauchline 1981), was in the range of 13.18 to 16.82 mm and did not differ significantly between the six moult stage groups (ANOVA, F=1.044, P=0.417). There was no predictable pattern with the nucleic acid-based indices, RNA:DNA, RNA per unit weight and DNA per unit weight, with regard to the moult cycle (Fig. 2.1). All three indices varied considerably between individuals. The RNA:DNA ratio was most variable between individuals with coefficient of variations (CVs) ranging from 0.32 to 0.76. Although the RNA:DNA ratio appeared elevated at stage A, there was no significant difference between the moult stages (Fig. 2.1 b, ANOVA, F=0.5428, P=0.7418). Neither did the amount of RNA per unit weight of fresh muscle differ significantly between the moult stages (Fig. 2.1 c, ANOVA, F=0.3425, P=0.8815). This was the same with DNA per unit weight, which showed no significant trends with the moult cycle (Fig. 2.1 d, ANOVA, F=1.3457, P=0.2508).

Growth rates and the tissue concentrations of nucleic acids

Twelve animals from each of the 4 IGR experiments, Exp 1, 3, 19 and 20, were selected and analysed. Exp 1 krill were caught on 30 January and Exp 3 animals on 4 February and these two represented the high growth group from the early part of the season. Exp 19 krill were collected on 14 March and Exp 20 animals on 16 March and they served as a low growth group of krill from late summer. The low growth group from the late season was represented mostly by females. Exp 19 animals in particular, were all spent females except for one male.

The body size of the experimental animals was measured by uropod length only. The animals of Exp 1 and 3 were slightly smaller by the mean uropod length than in Exp 19 and 20 and the differences between the groups were significant (ANOVA, df=47, F=7.573, $p=3.8\times10^{-4}$). A subsequent Tukey test, however, showed that the krill in

Exp 3, 19, and 20 did not differ significantly and also that there was no significant difference between the size of animals in Exp 1 and 3 (Table 2.1). The instantaneous growth rates of the 4 sets differed significantly (ANOVA, df=47, F=8.346, $p=1.7x10^{-4}$) and a post-hoc test demonstrated that the means of growth rates in Exp1, 3 and 20 were significantly higher than in Exp 19. The mean growth rates of Exp 1 and 3 were also higher than those of Exp 20, although the differences were not significant.

An ANOVA showed that the means of the 4 groups of krill were significantly different in RNA:DNA and RNA concentration (expressed as amount of RNA per unit weight of fresh muscle) (RNA:DNA; df=47, F=9.404, $p=6.4x10^{-5}$, RNA per wt; df=47, F=14.119, $p=1.4x10^{-6}$). A Tukey test revealed that the RNA:DNA ratio did not differ within each growth regime, high and low. The animals in Exp 1 and 3 exhibited higher mean RNA:DNA ratios than in Exp 19 and 20, however, the difference between Exp 3 and 20 was not significant (Fig. 2.2, Table 2.1). In terms of RNA per unit weight, there was a separation between the groups from the early and later part of the seasons. The animals from Exp 1 and 3 had significantly higher RNA concentration than in Exp 19 and 20. There was no significant difference within the growth regimes (Table 2.1). DNA concentration (expressed as amount of DNA per unit weight of fresh muscle) did not differ significantly between the experimental groups (df=47, F=1.568, p=0.211).

Plotting the individual nucleic acid indices against the individual growth rates determined by IGR measurement, there was an obvious trend of RNA content increasing with rising growth rates (Fig. 2.3 a, b). The relationships between the growth rates and both RNA-based indices were highly significant although the strength of the relationship was only modest (Table 2.2). The growth rates explained approximately 30% of the variation in RNA:DNA ratio. The tissue concentration of RNA correlated better with growth rates than the RNA:DNA ratio ($r^2=0.474$). As expected from the comparison between the growth groups, the correlation between the growth rates and DNA concentration was weak ($r^2=0.139$), notwithstanding the significance found.

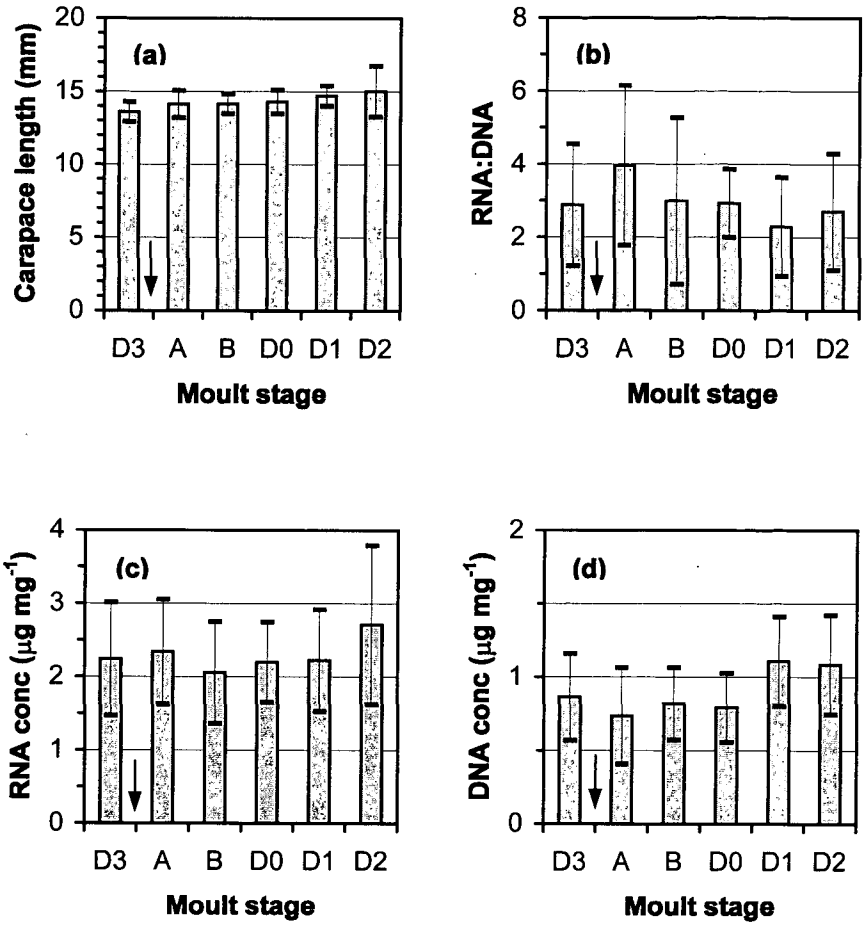


Fig. 2.1 The body size and nucleic acid levels in tissue of the krill by moult stages. (a) Carapace length, (b) RNA:DNA, (c) tissue concentration of RNA (µg RNA mg^{-1} fresh muscle) and (d) tissue concentration of DNA (µg DNA mg^{-1} fresh muscle). Error bars represent one standard deviation. Arrows indicate ecdysis

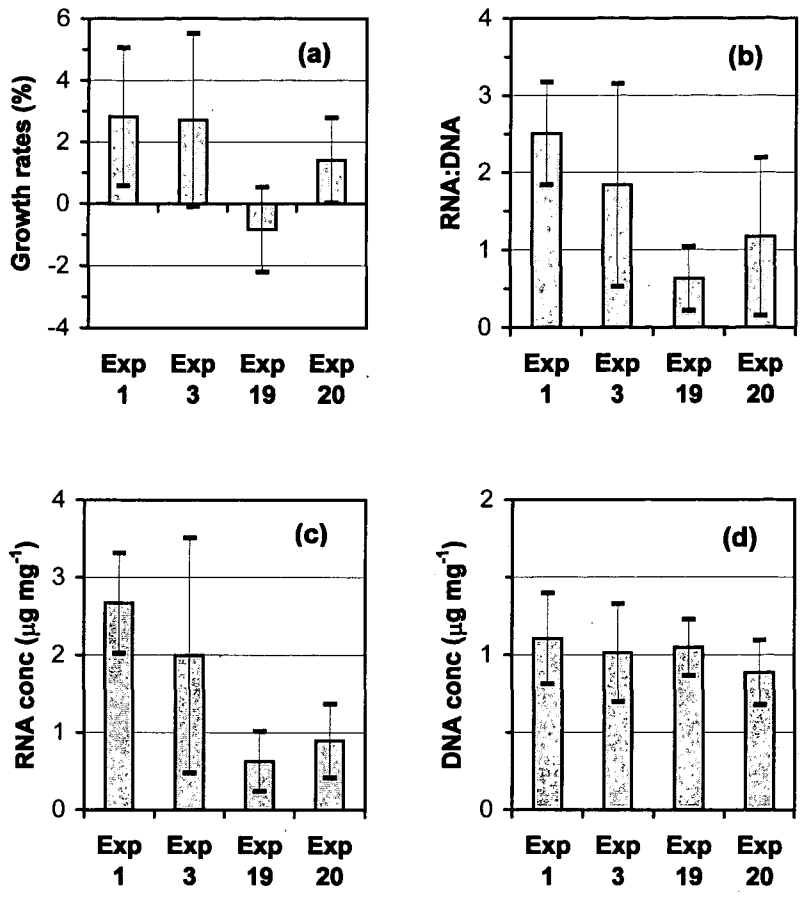


Fig. 2.2 Growth rates and nucleic acid levels in tissue of the krill from the IGR experimental groups. (a) Instantaneous growth rates, (b) RNA:DNA, (c) tissue concentration of RNA (µg RNA mg^{-1} of fresh muscle) and (d) tissue concentration of DNA (µg DNA mg^{-1} of fresh muscle). Error bars represent one standard deviation

Table 2.1 Matrix of pairwise comparison probabilities from multiple comparisons of the 'instantaneous growth rate' (IGR) experimental groups by body size, growth rate and nucleic acid-based indices. When connected by a line, there is no significant difference between these groups

Uropod length (mm)				
IGR groups	1	3	19	20
1	1.000			
3	.209	1.000		
19	.002	.208	1.000	
20	.001	.142	1.000	1.000
	1	3	20	19

Instantaenous growth rates (%)				
IGR groups	1	3	19	20
1	1.000			
3	.999	1.000		
19	.001	.001	1.000	
20	.337	.410	0.047	1.000
	1	3	20	19

RNA:DNA				
IGR groups	1	3	19	20
1	1.000			
3	.297	1.000		
19	.000	.013	1.000	
20	.005	.297	0.481	1.000
	1	3	20	19

RNA concentration (μg mg^{-1} fresh muscle)				
IGR groups	1	3	19	20
1	1.000			
3	.251	1.000		
19	.000	.002	1.000	
20	.000	.019	0.882	1.000
	1	3	20	19

DNA concentration (μg mg^{-1} fresh muscle)				
IGR groups	1	3	19	20
1	1.000			
3	.818	1.000		
19	.948	.987	1.000	
20	.173	.621	0.418	1.000
	1	3	20	19

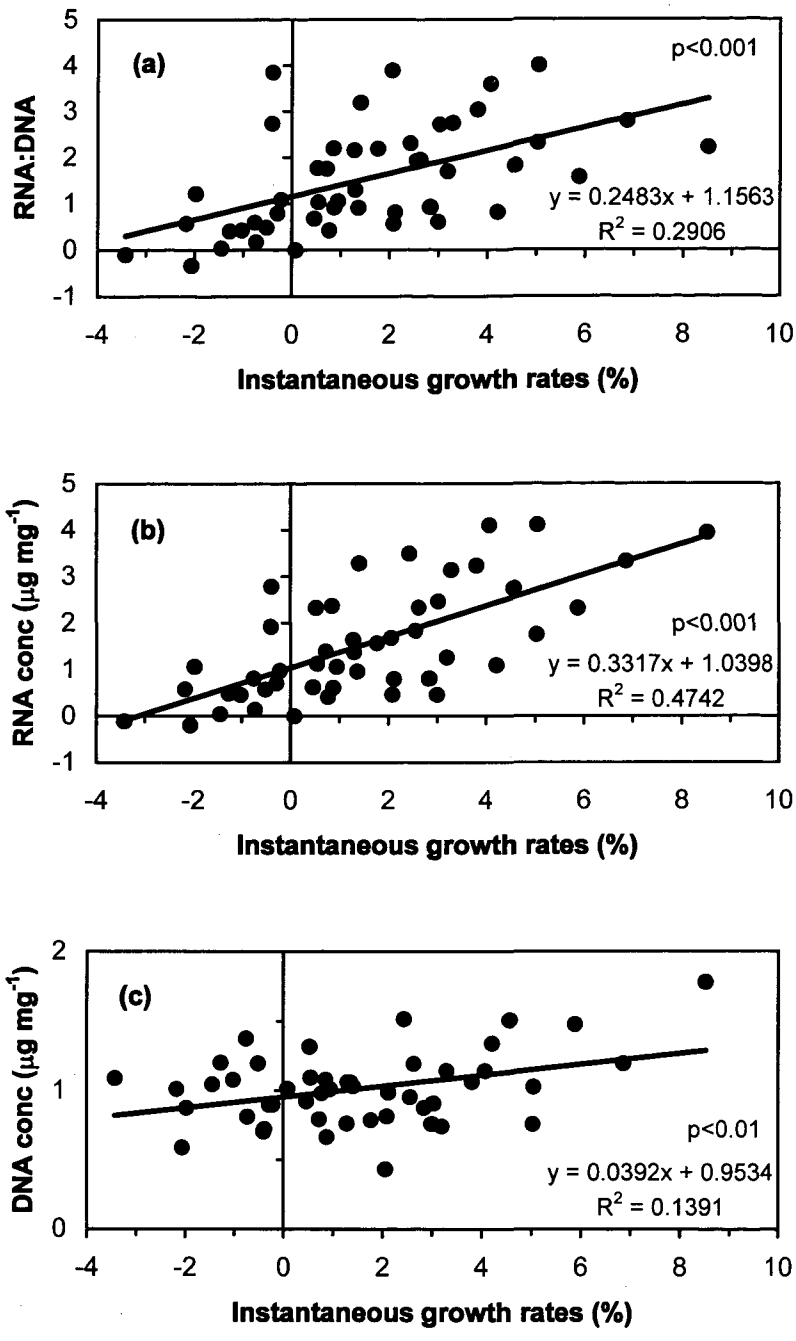


Fig. 2.3 Regressions of instantaneous growth rates (IGR) versus nucleic acid-based indices. (a) IGR vs RNA:DNA, (b) IGR vs RNA concentration, (c) IGR vs DNA concentration

Table 2.2 Regression statistics of the instantaneous growth rate (IGR) versus nucleic acid-based indices

	n	slope	r^2	significance
IGR vs RNA:DNA	48	0.248	0.2906	7.72×10^{-5}
IGR vs RNA concentrati	48	0.331	0.4742	6.29×10^{-8}
IGR vs DNA concentrati	48	0.039	0.1391	9.03×10^{-3}

2.5 Discussion

Nucleic acid contents of krill tissue varied considerably between specimens. The nucleic acid-based indices did not appear significantly affected by moult cycle of the animals. High growth krill and low growth krill from the early and later part of the season respectively, exhibited differences in RNA:DNA and RNA per unit weight of tissue. The relationships between the independently measured growth rates and the RNA-based indices were highly significant. The DNA content in tissue, on average, was relatively constant between the groups, despite the large degree of individual variation.

Juinio et al. (1992) reported significant effects of the moult cycle on nucleic acid contents in postlarval lobster. They indicated that the use of RNA:DNA ratios to estimate the relative nutritional state of postlarval lobsters must be qualified with respect to the period of the moult cycle and the temperature regime. On the other hand, Moss (1994a) found no significant effect of moult stages on nucleic acid contents in juvenile white shrimp. It is not clear if this disparity was due to the different stages in life history of the animals or inter-species differences. However, an association between the moult cycle and nucleic acid levels, which could complicate the relationship between growth and the nucleic acid contents, affecting the use of nucleic acid-based indices was not detected in this study on krill.

Ikeda (1989) determined the RNA content from whole krill, with a different procedure from that used in the present study and obtained a mean RNA content of 5 % of body protein. Assuming 75% water content and 40 % protein constituting dry weight, this would be equivalent to 5 μg RNA mg^{-1} fresh weight and is considerably higher than the RNA content measured in this study. This may not be contentious as his procedures for both extraction and quantification were different. However, he estimated a growth rate of 0.17-0.19 mm day^{-1} for krill larger than 30 mm body length, relying on equations obtained for other zooplankton species by Båmstedt and Skjodal (1980). This rate translates to well above 10 % growth per moult, probably the maximal individual growth rate obtainable from the field. Ikeda's conclusion was

that the species-specific RNA-growth rate relationship for krill needs to be established in the laboratory.

There existed a relationship between the growth of krill and the nucleic acid level. Unequivocally, actively growing individuals had more RNA in either DNA normalised, or weight-based terms, and shrinking animals had a smaller amount of RNA. RNA per unit weight was a better indicator than RNA:DNA ratio as far as the growth rate was concerned. This finding is not unique to Antarctic krill. Mathers et al. (1992) also reported that weight-based RNA concentrations were better correlated to the growth rates than were RNA:DNA ratios. The strength of the relationship demonstrated in this experiment, however, is probably insufficient to be a precise predictor of individual growth rates.

The usefulness of RNA-based indices to estimate growth rates in marine organisms has not always been convincing despite the solid theoretical underpinning. The constraints include the species-specific relationship, the effect of temperature and diel rhythms, and other factors (Ota and Landry 1984, Clarke et al. 1989, Chicaro et al. 1998). Selecting animals with known growth rates in this experiment, there could be no control exercised on the size, sex and maturity stage of the experimental animals, because this study relied on the animals that were caught in the net and moulted in the subsequent on-board incubations. The growth rate of krill will be a function of their size, nutritional status, and may well be affected by sex and the reproductive status. Theoretically, the amount of RNA necessary to synthesise proteins at a specific rate should be constant regardless of other factors. However this has not been empirically demonstrated. One of the low growth groups was represented by spent females late in the season which exhibited low growth rates. Presumably both low food availability and post-spawning conditions contributed to the low growth rates of these animals. It is not entirely clear whether the lower RNA-based indices were more due to the exhausted states of the animals or from the low intensity of growth.

The DNA concentration of krill was more or less constant irrespective of the moult stage and growth rates, although there was a large degree of individual variation. The

DNA levels were also similar in both the moult-staged sample and the sample with known growth rates. These two samples originated from different voyages in different years and locations. It appears that the tissue concentration of DNA in abdominal muscle represents cell number rather conservatively, at least when the animals come from a similar time of the year and are of similar developmental stage. Sex and maturity stages also seem to have little effect on the DNA content.

The length of the starvation period that the animals have been subjected to may affect the nucleic acid levels as it would affect their growth rates. However, once the krill are in an IGR experiment, they are kept in a food-limited state. As it was desired to relate the nucleic acid indicators to natural growth rates, only the animals that had moulted for the first few days were used. Therefore, the length of the starvation period was too short to be related to these indices, ranging from only 1 to 6 days.

Nucleic acid-based indices do give some indications of the growth rates of krill and the moult cycle does not seem to have a significant effect on the level of nucleic acids in tissue. However, the sex and maturity stages of the animals could not be controlled in this experiment. Although these indicators require reasonably costly and time-consuming measurements, they have the potential to provide information regarding recent growth of krill and their nutritional status. Further experimentation under controlled conditions is, therefore, warranted.

Addendum

Field growth rates of krill in this chapter were contributed by Robert King at the Australian Antarctic Division.

Chapter 3. Growth and nucleic acid contents of juvenile Antarctic krill in the laboratory

3.1 Abstract

Growth and nucleic acid levels in tissue were observed in a laboratory population of juvenile Antarctic krill under contrasting feeding regimes. Krill were either given excess food or were starved for 15 weeks, and then this feeding regime was reversed for the following 13 weeks. The krill that were growing at marginal rates at the start of the experiment responded to the excess food by active growth and to starvation by negative growth both in length and weight. The switch of food conditions was rapidly reflected in the individual growth rates and the growth trend was completely reversed in approximately 2 weeks, well within the duration of a single moult cycle. Growth in length was related to the growth in weight. Shrinkage in underfed juveniles, however, was less pronounced than in previously well-fed ones. The RNA: DNA ratio and tissue concentration of RNA were clearly different between the fed animals and the starved ones. There was a significant relationship between the RNA-based indices and the individual growth rates, although the predictability was modest. DNA per unit weight tended to be higher in starved, shrinking individuals, which implied cell shrinkage rather than loss of cells. Overall, there was a large degree of individual variation in nucleic acid-based indices. The RNA:DNA ratio, although not as precise as the 'instantaneous growth rate', may serve as an indicator of recent growth and nutritional condition of krill at the population level, and the tissue concentration of DNA may provide information regarding shrinkage of krill caused by starvation.

3.2 Introduction

The plasticity of growth in Antarctic krill (*Euphausia superba*, hereafter krill) is a key advantage to its success in the Antarctic marine environment, which is characterised by highly pulsed food supply and marked interannual variability. Krill are capable of rapid growth in the abundance of food (Buchholz 1991) and can even shrink, while dealing with extreme food shortage (Ikeda and Dixon 1982, Quetin and Ross 1991, Nicol et al. 1992a). Hence variable growth of krill, in itself is a response to a changing environment. Although there is now much information available on krill growth, the strong seasonality and geographical variation of the growth rates confound generalities. For this reason, there is a need to collect data over wider temporal and spatial scales.

The measurement of growth rates by the traditional method, i.e. length frequency analysis, is not free from possible errors. It is difficult to successively sample from the same population in a free-living pelagic species such as krill. Krill have also been shown to be able to shrink under food limitation both in the field and under laboratory conditions (Ikeda and Dixon 1982, Quetin and Ross 1991, Nicol et al. 1992a). This can potentially confuse the length frequency distribution. Mortality can also be size-related, making it difficult to interpret the observed changes in the mean size of the population. A recent approach termed the 'instantaneous growth rate' (IGR) technique, reliably provides individual growth rates from on-board experiments (Quetin and Ross 1991, Nicol et al. 1992a). This method uses fresh animals and thus is not restricted by such assumptions inherent in the repeated sampling of a population, but it is costly in labour and requires a large number of live animals.

If growth rates could be closely estimated without on-board incubations, it would provide the means for more extensive data collection and a better assessment of seasonally and regionally variable krill growth. The RNA:DNA ratio and RNA content of tissue proved to be indicators of recent growth and nutritional conditions in some marine invertebrates and fishes (Buckley 1984, Clemmesen 1994, Moss 1994a, b, Folkvord et al. 1996). The amount of RNA reflects, and varies with protein synthesis and hence growth, whereas DNA content is relatively constant per cell.

Some of these applications have obtained useful data that are very difficult to collect otherwise (Clemmesen 1994, Clemmesen and Doan 1996, Saiz et al. 1998).

Ikeda (1989) published an attempt to use the amount of RNA in krill tissue to estimate their growth rates, but by using calibration equations obtained from other species. In the previous chapter, reference measurements were made to the nucleic acid levels of field-caught animals to examine the relationship between the nucleic acid-based indices and growth rates and also the effect of moult stage on the nucleic acid contents. The moult stage did not have a significant effect on the content of nucleic acids. There was also a significant correlation between the RNA-based indices and the growth rates independently measured by IGR technique, although the predictability was only modest. One drawback of the experiment was that the effect of sex and maturity stages was not eliminated; the experimental groups of low growth were represented mostly by females with one group particularly by spent females. The low level of RNA may have resulted not solely from low rates of growth but also from other complications caused by exhaustion after a spawning event.

The response of krill growth to food conditions has been investigated a number of times (Ikeda and Thomas 1987a, Buchholz 1991, Nicol et al. 1992a, Ross et al. 2000). These studies, however, did not involve a controlled alteration of food regimes during the course of the experiment and the response of krill growth in changing food regime has not been studied. In experimental studies of krill, the animals are often put into food-limiting conditions, but recovery after a period of food limitation has not been examined. Also, the growth of krill has normally been expressed in length, whether from field or laboratory. It remains to be examined how the growth in length is related to the growth in mass.

A laboratory experiment was conducted with juvenile krill, to further explore the usefulness of nucleic acid levels for assessing nutritional conditions and estimating growth rates of krill and also to examine the growth of krill in food regimes where feeding and starvation were alternated. Contrasting long-term feeding conditions were imposed to generate regimes of high growth and negative growth and then

these were subsequently reversed and maintained. In this experiment, the growth rates as well as nucleic acid levels were determined through the period during which a switch of food regime occurred.

3.3 Materials and methods

Experimental animals

A large number of juvenile krill were collected on 15 April 1997 19:47 (local time) at 64°19' S, 110°57' E, just north of the ice edge in East Antarctica with an RMT 1+8. The krill were kept in a cold room on board while being transported to the aquarium at the Antarctic Division, Kingston, Tasmania. Then they were maintained in the dark at 2±0.5 °C with a low level feeding regime until the experiment started at the beginning of August 1997. A mixture of 3 cultured algal species, *Phaeodactylum tricornutun*, *Gemingera criophylum*, *Pyramimonas* sp. was given to krill in tanks approximately once a week. Limited capacities of the aquarium to process the waste load did not allow a high level of ration to support active growth of krill. The experimental animals were selected to cover a narrow size range (approximately 7.5 mm in carapace length, around 24 mm in total length) and kept individually in 2 litre plastic jars at 0 °C in the dark. One group was fed *Phaeodactylum tricornutum* at $5x10^5$ cells ml^{-1} to attain maximal growth rates (Ikeda and Thomas 1987a) and the other group was starved by being kept in filtered seawater. Water exchange and food provision were made every 3 to 4 days.

Organisation of the experiment

Phase 1

An initial group of 25 krill were sampled on Day 0. For half of each group (fed and starved), 12 animals were sacrificed after 5, 10 and 15 weeks; they were weighed and the carapace lengths measured. These animals comprised the Harvest Group and were used for nucleic acid level determination. Krill in their intermoult stages, rather than in immediate postmoult or premolt, were usually selected. A small portion of abdominal muscle was excised and put into a labelled tube and was then snap frozen in liquid nitrogen and stored in a -86 °C freezer until analyses. The other half of each group served as the Monitor Group. The krill, at each moult, were taken out and weighed and an image of each krill was taken with an image analysis system. The animals were then replaced in the experimental jars. From the image captured, the carapace length was measured. For the regular sampling of the animals, only animals which had not been disturbed in the image analysis procedure were intended to be

used. However, at week 15, due to the dwindling number of animals, about half of the animals came from the monitored and hence disturbed group.

Phase 2

After 15 weeks, the food regimes were reversed; the fed krill were starved and the starved krill began to receive food. Following the food regime switch, all krill were monitored at each moult and harvesting went on the monitored animals as required. Five krill were sampled from the 'fed and then starved' group on Day 3, 7, 14, 28 and 91 after the reversal and also 5 animals from the 'starved and then fed' group on Day 2, 5, 9, 14, 28 and 91.

Every morning the jars were inspected and if the animals had moulted, their cast exoskeletons were collected. Increments in the length of uropods between moults were used to determine individual growth rates.

Measurements of nucleic acid contents

The procedure for nucleic acid measurements in the previous chapter did not involve a purification process which removes interfering materials at a cost of extra steps. Although purification can improve the results, some applications were successful without employing the purification procedures. In this experiment, the protocol was replaced by the one that includes purification steps to minimise possible disturbance by non-nucleic acid materials. The analysis protocol for the nucleic acid measurement was modified from Clemmesen (1988, 1993) and Lemmens (1995). Approximately 10-20 mg of krill abdominal muscle was cut off and weighed and then placed into a 1.5 ml microcentrifuge tube. Fifty µl of 2% SDS (sodium dodecyl sulfate) in TNE buffer (Tris 10 mM, EDTA 1 mM, NaCl 100 mM) was added to the tube and the tissue was ground with a plastic pestle for 30 s. Four hundred and fifty µl of 2% SDS buffer was added and the tubes were vortexed for 30 s and left for 30 min on a shaker. The tissue sample was ground again for 30 s and vortexed for 30 s and shaken for a further 30 min. After brief vortexing, the tubes were centrifuged for 5 min at 3800 g. To the remaining supernatant, 20 mg ml^{-1} proteinase K was added to a final concentration of 200 µg ml^{-1}. Vortexed samples were shaken for 30 min. Four hundred µl of phenol: chloroform: isoamylalcohol (25:24:1) was added and mixed

for 5 min and then centrifuged at 3800 g for 10 min. Three hundred and fifty µl of the aqueous phase was collected and transferred to a new vial. Four hundred µl of chloroform: isoamylalcohol (24:1) was added and mixed for 5 min and then centrifuged for 5 min at 3800 g. One hundred µl of the aqueous phase was collected and transferred to a new tube for total nucleic acids (RNA+DNA) determination. Another 100 µl of the aqueous phase was collected and transferred to a new tube for DNA determination. For DNA determination, samples were treated with 1 µl of 10 mg ml^{-1} of RNase to a final concentration of 100 µg ml^{-1} at 37 °C for 30 min. Twenty five µl of the sample was mixed with 3 ml of ethidium bromide solution in a cuvette and the fluorescence was read at 365 nm for excitation and at 590 nm for emission. Concentrations of nucleic acids were determined by running standard curves with known concentrations of calf thymus DNA and yeast RNA. The contributions to fluorescence by washing the sample in chloroform was corrected for by subjecting the blank to the identical washing step.

3.4 Results

Growth rates of the Harvest Group

The experimental krill appeared to have been growing at marginal rates when the experiment started, reflecting their low maintenance food ration. Growth rates measured from specimens for the first 3 days of the experiment, representative of recent conditions, averaged –0.14 (±1.02) % per moult (n=7). The experimentally imposed contrasting food conditions generated differential growth rates in the animals. The growth rates of fed krill continued to rise up to 7.5 % per moult, during Phase 1 whereas the starved animals exhibited essentially negative growth rates between 0 and –2% per moult (Fig. 3.1 a). Fifteen weeks after the start of the experiment, the average growth rate of fed krill was 4.49 (±2.49) % and for starved krill, it was -0.96 (±0.85) % per moult. The ensuing switch of the feeding regime reversed this trend, and approximately 2 weeks was sufficient to deliver a complete reversal in the growth rates (Fig. 3.1 b).

Growth rates of the Monitor Group

The growth rates determined from the Monitor Group by increment of carapace length also showed that the reversal of food regime started to affect the growth rates very rapidly (Fig. 3.2 a, b). In approximately 10 days, the previously fed krill initiated negative growth and the growth rates of starved krill became positive. The growth rates of the two groups were completely reversed in about 2 weeks, significantly shorter than one moult cycle. The growth in fresh body weight exhibited a similar pattern to that of carapace length (Fig 3.2 b).

Growth in length and weight

Carapace length growth and fresh weight growth were obtained simultaneously from the Monitor Group. There was a highly significant linear relationship (r^2=0.6, $p=4.9x10^{-49}$) between the growth in length and weight (Fig. 3.3 a). However, it was apparent that in some cases, shrinkage in length and weight loss did not occur proportionately. The overall correlation was obtained with all available specimens. However, the length growth and weight growth of the starving krill during Phase 1, denoted by open symbols in Fig 3.3 a, seemed to be divergent from the general trend.

The experimental krill at the start of the experiment had been inadequately fed and this was reflected in their growth rates. This is partly because it is technically difficult to maintain a large number of krill under sufficient feeding condition in an aquarium. Besides, no new food was given to the animals for a week before the start of the experiment, as explained earlier. In Fig. 3.3 b, the two groups of starving krill that have been well fed or underfed are compared. The krill of the 'fed and then starved' group were given excess food during Phase 1. When the food supply was withdrawn from the krill as Phase 2 began, the animals started to shrink and lose weight proportionately, along the line of regression. However, the length change and weight change did not correspond well in the starving krill in Phase 1. These krill had already been underfed. While weight loss was occurring in virtually all individuals, the rates of length change were in a broad range, with some individuals even showing positive values.

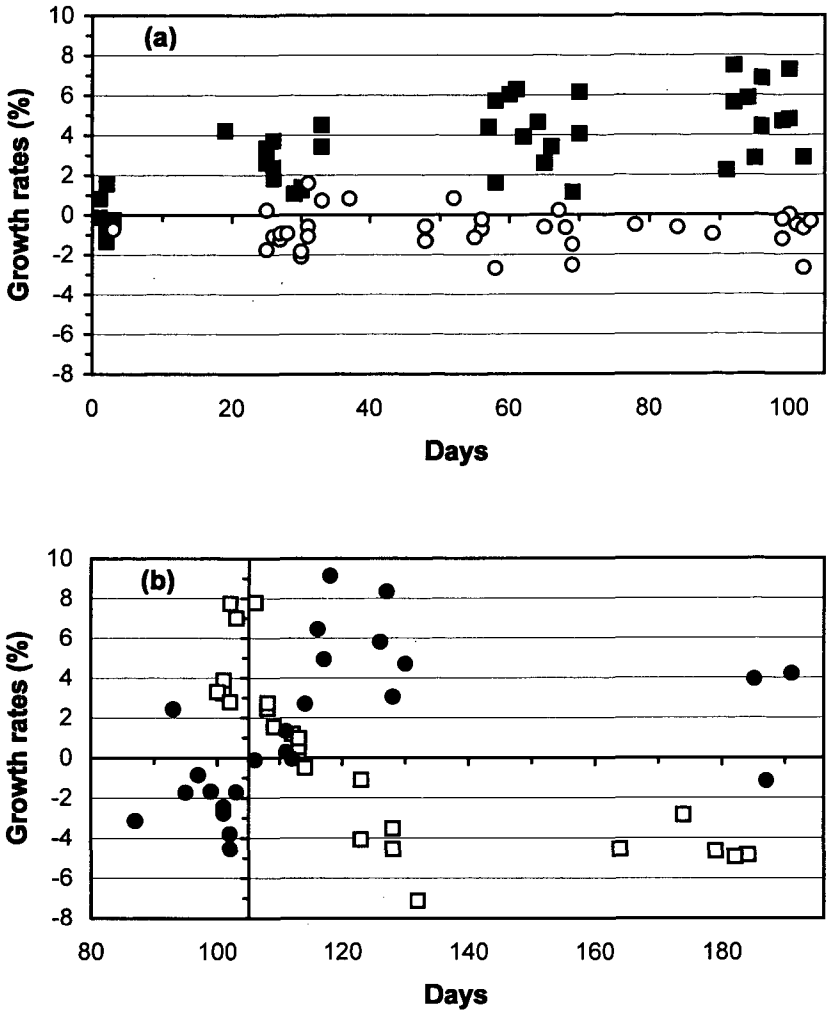


Fig. 3.1 Change in the growth rates of the Harvest Group krill over time in response to food conditions. (a) Growth rates of the krill sacrificed during Phase 1 of the experiment, which were determined by the incremental change in uropod length at moult. Growth rates of krill for the first 3 days of the experiment are also presented. Filled squares represent fed krill and open circles starved animals. (b) Growth rates of the krill sacrificed during Phase 2 of the experiment, determined from the incremental change in carapace length at moult. Day 105 is the point of food regime reversal and hence the point of change from Phase 1 to Phase 2. Open squares represent the 'fed and then starved' krill and solid circles the 'starved and then fed' animals. Note that growth rates are plotted on the day when moulting occurred, not when the animals were sacrificed.

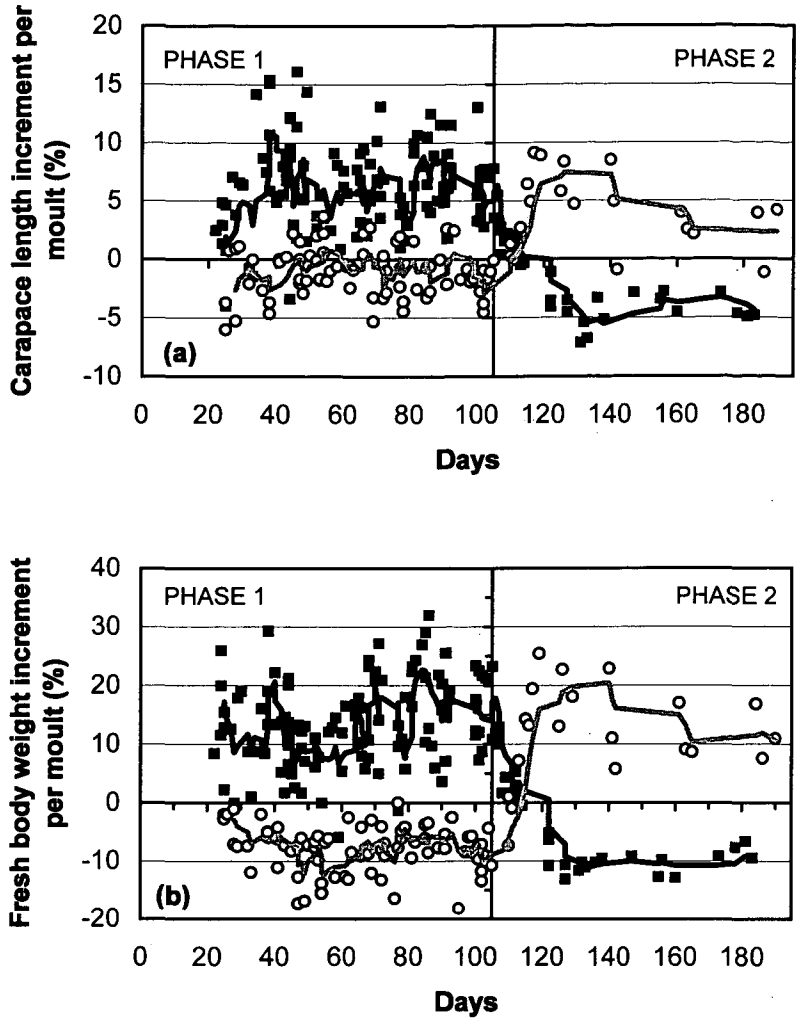


Fig 3.2 Growth rates in length and weight determined from the Monitor Group over time. (a) Growth in carapace length, (b) growth in fresh body weight. Day 105 is the point of food regime reversal and hence of the change from Phase 1 to Phase 2. Lines represent 'moving averages' drawn from the last 5 data points. Solid squares and solid line represent the 'fed and then starved' krill and open circles and shaded line indicate the 'starved and then fed' animals.

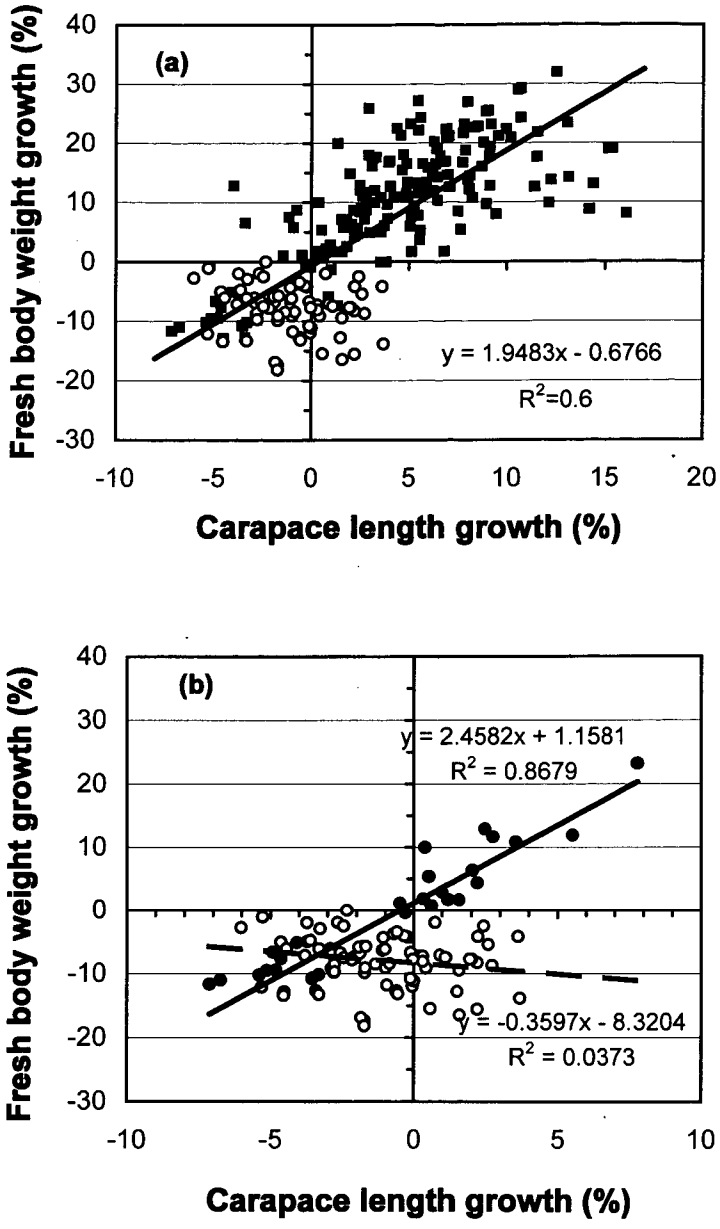


Fig. 3.3 The relationship between growth in carapace length and fresh body weight in percentage increase per moult. (a) All available specimens. Starving krill during Phase 1 are denoted by open circles. (b) Starving krill in Phase 1 and Phase 2. Solid circles represent starving krill during Phase 2 which had been previously well fed. Open circles denote starving krill during Phase 1 which had already been underfed

Changes in nucleic acid levels under varying food conditions

The RNA:DNA ratio became elevated when the animals were well fed whereas the ratio for the starved animals did not change greatly during Phase 1 of the experiment (Fig. 3.4 a). Five weeks after the start of the experiment, the ratio differed significantly between the fed animals and the starved ones (ANOVA, $F=9.64$, $p<0.005$). The gap between the two groups widened during the progress of the experiment. From the start of the experiment and throughout Phase 1, the RNA: DNA ratio of the starved krill remained around 1, while the ratio in the well-fed animals rose to nearly 3. The switch of the food regime after 15 weeks reversed this trend. The change in RNA:DNA ratio became clearly appreciable in 2 weeks after the switch in food regimes. The ratio for the starved animals, which had been previously well fed, became lower and eventually declined to a level similar to that at the commencement of the experiment. The RNA:DNA ratio of the 'starved and then fed' krill slowly recovered by the end of the experiment. It reached a similar level for the animals that had been well fed for about 10 weeks in Phase 1. Four weeks after the switch in feeding regimes, the 'fed and then starved' animals exhibited rather high ratios, which may imply that animals well fed for a long period can sometimes remain in good condition despite a few weeks of starvation. Similarly, the 'starved and then fed' krill, 4 weeks after the switch, showed lowered ratios, which may indicate that some individuals experienced difficulties in recovery after long starvation. Nevertheless, the RNA:DNA ratio of krill started to decline soon after a switch to starvation while improved food condition raised the ratio in a few weeks. This occurred in a time scale similar to that of the growth rate change.

The concentration of RNA in tissue, expressed as μg RNA mg^{-1} of fresh muscle, tended to increase in Phase 1 of the experiment (Fig. 3.4 b). Even the starved animals showed modest increases in RNA concentration until the switch. Still the RNA concentration of the fed krill remained higher than in the starved krill from week 5, the first measurement after the start of the experiment (ANOVA, $F=4.538$, $p=0.045$). A switch to starvation lowered the RNA concentration noticeably for the following 2 weeks. Although the RNA concentration rose again in 4 weeks following the switch, as was the case with RNA:DNA ratio, the RNA content remained at a low level until

the end of the experiment. The RNA concentration of the 'starved and then fed' krill became elevated following the switch to feeding, although the level at the end of the experiment was not especially high.

The DNA concentration expressed as μg DNA mg^{-1} of muscle tended to increase slightly with starvation and decreased considerably when the animals were well fed (Fig. 3.4 c). After 10 weeks, the concentrations of the fed animals and the starved ones became significantly different (ANOVA, $F=18.18$, $p=0.0003$) and the difference increased further at week 15. In the longer term, a switch of the food regime caused a change in DNA concentration similar to that in Phase 1 of the experiment; DNA content increasing with starvation and decreasing with feeding. However, the change was not readily appreciable in the first few weeks following the switch.

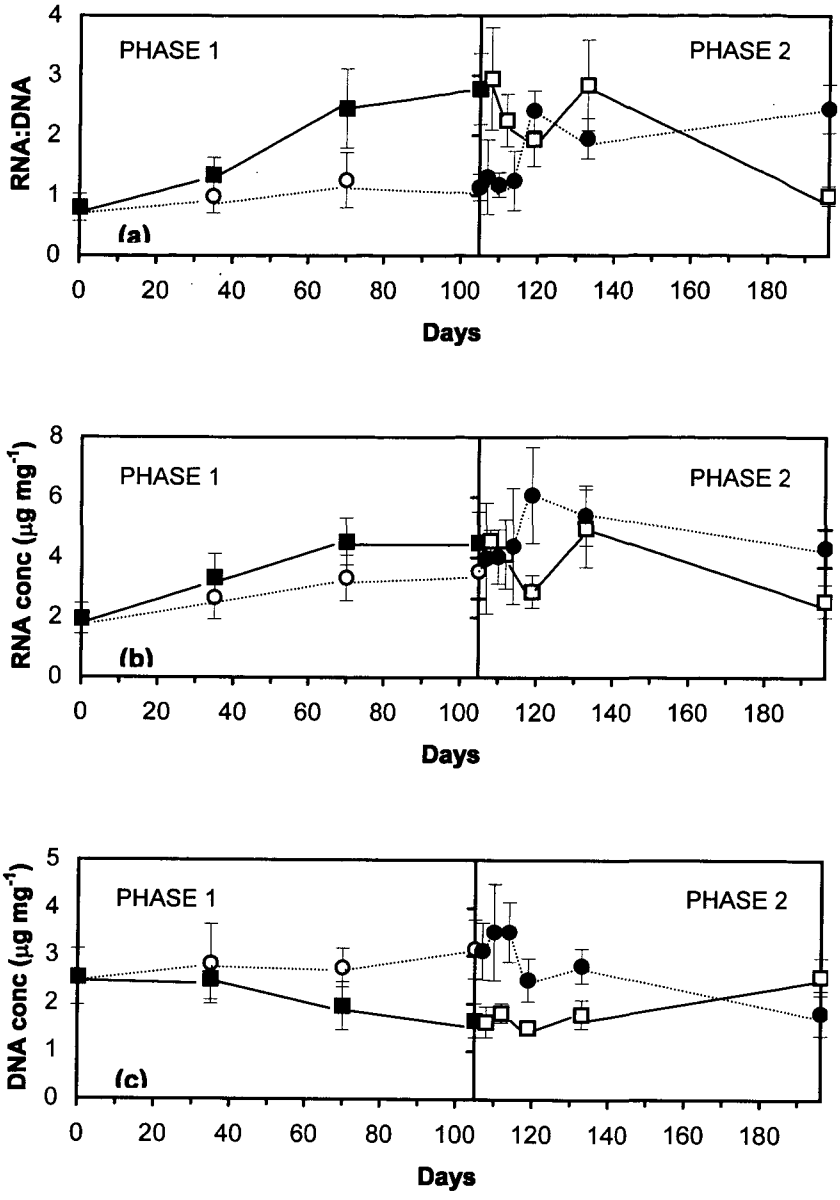


Fig. 3.4. Changes in nucleic acid levels of krill tissue during the experiment. (a) RNA:DNA, (b) RNA concentration (mg RNA mg^{-1} fresh muscle), (c) DNA concentration (mg DNA mg^{-1} fresh muscle). Day 105 is the point of food regime reversal and hence the point of change from Phase 1 to Phase 2. Squares represent the 'fed and then starved' krill and circles the 'starved and then fed' group. Solid symbols indicate that the krill are feeding and open ones denote that the animals are starving

The relationship between individual growth rates and nucleic acid levels

When the individual growth rates from Phase 1 of the experiment were plotted against the corresponding RNA:DNA ratio, a general trend of the ratio increasing with rising growth rates was evident, although the relationship was not very tight (r^2=0.518) (Fig. 3.5 a). When the animals were growing negatively, and also until the animals started to grow above 1 % per moult, the RNA:DNA ratio tended to remain in the range of 0.5 to 1.5. For the animals growing at less than 1% per moult, mostly starved krill, the relationship between the growth rates and the ratio does not seem to be linear, apparently levelling off. RNA concentration could be related to growth rates (Fig. 3.5 b). However, its variation was not as well correlated to growth rates as it was with the RNA:DNA ratio (r^2=0.286). DNA concentration showed a modest negative relationship to growth rates; the animals with low growth due to starvation, especially shrinking individuals, clearly had higher DNA content (Fig. 3.5 c). All these relationships were highly significant (Table 3.1).

Table 3.1 Regression statistics of the instantaneous growth rate (IGR) versus nucleic acid-based indices

	n	slope	r^2	significance
IGR vs RNA:DNA	70	0.210	0.512	3.42×10^{-12}
IGR vs RNA concentration	70	0.201	0.286	1.85×10^{-6}
IGR vs DNA concentration	70	-0.152	0.318	3.67×10^{-7}

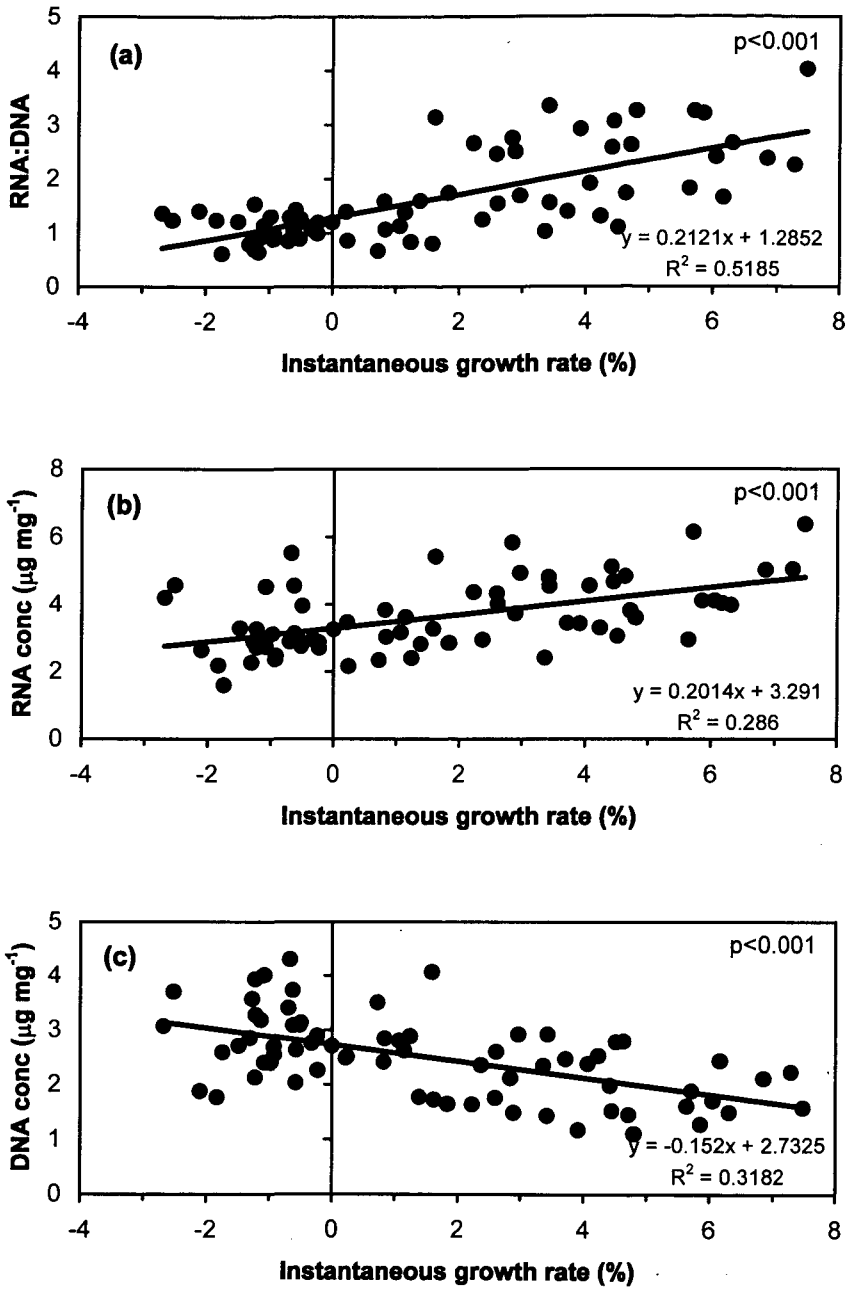


Fig. 3.5 Regressions of instantaneous growth rates (IGR) versus nucleic acid-based indices. (a) IGR vs RNA:DNA, (b) IGR vs RNA concentration and (c) IGR vs DNA concentration

3.5 Discussion

To generate contrasting growth rates, groups of juvenile krill were purposely fed and starved and then sampled over time. Krill growth responded to the prevailing food conditions and to changes in the food regime without much delay. These responses were also reflected in the nucleic acid-based indicators examined in this study.

The response of growth rates to the prevailing food regime was relatively prompt. The plasticity of krill growth has been demonstrated a number of times. Buchholz (1991) observed a large degree of variation in the growth rates of krill kept in the laboratory in response to the food supply. Nicol et al. (1992a) demonstrated that shrinkage of naturally growing krill that are deprived of food can occur after as little as 7 days. Ross et al. (2000) showed that field growth rates of young krill were related to food quality and quantity and that they varied on time scales of days to weeks. The present study demonstrates that the response of krill growth to food conditions can occur in either direction at time scales much shorter than a single moult cycle. In this experiment, the change in both directions was almost immediate. Approximately 2 weeks after the switch in feeding regimes, previously shrinking krill exhibited growth rates typical of well-fed krill, and krill which had been growing were clearly shrinking. This is well within the duration of a single moult cycle. Regaining zero growth rates following shrinkage and declining to zero growth rates from positive growth took even less time. Therefore, apparent positive growth during winter (Huntley et al. 1994) is not surprising as it can be achieved given a reasonable level of food supply for a relatively short period of time, i.e. 2 weeks. Similarly, shrinkage observed during winter need not be unusual (Quetin and Ross 1991). This short response time clearly demonstrates the highly adaptive nature of krill growth. In a food environment where the level of food supply can change considerably in the order of days to weeks, a slow response would not allow krill to exploit the food resource which becomes available at irregular intervals.

Growth in length was related to growth in weight. As krill would gain weight by continuous feeding, loss of weight under food shortage is inescapable and will proceed anyway. However shrinkage in length may not necessarily occur in a similar

fashion. Well-fed krill and underfed animals responded to starvation differently. Further starvation, when juveniles have already been under food limitation, seems to cause less pronounced shrinkage while loss of weight continues. Conversely, when the animals have been feeding adequately and hence growing well, loss of weight may be accompanied by shrinkage in length proportionately. Shrinkage of underfed juveniles may occur less readily compared to well-fed juveniles or adult animals. This implies some degree of adaptation to longer-term food supply.

Different food conditions imposed for an extended period led to changes in nucleic acid levels as well as in the growth rates. The RNA:DNA ratio not only responded to the prevailing food regimes but could also be related to individual growth rates. The resolution, however, may not be high enough to use it as a precise predictor of growth rates. The coefficient of determination was relatively low, implying the relation is not particularly tight. Unlike the observation in the previous chapter, this experiment was relatively free from the effect of sex and moult stages as only juvenile krill were used. The animals were uniformly subjected to known concentrations of food, or starvation, and were sampled at pre-determined periods. Animals just before or after ecdysis were avoided although the effect of moult stages has been shown to be insignificant. There was a highly significant relationship indeed, between the individual growth rates and the RNA levels. However, it was not much stronger than that obtained for field-caught animals without control over various biological factors.
It is noteworthy that below a certain level of growth rate, the RNA:DNA ratio appears to reach a more or less constant value. A similar observation was made for a fish species by Mathers et al. (1992). This level can be an indication that the animals are gaining only enough food to meet their basic metabolic needs and hence are growing only marginally, if at all.

A high concentration of DNA in muscle could be associated with a starvation of several weeks duration, although DNA per unit weight was highly variable between individuals. When the animals began to actively grow, the DNA concentration in the muscle became lower. The DNA level in the krill at the start of the experiment was already low and it rose only modestly with further starvation. This accords with the

fact that the experimental krill had been under sub-optimal feeding conditions before the experiment started. It is probable that a higher DNA concentration equates to an increased number of cells per unit body mass and this is most likely due to a reduction in cell size caused by starvation. Similarly, improved nutrition results in growth of cell size and therefore decreasing number of cells per unit body mass. It appears that the differential feeding conditions imposed on the animals, which were growing poorly at the start of the experiment, led to the above results. This is in agreement with the results of McGaffin (1998) obtained by histology. McGaffin (1998) showed that starvation would result in a decrease in cell size and that the cell number per unit mass was higher in starved animals. Juinio et al. (1992) also reported a decrease in cell biomass and a conservation of cell numbers during starvation of postlarval lobster. It seems likely that cells are not being lost but cells are shrinking with starvation. The decrease in the somatic mass appears to be accompanied by an increased DNA concentration.

Starvation maintained during the experimental period may well have been severe enough to cause a reduction in cell size. This could explain how the RNA per unit weight of muscle increased even modestly for starved individuals, whereas the RNA:DNA ratio remained low. The amount of RNA was not actually increasing but the cells were probably shrinking in volume. It is understandable that RNA concentration proved to be more poorly related to growth rates than the RNA:DNA ratio in this experiment; active growth, or shrinkage of cells, may have confounded the tissue concentration of RNA by altering the number of cells per unit body mass. The amount of DNA is probably cell-constant, but if the animals undergo active growth or severe starvation, it may not necessarily be mass-constant. It confounds one basic assumption for the application of RNA:DNA ratio; the amount of DNA should represent biomass and consequently should be somatic tissue-constant. Nonetheless, DNA as a denominator acts as a normalising factor with regard to body mass, and tissue concentration of DNA may reflect the nutritional stress that the animals have experienced. The RNA:DNA ratio will be a more reliable index than RNA content, particularly when animals are undergoing a marked change in cell growth, either due to drastic fluctuation in food supply or simply progressing through a series of development stages.

Krill growth is a process intimately linked to food supply, and the growth rate can be adjusted readily, well within a single moult cycle; a scale which may well be comparable to that of change in the abundance of food organism populations. Different, probably discernible, levels of RNA-based indices will be induced in the groups of animals subjected to different feeding conditions for varying length of time. There are some cases in which these indices have been successfully applied to crustacean species as estimators of growth rates with reasonable precision (Moss 1994a, b, Saiz et al. 1998). However, it should be noted that in many cases, the use of nucleic acid levels has been limited to simply an indicator of nutritional state or a detector of food limitation (Canino 1991, Malloy and Targett 1994, Westerman and Holt 1988, Wagner et al. 1998). There have been relatively few applications in which these nucleic acid indices were concluded to be, or employed as an estimator of growth rates. Even in these cases, the growth rates were usually not determined individually from the same specimens. In most cases, there were no other means to obtain such data, which prompted the investigators to examine the use of nucleic acid-based indicators. Growth rates, to which RNA:DNA ratio was related, were obtained from a group of organisms or from the trend of body size change over the experimental period (Juinio and Cobb 1994, Folkvord et al. 1996, Saiz et al. 1998). Then the growth rate is already a mean rate from a small population. Individual variation in the growth rates may well have been filtered and thus resulted in a better fit. There are relatively few cases where the individual growth rates and RNA-based indicators have been examined (Clarke et al. 1989, Mathers et al. 1992, Moss 1994a, b) and only a small number of studies have declared RNA-based indices as indicators of reasonable predictive power (Mathers et al. 1992, Moss 1994a, b). It is notable that, in the case of Moss (1994a, b), the juvenile shrimps were at early stages of their life history when maximal growth was occurring. At the upper end of the growth rates, the animals were growing at above 50% in weight per week and the RNA:DNA ratios were accordingly high and also varied widely. Thus, variability of individual growth could explain much of the variation in the RNA content and a strong relationship could be readily established between growth rates and nucleic acid ratios.

It appears that RNA levels in krill tissue are modified by a number of factors other than individual growth rates. These factors would be difficult to separate and quantify even in the laboratory. The nucleic acid-based indices can often be affected by environmental temperature, which was kept constant in this experiment. If the resolution were sufficiently high, it would warrant further investigation on the effect of temperature. To serve as a predictor of individual growth rates, however, this ratio appears to be of limited power and it is unlikely to replace other techniques for estimating growth such as 'instantaneous growth rate' measurement. In a varying food environment, which can encompass a massive bloom of phytoplankton and temporary cessation of food supply, RNA-based indicators may not be a fine-resolution estimator of growth rates for individual krill with varying physiology and feeding history. However, they should be able to differentiate relatively homogenous groups resulting from distinct seasonal differences, for instance, continued good feeding or sustained starvation with resultant contrasting growth rates. Thus they should be able to serve as a basic method to gauge what state the krill population is in, especially when the environments concerned are thought to be markedly different, e.g. mid-summer and mid-winter. Nucleic acid levels of tissue can thus form part of a repertoire of measurements that can provide descriptive information on the 'condition' of krill. However, growth rates of krill can respond to ambient food conditions in a scale of days to weeks, and the 'instantaneous growth rate' measurement is the best tool currently available to reflect the response of krill growth to environmental variables.

Chapter 4. Changes in the digestive gland of Antarctic krill during short-term starvation; mass, content of protein and lipid, and digestive enzyme activity

4.1 Abstract

The response of the digestive gland in Antarctic krill to short-term starvation was investigated with an aim of establishing indicators of recent feeding activity. The most significant change was in the decline of the overall mass of the gland, accompanied by corresponding loss of lipid and protein. The mass loss of the gland was immediate, following the removal of food supply, and continued for approximately 2 weeks. The calculated energy store in the mass loss indicates that the digestive gland of krill serves as a crucial storage organ, containing reserve material, which can provide against short-term food shortage. The mass loss of the digestive gland was also accompanied by a decrease in the length of the gland, which could be reliably measured from its outline viewed through the carapace before dissection. During starvation, the total digestive enzyme activities from a whole gland of krill declined on average as did the size of the gland, while activities per unit mass of the gland did not show significant changes. Enzyme activities exhibited high individual variation, which made the significance of any changes weak. The mass of the digestive gland in krill is a good indicator of the nutritional condition, which can complement other indices that reflect 'condition' such as 'instantaneous growth rate' measurements. The digestive glands of krill collected in early autumn were significantly smaller than those of high summer krill, which may indicate a seasonal difference in feeding intensity. The simple measure of the digestive gland size can provide information on the recent feeding of krill, especially under food limitation.

4.2 Introduction

Antarctic krill (*Euphausia superba*, hereafter called krill) has been shown to be highly flexible in its feeding strategy, which may have contributed to its role as the keystone species of the Antarctic marine ecosystem. Krill can utilise a variety of food resources, from detritus to copepods, despite being mostly a herbivore when there is a sufficient supply of phytoplankton (Kawaguchi et al. 1986, Price et al. 1988). In the laboratory it has been demonstrated that krill can even survive starvation of up to 7 months by breakdown of bodily protein and associated body shrinkage and this has been proposed as a key mechanism to cope with food scarcity particularly during winter (Ikeda and Dixon 1982). High tolerance to starvation and body shrinkage may be one of its major assets, which would enable krill to adapt successfully to the highly pulsed primary production in the Southern Ocean ecosystem. However, field results from winter are insufficient and so far have not yielded unequivocal evidence of starvation and associated shrinkage (McClatchie et al. 1991, Quetin and Ross 1991, Huntley et al. 1994). There is evidence to support winter feeding and there are reports that krill can, at times, find food sources during winter from sea-ice algae to copepods and even manage to sustain growth (Marchall 1988, Lancraft et al. 1991, Huntley et al. 1994). A number of possible mechanisms for a variety of feeding and growth strategies have been proposed for krill overwintering but only limited cases of supporting field or experimental evidence are available. It is most likely that krill will employ a variety of strategies in its repertoire in differing food environments. How commonly and also in what situation each of these strategies is adopted over the others in the wild remain to be investigated, particularly during food-limited season.

There have been few extensive studies examining the recent feeding history of krill under varying conditions and in fact, no standard methods have been established to examine krill feeding. Information on recent feeding status of the animals along geographical and environmental gradients will need to be obtained in a quantitative fashion to address questions concerning winter survival strategies (Anon 1994). However, the methodologies for determining the physiological condition of krill are limited. The condition of krill can be represented by *in situ* growth, of which the

rates can be measured using the 'instantaneous growth rate' technique (Quetin and Ross 1991). Ross and Quetin (1991) trialled lipid content as a measure of condition in larval krill in combination with instantaneous growth rates and carbon amount per volume. Nicol et al. (1992a) further proposed that 'instantaneous growth rates' coupled with time to onset of shrinkage may give some indication of the nutritional condition of the krill. The 'instantaneous growth rate' measurement, however, involves the shipboard experimental manipulation of a large number of live krill and this is not always possible.

Some features of the digestive system such as stomach content and fullness, colour of the digestive gland, and gut fullness have been used to examine the feeding activities of krill (Morris and Ricketts 1984, Antezana and Ray 1984, Endo and Kadoya 1991). However, these have not been widely applied as they have often failed to be reliable indicators of *in situ* feeding activity for various reasons. The digestive gland has been reported as the major organ of food assimilation and enzyme secretion in crustacean species (Dall and Moriatry 1983). The digestive gland of krill is a conspicuous organ, which would make individual measurements practical. In qualitative terms, a dark green digestive gland together with continuous production of faecal strings is cited as evidence of good feeding in laboratory experiments of krill (Buchholz 1991). The digestive gland of krill was also reported to play a dynamic role during short-term starvation in terms of lipid content and composition (Virtue et al. 1993). The digestive gland should offer further potential for use as an indicator of the nutritional condition of krill.

The contents of major biochemical constituents of krill have been examined for baseline descriptions, in relation to starvation, the moult cycle and seasonal changes (Clarke 1980, Ikeda and Dixon 1982, Nicol et al. 1992b, Torres et al. 1994). These studies, however, used whole animals and the changes within individual organs have generally not been examined. As krill experience change in food conditions, individual organs may respond more sensitively than others or than the whole animal. The change in the biochemistry of major organs may thus reflect this response better than the gross content of the whole body would.

Digestive enzyme activities have been examined in some zooplankton species as an index of feeding activities, with limited success (Hasset and Landry 1983, 1986, 1990 a, b, Harris et al. 1986). Although digestive enzyme activities have been measured from Antarctic krill (Mayzaud et al. 1985, McConville et al. 1986, Kolakowski 1989, Buchholz and Saborowski 1996, Saborowski and Buchholz 1999), these have not generally been examined as indicators of feeding activity and controlled experimental trials in the laboratory for such purposes have not been carried out. Most enzyme studies have also used whole animals, however, the large size of krill allows the dissection of the main digestive organ, the digestive gland, and the examination of changes in enzyme levels within it.

Development of a simple technique which can expeditiously determine the nutritional condition and growth potential of krill will greatly assist in understanding its seasonal growth and survival strategies. Changes in the digestive gland of krill during short-term starvation were investigated with the aim of exploring and establishing simple indicators of recent feeding history. The responses in terms of mass, protein and lipid content, and digestive enzyme activities in the digestive gland were examined.

4.3 Materials and methods

Field-collected summer krill were starved on board on 2 occasions, for 19 days and 7 days, respectively. The first set of starvation experiment examined the changes in digestive gland weight and content of protein and lipid in the gland. For the second experiment, the change in the size of the digestive gland, including the length and the digestive enzyme activities in the gland were determined. Field determinations of the digestive gland size in autumn krill were made for 2 seasons.

Starvation Experiment 1

Krill were collected with an RMT 8 net which was allowed to drift in the top 20 m of the water column. The experimental animals were collected at 01:40 (local time) on 14 February 1991 in the Prydz Bay region (66°30'83" S, 67°30'32" E) and were immediately placed in a 90 litre plastic tank containing freshly collected seawater at 0 °C which had been passed through a 0.45 μm filter. On the day of collection and on day 3, 6, 9, 12, 15 and 19, a sample of 20 large male krill of similar size was removed from the tank, their carapace lengths were measured and their digestive glands were dissected out whole. Each digestive gland was placed in a labelled tube and was frozen in liquid nitrogen for later analyses. For each day of the experiment, digestive glands were freeze-dried, weighed and the amount of protein and lipid were measured. Normally, the dry weight determination was made on 10 individuals, and 5 animals were used for protein and lipid measurement. The detailed lipid composition and its changes with starvation were examined separately and were reported in Virtue et al. (1993).

Protein measurement

For protein, the samples were analysed with a Bio-Rad DC protein assay kit which is based on Lowry et al. (1951), using bovine serum albumin as a standard. A weighed subsample of the freeze-dried digestive gland (1-15 mg) was digested in 3 ml 0.1 M NaOH at 60 °C for 12 hrs. One hundred μl of the digest was transferred to a clean test tube and 500 μl of alkaline copper tartrate solution was added and thoroughly mixed in. Four ml of Folin phenol solution was then mixed in and the tube allowed

to stand for 20 min. Absorbance was read at 750 nm against the standard curve constructed with a known amount of standard protein.

Lipid measurement

Lipid analysis was performed by a modification of the method of Bligh and Dyer (1959). A sample was homogenised and extracted in a mixture of chloroform, water, methanol and ethanol (6:2:1:1). Extraction was carried out at room temperature over 24 h. The chloroform layer was separated in a specially constructed micro-separation funnel, concentrated and dried at 70 °C under dry nitrogen flow, and the residue weighed to an accuracy of 0.01 mg.

Starvation Experiment 2

Animals

A large number of krill was collected at 17:15 (local time) on 31 January 1996 in East Antarctica (65°43'94" S, 79°59'64" E) with an RMT 8. After landing, krill were maintained in filtered seawater in the dark at 0±0.5 °C in a cold room on board for 7 days. Krill were sampled on the day of collection and day 1, 2, 3, 4, 5 and 7. Upon sampling, carapace length and digestive gland length (longest horizontal dimension) from the outline viewed through the carapace were measured with a caliper to the nearest 0.1 mm. Then digestive glands were dissected out and the length of the dissected glands measured again and placed in a labelled tube and stored in liquid nitrogen for later measurement of digestive enzyme activities.

Digestive enzyme assay

Ten krill were sampled for each day of the digestive enzyme measurement. Digestive glands were weighed frozen and then ground with a plastic pestle in a microtube containing 750 µl of distilled water. The homogenate was centrifuged at 10 000 g for 10 min and the supernatant was collected for use in enzyme assay, namely trypsin, amylase, laminarinase. In absence of a single buffer to provide optimal conditions for all 3 enzymes, the gland was homogenised in distilled water. However, the subsequent assay protocols included buffers to generate optimal conditions for each

enzyme. Some further dilutions, when necessary, were made in consideration for the working range of the assay protocols.

Trypsin activity was assayed in accordance with Erlanger et al. (1961). Trypsin hydrolyses the synthetic substrate, benzoylarginine-p-nitroanilide to give benzoylarginine and p-nitroaniline and the concentration of the product, p-nitroaniline liberated for 15 min was spectrophotometrically measured at 405 nm. One unit (U) is the enzyme activity that causes 1 μmole of substrate to react in 1 min at 25 °C.

Amylase activity was assayed using the Sigma amylase assay kit (No. 577) based on the method of Wallenfels et al. (1978). It relies on a coupled enzyme reaction; amylase and glucosidase hydrolyses 5 moles of 4,6-ethylidiene-G_7PNP into 4 moles of p-nitrophenol and 10 moles of glucose. The change in absorbance of p-nitrophenol during 2 min reaction was measured at 405 nm following a 2 min lag period. The unit was defined as mmoles of substrate to react at 37 °C per min.

Laminarinase activity was assayed by a modification of Head et al. (1984). The homogenate (50 μl) was incubated with 1 M sodium acetate buffer, pH 5.5 (100 μl) and 100 μl of laminarin solution (25 μg ml^{-1}) for 30 min at 37 °C and the reaction was terminated by placing the sample in boiling water for 3 min. The amount of glucose released by the reaction was measured with Sigma Glucose kit (No. 16). The unit was defined as release of glucose in μg per min.

Size of the digestive gland in autumn krill

The length of digestive gland was measured in the krill caught during autumn on 2 occasions. The gland length and carapace length were measured from 24 krill collected on 29 March 1993 at 64°49'02" S, 139°50'40" E and from 30 krill caught on 8 March 1996 at 65°07'54" S, 133°55'99" E.

4.4 Results

Starvation Experiment 1

Size of krill used.

Although the sizes of the krill (measured as carapace length; standard length 4 as in Mauchline 1981) used for the analyses of digestive glands differed between the days of the experiment at level of p=0.05, the difference was not highly significant (ANOVA, F=2.379, p = 0.039, df=68). A subsequent Tukey test failed to reveal between which days significant differences lay. The daily mean carapace lengths ranged between 14.82 and 16.18 mm and the size did not change consistently with time.

Change in dry mass of digestive gland with time

The digestive gland lost mass exponentially over the experimental period (Fig 4.1 a). Differences between the mean digestive gland dry mass on different days were highly significant (ANOVA, F = 30.166, p<0.0005, df=68). The greatest mass loss occurred over the first 3 days when the digestive gland lost on average 34% of its initial mass. After 12 days, further mass loss was virtually undetectable. The overall mean loss over 19 days was equivalent to 69% of the initial mass.

Absolute amounts of protein and lipid in the digestive gland

The total amount of protein and lipid present in the digestive gland declined as starvation progressed and the gland lost its mass (Fig. 4.1 b, c). The pattern of decline was similar to that of dry weight in that the greatest loss occurred over the first 3 days and after 12 days, further loss was hardly detectable. By simple deduction from mean values, the digestive gland lost, on average, 3.82 mg of protein and 1.98 mg of lipid between Day 0 and Day 19.

Changes in protein and lipid concentration with starvation.

Protein concentration did not differ significantly between days although there was a minor decline (ANOVA, F=1.87, p=0.122, df=34). Similarly lipid concentration between days did not differ significantly (ANOVA, F=2.001, p=0.099, df=34).

However, a decline throughout starvation was more noticeable in lipid, particularly for the first 9 days (Fig. 4.2).

Ratio of lipid to protein in digestive gland

The change in content of protein and lipid, both in terms of absolute amount and concentration (Fig. 4.1 b and c, Fig 4.2), seemed to suggest that lipid was lost more rapidly than protein during the early part of starvation, although the differences between days were not significant at level of $p=0.05$. The ratio of lipid to protein in the gland declined in the first 9 days and then rose again with a larger degree of individual variation (Fig. 4.3).

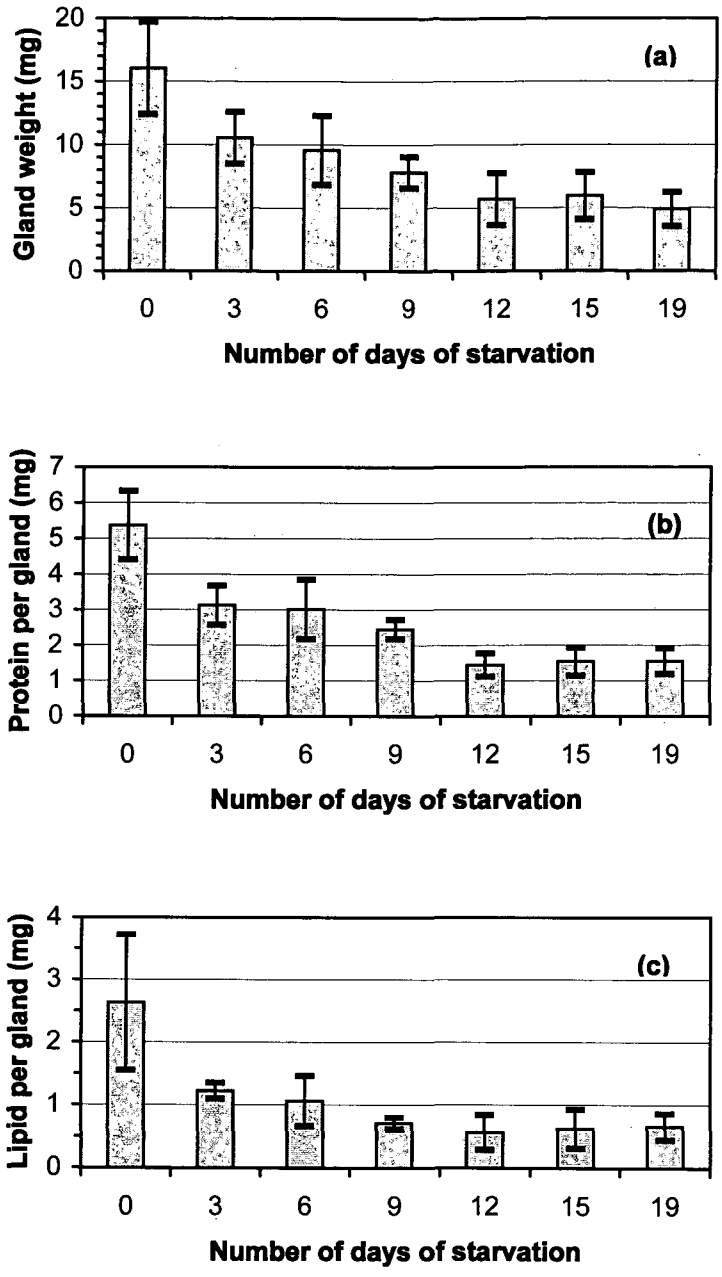


Fig. 4.1 Changes in mass, protein and lipid in the digestive gland of the krill during Starvation Experiment 1. (a) Dry weight, (b) amount of protein, (c) amount of lipid per digestive gland of the krill on the day of collection and following up to 19 days of starvation. Error bars denote one standard deviation

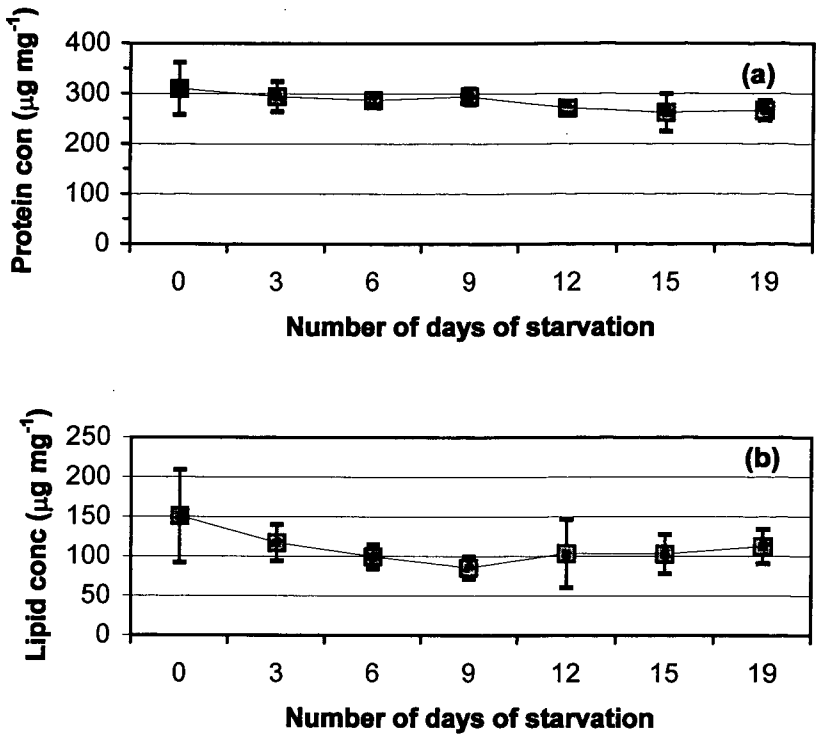


Fig 4.2 Changes in the concentration of protein and lipid (mg mg^{-1} dry weight) in the digestive gland in Starvation Experiment 1. (a) Protein, (b) lipid

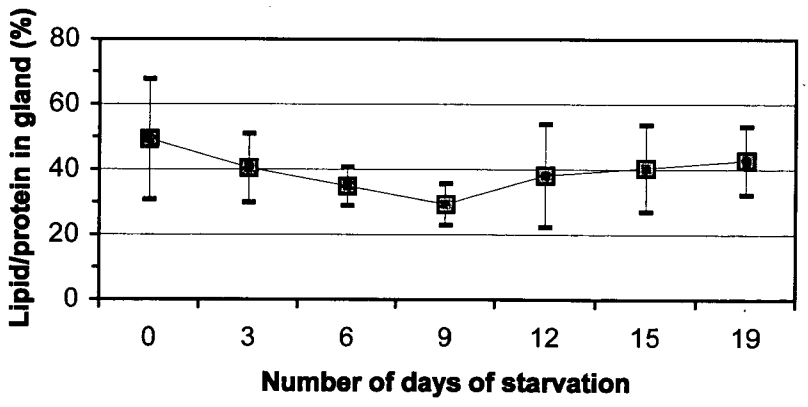


Fig 4.3 Changes in the ratio of lipid to protein in the digestive gland during Starvation Experiment 1

Starvation Experiment 2

The change in size of the digestive gland during a week-long starvation

The carapace length of the experimental animals on each day did not differ significantly during a week-long starvation experiment (ANOVA, $F=1.9504$, $p=0.0732$, $df=265$) (Fig. 4.4 a). For the same period, the digestive gland shrank to 75% of its initial length on average (Fig. 4.4 b). The relative gland length with respect to body size (expressed as percentage of the carapace length occupied by the digestive gland length) also declined from 56 % to 44% in a week (Fig 4.4 c). Differences in the absolute gland length between days were significant (ANOVA, $F=26.323$, $p<0.0005$, $df=265$) and a subsequent Tukey test showed that the absolute gland length became significantly different after just one day ($p=0.02$). The relative gland length was also significantly different between days. The difference of the relative gland length between Day 0 and Day 1 was even more significant than in the absolute gland length ($p<0.0005$). For the subset of the animals chosen for digestive enzyme activity measurements, the fresh weight of the digestive gland differed significantly between days (ANOVA, $F=7.793$, $p<0.0005$, $df=69$) and decreased substantially with time (Fig. 4.4 d). The fresh weight of the gland became significantly different in a day of starvation ($p=0.003$). The carapace length did not change significantly on each day (ANOVA, $F=0.6636$, $p=0.6792$). As body weight data were not available, there was no means of normalising the gland weight to body weight. Nevertheless, in one day during which significant loss of body weight is unlikely, the gland had already lost approximately 40 % of its initial weight (Fig. 4.4 d).

Digestive gland length measured from its outline viewed through the carapace before dissection was a good measure of the length of the dissected gland (Fig. 4.5 a). For the specimens selected for the enzyme activity measurements, both the gland length and fresh weight were available. The fresh weight of the gland showed a close exponential relationship with its length (Fig. 4.5 b).

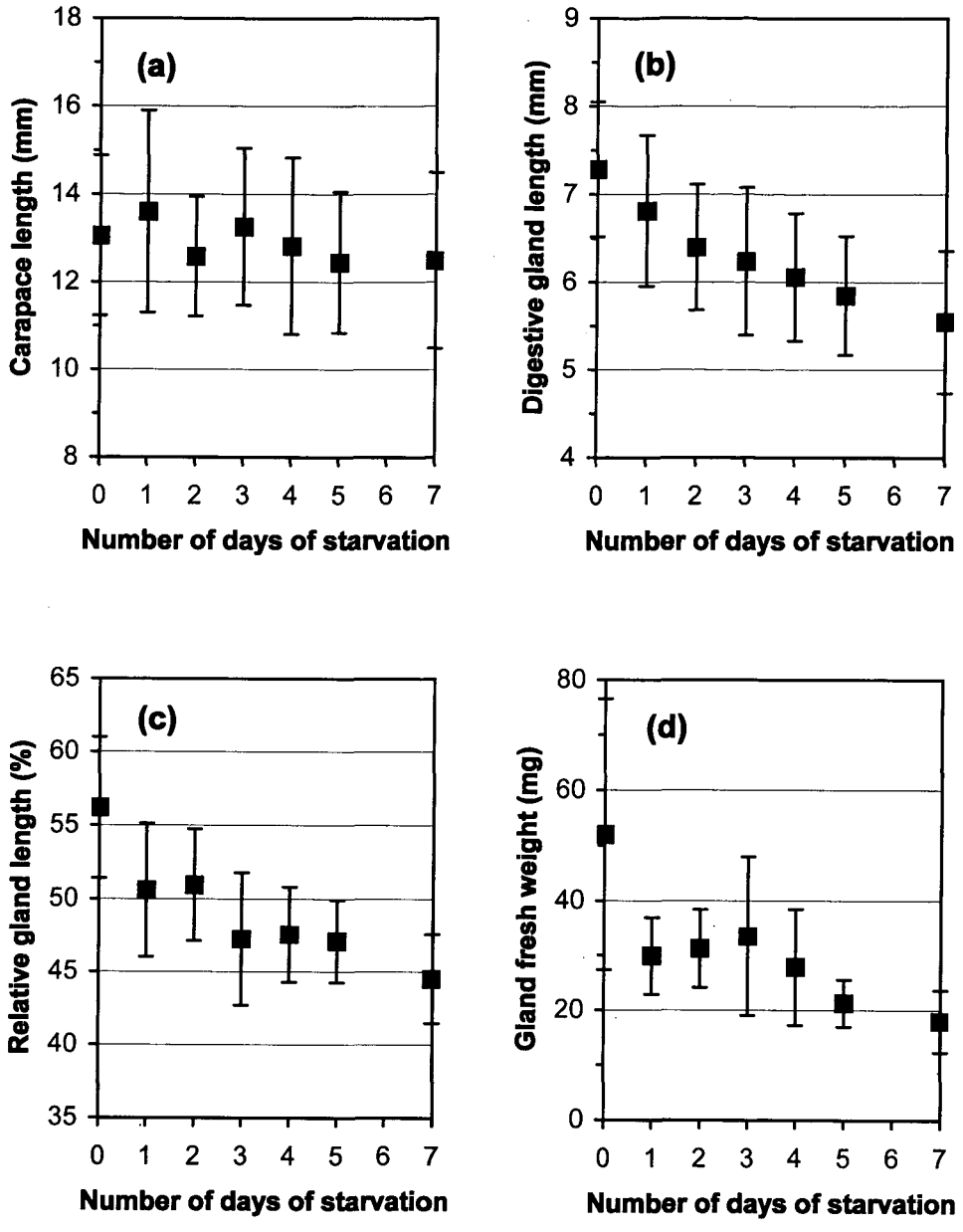
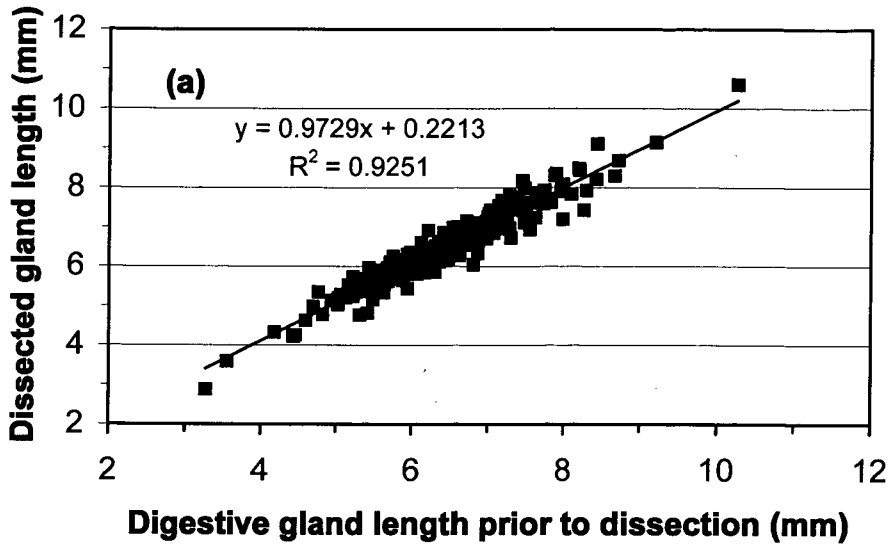


Fig. 4.4 Changes in the body size and the digestive gland size of the krill during Starvation Experiment 2. (a) Carapace length, (b) digestive gland length, (c) percentage of the carapace length occupied by the digestive gland, (d) fresh weight of the digestive gland



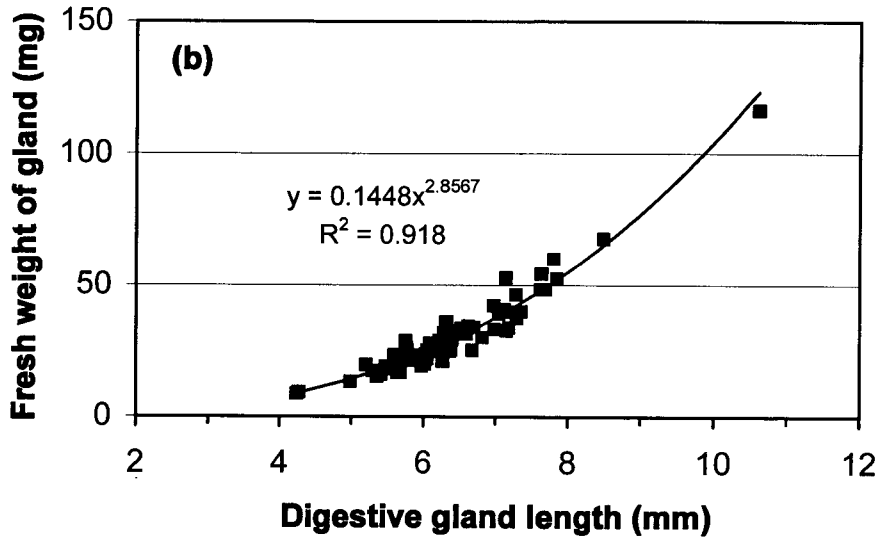


Fig 4.5 The relationships between different measures of the size of the digestive gland in krill. (a) Digestive gland length measured from its outline before dissection and the actual length of the dissected gland, (b) fresh weight of the digestive gland as a function of the length

Digestive enzyme activities

In terms of total activity from a whole gland, all three enzymes investigated showed a decrease in activity with time. The decline of the total activities was most appreciable between Day 0 and 1. Weight-specific activities, expressed as unit of activities mg^{-1} of the wet gland weight, tended to rise only slightly for the three enzymes. One of the most notable features of the digestive enzyme activities was a high degree of individual variation. Coefficient of variation in weight-specific activities was in the range from 32 to 82 %.

Trypsin

Total trypsin activity on average declined to 54 % of its initial level in a week. The decline in activity was readily noticeable in a day (Fig. 4.6 a). Following the second day, however, further decline was not substantial. Due to high individual variation in activities, differences in total activity between days were not significant (ANOVA, $F=1.374$, $p=0.239$). Weight-specific activity of trypsin tended to rise slightly in average terms (Fig. 4.6 b). Difference in weight-specific activity between days was even less significant than in total activity, again owing to high individual variation (ANOVA, $F=0.1617$, $p=0.7161$).

Amylase

Total amylase activity on average declined to 48 % of its initial level. As in trypsin, the activity declined noticeably in a day with the subsequent drop being rather minor (Fig. 4.6 c). Still total amylase activities did not differ significantly between days (ANOVA, $F=1.549$, $p=0.177$). Weight-specific activity did not show a clear trend (Fig. 4.6 d) and with a large degree of individual variation, the difference between days was not significant (ANOVA, $F=0.552$, $p=0.767$).

Laminarinase

Total lamainarinase activity showed the biggest decline among the three enzymes, 44 % of its initial level on average in a week (Fig. 4.6 e). As in the other two enzymes, total laminarinase activity exhibited a considerable decline in a day, resulting in a significant difference between days (ANOVA, $F=5.03$, $p<0.0005$). There was a significant difference between Day 0 and Day 1 ($p=0.021$). There was no obvious trend with the weight-specific activity of laminarinase (Fig. 4.6 f), and no significant difference was found between days (ANOVA, $F=1.6283$, $p=0.1541$).

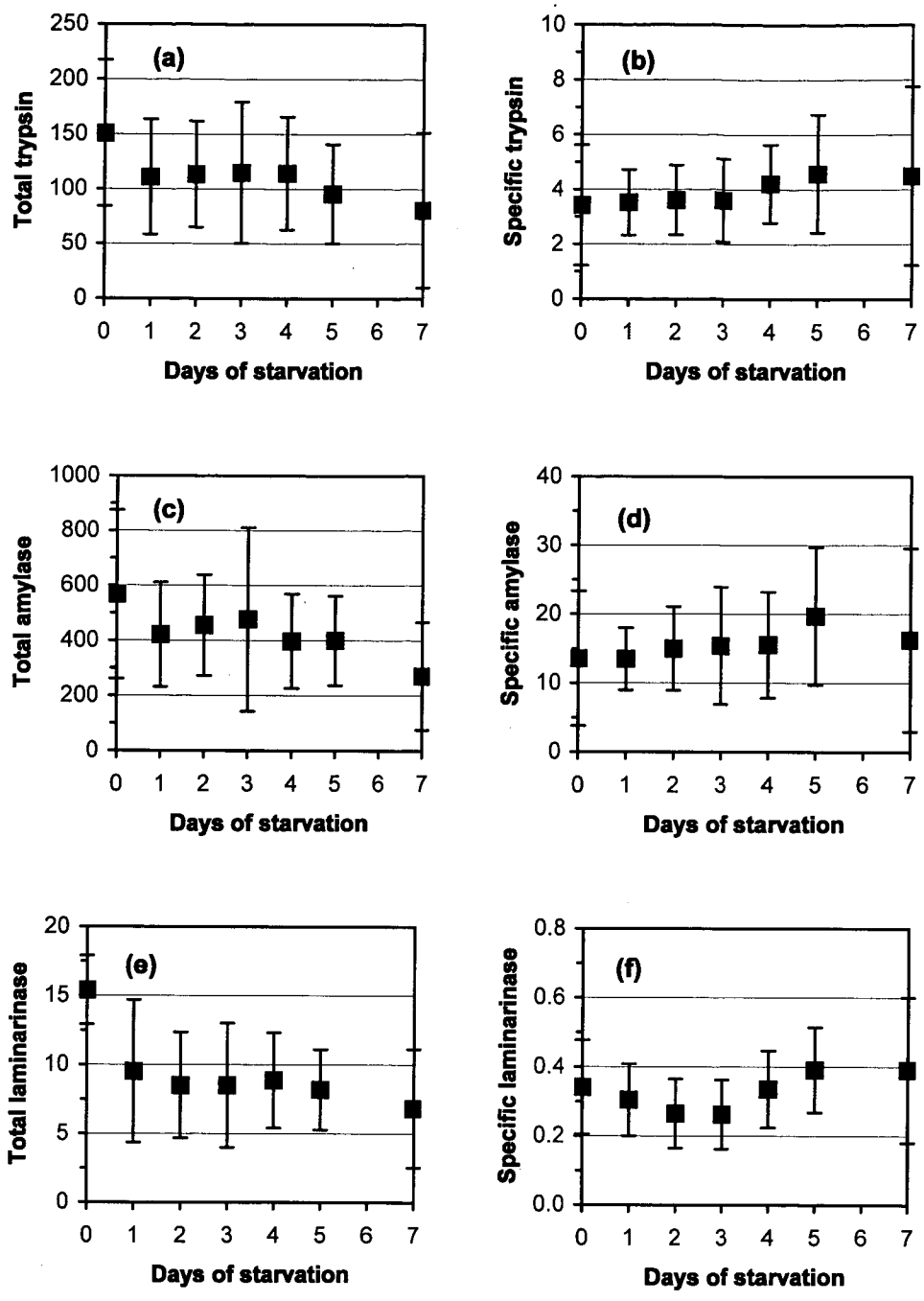


Fig 4.6 Digestive enzyme activities in the digestive gland of the krill during Starvation Experiment 2. Results are given in total activity from a whole gland (mU gland^{-1}) and weight-specific activity (mU mg^{-1} fresh weight). (a) Total trypsin, (b) weight-specific trypsin, (c) total amylase, (d) weight-specific amylase, (e) total laminarinase, (f) weight-specifc laminarinase

Digestive gland size of autumn krill and starved krill

To explore the applicability of the digestive gland length as an index of feeding activity, krill caught in different seasons, presumably with different food availability, were compared to see whether there were differences in terms of digestive gland length. The krill caught during the same cruise as for Starvation Experiment 2 but later in March, had a considerably smaller digestive gland for a given carapace length (Fig. 4.7 a). The lengths of digestive gland in the krill caught in late March 1993 were also between those of fresh summer krill and summer krill starved for a week in Starvation Experiment 2 (Fig. 4.7 b, c). The length of the carapace and the digestive gland showed a significant linear relationship in all 3 cases. However, a single equation could not describe this linear relationship both in summer krill and autumn krill. This was the same case with freshly collected krill and starved ones. Comparing the slopes and elevations of the relationship between the carapace length and the digestive gland length, the slopes were not significantly different between summer and autumn krill or between fresh summer and starved summer krill (t test, $p > 0.05$). However, the elevations were significantly higher in fresh summer krill than in autumn krill or starved summer krill (t test, $p < 0.001$). That is, the difference between summer and autumn and between the immediate post-capture and a week of starvation is a mere decline of the digestive gland length for a given body size. The digestive gland of krill simply shrank in its length without altering the relationship between its length and body length.

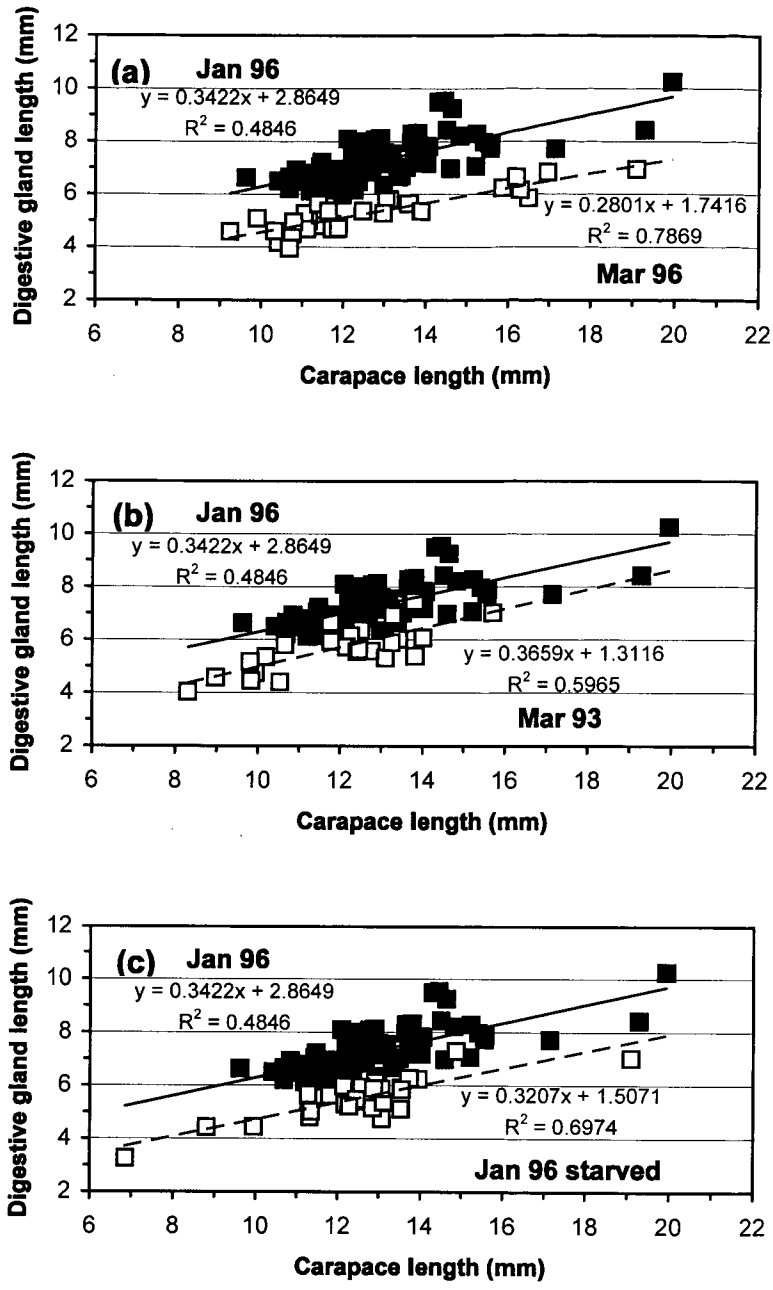


Fig 4.7 Carapace length and digestive gland length of krill collected in mid summer and early autumn, and starved summer krill. (a) Krill sampled in January 1996 and caught later in the cruise, March 1996, (b) krill collected in January 1996 and in late March 1993, (c) fresh summer krill caught in January 1996 and the krill starved for 7 days in Starvation Experiment 2. Solid symbols represent the summer krill and open symbols indicate the autumn or starved specimens

4.5 Discussion

The greatest and most significant change over the period of starvation was in the overall mass of the digestive gland, which confirms visual assessments that the gland was decreasing in size. The mass loss of the digestive gland was immediate once the food supply was withdrawn and evident for the first 3 to 6 days. The absolute amount of protein and lipid in the gland exhibited a similar pattern of decline. Moreover, the decline of lipid seemed more prompt than that of protein. Protein and lipid in the gland will serve both a structural and storage function. The mass loss with starvation indicates utilisation of stored reserve, but in the longer term may reflect breakdown of some structural components. The low level of digestive gland mass reached in 12 days in this experiment indicates an apparent depletion of the reserve material. Lipid appears more readily mobilised during the early phase of the starvation period and this is not surprising given its higher energy content.

The protein and lipid content of the gland at the end of the starvation experiment could be regarded as a baseline level, mostly representing structural components as little further loss was detectable after 12 days. One thing to be noted is that the two major components added up to about half of the dry mass of the digestive gland. As carbohydrate and ash are likely to account for far less than the remaining portion, the current absolute amounts of protein and lipid are likely to be underestimates. This is mostly due to technical aspects of the measurement. For instance, the protein content is determined against the standard protein of known amount, of which the characteristics are not necessarily the same as the specimen protein. The completeness of extraction will also have an effect on the final amount of protein determined and this is the case with lipid as well. Combined contents of protein, lipid, carbohydrate and chitin from whole krill by Torres et al. (1994) also fell well below 100%. Nevertheless, this would not affect the overall trend, i.e. the decline of protein and lipid in the gland, and the apparently more immediate loss of lipid.

The loss over the whole 19 day period can be viewed as a measure of mobilisable reserve. When 3.82 mg of protein and 1.98 mg of lipid are combusted, it would generate 37.05 cal, applying 4.8 cal mg^{-1} protein and 9.45 cal mg^{-1} lipid (Brett and

Groves 1979), and this can be converted to 155.24 J using 1 cal=4.19 J (Pennycuick 1988). A male krill measuring 14.8 mm in carapace length, the average size in Starvation Experiment 1, will be 49.7 mm in total length and 225 mg in dry weight, using the formula for pooled males by Morris et al. (1988). The respiration rate of a krill of this size is estimated to be 2.2 ml O_2 day^{-1} from $QO_2 = 0.985\ W^{0.837}$ in µl O_2 hr^{-1}, the formula compiled by Quetin et al. (1994). The oxygen consumption rate of krill can be converted to an energy requirement of 42.68 J day^{-1}, using an equivalent of 19.4 J ml^{-1} O_2 (Brett and Groves 1979). The calories generated by expending this reserve alone would last the krill for 3.64 days. As already discussed, the amounts of protein and lipid measured in this analysis are almost certainly underestimates, falling short of the dry mass lost. Furthermore, the energetic gain by combusting carbohydrate was not included in this calculation, although it would be much smaller than calculated for protein or lipid. Thus this calculation of calories should be a minimal estimate and normal krill are likely to last somewhat longer by relying on the reserve in the digestive gland.

Instantaneous growth rates of individual krill are estimated by keeping freshly caught krill individually and measuring the uropod (or telson) length increment of the animals that moulted within the first 4 days of capture (Quetin and Ross 1991). The growth rates for the first 4 days are assumed to be representative of natural conditions and the growth rates after this period are considered to have been affected by the laboratory conditions. Given the calorific store in the digestive gland and its rate of utilisation determined in this study, using the growth rates of the first 4 days for representative *in situ* growth appears sound.

The decrease in size of the digestive gland with starvation was evident not only in weight but also in length. Starvation Experiment 2 showed that a decrease both in length and weight takes place immediately with the suspension of food supply. The size decline of the digestive gland appeared to be more pronounced in weight than in length, simply in terms of scale. However, a close relationship between the gland length and weight indicates that the decrease in length and weight are tightly linked. In Starvation Experiment 2, with a shorter duration and with more frequent sampling interval, the change of gland size in a week was substantial and the greatest loss

actually occurred in a single day. Relative digestive gland length as a percentage of carapace length depicted the decrease in its size reliably. The gland length measured from its outline viewed through the carapace was nearly as good as measurement of the length of the actual gland. Dissecting the digestive gland, to measure the decrease in the length, was not essential.

In the short-term starvation experiment, total digestive enzyme activities from a whole gland of krill declined as did the size of the gland. However, the significance of the decline was weak due to high individual variation between animals. The weight-specific activity exhibited even higher variation, which was difficult to explain. There was no obvious difference between days during the experiment and hence no clear trend was shown. Digestive enzyme activities have been examined in a number of zooplankton species, mostly in copepods, in an attempt to develop indicators of feeding activity (Hasset and Landry 1983, 1988, 1990a, b, Head et al. 1984, Harris et al. 1986). However, activities of digestive enzymes have not proved to be a simple and straightforward index of feeding rates (Båmstedt 1988). This experiment used dissected digestive glands and therefore, variation is unlikely to be due to other somatic factors.

Enzyme activities are results of an integration of short-term and long-term acclimation to food environments and to probably diurnal rhythm as well. In this experiment the enzyme activities were measured from the major enzyme secretion organ of the individual animals. The overall trend in enzyme activities was not particularly different from that of the gland size, but with larger individual variation. Apparently, short-term starvation did not lower digestive enzyme activities per unit mass of the gland and the decreases in total activities of the gland most probably occurred by the decrease in the gland size.

The digestive gland mass of krill appears to be the result of recent feeding and is also an indication of size of the reserve. Hence the gland size may well represent the nutritional condition of the individual, and population means of digestive gland parameters could be linked to other relevant features. Nicol et al. (1992a) carried on 'instantaneous growth rate' experiments over an extended period and found that

population growth rates became negative in food-limited conditions after as little as 7 days. They suggested that the initial growth rate coupled with the length of period between capture and the onset of shrinkage may give some indication of the nutritional condition of the krill. The growth rate profile with time from an extended 'instantaneous growth rate' measurement would be a figure, which is dependent on the nutritional condition of the sampled population. The shape of this figure will be determined by the initial growth rates, time to onset of the decline in growth rates and time to onset of shrinkage. Presumably the growth rates will become negative when the immediate energy store becomes depleted. The result from the 'instantaneous growth rate' experiment, which was run parallel with this starvation experiment is presented in Fig. 4.8. Locating the point of decline and the point of reaching zero growth, in the growth rate profile with time, requires some subjective judgement. The working definition of the decline may be set as 2 consecutive drops in the mean growth rates after 4 days and similarly, the onset of shrinkage may be defined as 2 consecutive mean negative rates. The growth rates became negative in about 12 days, a point at which further loss of mass in digestive glands became undetectable. The growth rates started to decline from day 7, after the initial substantial mass loss occurred. To generalise, the initial growth rates ((1) in Fig. 4.8) will be higher in a better-fed population with larger reserve, which will prolong the time to onset of decline in growth rates ((2) in Fig. 4.8) and the time to onset of shrinkage ((3) in Fig. 4.8). Conversely, food shortage will do the opposite, probably resulting in a starting point of a decline which is less clear-cut. The outcome of 'instantaneous growth rate' measurement conducted for an extended period may well be determined by the nutritional condition of the population, which can be indicated by the size of the reserve, the digestive gland.

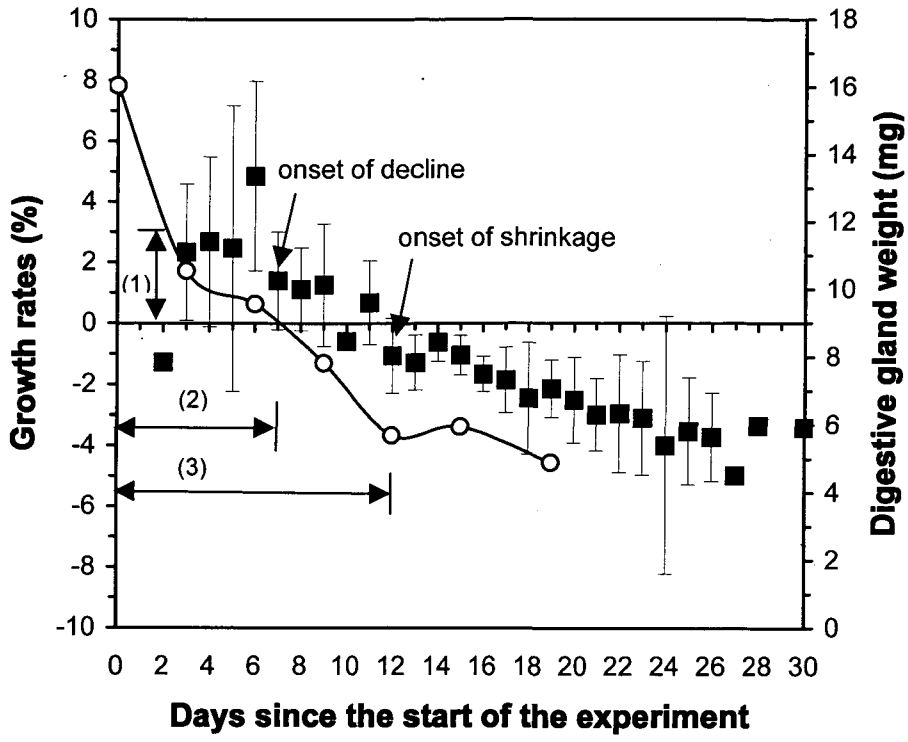


Fig. 4.8 Growth rate-time profile from the 'instantaneous growth rate' experiment run parallel with Starvation Experiment 1 and the decline in the mass of the digestive gland. Solid squares represent the average growth rate of the day with error bars indicating one standard deviation. Open circles represent dry weight of the digestive gland from the animals in Starvation Experiment 1. (1) initial growth rates; the average of the growth rates for the first few days before the decline of growth rates, (2) time to onset of the decline in growth rates, (3) time to onset of shrinkage

There have been a few attempts to use features of the digestive organs of krill as an indication of feeding activity. Morris and Ricketts (1984) used visual assessments of stomach fullness and colour, digestive gland colour and gut fullness as indicators of feeding states while examining the link between feeding, swarming and vertical migration. There was a clear increase in plant pigment with increase in visual classification number for both stomach and digestive gland, in spite of considerable variation in pigment levels within each class, which the authors partially attributed to the size variation of the krill. Antezana and Ray (1984) compared the fluorometric estimates of total pigments for the extreme visual ranks of stomach colour, yellow and black green. While yellow stomachs had significantly lower median values of extracted pigments, the commonest stomach colour, green was not significantly different in fluorescence from either yellow or black green. They also evaluated the relationship between visual differences in stomach fullness and the total stomach pigments, but no significant differences were found. They concluded that the visual estimate of stomach fullness seems inaccurate and imprecise. Visual estimates of stomach fullness have been shown to be correlated with the mass of stomachs of the North Atlantic euphausiid *Meganyctiphanes norvegica* by Nicol (1984). The size of the digestive gland, however, has not been used as an indicator of recent feeding history in any species of euphausiid.

Reported pigment levels in the krill stomach are typically low compared to energetically required ingestion rates. According to estimates by Morris (1985), turnover time of pigment levels is every 3.4 min in the stomach and every 7.3 min for the digestive gland. These high rates, in fact, seriously compromise the usefulness of stomach pigments or related features to serve as an index of recent feeding. Stomach content or fullness can also possibly be affected by immediate past events such as cod-end feeding which has little to do with genuine feeding history. Evidence of net-feeding by euphausiids (Hirota 1984, Nicol 1984) renders the use of stomach fullness as a measure of recent feeding activity more unreliable. As the food does not reach the digestive gland instantly, the size of digestive gland would not respond too sensitively to an immediate past event, particularly when it is of short duration, such as cod-end feeding.

Even though the size of the gland was not their major concern, Endo and Kadoya (1991) reported that the quantity of the plant pigment in the digestive gland of krill dropped off during the first 6 days of starvation and appeared to level off over the next 6 days. The purity (vividness) of the digestive gland colour followed the same trend as for the pigment quantity, and phaeopigments represented 94-99 % of the total pigments present in the gland. These results are in good agreement with the findings from this study. A range of 12 colouration types have been described for determining feeding intensity in Antarctic krill by scientific observers on krill fishing vessels (CCAMLR 1999) but the relationship between the colour and the previous feeding history has not been quantitatively examined. Recently, Kawaguchi et al. (1999) showed that the proportion of krill that have green digestive glands can be an indication of the size of algal species available to krill as food. Probably the size of the digestive gland in combination with its colour will provide more information, indicating feeding intensity and possibly the type of food.

The digestive gland of krill is a crucial storage organ, containing reserve material for immediate use, which can back up short-term food shortage. It is a large, easily identifiable organ which can be dissected out simply from freshly caught or experimentally manipulated krill. Information on the recent feeding history of krill can be obtained from the digestive gland and it is more likely to yield a clear signal than more traditional methods that utilise whole animals. From the present study, it is evident that the simple measure of digestive gland size, the weight of the dissected gland or even the length as measured by its *in situ* outline can provide information on the recent feeding of individual krill covering the period of a few days to a week's duration. As an indicator, it will be more reliable than measuring digestive enzyme activities. There was a clear difference in the digestive gland length between the summer animals and the autumn specimen, and this may well reflect a seasonal difference in feeding intensity. Further experimentation involving the manipulation of food regimes will provide more insight in the longer term. Yet this measure appears to be sufficiently robust to qualify it as a standard field index for determining the 'condition' of krill.

Chapter 5. Changes in the digestive gland of juvenile Antarctic krill during a long-term feeding experiment

5. 1 Abstract

Changes in the size and digestive enzyme activities in the digestive gland of Antarctic krill were investigated during a long-term feeding experiment. Krill were either given excess food or starved for 15 weeks and then this feeding regime was reversed for the following 13 weeks. The length and weight of the digestive gland of fed krill increased substantially whereas the gland of starved krill remained small. With the switch of the food regime, the gland of the 'previously fed and then starved' animals shrank, and that of 'starved and then fed' krill became enlarged rapidly over a few days. Digestive enzyme activities showed very high individual variation. On average, total digestive enzyme activities from a whole gland changed in response to food condition - this appeared to be largely a reflection of the changes in the gland size. Activities per unit weight of the gland failed to show clear trends under constant conditions. Starvation, even for long periods, does not seem to depress weight-specific enzyme activity. When constant feeding regimes were abruptly reversed, amylase and laminarinase tended to show short-term responses: enhancement under starvation and decline in response to feeding. This created a shift in ratios between different enzyme activities, notably of trypsin to laminarinase with the change of the food regime. The mass of the gland appears to matter most in determining the capacity of krill in terms of digestive enzymes under most conditions. Prior acclimation may well affect the enzyme index strongly but it does not seem to play a role in the mass gain or loss of the gland. The size of the gland is a simple and direct integration of recent feeding history. These results indicate that the digestive gland of krill can serve as a cushion against short-term starvation, which is readily expendable and quickly re-buildable. At a minimum, the size of the digestive gland should be a simple measure of whether krill have recently undergone sustained starvation and thus is applicable to studies examining seasonal and regional feeding states.

5.2 Introduction

Antarctic krill (*Euphausia superba*, hereafter krill) is a keystone species in the Antarctic marine ecosystem, being relied upon by most of the higher predators and sometimes exerting significant impacts on lower levels of the food web (Laws 1985, Miller and Hampton 1989, Atkinson et al. 1999). As in any other animal species, the rate of feeding will essentially determine growth rates and the physiological condition of krill. Since krill does not have a sufficiently large lipid reserve, it has developed physiological plasticity to deal with the highly pulsed primary production of the Antarctic marine system. A variety of strategies, from carnivory to starvation, have been suggested which would enable Antarctic krill to exploit varying food environments (Ikeda and Dixon 1982, Price et al. 1988). However, investigations of the feeding status of krill in the wild have largely been lacking. Recently it has been suggested that sea ice extent during winter and the winter biology of krill are critical to the success of krill populations in the following years (Siegel and Loeb 1995, Loeb et al. 1997). It has become established that krill can utilise sea-ice biota as a food source (Marschall 1988, Stretch et al. 1988) but the extent and importance of this during winter remains to be clarified. Indeed, there are a number of different circumstances for which the knowledge of how actively krill are feeding and how well they are likely to grow is crucial.

While experimentation has helped greatly to find explanations and mechanisms for many aspects of krill biology, compilation of field-collected evidence is necessary to address the above questions. On-board experimentation often involves labour intensive effort and is unsuitable for assessing a large number of samples encompassing the whole range of areas and timings investigated. To have a large scale depiction along the gradient of the factors concerned, it is necessary to have relatively easy ways of assessing the feeding related features of krill.

In the previous chapter it was shown that the digestive gland of krill serves as a crucial storage organ containing a reserve that can back up short-term food starvation. The size of the gland will indicate the size of the reserve that krill have accumulated for immediate use. The size of the gland can potentially be a simple

indicator of recent feeding activity, particularly when krill experienced a food shortage.

The long-term response of the digestive system in krill has not been studied so far. The effect of sustained absence or inadequacy of food on the digestive enzyme system is unknown, and its recovery after a period of food limitation is yet to be examined. The digestive enzyme activities may not behave, at least in the short term, in an easily predictable fashion as they integrate over a number of factors, particularly acclimation to changing food conditions. However, when they are measured from contrasting settings that have been maintained for extended periods, they may reflect past feeding history over the longer term. The response of the digestive enzymes to long-term starvation has not been investigated and may provide clues regarding the ability of krill to survive food shortage.

To understand how the digestive gland and its enzyme system respond to changing food environments, it is necessary to establish some reference points in the laboratory, for possible use in the field. This study investigated the changes in digestive gland, including enzyme activities, upon being given excess food and starvation, and subsequent reversal of the food regime, with an ultimate goal of developing and establishing indicators which could provide information on the long-term seasonal food environment krill have experienced.

5.3 Materials and methods

Experimental animals

A large number of juvenile krill were collected on 15 April 1997 19:47 (local time) at 64°19' S, 110°57' E, just north of the ice edge in East Antarctica with an RMT 1+8. The krill were kept in a cold room on board while being transported to the home aquarium. Then they were maintained in the dark at 2±0.5 °C under sub-optimal food concentrations until the experiment started at the beginning of August 1997. A mixture of 3 cultured algal species, *Phaeodactylum tricornutun*, *Gemingera criophylum*, *Pyramimonas* sp. was given to krill in tanks approximately once a week. Limited capacities of the aquarium to process the waste load did not allow a high level of ration to support active growth of krill.

Organisation of the experiment

The experimental animals were selected to cover a narrow size range (approximately 7.5 mm in carapace length, around 24 mm in total length) and kept individually in 2 litre plastic jars at 0 °C in the dark. An initial group of 22 krill were removed at the start of the experiment. For a week before the start of the experiment, no new algal food was given from a concern that it might induce an unusual boost in the initial group of animals.

Phase 1

One group was fed *Phaeodactylum tricornutum* at $5x10^5$cells ml^{-1} to attain maximal growth rates (Ikeda and Thomas 1987a) and the other group was placed in filtered seawater. Water exchange and food provision were made every 3 to 4 days. For half of each group, fed and starved, 12 animals were sacrificed after 5, 10 and 15 weeks. The animals were weighed wet, and the carapace length and the length of the digestive gland from the outline viewed through the carapace were measured. Then the digestive glands were dissected out and placed in a labelled tube, quick frozen in liquid nitrogen and stored in a -86 °C freezer until analyses. For the other half of each group, the animals, at each moult, were taken out and weighed and their pictures were taken with an image analysis system. The krill were then replaced in the experimental jars. From the image captured, carapace length and length of the digestive gland were measured. For the regular sampling, only animals that had not

been distressed in the image analysis procedure were meant to be used, however, at week 15, about half of the animals came from the monitored and hence disturbed group.

Phase 2

After 15 weeks, the food regime was reversed; animals which had been fed were starved and the previously starved animals were fed. The switched food regimes were maintained for a further 13 weeks. Up to 5 krill from the 'fed and then starved' group were sampled on Day 3, 7, 14, 28 and 91 after the reversal, and 5 animals from the 'starved and then fed' group on Day 2, 5, 9, 14, 28 and 91. All the krill were monitored at moult and 5 selected krill were monitored more frequently for the first 4 weeks following the switch.

Enzyme activity measurement

Digestive glands were weighed frozen and then ground with a plastic pestle in a 1.5 ml microtube containing 750 µl of distilled water. The homogenate was centrifuged at 10 000 g for 10 min and the supernatant was collected for use in enzyme assay. In absence of a single buffer to provide optimal conditions for all 3 enzymes, the gland was homogenised in distilled water. However, the subsequent assay protocols included buffers to generate optimal conditions for each enzyme. Some further dilutions, when necessary, were made in consideration for the working range of the assay protocols.

Trypsin activity was assayed in accordance with Erlanger et al. (1961). Trypsin hydrolyses the synthetic substrate, benzoylarginine-p-nitroanilide to give benzoylarginine and p-nitroaniline and the concentration of the product, p-nitroaniline liberated for 15 min was spectrophotometrically measured at 405 nm. One unit (U) is the enzyme activity that causes 1 µmole of substrate to react in 1 min at 25 °C.

Amylase activity was assayed using the Sigma amylase assay kit (No. 577) based on the method of Wallenfels et al. (1978). It relies on a coupled enzyme reaction; amylase and glucosidase hydrolyses 5 moles of 4,6-ethylidiene-G_7PNP into 4 moles of p-nitrophenol and 10 moles of glucose. The change in absorbance of p-

nitrophenol during 2 min reaction was measured at 405 nm following a 2 min lag period. The unit was defined as mmoles of substrate to react per min at 37 °C. Laminarinase activity was assayed by a modification of Head et al. (1984). The homogenate (50 µl) was incubated with 1 M sodium acetate buffer, pH 5.5 (100 µl) and 100 µl of laminarin solution (25 µg ml^{-1}) for 30 min at 37 °C and the reaction was terminated by placing the sample in boiling water for 3 min. The amount of glucose released by the reaction was measured with Sigma Glucose kit (No. 16). The unit was defined as release of glucose in µg min^{-1}.

5.4 Results

Growth of the animals

Although the animals were selected to cover a narrow size range at the start of the experiment, there was no control over the size of the animals harvested on subsequent sampling occasions. However, it was evident that animals grew both in length and weight upon being well fed and when starved, showed negative growth, particularly in weight (Fig. 5.1 and Table 5.1). The initial group of krill were 7.5 (±0.6) mm in average carapace length and weighed 137.7 (±43.85) mg on average. After 15 weeks, animals of the fed group averaged 9.9 (±0.5) mm and 258.2 (±42) mg while the starved ones averaged 7 (±0.6) mm and 100.8 (±28.2) mg. When the feeding regime was reversed after 15 weeks, it was again apparent, despite the smaller sample size, that the animals grew or shrank in response to the food regime.

Changes in the size of the digestive gland

In Phase 1, the mean length of digestive gland of well-fed krill increased in 15 weeks from 2.8 (±0.3) mm to 5.0 (±0.3) mm. The fresh weight of the gland also increased steadily from 4.2 mg to 17.6 mg. The digestive gland length of starved krill declined slightly from 2.9 to 2.6 mm during Phase 1 and the average gland weight was between 2.8 and 4.2 mg (Fig. 5.2 and Table 5.1). During Phase 2 following the switch of the food regime, the gland of the 'previously fed and then starved' animals shrank back to 2.9 mm and 5.2 mg. The gland of the 'starved and then fed' krill grew in size to 4.3 mm and 11.4 mg by the end of the experiment, 13 weeks after the switch. Fig 5.2 shows that the change in digestive gland size, particularly in length, in response to the switch of the food regime occurred rapidly.

The relative size of the digestive gland with respect to body size also responded in a similar fashion. The percentage of the gland weight to body weight was initially around 3 %. It rose to the level of nearly 7 % in 10 weeks of feeding, and then levelled off. Under continued starvation, it remained around 3 % for 15 weeks (Fig. 5.2 and Table 5.1). The relative sizes of the gland were significantly different

between fed and starved krill by 5 weeks (ANOVA, $F=118.7$, $p=2x10^{-10}$, $df=23$). The relative gland weight of the well-fed krill, in response to a switch to starvation, decreased substantially in 2 weeks and weight percentage declined to the initial level of 3% for that period. Krill that had been starving also appeared to recover when fed well, reaching the level of 7 % of body weight in 2 weeks. However, animals sacrificed 4 and 13 weeks after the switch, had rather low percentages (5.46 and 4.97 % of body weight), suggesting that prolonged starvation may have hindered a full recovery in some individuals.

The change in the gland length displayed a similar pattern to that of the weight. The proportion of digestive gland length over carapace length substantially increased during the first 10 weeks of feeding from 38 % to 51%. Then it remained around that level until week 15, the end of Phase 1. With a switch to starvation in Phase 2, it started to decrease promptly and in 4 weeks, came down to 37%, which was almost the same as the initial level. By the end of the experiment, it decreased further to 33%. For the initially starved animals, the proportion of gland length to carapace length stayed at the initial level during the whole 15 weeks of starvation and immediately started to rise when switched to full feeding, reaching the level of 50% in 4 weeks. When the experiment was completed, the relative gland length was slightly below 50%.

Under the experimental conditions it took only a few weeks for the gain or loss of the digestive gland size to be clearly apparent. The response of the digestive gland size to feeding or starvation was relatively rapid and constant.

Digestive glands became enlarged in feeding animals of Phase 1, since they had been feeding only marginally prior to the beginning of the experiment. Frequent monitoring of newly fed animals in Phase 2, that had been previously starving in Phase 1, confirmed this and the build-up of the digestive gland occurred rapidly in a matter of days (Fig 5.3). The digestive gland was quickly re-built in 2 to 4 days and then continued to increase slowly for approximately 3 weeks. The change in body length at moult did not seem to greatly affect the overall trend.

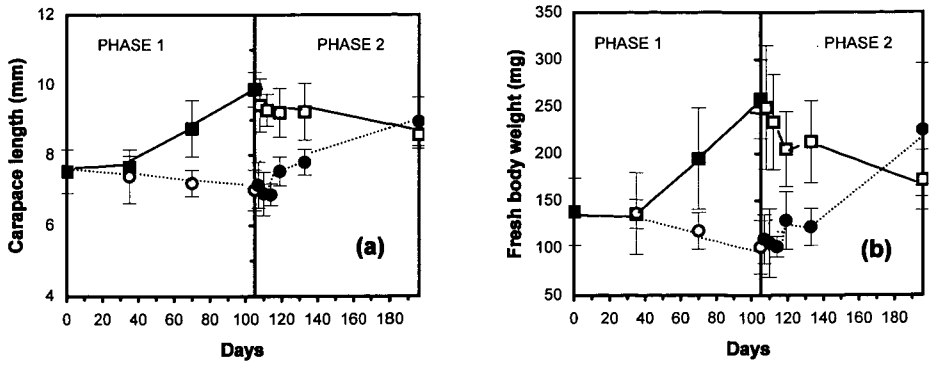


Fig 5.1 Change in body size of the krill. (a) Carapace length and (b) fresh body weight. Day 105 is the point of food regime reversal and hence the point of change from Phase 1 to Phase 2. Squares represent the 'fed and then starved' group and circles the 'starved and then fed' krill. Solid symbols indicate fed specimens and open ones starved animals. Error bars denote one standard deviation

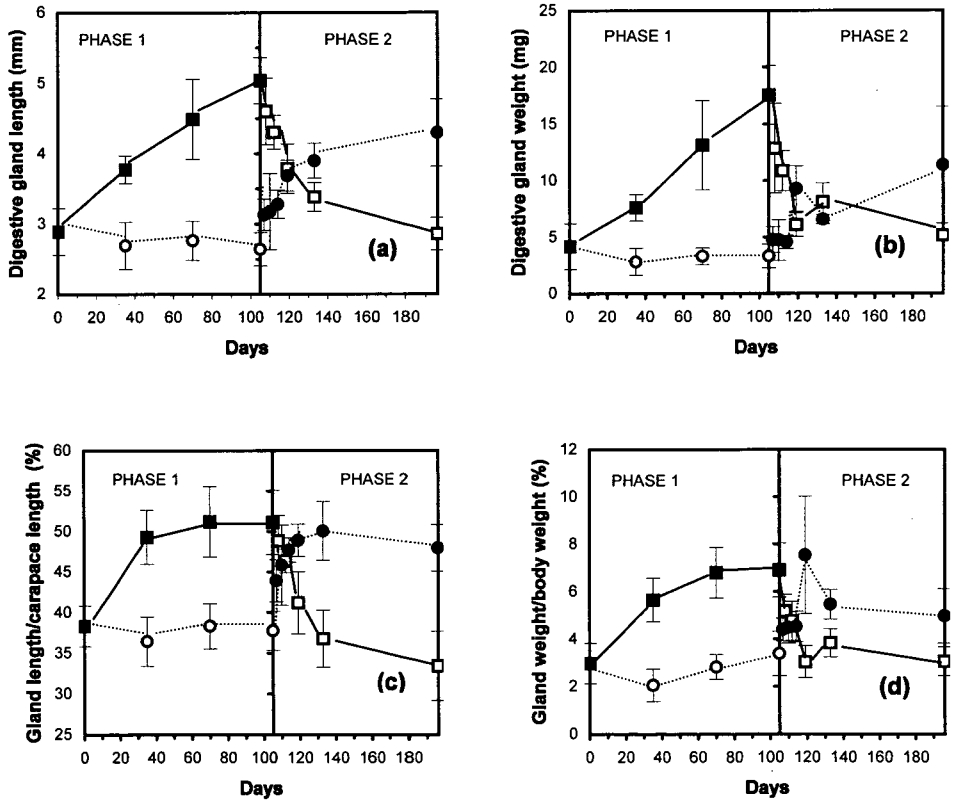


Fig 5.2 Change in digestive gland size of the krill. (a) Gland length and (b) fresh weight of gland, (c) percentage of carapace length occupied by the gland, (d) percentage of gland weight to body weight. Day 105 is the point of food regime reversal and hence from Phase 1 to Phase 2. Squares represent the 'fed and then starved' krill and circles the 'starved and then fed' animals. Solid symbols indicate fed specimens and open ones starved animals. Error bars denote one standard deviation

Table 5.1 Summary of changes in body size and digestive gland size of the experimental krill

		Start	Phase 1			Phase 2							
	Day	0	35	70	105	107	108	110	112	114	119	133	196
	Number of animals												
Fed and then starved	n	22	12	12	11		5		5		5	5	2
Starved and then fed	n	22	12	11	12	5		5		3	5	5	3
	Carapace length (mm)												
Fed and then starved	Mean	7.54	7.67	8.77	9.87		9.42		9.28		9.21	9.24	8.58
	SD	0.62	0.32	0.80	0.50		0.76		0.45		0.70	0.81	0.40
Starved and then fed	Mean	7.54	7.39	7.20	7.00	7.14		6.90		6.87	7.54	7.80	8.95
	SD	0.62	0.77	0.37	0.58	0.65		0.61		0.31	0.41	0.37	0.70
	Fresh body weight (mg)												
Fed and then starved	Mean	138.3	135.8	194.7	258.2		249.2		233.6		205.0	212.8	171.5
	SD	35.7	15.2	53.9	42.0		65.8		50.6		39.8	43.7	31.8
Starved and then fed	Mean	138.3	136.5	117.9	100.8	109.4		105.2		101.3	129.2	122.2	224.7
	SD	35.7	43.2	19.3	28.2	25.9		36.3		11.0	30.6	19.6	70.7

Table 5.1 (continued)

	Digestive gland length (mm)										
Fed and then starved	Mean	2.9	3.8	4.5	5.0	4.6	4.3	3.8	3.4	2.9
	SD	0.3	0.2	0.6	0.3	0.5	0.2	0.4	0.2	0.2
Starved and then fed	Mean	2.9	2.7	2.8	2.6	3.1	3.2	3.3	3.7	3.9	4.3
	SD	0.3	0.3	0.3	0.2	0.2	0.5	0.2	0.2	0.2	0.5
	Digestive gland weight (mg)										
Fed and then starved	Mean	4.2	7.6	13.1	17.6	12.9	10.9	8.1	6.1	5.2
	SD	2.0	1.2	3.9	2.6	4.0	1.8	1.7	1.0	0.1
Starved and then fed	Mean	4.2	2.8	3.3	3.3	4.8	4.7	4.6	6.6	9.3	11.4
	SD	2.0	1.2	0.7	1.0	1.1	1.8	0.6	0.4	2.0	5.2
	Digestive gland length/carapace length (%)										
Fed and then starved	Mean	38.3	49.3	51.2	51.1	48.8	46.4	41.2	36.8	33.4
	SD	2.5	3.3	4.3	4.0	3.1	1.5	3.8	3.5	3.0
Starved and then fed	Mean	38.3	36.4	38.3	37.8	43.9	45.9	47.7	48.9	50.1	48.0
	SD	2.5	3.0	2.8	2.4	2.6	5.0	1.5	2.0	3.6	2.9
	Digestive gland weight/body weight (%)										
Fed and then starved	Mean	3.0	5.7	6.8	6.9	5.2	4.8	3.0	3.8	3.1
	SD	0.9	0.9	1.1	1.1	0.7	0.8	0.7	0.6	0.6
Starved and then fed	Mean	3.0	2.1	2.8	3.4	4.4	4.5	4.5	7.5	5.5	5.0
	SD	0.9	0.7	0.5	0.9	0.5	0.7	0.6	2.5	0.6	1.1

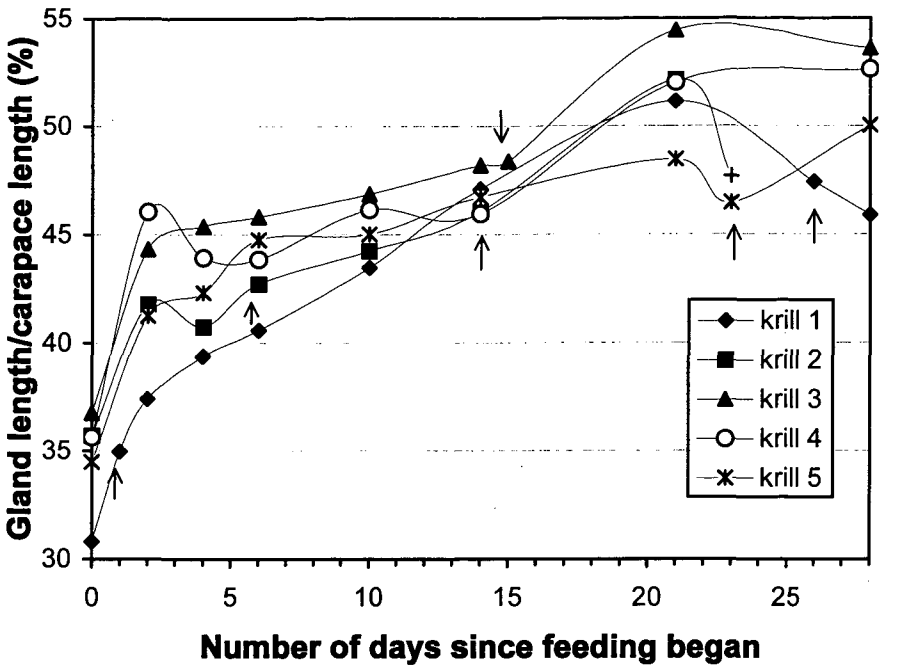


Fig. 5.3 Enlargement of the digestive gland of the newly fed krill in Phase 2 after a long period of starvation during Phase1. Arrows indicate moulting and a cross denotes the death of the animal

Digestive enzyme activities

All three of the digestive enzyme activities exhibited a high degree of individual variation. The coefficient of variation (CV) in enzyme activities often reached 70%, whereas the CVs of the relative gland size were usually below 10 % in the case of length and below 30 % in weight.

Trypsin

Total trypsin activity in the gland of the individual animal increased with feeding by a factor of 7 in 15 weeks (from 11 mU $gland^{-1}$ to 78 mU $gland^{-1}$) and did not change substantially with starvation, ranging between 8.5 to 12 mU $gland^{-1}$ (Fig. 5.4 a and Table 5.2). This trend in total activity appears to be mostly due to the change in size of digestive gland mass. Weight-specific activity expressed as activity per unit weight of the gland increased moderately with feeding (2.4 to 4.4 mU mg^{-1}) and to a lesser degree with starvation (2.4 to 3.7 mU mg^{-1}) (Fig 5.4 b). Overall, the change in weight-specific activity in relation to feeding regime was not as pronounced as in total activity.

With the reversal of the food regime, total enzyme activity of a whole gland again showed a pattern of decreasing with starvation and increasing with feeding. Following a switch to starvation, total activity of the previously fed krill decreased to 38 mU $gland^{-1}$ in 2 weeks. Weight-specific activity after the reversal, however, remained high for the following 4 weeks, around 5-6 mU mg^{-1}. With a switch to full feeding, total activity of starved animals started to rise in 2 weeks from 10 to 40 mU $gland^{-1}$ in a similar pattern to the size of digestive gland. Weight-specific activity also increased to 4.5 mU mg^{-1} from 2.5 mU mg^{-1} in 2 weeks.

Amylase

When krill were well fed, total amylase activity increased substantially from 30 to 150 mU $gland^{-1}$ in 15 weeks (Fig 5.4 c and Table 5.2). Total activity of starved animals remained around 30 to 44 mU $gland^{-1}$. As with trypsin, the total activity appears to be much related to the mass of the digestive gland. Full feeding did not bring about a great change in weight-specific activity; the activity ranged between 5

and 10 mU mg^{-1} for the 15 weeks, lacking any clear upward or downward trend. On the other hand, weight-specific activity of starved animals gradually increased from 7.7 to 13.4 mU mg^{-1} in 15 weeks but with a large individual variation.

Responding to a switch to starvation, weight-specific activity rose from 10 to above 20 mU mg^{-1} (Fig 5.4 d). Despite the cut in food supply, total activity seemed to be on a rise for the first few days and reached a level of 217 mU $gland^{-1}$ a week after the switch. Although there was a rather irregular drop after 2 weeks following the switch, this enhanced level of amylase activity appeared to have been maintained for the first 4 weeks after the switch. However, total activity eventually decreased below 50 mU $gland^{-1}$, the initial level, by the end of the experiment, 13 weeks after the reversal. Starved animals subjected to full feeding exhibited a slight decline in weight-specific amylase activity, down to 7 from 13 mU mg^{-1}. Total activity rose slowly and it took 2 weeks to become elevated to the level of 80 mU $gland^{-1}$ and then slightly decreased again. It is notable that the highest level of amylase activity was encountered when the animals were subject to an abrupt cut in algal food supply.

Laminarinase

Total laminarinase activity of well-fed krill rose considerably from 2.75 to 9.75 mU $gland^{-1}$ in 10 weeks, which was even higher than 7.13 mU $gland^{-1}$ at week 15 (Fig 5.4 e). Total activity of starved animals remained between 2.3 and 4.1 mU $gland^{-1}$ level. Total laminarinase activity also appeared to be dependent on the size of the gland. Weight-specific activity of laminarinase decreased slightly from 0.64 to 0.42 mU mg^{-1} when the krill were well fed. Upon starvation it slowly rose from 0.64 to 1.26 mU mg^{-1} level.

With a switch to starvation, weight-specific activity started to rise readily to 1.54 mU mg^{-1} in 2 weeks, and was, overall, on a rise for 4 weeks following the switch (Fig 5.4 f). Because of this enhanced specific activity, total activity exhibited no great change for this period, rising first and then declining only slightly. However, weight-specific activity fell to a low level of 0.35 mU mg^{-1} eventually, by the end of the experiment. Accordingly, total laminarinase activity was at as low a level as in the initial stage of the experiment, 13 weeks after the switch to starvation.

Starved krill when switched to full feeding, responded with a significant short-term decline in weight-specific activity. It fell to 0.42 mU mg^{-1} in 2 weeks after the switch from 1.26 mU mg^{-1}, which was a peak value after 15 weeks of starvation. As a result of this lowered specific activity upon a switch to feeding, total activity remained at a similar level for the first 4 weeks, still around 3 mU $gland^{-1}$ but eventually became far higher at 6.47 mU $gland^{-1}$ until the end of the experiment, 13 weeks after the switch.

Ratio between different enzyme activities

Trypsin activity tended to remain rather stable in weight-specific terms whether well fed or starved. Its total activity from a whole gland rose and fell as the gland gained and lost its mass. On the other hand, activity of carbohydrate-degrading enzymes, particularly laminarinase, tended to become elevated for a few weeks when facing starvation. This created a change in the ratios between different enzyme activities (Fig 5.5 and Table 5.3). The ratio of trypsin activity to that of laminarinase started with 3.8 and became elevated to 10.3 in 15 weeks of feeding. Then, with a switch to starvation, it rapidly declined to the level of just above 4 in 2 weeks. Further on, it remained at that level until 13 weeks after the switch. Meanwhile the ratio of starved animals remained below 4 during Phase 1. With the commencement of feeding, the ratio of the 'starved and then fed' group rapidly rose to the level of 10 in 2 weeks. Although the ratio started to decline then, it still remained above 5, by the end of the experiment. Generally, full rations resulted in higher trypsin/laminarinase ratio and starvation was associated with low trypsin/laminarinase.

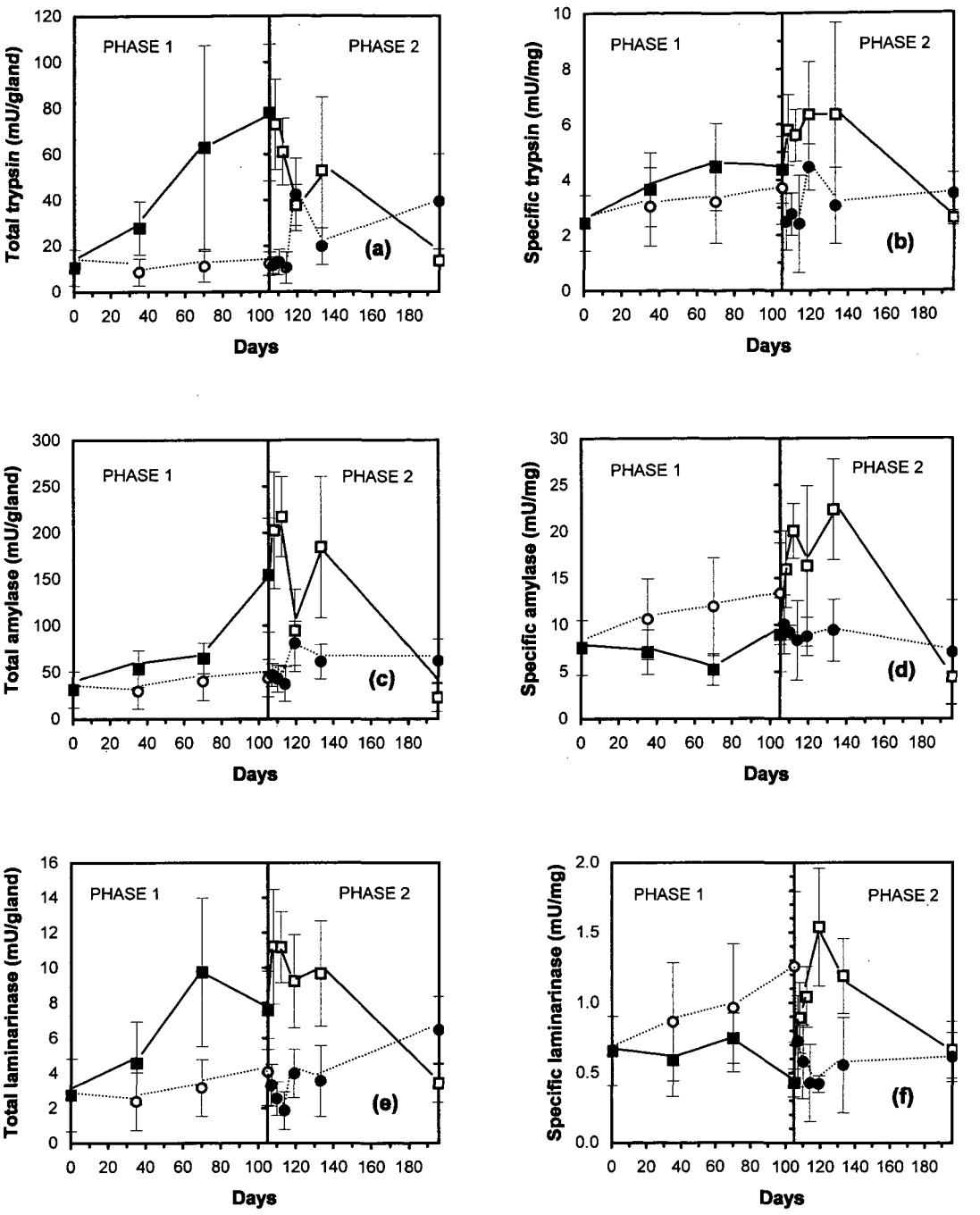


Fig 5.4 Changes in digestive enzyme activities in the digesive gland of the krill. Results are given in total activity from a whole gland (mU gland^{-1}) and weight-specific activity (mU mg^{-1} fresh wt). (a) Total trypsin, (b) weight-specific trypsin, (c) total amylase, (d) weight-specific amylase, (d) total laminarinase, (e) weight-specific laminarinase. Day 105 is the point of food regime reversal. Squares represent the 'fed and then starved' group and circles the 'starved and then fed' krill. Solid symbols indicate fed krill and open ones starved animals. Solid lines follow the change in the 'fed and then starved' group and broken lines track the change in the 'starved and then fed' animals. Error bars denote one standard deviation

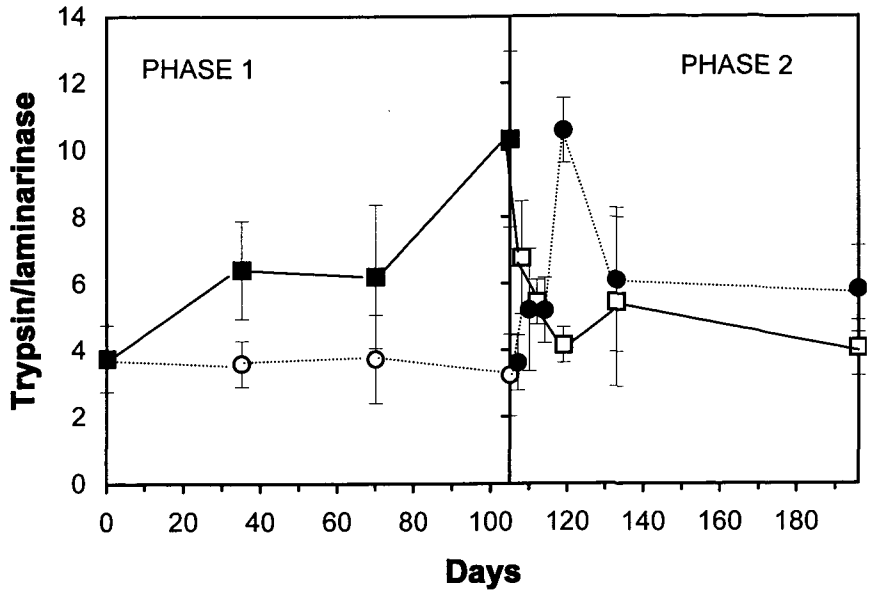


Fig 5.5 Change in the ratio of trypsin activity over laminarinase activity in the digestive gland of the krill. Day 105 is the point of food regime reversal and hence the point of change from Phase 1 to Phase 2. Squares represent the 'fed and then starved' krill and circles the 'starved and then fed' animals. Solid symbols indicate fed krill and open symbols starved animals. Solid line follows the change in the 'fed and then starved' group and broken line tracks the change in the 'starved and then fed' krill

Table 5.2 Changes in the digestive enzyme activities in the digestive gland of the experimental krill

		Start	Phase 1			Phase 2							
	Day	0	35	70	105	107	108	110	112	114	119	133	196
	Total trypsin (mU/gland)												
Fed and then starved	Mean	10.5	27.8	62.9	78.0		73.0		60.9		37.6	52.8	13.4
	SD	7.9	11.7	44.4	29.8		19.7		14.5		9.0	32.0	1.5
Starved and then fed	Mean	10.5	8.5	11.0	12.1	11.4		12.9		10.4	42.3	19.7	39.3
	SD	7.9	5.8	6.7	5.1	3.6		5.5		6.9	15.8	8.0	20.8
	Weight-specific trypsin (mU/mg fresh weight)												
Fed and then starved	Mean	2.4	3.6	4.5	4.4		5.8		5.6		6.3	6.3	2.6
	SD	1.0	1.3	1.6	1.2		1.3		0.9		1.9	3.3	0.3
Starved and then fed	Mean	2.4	3.0	3.2	3.7	2.5		2.8		2.4	4.5	3.1	3.5
	SD	1.0	1.4	1.5	1.3	1.0		0.8		1.8	0.8	1.4	0.8
	Total amylase (mU/gland)												
Fed and then starved	Mean	31.5	53.7	64.9	154.3		202.7		217.3		94.5	184.5	22.8
	SD	19.3	19.7	16.3	61.5		63.0		43.3		44.0	76.2	14.9
Starved and then fed	Mean	31.5	29.5	40.2	43.7	46.9		42.8		37.2	80.8	61.1	62.1
	SD	19.3	18.5	18.5	20.0	12.4		14.3		18.5	23.7	18.5	23.2
	Weight-specific amylase (mU/mg fresh weight)												
Fed and then starved	Mean	7.6	7.1	5.2	9.0		15.9		20.1		16.3	22.4	4.4
	SD	2.9	2.4	1.7	4.0		4.1		2.9		8.6	5.4	2.9
Starved and then fed	Mean	7.6	10.6	12.0	13.3	10.0		9.2		8.4	8.8	9.4	7.1
	SD	2.9	4.3	4.4	5.4	3.1		0.8		4.2	2.0	3.3	5.5

Table 5.2 (continued)

	Total laminarinase (mU/gland)											
Fed and then starved	Mean	2.75	4.57	9.75	7.58		11.21		11.17		9.24	9.67	3.43
	SD	2.08	2.36	4.23	2.21		3.27		2.03		2.65	2.99	1.09
Starved and then fed	Mean	2.75	2.39	3.17	4.07	3.31		2.54		1.86	3.98	3.56	6.47
	StDev	2.08	1.66	1.62	1.92	1.19		0.97		1.08	1.38	2.02	1.89
	Weight-specific laminarinase (mU/mg fresh weight)											
Fed and then starved	Mean	0.66	0.59	0.74	0.43		0.89		1.04		1.54	1.19	0.66
	SD	0.25	0.26	0.18	0.10		0.25		0.22		0.42	0.27	0.20
Starved and then fed	Mean	0.66	0.86	0.96	1.26	0.73		0.58		0.43	0.42	0.56	0.61
	SD	0.25	0.42	0.46	0.54	0.32		0.26		0.28	0.06	0.34	0.18

Table 5.3 Change in trypsin/laminarinase ratio of the experimental krill

		Start	Phase 1			Phase 2							
	Day	0	35	70	105	107	108	110	112	114	119	133	196
Fed and then starved	Mean	3.8	6.4	6.2	10.3		6.8		5.4		4.1	5.4	4.1
	SD	1.0	1.5	2.2	2.6		1.7		0.7		0.5	2.5	0.8
Starved and then fed	Mean	3.8	3.6	3.7	3.2	3.6		5.2		5.2	10.6	6.1	5.8
	SD	1.0	0.7	1.3	1.2	0.8		1.8		1.0	1.0	2.2	1.3

5.5 Discussion

It was shown in the previous chapter that the digestive gland of krill serves as a short-term storage organ and that its size both in length and weight responds sensitively to food shortage. At the start of this experiment, the digestive gland of the krill appeared to have been already at a baseline level with little reserve, as further starvation did not cause substantial change. The size of the gland increased or decreased significantly in response to either favourable food conditions or starvation maintained for an extended period. These changes in size occurred over a relatively short period of time, while changes in digestive enzyme activities failed to show a distinct, predictable pattern.

The digestive gland receives macerated food material from the stomach and conducts further processing, mainly nutrient absorption (Ullrich et al. 1991). Without constant input, the gland will begin to lose its mass. According to Perissinotto and Pakhomov (1996), gut passage time is estimated to be 2.3 to 9.9 h under continuous feeding conditions. This period may be lengthened when the food supply is suspended. Still, the ingested material will leave the krill's gut in a matter of a few days at the longest, after the nutrition is absorbed. When there is a constant input of food, nutrient absorption will continue, which will lead to a mass increase of the gland. In this respect, the gland mass is not quite the same as the stomach fullness or gut fullness, which is merely the extent of filling.

In a week-long starvation experiment described in the previous chapter, the digestive enzyme activities per unit mass of the digestive gland in field-caught krill was not greatly affected. However, the mass loss itself was substantial and thus total activities from a whole gland declined with starvation. In this experiment, extreme food treatments were maintained for a much longer period, which might generate more fundamental, longer lasting effects. The result from this long-term starvation experiment, in general, is that total digestive enzyme activities from a whole gland rose and declined in response to food conditions as did the size of the gland. However, the significance of the increase or decrease in the enzyme activities was made weaker due to high individual variation. The weight-specific activity exhibited

even higher variation than for the total activities as was the case in the short-term starvation experiment. This individual variation is difficult to explain. Fed juvenile krill tended to have elevated weight-specific activities when they faced starvation. The krill which had been well fed during Phase 1, in particular, showed sharply enhanced activities upon sudden starvation. This was more pronounced in amylase and laminarinase than in trypsin. Cox (1981) showed that, in *Euphausia pacifica*, the withdrawal of algal supply but replacement with *Artemia* nauplii brought a clear decline in laminarinase activity, which was enhanced again by the resupply of algal culture. A similar decline was not observed in this experiment when the algal supply was removed, although the algae had not been replaced by other food. On the other hand, the weight-specific activities of these two enzymes in the previously starved krill declined when the food supply was resumed. McConville et al. (1985) observed that laminarinase activity in krill that were feeding for 5 days but had been starved for 7 days prior to feeding was lower than that of krill that were starving for 12 days throughout. This is in agreement with the current results.

Evidently starvation, even for a long period, does not seem to depress the weight-specific activities of digestive enzymes. Generally speaking, poorly fed krill, which have been acclimated to low food concentration, did not show an increase in weight-specific activity when sufficient food became available, but their enlarged gland size covered the situation. This relatively unchanging weight-specific activity under a long starvation may imply that the digestive system of krill is in a state of permanent readiness. The digestive system of krill does not seem to enter a dormancy-like state even after a sustained absence of food. Their total digestive enzyme activity will rise when there is a sufficient food supply as the size of the gland increases accordingly. The magnitude and change of total activities were largely a reflection of the gland size. The gland size rather than enzyme activities provided much clearer evidence of recent active feeding or starvation. Without knowledge of the feeding history, the results of enzyme activity measurements would be very difficult to interpret. Kolakowski (1989) reported a marked increase in the activity of proteinase determined in whole krill with the onset of feeding season. Although the author interpreted this as a lack of autoproteiolysis during winter, this is more likely to be the reflection of enlarged digestive gland size due to intensified feeding. Mayzaud et

al. (1985) measured activities of a range of digestive enzymes in the cephalothorax of krill collected in the Indian sector of the Southern Ocean. They found that smaller subadult animals from western stations had lower activities than the larger animals found in the eastern area, which they could not relate to their food availability index but attributed to different physiology resulting from different developmental stages. In a follow-up study (Mayzaud et al. 1987), they stated that the effect of growth stage on either enzyme activity or concentration is inconsistent. Buchholz (1989) investigated the chitinolytic enzyme activities of krill in the digestive tract and showed that the activities changed little in relation to moult activities. The author concluded that the chitinolytic system did not appear to be sensitive to changes in nutritional quality and quantity but rather shows the ability to digest chitin irrespective of its source. Buchholz and Saborowski (1996) found that chitinase activities in the stomach and midgut gland of the field-caught krill were characterised by high individual and spatial variations and were not well correlated to Chl *a* and phytoplankton protein in the surface water. Saborowski and Buchholz (1999) confirmed that the weight-specific enzyme activities are higher in the stomach than in the digestive gland and found that the enzyme activities decreased after 4 days of starvation and recovered subsequently.

The general conclusion from these enzyme studies of krill is that krill digestive enzymes provide the species with the capability to adapt to changing food conditions, but that their activity levels are not particularly useful as a basis for a feeding activity indicator. Also, the activities tended to be highly variable individually. The digestive gland is believed to be the primary digestive organ which responds to the prevailing food conditions. Although mass-specific activities are higher in the stomach than in the digestive gland, the gland is simply much larger than the stomach. Digestive enzymes in the stomach will certainly have an important role to play, but they would have been originally supplied from the digestive gland . As the digestive fluid flows from the digestive gland into the stomach and accumulates, it should have higher mass-specific activity. In many cases, geographical differences in enzyme activities could not be well explained by other food-related factors such as ambient chlorophyll concentrations or particulate protein (Mayzaud et al. 1985, Buchholz and Saborowski 1996). This is understandable considering the krill's ability to graze

down algal patches in a relatively short time and to move on to a new location. The enzyme systems may well respond at slower rates and the enzyme activities determined need not reflect the food conditions on the same temporal and spatial scales, i.e. the condition at the timing and location of the sample collection.

Digestive enzyme activities have been determined as measures of feeding activity in other zooplankton species, mostly copepods. However, a positive relationship between enzyme activities and food concentration has not always been found (Hassett and Landry 1983). Direct links between enzyme activities and ingestion rates have been difficult to establish (Head et al. 1984, Bamstedt 1988, Hassett and Landry 1988). The response of the digestive enzyme system to food conditions appears to be governed by a number of factors. The food environment the animals have recently been subject to would affect the subsequent enzyme activity for a variable length of time (Hassett and Landry 1988, 1990a). There seems to be a compensatory mechanism between digestive enzymes and the substrate ingested (Harris et al. 1986). The production of enzymes and their release for use are not necessarily linked and may not occur on similar time scales (Head et al. 1984). Generally, the time span needed for the enzyme system to reach an equilibrium seems to be longer than the scale for the food conditions to change. Detection of general seasonal differences in feeding activity may well be achieved by the determination of enzyme activities from samples across seasons (Hassett and Landry 1990b). So far it appears that digestive enzyme activity may indicate feeding potential of the animals, but is unlikely to serve as a simple measure of *in situ* feeding activity. The present study differs from the earlier studies, which are mostly on copepods, in that the activities were measured from a major individual organ dissected from an animal whereas the other studies used a whole animal or a number of whole animals. As shown, the total enzyme activity from krill's digestive gland was largely determined by the size of the organ. Although krill and copepods perform in different food environments in different spatial and temporal scales, the varying level of enzyme activity in other studies may well have been a reflection of the change in the size of the digestive organ of the species. It is possible that the reported time scale for the digestive system to acclimate was actually the time during which the digestive organ was enlarging or shrinking. Also, if the body size of the

animals had not been reasonably tightly controlled, the effect of body size would have been considerable. It was not possible to follow the change in mass-specific activity of the digestive organ in these studies. Thus the fine-scale adaptive process occurring in the digestive system, particularly during the time of food regime change would have been extremely difficult to deduce.

The results of this study indicate that simply to measure the enzyme activities from the digestive gland or from a whole krill, as an indicator of feeding activity, may not be a fruitful exercise since the changes in activity are strongly related to the mass change of the gland. Nonetheless, the ratio between different enzyme activities, notably of trypsin to laminarinase, at least in juvenile krill, may respond to changes of feeding regime and undergo a significant change when the food supply level changes abruptly. This has some potential to provide detailed or supporting information regarding the feeding status and history of the animals, particularly when combined with measurements of the relative size of the digestive gland. A high relative gland size will mean that the krill have been subject to relatively favourable feeding conditions for a week or more. If the ratio of trypsin to laminarinase is low in these animals, this may well indicate the very recently interrupted food supply. If the animals have a small gland and also a low trypsin/laminarinase ratio, they may have had a very low level of food for a while. When the animal has a still small gland, yet displays a high level of this ratio, it may have found a new food source very recently. Other observations such as stomach fullness may provide limited additional support to the findings. If the diet is overwhelmingly carnivorous, its effect on this ratio is a distinct possibility, which is yet to be investigated.

Because of the relatively large size of krill, it was possible to examine the enzyme activities of the most conspicuous digestive organ and at an individual level. It should be remembered, though, that the krill in the current experiment were subjected to a constant nutritional regime. Krill in the wild may experience interruptions to their food supply over a few days, in which case the enzyme index may not respond similarly as in this experiment. Hence the above enzyme index, the ratio of trypsin to laminarinase, needs to be viewed with caution. It should be noted that, in the previous short-term experiment, krill showed, upon starvation, neither an

enhancement in enzyme activities nor a shift in ratio of the activities between trypsin and laminarinase. These krill were most probably feeding actively when they were captured, given the large size of their digestive gland. It should also be noted that the current long-term experimental krill were juveniles and the short-term on-board starvation experiment was conducted with adults. This could have affected the results if there were ontogenetic differences in the function and adaptive mechanism of the digestive enzyme systems.

Evidence of recent active feeding, or starvation, was much more clearly illustrated from the gland size rather than from digestive enzyme activities. The gland size also recovered rapidly over a few days by the resumption of feeding after a period of starvation. The current results, together with the demonstrated role of the digestive gland as a reserve in the previous chapter, indicate that the digestive gland of krill can serve as a kind of battery against short-term starvation, which is readily expendable and quickly rechargeable. The mass of the gland appears to matter most in determining the capacity of krill in terms of digestive enzymes under most conditions. Prior acclimation may well affect the enzyme index strongly but it does not seem to play a role in the mass gain or loss of the gland. It appears that digestive enzyme activities in the krill digestive gland are unlikely to be useable as an indicator of field ingestion rates or reflect the most current prevailing food conditions in their own right. Rather, the relative size of the gland is a simple and direct integration of recent feeding history, hardly affected by immediate past events such as cod-end feeding and not subject to varying degrees of destruction as is the case with pigment content. The relative gland length expressed in the portion of the carapace length occupied by the digestive gland was not highly sensitive to the body length change at moult and appears suitable for the measurement of field-collected specimens and this may also be suitable for use on preserved specimens.

It is noteworthy that the krill given excess food for a long period in this experiment had smaller glands than field-caught animals that were used for the previous short-term starvation experiment. It is thus not appropriate to suggest any set numbers here, that would be universally applicable as criteria for determining the level of feeding intensity in the recent past. The effect of body size on the gland size may also need

to be explored. However, it could still be reasonably assumed that krill in which the digestive gland occupies more than 50 % of their carapace length have been relatively well fed and that krill with below 40 % of gland length/carapace length have experienced serious food shortage. These findings warrant further field tests and repeated measurements, ideally compiled from a range of contrasting food environments over different seasons. Nevertheless, if there is any sustained starvation, the nutritional stress caused by it should be apparent in the size of the digestive gland, and this is easily measurable in field-caught krill and even in live animals if aided by an image analysis system, and possibly from preserved specimens.

Chapter 6. The potential of using eye diameter as an indicator of shrinkage in Antarctic krill; an experimental appraisal

6.1 Abstract

Shrinkage may be one of the strategies that Antarctic krill use to cope with food scarcity, particularly during winter. Although krill has been shown to shrink under laboratory conditions, there are only very limited data to determine how commonly shrinkage occurs in the wild. It has been shown previously that laboratory-shrunk krill tend to conserve the size of the eye. This study examined whether the relationship between the eye diameter and body length could be used to detect whether krill have been shrinking. By tracking individuals over time and examining specimens sampled as groups, it was demonstrated that fed and starved krill are distinguishable by the relationship between the eye diameter and body length. The eye diameter of starved krill did not decrease, even when the animals were shrinking in overall body length. The eye diameter of well-fed krill continued to increase as overall length increased. This created a distinction between fed and starved krill, while no simultaneous separation was detected in terms of the body length to weight relationship. Eye growth of krill re-commenced with re-growth of krill following shrinkage although there was some time lag. It would take approximately 2 moult cycles of shrinkage at modest rates to significantly change the eye diameter to body length relationship between normal and shrunk krill. If krill starve for a prolonged period in the wild, and hence shrink, the eye diameter to body length relationship should be able to indicate this. This would be particularly noticeable at the end of winter. How often krill are forced to starve in the wild and have to invoke shrinkage could be investigated by examining field-collected specimens using this method.

6.2 Introduction

Antarctic krill (*Euphausia superba*, hereafter krill) has been shown, both from laboratory experiments and field studies, to be able to undergo negative growth under starvation or severe food limitation (Ikeda and Dixon 1992, Quetin and Ross 1991, Nicol et al. 1992a). Shrinkage is one of the strategies that krill may use to cope with food scarcity, particularly during winter, but there are only very limited data to determine how commonly shrinkage occurs in the wild. One of the major problems with body shrinkage is that it can interfere with the relationship between body length and age and thus affect length frequency analysis traditionally used in population dynamics (Ettershank 1984). As there are currently no reliable independent measures of age in krill (Nicol 1990), this can introduce significant uncertainty. Shrinkage has been reported in a number of other euphausiid species (Dalpadado and Ikeda 1989, Hosie and Ritz 1989, Marinovic and Mangel 1999). However, because of the longer life span and extended food-limited periods, shrinkage is more likely to pose a problem in studies of Antarctic krill than for other euphausiids. Additional measures of age which are insensitive to shrinkage would assist in establishing age classes with more confidence.

The controversies surrounding winter growth and shrinkage of krill have not been resolved. The only available year-round length frequency data have been interpreted in different ways (Stepnik 1982, McClatchie 1988, McClatchie et al. 1991) and scarce direct field observations of winter growth do not agree, illustrating both shrinkage and apparent growth (Quetin and Ross 1991, Huntley et al. 1994). Nevertheless, population processes during winter when shrinkage might occur, are thought to be critical in determining the krill populations' success in the following season (Smetacek et al. 1990). Recently, the extent and duration of sea ice cover, particularly during winter, has been proposed as a major factor governing such processes (Siegel and Loeb 1995, Loeb et al. 1997). Regional differences in overwintering strategies of krill in relation to the physical environment have been identified as one of the major questions in the Southern Ocean GLOBEC (Global Ocean Ecosystem Dynamics) program (Anon 1994, 1997). While knowledge about events during winter is of great importance, standard methods to gain such

knowledge of winter population processes, including the occurrence of shrinkage, have not been well established.

Sun et al. (1995) suggested that the relationship between the body length and the crystal cone number of the krill eye might indicate whether they had experienced a period of shrinkage. They showed that when a krill shrinks in length, the number of crystalline cones in the compound eye remains constant. Sun (1997) later indicated that the diameter of the eye may also remain constant during extended body shrinkage and suggested that the ratio of the body length to the eye diameter could be used to detect the effect of shrinking in natural populations of krill. In essence, shrunk krill should have more cones in their eyes, and thus should have larger eyes, for a given body length than krill which have not shrunk. These results were obtained by comparing field-caught summer specimens and laboratory-shrunk animals. Although these results were convincing, the same group of animals was not sampled for comparison. In addition, the laboratory population was under food limitation for 8 months, which is unlikely in the wild. It remains to be determined under what conditions the eye diameter to body length relationship would become differentiable between fed animals and starved ones; how much shrinkage is needed and for how long such shrinkage needs to be maintained.

Sun et al. (1995) also questioned if the eye would start to grow immediately once the body growth re-commenced following a period of shrinkage or whether eye growth would begin only after the original body length had been regained. Sun (1997) suggested that the eye diameter would remain constant while the rest of the body was recovering to the original size. Whether it does, however, has a significant bearing on the potential use of the eye size as an indicator of shrinkage. If eye growth re-commences with body growth, this modified relationship would be preserved. On the other hand, if krill have to re-grow to the original pre-shrinkage body size before the eye growth re-starts, then any altered relationships between eye size and body size due to shrinkage will not last.

This study aimed to examine, from direct observation of individuals over time and from inspection of animals sampled as groups, whether the eye diameter of krill is

unaffected by starvation and accompanying shrinkage of the other body parts. It was also designed to determine if resumed food supply following starvation re-initiates growth both in body length and in eye diameter. It also attempted to assess what conditions are required for an appreciable difference to be generated in the eye diameter to body length relationship between normal and shrunk krill.

6.3 Materials and methods

Experimental animals

A large number of juvenile Antarctic krill were collected on 15 April 1997 19:47 (local time) at 64°19' S, 110°57' E, just north of the ice edge in East Antarctica with an RMT 1+8. The krill were kept in a cold room on board while being transported to the home aquarium. Then they were maintained in the dark at 2±0.5 °C under sub-optimal food concentrations until the experiment started at the beginning of August 1997. A mixture of 3 cultured algal species, *Phaeodactylum tricornutun*, *Gemingera criophylum*, *Pyramimonas* sp. was given to krill in tanks approximately once a week. Limited capacities of the aquarium to process the waste load did not allow a high level of ration to support active growth of krill.

Organisation of the experiment

The experimental animals were selected to cover a narrow size range (approximately 7.5 mm in carapace length, around 24 mm in total length) and were kept individually in 2 litre plastic jars at 0 °C in the dark. An initial group of 25 krill was removed at the start of the experiment. For a week before the start of the experiment, no new algal food was given.

Phase 1

One group was fed *Phaeodactylum tricornutum* at $5x10^5$ cells ml^{-1} to attain maximal growth rates (Ikeda and Thomas 1987a) and the other group was placed in filtered seawater. Water exchange and food provision were made every 3 to 4 days. Half of each group, either fed or starved, was assigned to either the Harvest Group or the Monitor Group as they moulted. From the Harvest Group, 12 animals were sacrificed after 5, 10 and 15 weeks and then weighed and the carapace length and the eye diameter were measured. For the other half, the Monitor Group, the animals at each moult were taken out and weighed, and an image of each krill was taken with an image analysis system. The krill were then replaced in the experimental jars. From the image captured, the eye diameter and carapace length were measured at each moult. Initially it was aimed to use only unharassed animals from the Harvest Group for the regular sampling. However, at week 15, about half of the animals came from the monitored, and hence disturbed group, due to a dwindling number of animals.

Phase 2

After 15 weeks, the food regime was reversed; the fed group was starved and the starved group began to receive food. Following the switch, all animals were monitored at each moult and up to 5 krill were sampled at regular intervals for other experimental purposes. The experiment continued for a further 13 weeks following the switch of feeding regimes.

6.4 Results

Body length to weight relationship and body length to eye diameter relationship

Throughout the course of the experiment, starved juvenile krill shrank in length and lost weight, whereas the well-fed animals grew both in length and weight (Fig. 6.1). The experimental krill had been on sub-optimal food levels before the start of the experiment. Thus the starved animals in Phase 1 did not shrink substantially in length although they lost weight. Fed and starved groups of harvested krill were indistinguishable in terms of the body length to weight relationship, even after 15 weeks of extreme treatments (Fig. 6.2 a). The linear regressions of the log-transformed body length versus weight of the fed and starved groups were compared. Comparing the regression equations involved the use of t test. Equality of the slopes was tested first and a common slope was calculated, if the slopes were not significantly different. Then these lines were assumed to be parallel, and it was determined whether the regressions had the same elevation and thus coincided. Neither the slopes nor the elevations of the two regression lines from the fed and starved groups were significantly different (t test, $0.2<p<0.5$); sharing a common slope and a common elevation. That is, a single equation could describe the body length to weight relationships of both groups.

In terms of the carapace length to eye diameter relationship, the starved krill were separable from the well-fed animals after 15 weeks (Fig. 6.3 a). The two groups of krill could not be placed on a single regression line in the body length to eye diameter relationship. The slopes of the linear relation between the carapace length and eye diameter from the fed krill and the starved animals were not significantly different (t test, $0.2<p<0.5$), but the elevations were significantly different (t test, $0.02<p<0.05$); starved krill had larger eyes compared to the fed krill of similar lengths or starved krill were shorter than the fed krill of similar eye diameters.

The body length to weight relationships in krill fed or starved for 15 weeks were not only similar but also had not greatly changed from that of the krill at the start of the experiment. Overall, there was little difference in the length to weight relationship

between the initial krill and the krill fed or starved for 15 weeks (Fig. 6.2 b). In terms of the body length to eye diameter relationship, however, the initial group was much closer to the fed group than to the starved animals (Fig. 6.3 b). Starved krill were becoming separated from the fed group as shrinkage was occurring in the body length but not in the eye diameter.

Because the specimens were selected in a narrow range of body size, the coefficient of determination from the relationships derived tended to be relatively low. However, the separation of fed and starved animals in terms of the carapace length to eye diameter relationship was obvious by week 15 and also appears to have become already manifest 10 weeks into the experiment. Two individuals of the 10 week-fed group showed a relationship dissimilar to the rest of the group, being more like starved krill in terms of the carapace length to eye diameter relationship. By removing these 2 data points, a trend could be established for the reduced data set of krill fed or starved for 10 weeks, similar to that seen in week 15; a common slope but significantly different elevations (t test, $0.02<p<0.05$). A gradual separation of fed krill and starved animals was also taking place with time in the Monitor Group (Fig. 6.4). Despite the small number of specimens, a similar separation was seen to have occurred again, 13 weeks after the reversal of food regime; the animals starved for 13 weeks after a switch from full rations became shorter for a given eye diameter, compared to the animals that were well fed following a long period of starvation (Fig. 6.4 d).

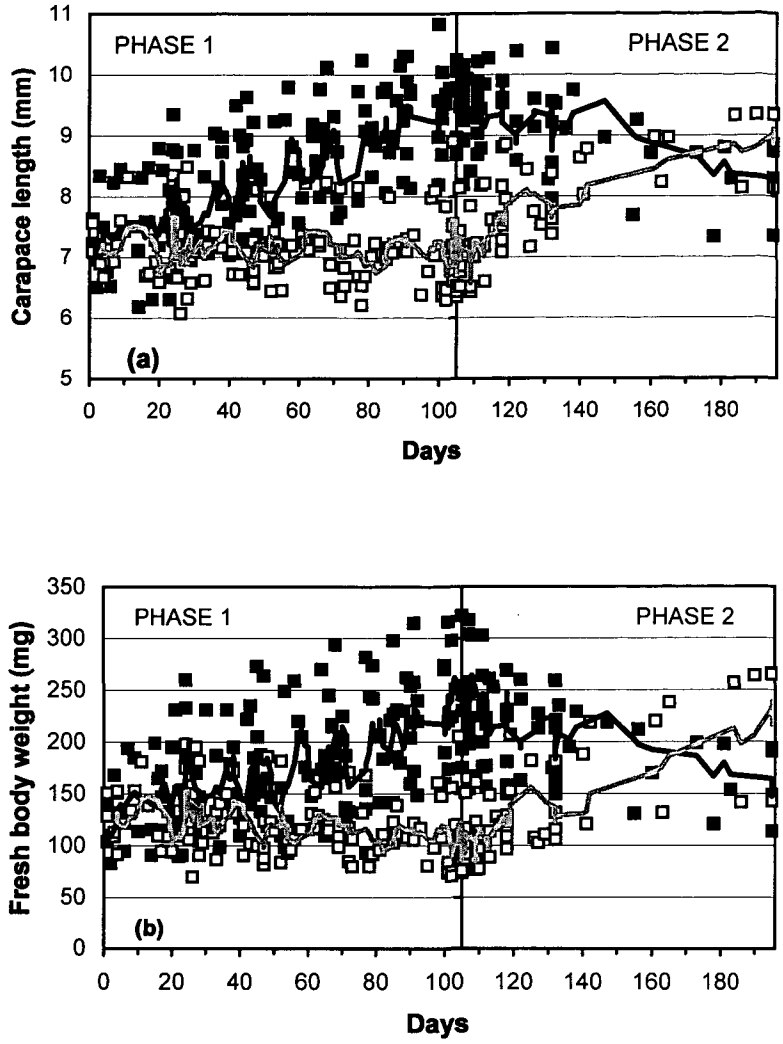


Fig. 6.1 Change in the body size of the krill from the Monitor Group.
(a) Carapace length, (b) fresh body weight. Each data point is a measurement at each moult or at the point of sampling. Day 105 is the point of food regime reversal and hence the point of change from Phase 1 to Phase 2. 'Moving average' lines are drawn from the mean of the last 5 data points. Filled squares and solid line represent the 'fed and then starved' krill and open symbols and shaded line the 'starved and then fed' animals

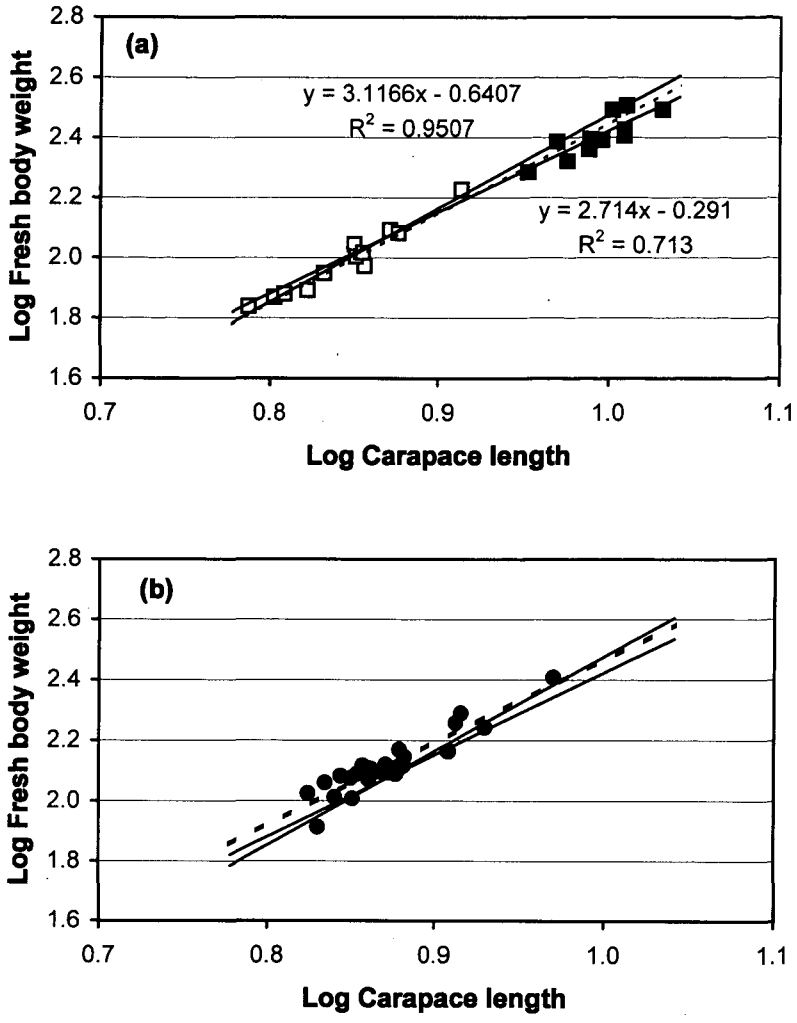


Fig. 6.2 The relationship between the body length and body weight of well-fed or starved krill. (a) Log carapace length and Log fresh body weight of the Harvest Group animals sampled after 15 weeks. Filled squares indicate fed krill and open ones starved animals. A new regression line (broken) was drawn with the common slope and the common elevation. (b) The length and weight data of the initial group of krill (solid circles) and their regression line (broken) superimposed on the regression lines of the 2 groups of animals fed or starved for 15 weeks

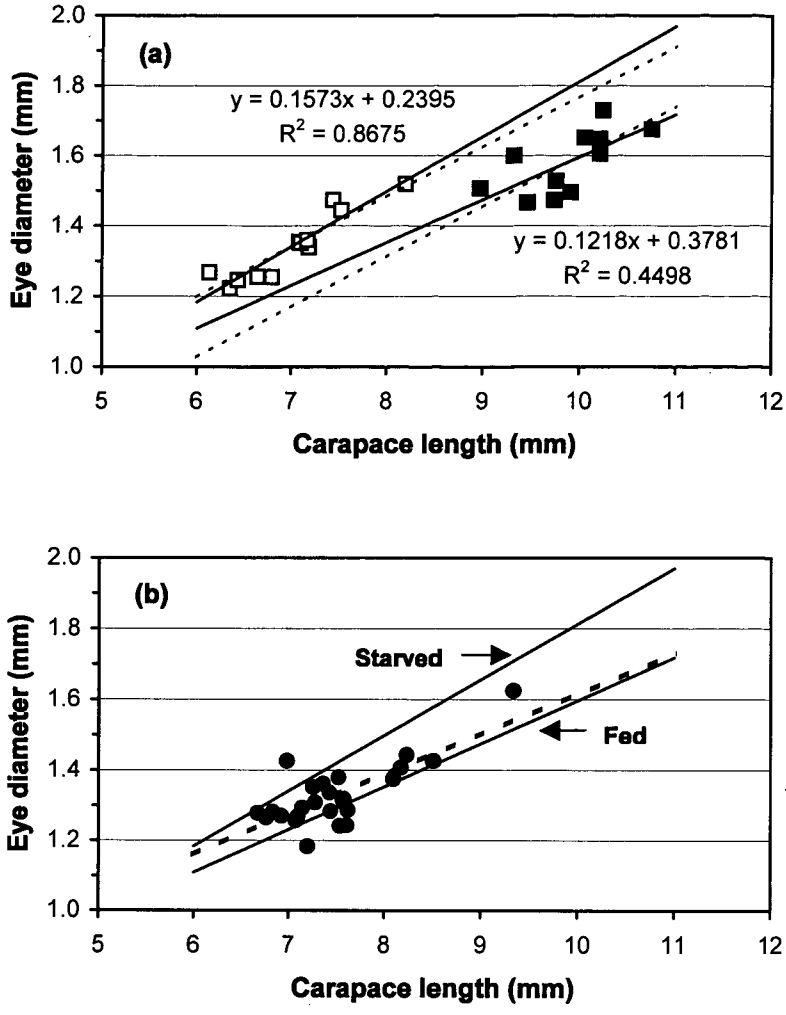


Fig. 6.3 The relationship between carapace length and eye diameter of the fed or starved krill. (a) The carapace length to eye diameter relationship from the Harvest Group after 15 weeks. Solid squares indicate the fed krill and open squares the starved animals. Broken lines are drawn from the common slope and corrected elevations. (b) The carapace length to eye diameter relationship of the initial group (circles) and its regression line (broken) overlaid on the regression lines of the 2 groups of krill fed or starved for 15 weeks

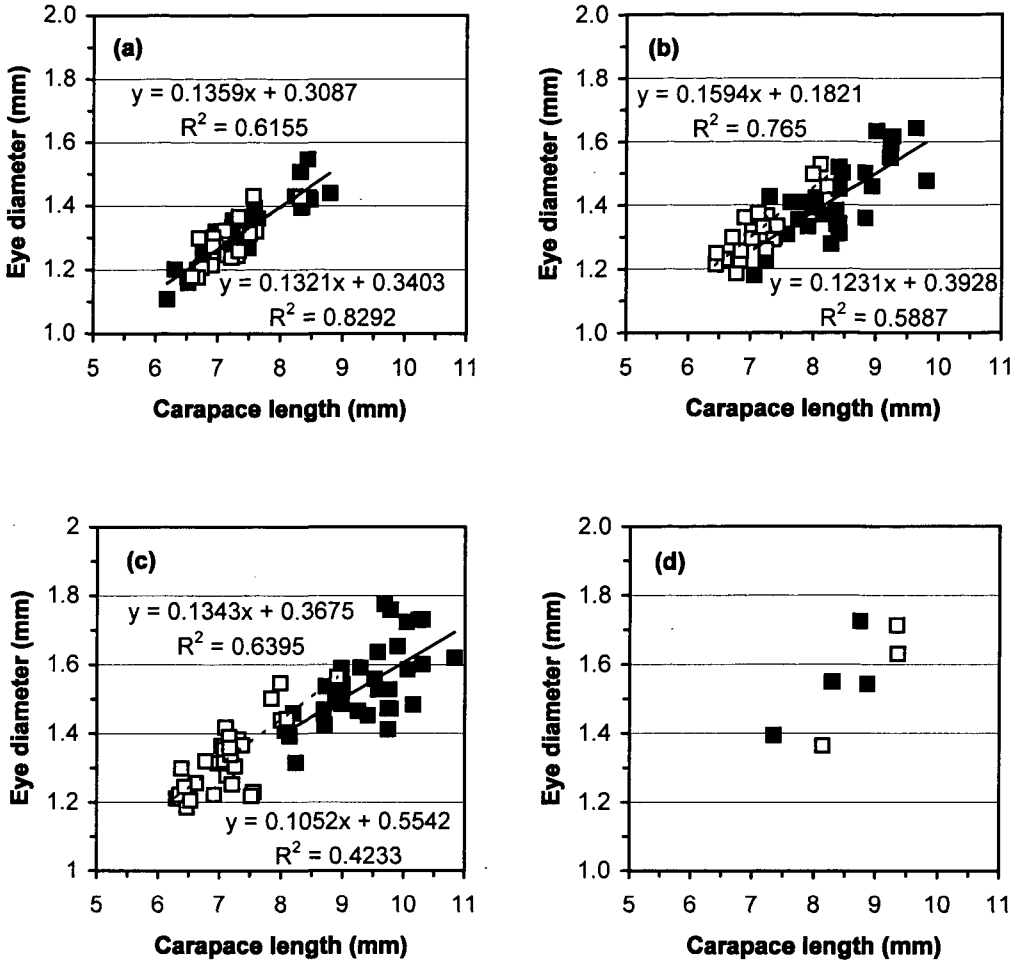


Fig. 6.4 The carapace length to eye diameter relationship over time from the animals of the Monitor Group. (a) The carapace length to eye diameter relationship during the first 3 weeks of Phase 1, (b) the carapace length to eye diameter relationship during the mid 3 weeks of Phase 1, (c) the carapace length to eye diameter relationship during the last 3 weeks of Phase 1, (d) the carapace length to eye diameter relationship at the end of Phase 2. Solid squares represent the krill fed during Phase 1 and then starved during Phase 2. Open squares represent the animals starved during Phase 1 and then fed during Phase 2

Change in the eye diameter and body length of individually monitored krill

The eye diameter of the well-fed krill continued to increase as overall length increased. When the previously well-fed krill were starved, eye diameter growth was halted but the diameter of the eye did not decrease. The percentage increments per moult of the eye diameter and of the carapace length are presented from the animals of the Monitor Group and these demonstrate the overall trend (Fig. 6.5 a, b). These increment rates represent the growth that occurred during one moult cycle, i.e. between the previous moult and the current moult. As the measurements had to be made quickly on live animals, a small amount of measurement errors was unavoidable, which may be responsible for some of the variations. Nevertheless, a general pattern emerged. During Phase 1 of the experiment, the eye diameter increment per moult of the starved animals approximated zero, whereas, at the same time the animals were shrinking marginally in carapace length. When the previously well-fed animals started to starve and thus shrink, the eye growth fell and settled close to zero.

Krill that had been starving responded to food by resuming growth both in body length and eye diameter. The response of the eye diameter to the change in food regimes, whether in a positive or negative direction, tended to be slower than that of the carapace length with some degree of individual variation (Fig. 6.5 a, b). Some krill re-started eye growth after a time lag. Similarly, the eye diameter of others continued to grow for a short period after body shrinkage commenced. However, these time lags usually lasted only a few weeks, a similar length of time to a single moult cycle. Fig. 6.6 presents the individual tracks of the eye diameter and carapace length in some krill that were allowed to carry on through the whole period of the experiment. This depicts the varying degree of promptness in the re-initiation of eye growth between individuals. Krill 1 exhibited a cessation of eye growth almost immediately following the switch to starvation, whereas the eye diameter of krill 2 continued to grow for almost 2 more moult cycles. Krill 3 which had been starved first and then fed, needed another moult cycle to resume eye growth, while its carapace length started to increase with the switch of the food regime.

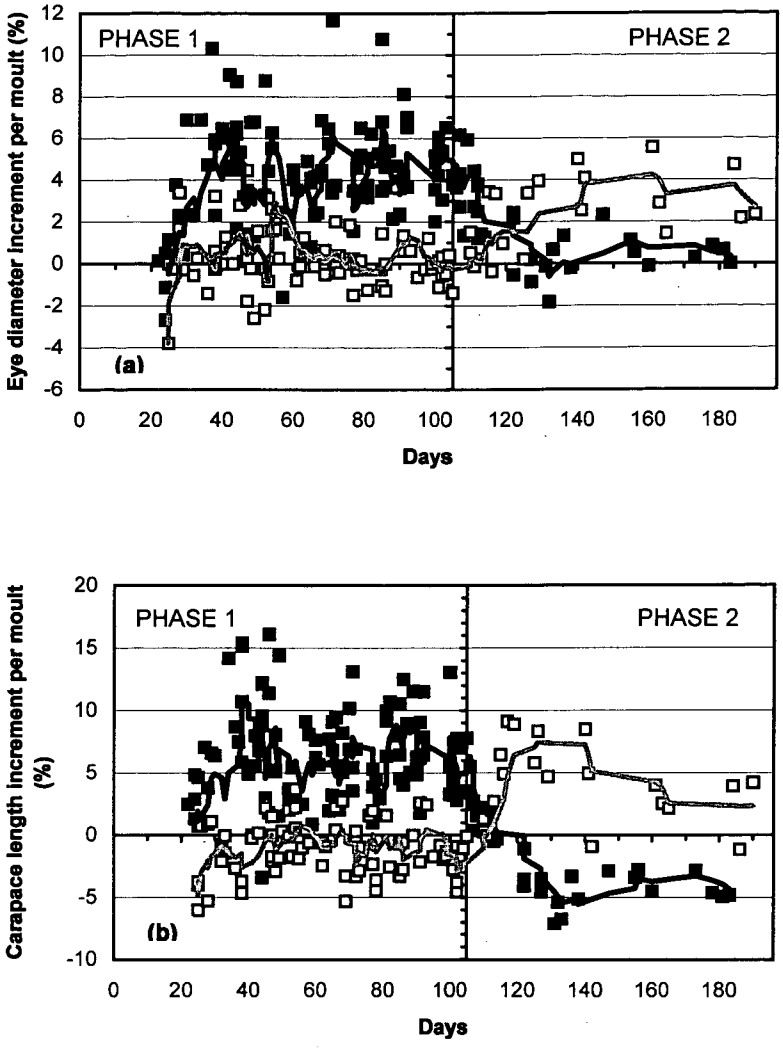


Fig. 6.5 Eye diameter and carapace length increment in percentage per moult of the individually monitored krill throughout the experiment. (a) Eye diameter growth, (b) carpace length growth. Each data point is a rate of growth that occurred between the day of current moult and the previous moult. Day 105 is the point of food regime reversal and hence the point of change from Phase 1 to Phase 2. 'Moving average' lines are drawn from the average of the last 5 data points. Solid line represents the 'fed and then starved' group and shaded line the 'starved and then fed' group

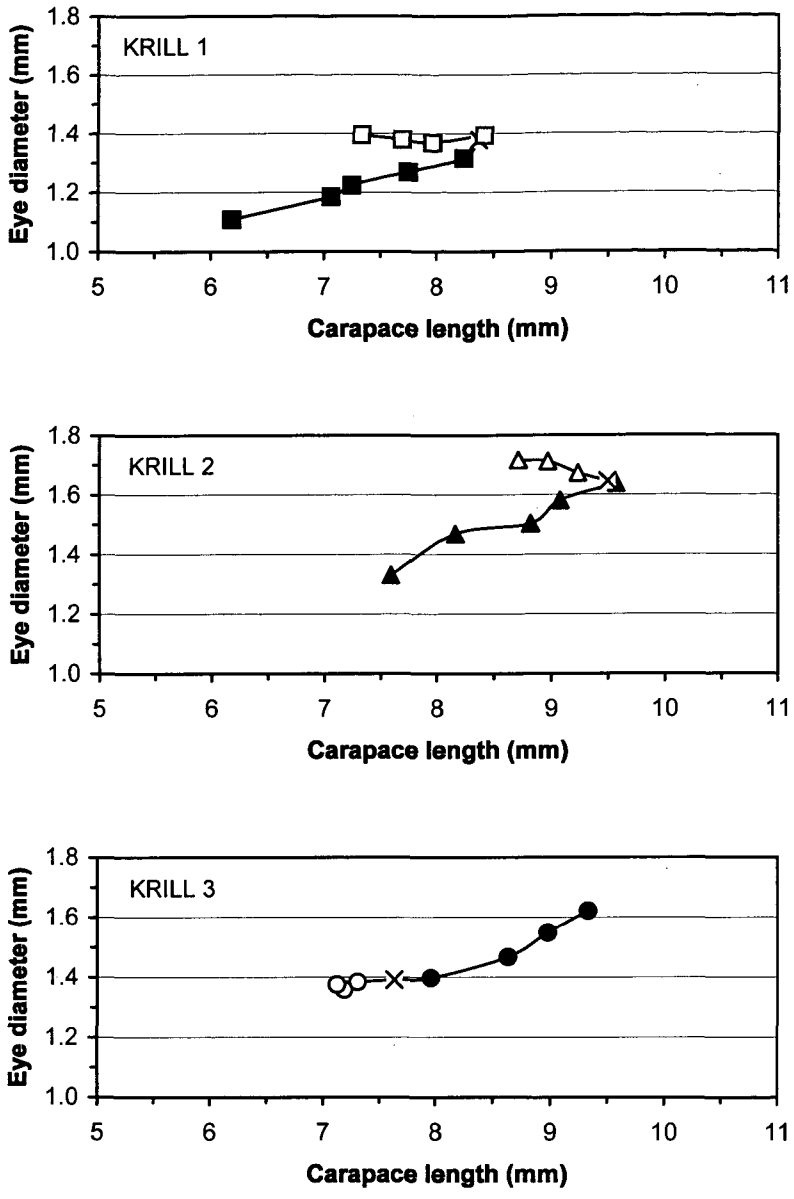


Fig. 6.6 Trajectories of carapace length and eye diameter of 3 representative krill that completed both feeding and starvation phase of the experiment. Krill 1 and 2 are from the 'fed and then starved' group and krill 3 is from the 'starved and then fed' group. Solid symbols represent feeding krill and open symbols starving animals. The point of food regime reversal is indicated by crosses. Each data point resulted from a moulting event

6.5 Discussion

This experiment demonstrated, from both tracking individuals over time and examining specimens sampled as groups, that fed and starved krill became distinguishable by the body length to eye diameter relationship. Separation of the fed and starved groups by the body length to eye diameter relationship stems from the response of the eye diameter to the food regime. Eye diameter increases as the animals grow but does not decrease while the overall body length decreases during shrinkage. The body length to weight relationship, which is regarded as a condition indicator, did not exhibit a difference in the experimental krill, even after 15 weeks of extreme treatments. Differences in terms of the body length to weight relationship have been noted from the field-caught specimens between summer and winter (Siegel 1989, Quetin and Ross 1991) and also within seasons (Nicol et al. 2000). Krill in this experiment were juveniles and it is possible that juvenile krill cannot afford to lose too much body mass while adults have more reserve to dispense with. Therefore a change in the length to weight relationship may not be as readily apparent in juveniles as in adults. Nevertheless, if krill shrink in length while losing weight, they would have to lose weight more quickly than they decrease in length, in order to change the length to weight relationship. In this experiment, when the well-fed krill started to lose weight and shrink in response to starvation, it seems to have occurred in the same way, as they had gained weight and had grown in length, but only in a reverse direction. This resulted in krill of the 'fed and then starved' group moving along the same path on the length versus weight plane while growing and then shrinking (Fig. 6.7). These krill may have been optimising the body length for a given weight.

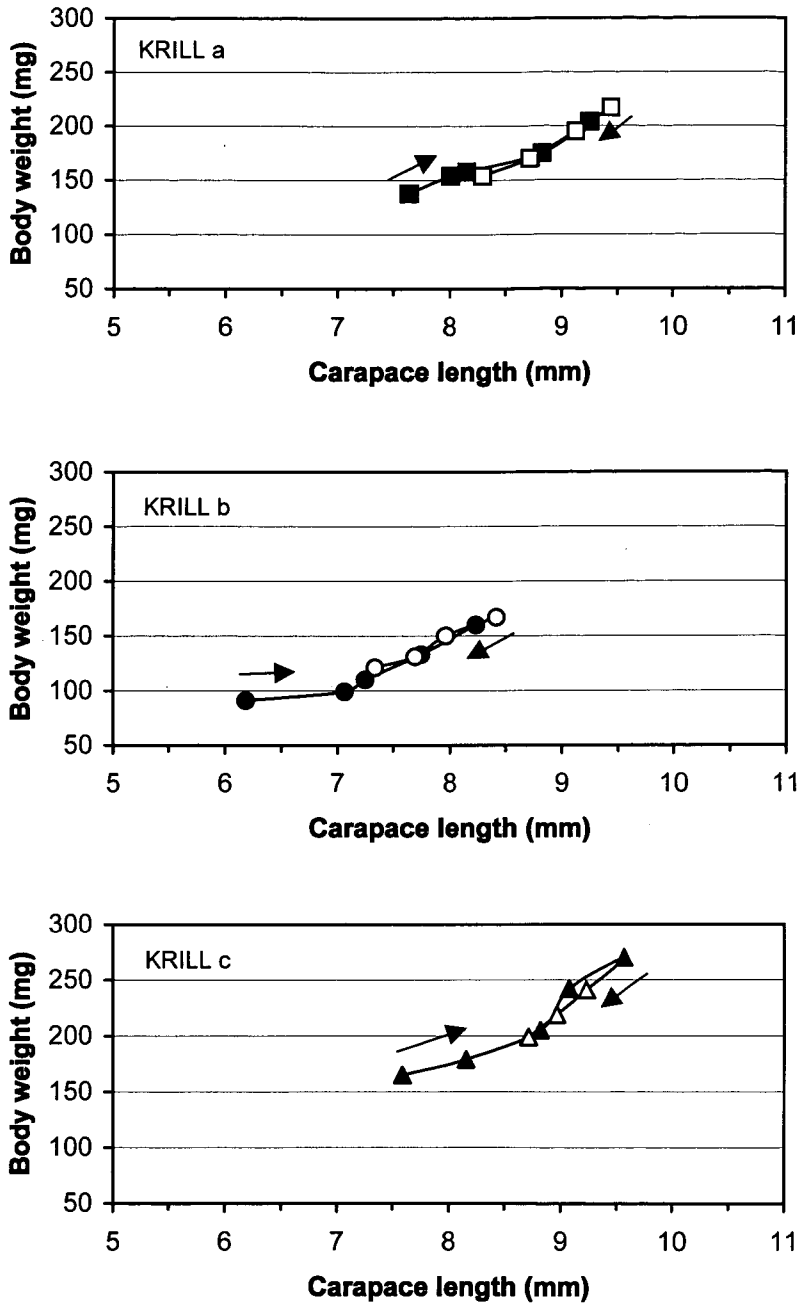


Fig. 6.7 Trajectories of carapace length and body weight of representative individual krill from the 'fed and then starved' group. Upward arrows inidcate the start of the experiment and downward arrows a decline to negative growth caused by starvation. Open symbols represent the starved specimen after the switch to starvation

While the eye size has the potential to serve as an indicator of shrinkage by staying constant during negative growth of the body, it remains to be determined under what circumstances a detectable change might occur. In this experiment, starving krill during Phase 1 were shrinking at rates between 0 and -2% per moult. Separation of fed and starved krill in terms of the carapace length to eye diameter relation became evident by week 15 and this was detectable from around week 10 excluding a few outliers. By week 10, the starved krill had moulted twice except for one animal. Krill that shrank for 2 moults at an average rate below -1% per moult became discernible from the well-fed, normally growing krill, by the body length to eye diameter relationship.

Resumed food supply after a long period of starvation promoted growth both in carapace length and eye diameter although there was a time lag (Fig. 6.5 and 6.6). Also the eye diameter of poorly fed krill, at the start of the experiment, grew together with the carapace length in response to improved food conditions. Therefore it appears that krill do not have to regain their original body length before the growth of the eye diameter re-commences. This will make the application of eye size to the population study of krill even more practical because krill will retain the modified body length to eye diameter relationship when they start to grow again.

To illustrate how body shrinkage would affect the body length, the eye diameter and the relationship between the two, a simplified hypothetical growth model was constructed for krill that shrink or do not shrink during winter. The following assumptions were made.

. Krill live for 5 years and moult 12 times a year.

. Krill grow at high rates for the first 4 moults during summer.

. For the next 3 moults in autumn, krill grow at low rates.

. During winter krill moult 2 times and they either stop growing or shrink by 2% per moult. However, krill in their first winter after hatching, i.e. larvae, do not shrink.

. In spring krill start to grow at the same low rates as in autumn and they moult 3 times.

Except for the non-growing winter season, growth rates of krill between 1 to 5 % per moult were assigned to the different seasons each year. The growth rates were set at

modest levels and within the range of published values so that the resultant growth curve is in agreement with current knowledge. The actual value of growth rates was not deemed significant, as adjusting the growth rates may refine the growth curve but will have no effect on examining the consequences of shrinkage or absence of shrinkage. The function describing the initial relationship between the body length and the eye diameter was modified from Sun (1997), as the current experiment dealt with only juvenile krill in a narrow size range. Although Sun (1997) obtained the relationship from a wide size range of animals, he described an exponential function between the body length and the eye diameter because it was a better fit than a linear regression. This leads to the proposition, however, that, as krill grow larger, the eye diameter growth continues at the same rate while the growth in body length slows down. In other words, the growth of eye size accelerates while body length increases by a constant rate. This does not appear biologically realistic, therefore, a linear relation was assumed instead. A linear relation was established from the combination of body length and eye diameter, which would have fitted Sun's (1997) exponential relationship, in the size range he observed. It resulted in a regression line which had a similar slope to the one from this experiment on juveniles. For krill that do not shrink, the eye diameter of the animal was derived from this relationship throughout. For shrinking krill, the eye diameter was obtained in the same way but kept constant when the animals were shrinking. When the animals re-commenced growth in spring, the eye diameter also began to grow.

The maximal body length attained by the krill that shrink for 2 moults every year was shorter than that of the krill that do not shrink (Fig. 6.8 a). The eye diameter, however, was not greatly different between shrunk krill and non-shrunk animals even after 5 years (Fig. 6.8 b). When krill shrink in body length but conserve the size of their eyes, the relationship between the body length and the eye diameter becomes altered. Each time krill shrink, the body length to the eye diameter relationship diverges from the one that would have been the case if the krill had not shrunk (Fig. 6.8 c). Fig. 6.8 d presents a magnified view of the year between the first summer and the second summer since hatching, which depicts how shrunk krill have developed large eyes for a given body length or how the krill have become longer for a given

eye diameter. The output from this simplified model agrees with what was observed in the current experiment (Fig. 6.3 and 6.4).

The change in this relationship can also be shown in the ratio of the body length to the eye diameter (hereafter BL/ED ratio), the index suggested by Sun (1997). The BL/ED ratio will drop each time krill shrink (Fig. 6.9). The difference in this ratio between the shrunk krill and non-shrunk ones will be conserved even if the krill re-commence growth in body length and it will also become greater each year if shrinkage occurs every winter. If the eye growth of krill is suspended not only for the period of body shrinkage but until the re-attainment of the pre-shrinkage body length, this altered relationship will eventually return to the original one, with the recovery of body length (Fig 6.9). This restoration can also be envisaged in Fig 6.8.c; the altered relationship caused by starvation would be fused back into the initial 'no-shrinkage' regression line (Fig. 6.8 c). If the altered eye to body size relationship induced by shrinkage becomes lost with re-growth of the krill, the eye size is not a more conservative measure of age than the body length except for the winter season and for some period in spring. By high summer, there would be no detectable difference between eye diameter and body length as an indicator of age for demographic studies.

Given the finding that eye growth is reactivated almost simultaneously with re-growth of krill, this may prescribe an alternative explanation for Sun's (1997) description of an exponential relationship between body length and eye diameter. As krill grow older, they may experience more years of food shortage and hence shrinkage. Consequently, a variety of altered eye size to body length relationships may be generated in the population with time. That is, as krill grow longer, more regression lines of body length and eye diameter will develop on top of the initial 'no-shrinkage' regression line (Fig 6.10). Should these multiple lines fuse together, an apparent exponential relationship would appear. This illustrates the fact that eye growth is decoupled from body shrinkage while its re-activation proceeds with the recovery of body length growth. The eye size, therefore, is more conservative as a measure of age than the body length.

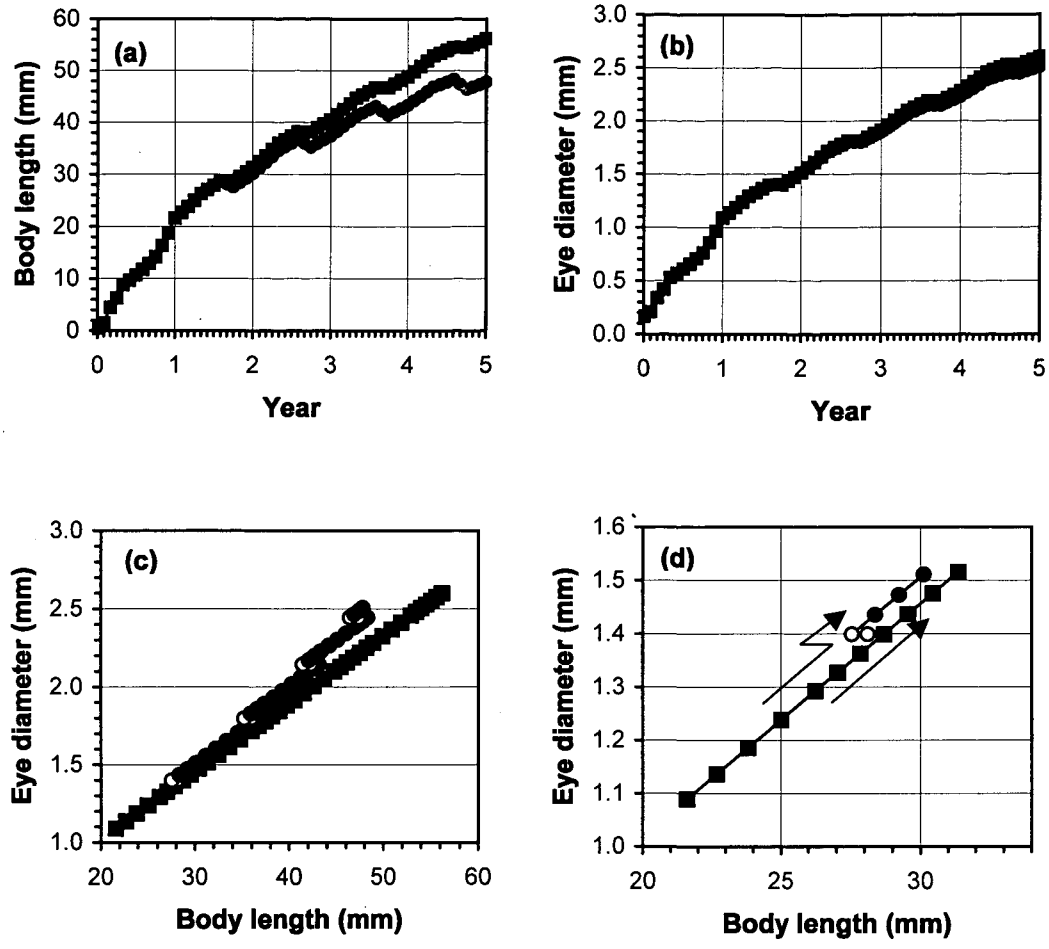


Fig 6.8 Hypothetical 5-year growth of krill that shrink over winter (●) or do not shrink (■). (a) Body length, (b) eye diameter, (c) the relationship between body leng and eye diameter, (d) a magnified view of (c), but between the first summer and second summer after hatching. Krill shrink in body length only, not in eye diameter. Open circles indicate that shrinkage has occurred. Arrows indicate how the body length to eye diameter relationships change sequentially with initial normal growth, shrinkage or zero growth, and then re-growth

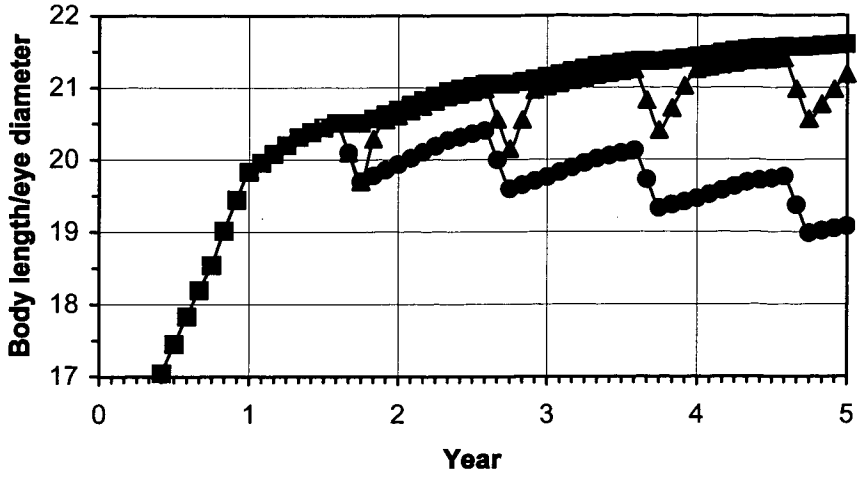


Fig 6.9 The change in the ratio of body length to eye diameter over 5 years as modelled in Fig. 6.8. Squares represent the krill that do not shrink. Circles represent the shrinking krill whose eye growth re-commences before gaining its original pre-shrinkage body length and triangles the shrinking krill whose eye growth recommences only after regaining its original pre-shrinkage body length

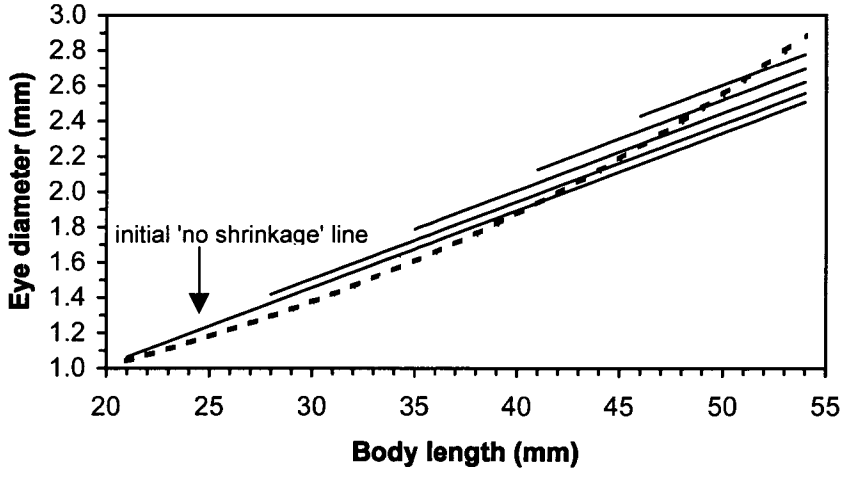


Fig 6.10 Multiple regression lines of body length versus eye diameter generated by shrinkage on top of the initial 'no shrinkage' line. Broken line represents the exponential relationship between the body length and eye diameter as suggested by Sun (1997)

It appears that should starvation proceed for more than 2 moult cycles at modest rates, a detectable change is likely to be induced in the body length to the eye diameter relationship. Shrinkage by 2 % per moult as used in the model is not an extreme or unlikely phenomenon. Quetin and Ross (1991) reported negative growth of -0.16 to -2.03 % per moult during winter. Nicol et al. (1992a) conducted 'instantaneous growth rate' measurement over an extended period and the krill showed negative growth under food limitation after as little as 7 days. The growth rates of krill were around -2 % per moult in approximately 10 days. Buchholz (1991) also reported similar rates of shrinkage in food-deprived krill. In the starvation experiments of Ikeda and Dixon (1982) and Virtue et al. (1997), krill continued to shrink for a number of months at a rate of -2 to -3 % per moult. Nevertheless, how clearly these changes become manifested will depend on a number of factors. The more severe the shrinkage is, i.e. the higher the shrinking rate of krill is and the more often krill moult while shrinking, the more evident this alteration will be. Separate groups of different body length to eye diameter relationships will also appear in mixtures. The timing of spawning, the hatching date and the time needed to complete larval stages will vary individually, regionally and interannually, as do the growth rates of krill. All these factors will contribute to an age group in a range of body size, overlapping with the next age group. How well these mixtures can be separated depends on how strongly they overlap and this would be shown from the examination of the body length to eye diameter relationship from field-collected specimens. However, this should not be a simple extraction of the BL/ED ratio, which is unlike other morphometric indices such as gonado-somatic index or hepato-somatic index. These indices can indicate what stage an individual has reached in certain biological aspects. It needs to be remembered, however, that this BL/ED ratio should not be used in isolation but applied in comparison to the rest of the population.

The significance of the suspension in eye diameter growth only for the period of body shrinkage, not beyond, has been discussed. If the simplified model suggested here is valid in field situations, then the altered body length to eye diameter relationship should remain and the effect of shrinkage should be detectable even in summer. But it is uncertain whether this would be the case in the field. By high summer, krill would have benefited from improved food supply and would have

already experienced some growth. Any imprints left by winter shrinkage, would probably be blurred. It is possible that the distinction between shrunk and non-shrunk krill becomes unclear as krill start active growth. For the eye diameter to body length relationship to leave a detectable signal in summer, krill not only have to shrink, but more importantly, a substantial portion of the starved and hence shrunk krill need to survive through this unfavourable period and the rest of the year and then be strongly represented in the population. For these reasons, the effect of starvation and shrinkage would still be best seen at the end of winter, after the most likely period of extended food limitation. Concurrent examination of body size and eye diameter, and comparison between summer and winter will provide clues to whether food shortage has occurred overwinter.

Very young krill such as larvae are likely to be much less tolerant to starvation than older krill. The majority of larval krill that have had to starve severely would probably die. For larval krill to be seen in the coming summer, they would be the ones that have had access to reasonable food sources. In the case of larval krill, it will most likely be a matter of 'grow or die', not a matter of 'grow or shrink'. By the time larval krill reach their first summer after hatching, juveniles entering their second summer would probably have already grown out of winter shrinkage. Hence there would be significant differences in body length between age group 1+ and group 2+ in most cases and it may not be necessary to resort to the eye diameter measurements in order to separate new recruits from 2+ aged krill and older, although this may well provide clearer demarcation as stated by Sun (1997). Nevertheless, this method may prove useful in the separation between 2+ krill and older ones. Further information will come from inspection of winter specimens. A comparison of population structures both in eye diameter and body length, between high summer and the end of winter is also necessary to define the strengths and limitations of this method.

From an evolutionary perspective, if krill did not have to deal with severe food shortage and if their life history was not food-limited, they would not have developed such a high tolerance to starvation. Food limitation and body shrinkage are not exclusive to Antarctic krill. Other euphausiid species have been shown to be

able to shrink in the laboratory (Lasker 1966, Hosie and Ritz 1989, Dalpadado and Ikeda 1989, Marinovic and Mangel 1999). Mean size reductions during winter have also been reported from field studies, although this has been attributed to other factors such as increased over-winter mortality of larger animals by selective predation (Falk-Petersen 1985, McClatchie et al. 1991). This, however, has not been quantified and its significance remains as unsubstantiated as that of shrinkage. The shrinking rate of large adult krill appears to be higher than that of small krill. The size structure of laboratory krill populations tends to have less peaks and becomes skewed to the smaller size after an extended period of inadequate food supply (Nicol et al. 1991, Sun 1997). It is not impossible, therefore, that both shrinkage and selective predation contributed to the observed mean size reduction in the field population. It may be that high tolerance to starvation and ability to shrink are general features of most euphausiid species. In the case of Antarctic krill, sustained starvation and continued shrinkage do not seem to irreversibly affect the subsequent growth of krill (Thomas and Ikeda 1987). If starvation is a persistent, regularly repeated problem which lasts a whole season, Antarctic krill might have opted for more secure means such as accumulation of a lipid reserve which is sufficiently large to cover the most part of a whole winter. It has also become known that krill can, and do utilise a variety of food sources whenever they become available. Starvation may be an actual, recurring but unpredictable obstacle occurring in some years and areas and to some populations. How frequently and systematically shrinkage occurs in natural populations and whether it occurs to such a degree that it disrupts the length frequency distribution are yet to be shown from field studies. Even if the population structure remains relatively intact, the extent of food shortage can be studied in relation to changing environments. If starvation and shrinkage occurred as a local, short-duration incidence, it is not likely to deliver significant changes and thus will be hardly detectable. However, food limitation and shrinkage over wider temporal and spatial extents brought about by a large scale change in physical regimes, are likely to be detected.

If krill starve for a prolonged time in the wild, and hence shrink, the eye diameter to body length relationship should be able to indicate this, at least at the end of winter. How often krill are forced to starve and have to invoke the shrinkage strategy could

be investigated by examining specimens using this method. If a population contains juvenile krill and similarly sized krill regressed from adults, they should be discernible by the eye diameter to body length relationship. This technique gives us a new tool to collect information that could contribute to resolving the controversy of shrinkage and to advancing knowledge of the overwintering biology and recruitment of krill.

Chapter 7. Concluding remarks

7.1 Summary of results

Antarctic krill has been subject to a considerable amount of research effort which has produced much detailed information on its basic biology, however, the linkages between krill populations and the highly seasonal, annually fluctuating environment are yet to be systematically investigated. Although measures of performance such as spawning and recruitment indices have been developed (Cuzin-Roudy and Amsler 1991, de la Mare 1994a, b), biological indicators that can describe the 'condition' of krill in relation to seasonal cycles and shifts in physical regime are not well established. This study presents the results of the efforts to develop such indicators for krill 'condition' which reflect their recent growth and feeding activities.

The amount and ratio of nucleic acids in tissue as a growth rate estimator were measured from field-caught and laboratory-reared animals. Changes in the size, protein and lipid content and digestive enzyme activities of the digestive gland of krill were examined in relation to changing food regimes. The relationship between eye diameter and body length as a long-term starvation indicator was experimentally examined.

The amount of RNA and RNA:DNA ratio in krill muscle exhibited a significant relationship with individual growth rates determined by the 'instantaneous growth rate' (IGR) technique, although the predictability was only modest. This was the case with both field-collected specimens and experimental juveniles. Starved krill with lower growth rates tended to show a lower RNA:DNA ratio and the RNA content levelled off when the growth rates became negative. The moult stage had no significant effect on the nucleic acid contents. Overall, the amount of nucleic acids varied considerably between individuals. Starved krill tended to have higher DNA per unit biomass, which implies shrinkage of cells. The response of the experimental krill in their growth rates to the food conditions was fairly rapid either in a positive or negative direction, and well within the course of a single moult cycle.

The digestive enzyme activity in the digestive gland of field-caught adults decreased substantially within a week of starvation. The size of the gland decreased both in length and weight, accompanied by a substantial loss of lipid and protein, with the former being more readily utilised. In the laboratory experiment, where juvenile krill were alternately fed and starved, the digestive enzyme activities changed in response to the food regime. These changes largely mirrored the mass gain and loss of the digestive gland. The gland size-specific activities of digestive enzymes showed no consistent trends even after a long period of starvation. When the food supply was resumed, the gland regained its mass and enzyme activities. The digestive gland appears to serve as a reserve, which can provide against a few days' starvation and be rebuilt relatively quickly. Its size showed a prompt and steady response to short-term changes in feeding regimes, proving a reliable and easily measurable indicator of recent feeding activities.

By tracking individuals over time and examining specimens sampled as groups, it was demonstrated that fed and starved krill are distinguishable by the relationship between the eye diameter and body length. The eye diameter of starved krill did not decrease even when the animals were shrinking in overall body length. The eye diameter of well-fed krill continued to increase as overall length increased. This created a distinction between fed and starved krill while there was no simultaneous separation in terms of the body length to weight relationship. It would take approximately 2 moult cycles of shrinkage or more at modest rates for the eye diameter to body length relationship to significantly change. Whether this feature is manifested in the wild would be best seen at the end of winter, after the most likely period of extended food limitation.

RNA-based indices have only limited predictive power as an estimator of growth rates. Nucleic acid levels can be used to indicate the nutritional status of the animals, however, they are unlikely to provide accurate, alternative estimates of individual growth rates, but they may demonstrate trends in the population. Growth rates measured by the 'instantaneous growth rate' technique are still the best representation of *in situ* growth, which is apparently determined by the nutritional condition during the period since the last moult, as observed similarly by Ross et al.

(2000). In this study, the prevailing food condition became reflected rapidly in the growth rates and approximately 10 days of new food condition was sufficient to alter the growth rates, either raising them or initiating negative growth. The size of the digestive gland of krill, a crucial short-term storage organ, was more responsive to food condition than enzyme activities. The gland size is a result of feeding activities over the past few weeks and should not be affected by immediate past events such as cod-end feeding. The digestive gland size should, at a minimum, be a simple measure of whether krill have recently undergone severe, sustained food shortage. Long-term, seasonal starvation and the shrinkage it can cause over a few moult cycles can be seen in the eye diameter to body length relationship more obviously than the traditional body length to weight relationship (Siegel 1989, Quetin and Ross 1991).

7.2 Suggestions for future application

A suite of measurements can be employed on krill populations, to produce a matrix of krill 'conditions' in time scale ranging from a week to a few months. Although a large amount of effort is required, the 'instantaneous growth rate' measurements from fresh animals will directly provide the rates of actual *in situ* growth without extra procedures. The recently modified IGR protocol uses smaller perforated jars in through-flow system to hold the krill and considerably eases the experimental effort while substantially increasing the sample size (Nicol et al. 2000). A series of growth rate measurements conducted over a geographical range or time period will supply a useful comprehensive index of recent conditions. In addition to this, as proposed by Nicol et al. (1992a), growth rate measurements, when conducted for an extended period, can be coupled with time to onset of shrinkage and provide information regarding the nutritional states of krill.

Because the size of the digestive gland offers a considerable potential as feeding activity indicator, it is worthwhile measuring the size of the digestive gland from a large number of individuals and schools immediately upon capture and this can be done with a relatively small amount of effort. The size of the digestive gland might be measurable from preserved specimens which would then greatly enhance its

applicability. As long-term, sustained food limitation would become detectable from the gland size, a gland of very large size may only be achieved by continually maintained favourable food conditions. There may well be a distinction in terms of the digestive gland size among catches of krill spread in time and space. If data are collected on a larger scale, they could identify favourable feeding grounds and also indicate local deterioration of food conditions. This measure may also help to test hypotheses involving feeding activity of krill. For instance, whether krill are in direct competition for food with other opportunistic species, and outcompeted by them (Siegel and Loeb 1995, Kawaguchi et al. 1998), could be examined using an index based on the gland size. Although this study did not deal with the colour of the gland, it may deserve further attention. Recently Kawaguchi et al. (1999) showed that the greenness of the digestive gland of krill can indicate micro- and nano-size phytoplankton availability, probably because its greenness was affected by the reduced feeding rates caused by the small size of the algal food. The colour of the digestive gland might be affected by feeding intensity and in some cases, the type of diet. Parallel determination of the gland size and colour may provide further information, possibly concerning switching of food sources.

Despite the potential of nucleic acid-based indicators for assessing the nutritional conditions of the animals, the size of the digestive gland can provide a much simpler yet robust alternative, if the purpose is the simple, expeditious evaluation of the nutritional status. Furthermore, growth rates and the size of the digestive gland may well be correlated, both being determined by recent food conditions at a similar time scale, since the digestive gland of krill serves as a storage organ for immediate use. The protocol for measuring individual growth rates requires the confinement of animals in a small container for a few days, which is long enough for the krill to utilise a substantial portion of their reserve, i.e. the contents of the digestive gland. Thus the initial gland size of an experimental animal at the time of capture, cannot be obtained after the individual moults and provides the instantaneous growth rate. An alternative approach would be to determine the average growth rate of the school and compare this to the average digestive gland size obtained from the same school. This will give more insight and if a significant relationship exists between the

growth rate and the gland size, the gland size may be useful as a crude index of growth potential at the population level, which can be more easily measured.

If young krill have survived a severe food shortage during winter, the relationship between the eye diameter and body length of the samples collected at the end of winter should be able to provide information on whether shrinkage has occurred. The eye diameter to body length relationship in the following summer may reflect winter shrinkage. There is a need to conduct winter surveys and apply this index, ideally at the end of winter. Nonetheless it would be useful to apply this measurement for small sized krill sampled during the early part of summer and determine if there are distinguishable groups based on this index. This should make it possible to determine whether there has been a severe food shortage during winter and hence shrinkage at population level, and if further separation of the recruitment indices obtained from body length measurement alone is necessary. Regional and long-term studies may indicate spatial and temporal patterns in overwintering strategies.

Applying a suite of measurements to determine krill 'condition', will yield a data matrix from short term to long term. These different measures; instantaneous growth rates, digestive gland size and the relationship between the eye diameter and the body length, can now be applied to field studies over wider temporal and spatial extent in order to unravel the interaction between krill population and the environment.

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