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Diel vertical migration and feeding by krill Meganyctiphanes norvegica

Dawdry , Nicola Elizabeth

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Nicola Elizabeth Dawdry

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ABSTRACT

The diel vertical migration (DVM) of zooplankton has been extensively studied and reviewed. Yet the controlling mechanisms for DVM are still uncertain, although several hypotheses, e.g. predator evasion, hunger – satiation, light avoidance, have been proposed. This is particularly so for krill. An important part of understanding krill DVM depends on explaining the factors which drive krill to the surface waters at night. It is frequently speculated that krill migrate to the surface layers to feed. Although there is a vast literature on krill feeding (and the pattern of krill DVM) there has been little attempt to establish the role of feeding in DVM. Consequently, the main aims of this thesis were to further explore the mechanisms for krill DVM and also to explain the feeding strategy of krill in order to understand the role of feeding in DVM, using Northern krill *Meganyctiphanes norvegica* as a model system. These aims were achieved by examining the following: whether krill are selective feeders and also whether the morphology of the feeding basket constrains the food types that can be handled by krill; whether krill feed throughout DVM; the relationship between krill metabolism and feeding during DVM. Krill showed significantly greater feeding rates with larger food types compared with smaller food types and this size selection appeared to be at least in part related to the morphology of the feeding basket. Above all it seemed that krill were opportunistic omnivores and the food types handled by krill were affected by the morphology of the feeding basket. Krill also showed significantly greater feeding rates when offered food types available during the night compared with during the day. Gut contents from field caught individuals supported that krill did not feed extensively during the day as day caught individuals had significantly less stomach pigment content compared with night caught individuals. As krill appeared to not feed extensively on day time available food types it raised the question 'is there a cost to not feeding extensively during the day'. There did appear to be a cost to the lower daytime feeding than compared with the greater feeding shown both with night time available food types and from night captured individuals. It was hypothesized that krill may break down their respiratory pigment, haemocyanin (Hc) possibly for nutrition during these periods of low feeding during the day. In a

field experiment, day captured krill had significantly lower Hc concentrations ([Hc]) than individuals captured at night. There was a clear cost to the lower [Hc] of day caught krill as concentrations of lactic acid in the haemolymph (indicating an O_2 debt) were significantly greater in these day captured krill than compared with night captured krill. Consequently it seems that krill break down Hc during the day probably for nutrition because, for whatever reason, they do not feed extensively on the food types available to them in the deeper depths they reside within during the day. As they ascend to the surface layers at night, where they feed to significantly greater levels on the available food types, they appeared to rebuild their [Hc] and recover from the O_2 debt they incurred during the day. Feeding experiments examining the recovery of [Hc] with food types available during either the day or night showed that after starvation krill recovered their [Hc] significantly quicker (and possibly to higher levels) with night available food types compared with day available food types. As they appear to be opportunistic omnivores it is proposed that this feeding strategy would facilitate the recovery of their daytime incurred debts. Krill appeared to show an asynchronously DVM and in particular female krill appeared to ascend to the surface layers of the water column earlier than males. In fact female krill showed a more extreme pattern of metabolism during DVM, with significantly greater [Hc] (ca. twice that of males) but also greater lactate debts with the breakdown of their Hc during the day. The earlier ascent to the surface layers and also the much greater [Hc] of females may indicate that they have greater metabolic demands than males. The asynchronous pattern of krill DVM supports the hunger – satiation hypothesis for DVM. If satiation is modified to also include the recovery of daytime incurred debts the findings of this thesis do indeed fit this hypothesis. A tentative model is proposed for krill DVM where krill break down their Hc during the day and then recover at night with feeding in the surface layers of the water column.

CONTENTS

FIGURES

TABLES

PLATES

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At no time during the registration for the degree of Doctor of Philosophy has the author been registered for any other University award.

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KMRS seminar 'Factors affecting feeding in the krill, *Meganyctiphanes norvegica*, during diel vertical migration.'
BES oral presentation 'Feeding behaviour of *Meganyctiphanes norvegica* in relation to food type: comparison of herbivorous and carnivorous feeding.'
BES poster presentation 'Relationship between diel vertical migration and feeding in the krill *Meganyctiphanes norvegica* (M. Sars).'
Relevant scientific seminars were attended.

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Chapter 1

General Introduction:
Krill and diel vertical migration

1.1 Mechanism for krill diel vertical migration

Diel vertical migration (DVM) by zooplankton has been widely studied since the early part of the 19th century and has been reviewed extensively (Wyville Thompson and Murray, 1878; Michael, 1911; Esterly, 1919; Russell, 1925, 1927, 1930; Worthington, 1931; Cushing, 1951; Hardy and Bainbridge, 1954; Banse, 1964; Iwasa, 1982; Gabriel and Thomas, 1988; Lampert, 1989, 1993; Ohman, 1990; Andersen and Nival, 1991; Alonzo and Mangel, 2001; De Robertis, 2002; Hays, 2003; and in particular, the excellent recent review by Pearre, 2003). Nocturnal DVM (NDVM) where zooplankton ascend to the surface at night and then descend to deeper depths during the day is the most usual form of movement (Pearre, 2003). Hays (2003) recently reviewed the adaptive significance and ecosystem consequences of these migrations, considering various hypotheses for the ultimate reasons for NDVM. Hays (2003) suggested there is little empirical evidence and often opposing evidence for the hypothesis that migrating into warmer surface waters at night, compared with residing in colder water during the day, gives the migrant any metabolic advantage. He goes on to say that he believes that there is, in contrast, considerable evidence for the predator evasion hypothesis where migrants avoid shallow depths during the day because they would have a high chance of being seen by visual predators (Zaret and Suffern, 1976). The phrase 'better hungry than dead' has been used to explain the advantages of reduced predation risk balanced against the cost of lower feeding during the day (Kremner and Kremner, 1988). For example, Tarling (2002) suggested that copepods descended to deeper depths of the water column on the arrival of krill, *Meganyctiphanes norvegica*. This said, as Pearre (2003) suggested, it actually appeared that copepods descended earlier than the onset of krill migration. Thus he suggested that this was due to the satiation of individuals

after feeding. Even if krill do migrate to deeper depths during the day to avoid predators, the predator evasion hypothesis only partially explains DVM as the question remains as to why krill migrate to shallower depths at night. The movement of plankton to the surface layers to feed is often referred to in these hypotheses but not as part of the mechanism for DVM. One potential mechanism for feeding during DVM, the hunger – satiation hypothesis has, however, been extensively reviewed and commented on by Pearre (2003). The hunger – satiation hypothesis proposed by Pearre (1979) suggests that migrants ascend to feed and then when satiated they return to deeper depths of the water column.

Although not an ultimate reason for DVM the effect of light is one of the most documented proximal cues for NDVM (e.g. Michael, 1911; Russell, 1926; McLaren, 1963; Backus *et al.*, 1965; Kampa, 1975; Haney, 1988; Ringelberg 1995, 1999; Tarling *et al.*, 1999). For example, Strömberg *et al.* (2002) investigated the DVM of krill, *Meganyctiphanes norvegica* in relation with the last eclipse of the millennium finding that the upward and downward movement of *Meganyctiphanes norvegica was* related to changes in light intensity. They suggested that the observation of a midday ascent of krill with reduced light intensity from the eclipse confirms light as an important trigger for DVM. Anderson and Nival (1991) modelled the DVM of euphausiids and suggested that both light and food influenced migration. Pearre (2003) claimed that if the ultimate purpose of upward migration is feeding then a feeding-related signal should also affect migratory behaviour.

Although it is important to investigate proximate cues for DVM such as light there is a great lack of empirical data for the ultimate reasons for DVM particularly with regards feeding (see Tarling *et al.*, 2003 in agreement with Pearre 2003; Anderson and Nival 1991). This lack of consideration of feeding as part of the mechanism for DVM seems surprising considering that Manteyfel (1958 as cited in Pearre, 2003)

commented that 'research on plankton vertical migration...shows that biotic factors are often the most important ones which occur against the background of changing abiotic factors that many researchers consider the leading ones' and also the suggestions by Kozhov (1963) that basing theories of migration only on light or temperature effects were one sided and could produce only a rather primitive concept of a complex biological phenomenon. (The most extreme version of Kozhov's view was taken by Haney (1988) who suggested that each system was so different that there was no common mechanisms, and even within a system there were so many different mechanisms operating that it was impossible to disentangle them!)

Despite the importance of the phenomenon, and the fact that the pattern(s) seem well documented and established, literature on mechanisms for krill DVM is scarce and indeed highlights the need for more studies considering the influence of biotic factors such as feeding on DVM. The lack of empirical evidence for krill DVM seems surprisingly considering that, as highlighted by the comments of Pearre (2003), 'next to copepods, the euphausiids are probably the best – studied group of marine zooplankton and undoubtedly the best – studied of the marine macroplankton.' Even those krill studies that appear to investigate DVM (or claim to) (Sameoto, 1980; Simard, *et al.*, 1986; Onsrud and Kaartvedt, 1998; see also Tarling *et al.*, 2002 on the copepod *Calanus finmarchicus*) either do not examine the ultimate reasons for DVM or they are descriptive and therefore speculative about the reasons for DVM or they make predictions based upon models. Although, models may make useful predictions about DVM (e.g. Alonzo and Mangel, 2001; Burrows and Tarling, 2004) there is still a great need for empirical evidence to support these models and also to investigate the mechanisms for DVM and therefore provide information to formulate models.

1.2 The role of feeding in DVM

The pattern of ascending at night, feeding and then returning to deeper depths has been recorded in krill; for example, *Meganyctiphanes norvegica* (Simard *et al.*, 1986; Onsrud and Kaartvedt, 1998) *Euphausia pacifica* (Nakagawa *et al.*, 2003) and *E. lucens* (Gibbons, 1993). All these studies have suggested nocturnal feeding deduced from an increase in stomach pigment content at night. Given that feeding appears to increase at night with ascent to the surface layers of the water column, it suggests that feeding must form, or affect, part of the strategy for DVM. There are, on the face of it, a great number of studies investigating, to a greater or lesser extent, aspects of krill feeding in the field, and for a variety of krill species: *M. norvegica* (Sameoto, 1980; Simard *et al.*, 1986; Onsrud and Kaartvedt, 1998; Lass *et al.*, 2001; Kaartvedt *et al.*, 2002); *Euphausia superba* (Morris and Ricketts, 1984; Pakhomov *et al.*, 1997; Perissinotto *et al.*, 1997; Atkinson and Snyder, 1997; Atkinson *et al.*, 1999; Ligowski, 2000; Perissinotto *et al.*, 2000; Hernandez – Leon *et al.*, 2001); *Euphausia spinifera* (Perissinotto *et al.*, 2001); *Euphausia pacifica* (Nakagawa *et al.*, 2001, 2002, 2003, 2004); *Euphausia lucens* (Stuart and Pillar, 1990; Gibbons *et al.*, 1991; Gibbons, 1993); *Nyctiphanes australis* (Ritz *et al.*, 1990); *Thysanopoda aequalis* (Schnetzer and Steinberg, 2002) and others. There have also been studies examining krill feeding in relation to their DVM: *M. norvegica* (Sameoto, 1980; Simard *et al.*, 1986; Onsrud and Kaartvedt, 1998; Lass *et al.*, 2001); *Euphausia pacifica* (Nakagawa *et al.*, 2003); *Euphausia* species (Hirota and Nemoto, 1990). Yet despite this comparatively large literature on krill feeding, even in relation to DVM, it is still difficult to interpret the data presented, and thus assess or understand the role of feeding in DVM, as many of these studies are descriptive and not experimental.

Northern krill, *Meganyctiphanes norvegica* have been frequently used as a 'temperate waters model organism' to investigate or model the DVM of krill (e.g.

Sameoto, 1980; Simard *et al.*, 1986; Onsrud and Kaartvedt, 1998; Spicer *et al.*, 1999; Strömberg and Spicer, 2000; Tarling *et al.*, 2000 Lass *et al.*, 2001; Spicer and Strömberg, 2002; Tarling, 2003; Burrows and Tarling, 2004) as they exhibit a strong DVM pattern (Liljebladh and Thomasson, 2001). Some of these studies have made suggestions about the mechanism for DVM.

Onsrud and Kaartvedt (1998) suggest that nocturnal avoidance of surface waters may have been due to predator avoidance as fish schools invaded the surface layer at night. Lass *et al.* (2001) go further and suggest that the diel rhythm in feeding activity of *M. norvegica* in the Clyde Sea and Kattegat is an adaptive response to minimize predation risk. They even suggest predation pressure may be an explanation for when and where food is consumed. Another suggestion for krill DVM has been related to energy gain. Tarling (2003) suggested that female *M. norvegica* undertook a riskier DVM than males and attributed this difference to the demand for energy for reproduction. Yet the role of feeding in even the DVM of a relatively well studied species such as, *M. norvegica*, remains uncertain because either feeding has not been the focus of the above investigations or they are descriptive field studies and therefore it is difficult to establish whether there is a relationship between feeding and DVM. Essential to understanding the role of feeding in DVM is gaining an understanding of the feeding strategy of krill.

Of primary importance is whether krill are selective feeders as it determines the basis for the relationships between krill and their food types during DVM. In surface waters phytoplankton and zooplankton may be more abundant than in the deeper depths of the water column (e.g. Onsrud and Kaartvedt, 1998). Phytoplankton is also mainly concentrated in the surface layer of the water column at night. Consequently, when ascending to the surface layers of the water column both phytoplankton and zooplankton would be available to krill. The extent to which krill feed upon phytoplankton and zooplankton may be a key part of

understanding the feeding strategy of krill and consequently perhaps their DVM strategy as it may explain why krill migrate to the surface waters. Determining whether krill are selective feeders is fundamental to determining the feeding strategy of krill as it forms the basis for the relationships between krill and their food types.

Furthermore, it is also important to understand the basis for selecting or not selecting food types. Experimental evidence suggests that the size and shape of food types may affect feeding rates by krill (e.g. Quentin and Ross, 1985). Both Ikeda and Dixon (1984) and Quentin and Ross (1985) have suggested that *E. superba* feeds more efficiently on larger food types. There have also been studies investigating the functional morphology of the feeding basket (e.g. Artiges *et al.*, 1978; McClatchie and Boyd, 1983; Boyd *et al.*, 1984; Suh and Nemoto, 1987; Hamner, 1988; Suh and and Choi, 1998), although most of these studies focus on *Euphausia* species. While many of the key features of the feeding basket of *M. norvegica* have been well described by Artiges *et al.* (1978) it would be beneficial to examine the finer structure of the feeding basket using scanning electron microscopy to investigate the possibility that the morphology of the feeding basket relates to the food types eaten by krill.

Also it is of key importance (irrespective of the basis) to establish whether krill feed throughout DVM or only nocturnally during DVM as if krill only feed in the night it may explain why krill ascend to the surface layers of the water column. Although it is difficult to ascertain whether krill feed throughout DVM or only at night because field studies investigating krill feeding during DVM are based upon gut contents often with conflicting conclusions about whether krill feed throughout DVM or only nocturnally. For example, Sameoto (1980) suggested that one reason for the upward migration at night of krill may be to locate high densities of food and then goes on to pose the question of why *Meganytiphanes norvegica* migrates when it

feed on copepods at the deeper daytime depth is uncertain. Sameoto (1980), however, did not consider that the copepods may have been remnants of the previous night feeding. Also the lack of accurate identification of the copepod species available to krill at different depths, or copepod species in the krill guts in, means it is impossible to determine whether krill were feeding during the day. This use of gut contents can lead to misinterpretations of the timescale for krill feeding when gut residence times are not taken into account. Sameoto (1980) and Onsrud and Kaartvedt (1998) have both suggested that *M. norvegica* feeds during the day and night whereas Lass *et al.* (2001) suggested feeding ceased during the day. Simard *et al.* (1986) even suggested that *M. norvegica* shifted to a herbivorous diet at night. Sameoto (1980) suggested that the percentage of copepod remains in the stomachs of *M. norvegica* were greater during the day than at night. As both the studies by Sameoto (1980) and Onsrud and Kaartvedt (1998) are primarily based upon gut contents and therefore cannot suggest when food types have been eaten as they may have remained in gut content. As neither Sameoto (1980) or Onsrud and Kaartvedt (1998) identify the copepod species available to krill with depth during the day and night it is difficult to conclude from their studies whether krill feed throughout DVM. Onsrud and Kaarvedt (1998) found higher numbers of copepod mandibles during the day compared with the night but that does not, however, confirm that krill are feeding throughout DVM. Higher numbers of copepod mandibles in the gut may have occurred after the sampling time during the night and then remained in the gut the next day. Lass *et al.* (2001) explained the copepod mandible content of day – caught krill retention of mandibles in gut from feeding during the previous night. Kaartvedt *et al.* (2002) suggested that *M. norvegica* fed selectively on the copepod *Temora longicornis* during their nocturnal migration to surface layers. Therefore the conclusions from these studies are conflicting regarding whether krill feed throughout their DVM or only nocturnally

(and is a key question I will address in this thesis). Indeed, Pearre (2003) has also highlighted that 'if it can be shown from even a single sample from deep water at any time of day, that some organism contains remains of prey which is not known to occur in that depth stratum, it is often taken as evidence for migration of either predator or prey.' Determining whether krill can and/or do feed on various food types is extremely important to both understanding the feeding strategy of krill and also the relationship between krill feeding and their DVM.

1.3 'Metabolic' status and feeding during DVM

Determining whether krill feed throughout DVM is key not only to understanding their feeding strategy but also to understanding their DVM strategy. Spicer and Strömberg (2002) found that starved *M. norvegica* had lower haemocyanin concentrations ([Hc]) than compared with fed individuals. Although, the effect of food availability on [Hc] has been investigated in other crustaceans (Uglow, 1969; Djangmah, 1970; Dall, 1974; Hagerman, 1983) Spicer and Strömberg (2002) noted a change in [Hc] in an unprecedented short timescale. Therefore, if krill cannot, or do not, feed for part of their DVM it could have an effect on their [Hc]. Spicer and Strömberg (2002) suggested that the breakdown of Hc by krill could be for nutrition. Consequently, [Hc] in krill may not only be affected by whether krill feed throughout their DVM but they may also break down their Hc if they are not feeding. The role of Hc in nutrition together with feeding during DVM therefore requires further investigation.

1.4 Thesis aims

Northern krill, *Meganyctiphanes norvegica*, provide a useful model system for examining mechanisms for krill diel vertical migration as they exhibit a strong DVM behaviour. They have an extremely wide distribution in the northern hemisphere, ranging from the Arctic down to the Mediterranean and across the Atlantic from Western Europe to Canada (Mauchline and Fisher, 1969). They are found (and have been investigated) within a range of habitats, for example: fjords (e.g. Liljebladh and Thomasson, 2001); estuaries (e.g. Sameoto, 1980) and the open ocean (e.g. Lindley, 1977). The main breeding areas are equally diverse and are thought to be: the Gulf of Maine, Gulf of St. Lawrence, south-western and southern Iceland; and the Norwegian sea up to ca. 70° North (Everson, 2000). The development of krill occurs in stages. Heegaard (1948) examined the progressive stages of *M. norvegica* larvae development and suggested two Nauplius stages followed by, one Metanauplius stage, three Calytopis stages and 4 Furcilia stages. Like all crustaceans *M. norvegica* have an exoskeleton, therefore to grow they must moult to achieve a larger body size. Their body size is the largest amid euphausiid species found in the northern hemisphere (Buchholz and Saborowski, 2000), reaching up to approximately 40 mm in body length.

The role of feeding in krill DVM was investigated using *M. norvegica* as a model. As feeding has been shown to increase at night it may be expected that feeding plays an important role in DVM. Many of the hypotheses proposed for DVM do not, however, really consider the role of feeding in DVM and although feeding may be mentioned it is not investigated and considered as part of the mechanism for DVM. One hypothesis in particular, the hunger – satiation hypothesis, does consider the direct role of feeding in DVM. The main aims of this thesis were to explain the role of feeding in the DVM of krill and ultimately the DVM of krill as a

whole. Consequently I am interested in investigating the role of feeding in the DVM of krill within a context, which allows me to test the hunger – satiation hypothesis. In order to address these main aims it was necessary to both examine the feeding strategy of krill, the pattern of krill feeding during DVM and the consequences of the pattern of feeding in relation to krill metabolism during DVM. Therefore the main aim of the thesis was investigated by pursuing a number of subsidiary aims, outlined below, and presented in each of the following chapters.

Chapter 2

Aims

To:

(1) further explain the extent to which *M. norvegica* feeds upon both phytoplankton and zooplankton food types and thus

(2) whether they are selective feeders and also

(3) whether they are primarily carnivorous or herbivorous.

These aims were achieved by;

- quantifying feeding by measuring ingestion and clearance rates of *M. norvegica* with various different food types chosen to represent a range, morphologically different food types to examine size and morphological selectivity which also included both phytoplankton and copepods to compare herbivorous and carnivorous feeding food types

Chapter 3

Aim

To describe aspects of the morphology of the feeding basket in order to investigate how structure may determine/constrain feeding

This aim was achieved by;

- investigating the intersetal distances for both primary and secondary setae.
- measuring the lengths of both primary and secondary setae on the first and second thoracic appendage for a range of krill body lengths.
- quantifying feeding basket length and width in relation to krill body length and krill sex.

Chapter 4

Aim

To determine whether male and female krill feed throughout their DVM in order to explain and compare the feeding and DVM strategy of krill sexes during DVM.

Using a combination of field investigations together with laboratory studies the aims of this chapter were achieved by investigating;

- the effect of food types available during the day or night on feeding quantified by measuring clearance and ingestion rates by krill.
- the effect of food types available during the day or night on the functional response of feeding.
- the pattern of krill feeding during DVM (*in situ*) by examination of krill gut content.
- the pattern of male and female migration and feeding during DVM.

Chapter 5

Aim

To determine whether male and female krill metabolism is related to feeding during DVM in order to explain and compare the feeding and DVM strategy of krill.

Using a combination of field investigation/experiment together with a laboratory experiment the aims of this chapter were achieved by investigating;

- feeding *in situ* during the day and night of krill performing DVM and krill prevented from performing DVM (i.e. placed in cages) was measured.
- haemocyanin, glucose and lactate concentrations of krill during the day and night of krill performing DVM and krill prevented from performing DVM (i.e. placed in cages) was measured.
- recovery of haemocyanin concentrations of starved krill then subsequently fed on diets either available during the day or night was investigated in the laboratory.

Appendix A

Aims

To determine a pattern for krill feeding during DVM in order to provide vital background information, informing the design the studies presented in this thesis. And to explore potential experiments that could be useful in determining the feeding strategy of krill.

Experiments recorded in the Appendix were valuable in forming the ideas, direction and methods for the experiments in the chapters of this thesis.

Consequently, it may help to refer to Appendix A prior to reading the chapters of this thesis.

1.5 Rationale in addressing the thesis aim

My overriding approach to addressing the aim of the thesis has been question driven. Therefore, I have not been primarily interested in exploring and detailing material that fits neatly into particular disciplines (e.g. ecology, physiology, behaviour). Rather the thesis has the form of answering a stream of questions, each emerging from the answer to the one posited before it, and all geared to addressing my overall question. Consequently, the chapters of this thesis are sequential and hopefully flow into one another, pushing towards the overall aim. This has the effect of reducing the sort of (discipline-based?) discussion of 'peripheral' detail and comparison one might expect to find if each chapter was a separate entity and, as such, similar in its form to a stand-alone, scientific paper. It does mean that, if I have succeeded, the thesis is one story, as opposed to a number of related-short stories. It also mirrors my own 'voyage of discovery' while investigating just a little bit of a very big and fascinating question – why do aquatic animals migrate vertically?

Chapter 2

Is the northern krill, *Meganyctiphanes norvegica*, a selective feeder?

ABSTRACT

Krill are of central importance in pelagic marine food webs, consequently a knowledge of their feeding biology is essential to understand how these food webs function. Whether feeding is a selective or non-selective process is fundamental to understanding the feeding strategy of krill as it forms the basis for any trophic relationships between krill and their food types. Therefore, phytoplankton and zooplankton food types were used to assess whether feeding was a selective process in northern krill, Meganyctiphanes norvegica. *Krill were offered one of a range of morphologically different food types including three diatom species and a high and ambient density of a natural copepod assemblage. Clearance and ingestion rates were estimated from laboratory feeding experiments. Herbivorous mean clearance rates were variable with small diatom food types and less variable, with the mean highest herbivorous ingestion rates shown with the largest diatom food type. Carnivorous clearance rates were not significantly different (94.8 ± 36.3 and 92.4 ± 45.5 copepods ml $^{-1}$ h $^{-1}$) with respect to prey density but were significantly greater than herbivorous rates (40.94 ± 10.14 cells ml $^{-1}$ h $^{-1}$). Carnivorous and herbivorous ingestion rates were comparable when krill were offered either an ambient density of copepods (5.3 ± 2.2 copepods ind. $^{-1}$ h $^{-1}$) or a large diatom food type (7.36 ± 1.63 cells ind. $^{-1}$ h $^{-1}$). Therefore clearance and ingestion rates were greater with food types which had a longer length or diameter and were cylindrical shaped. Therefore, it seems more likely that more likely that* M. norvegica *do not actively seek certain food types for which they have a preference but that they can only effectively handle food types of a certain length and morphology.*

2.1 INTRODUCTION

2.1.1 The importance of krill in food webs

Zooplankton, including krill, have been suggested to carry out extensive diel vertical migrations into food rich surface layers in search of food at night (Sameoto, 1980; Lass *et al.*, 2001). During this nocturnal migration both phytoplankton and zooplankton are available food types to krill in the surface layers of the water column. Most krill species are thought to be omnivorous (Mauchline, 1980). Therefore both phytoplankton and zooplankton are important food types to krill. The relative importance of carnivorous and herbivorous feeding in the diet of tropical euphausiids has been shown to vary with species from herbivorous to strictly carnivorous (Roger, 1973). The relative importance of food types with various species has also been shown by Bamstead and Karlson (1998), when they assessed the importance of carnivory in the diet of euphausiids in the Northeast Atlantic. They found that copepods were an important food item and ranked the degree of carnivory for each species as follows; *Meganyctiphanes norvegica* ≥ *Thysanoessa inermis* = *Thysanoessa longicaudata* > *Thysanoessa raschii*. They also suggested that carnivory was less important in the Skagerrak than in the northern area for species occurring in both areas. Lass *et al.* (2001) also found that the importance of carnivory in *Meganyctiphanes norvegica* varied regionally. They suggested that a higher degree of carnivory in the Kattegat than in the Clyde Sea correlated with a higher ratio of copepod to phytoplankton biomass in the Kattegat compared with the Clyde Sea. It has also been suggested that the Antarctic krill *Euphausia superba* feed omnivorously around South Georgia during non – phytoplankton bloom periods (Atkinson and Snÿder, 1997). Therefore it seems that krill generally are flexible feeders able to ultilize

both phytoplankton and zooplankton to varying extents depending on the relative abundance of these food types. Fevolden (1982) suggested that *M. norvegica* had a highly varied diet and in support, suggested that they had a higher heterozygosity of enzymes to deal with a more varied diet. Saborowski and Buchholz (2002) investigating the metabolic properties of *M. norvegica* from different climatic zones suggested that the enzyme characteristics of *M. norvegica* were more influenced by trophic conditions and nutritive state than temperature. Although, these studies have indicated that krill do feed upon both phytoplankton and zooplankton to various degrees depending on species and region, why krill feed on certain food types at given times, and thus why given trophic relationships exist remains largely unresolved. Consequently, one of the most important questions that may be posed is what factors determine the feeding behaviour of krill? More specifically I would like to know are krill selective or non – selective feeders? Whether krill are selective or non – selective feeders is fundamental to understanding food web function, as it forms the basis for any trophic relationships between krill, zooplankton and phytoplankton and thus is a pre – requisite to questions concerning what factors determine the feeding behaviour of krill.

2.1.2 The 'model' species; a selective feeder?

Northern krill, *Meganyctiphanes norvegica* (M. Sars) is thought to be omnivorous, feeding upon both phytoplankton and zooplankton (e.g. Lass *et al.*, 2001; Kaartvedt *et al.*, 2002). However, the extent to which *M. norvegica* utilizes either (or both) of these food types during diel vertical migration (DVM) is unclear. This uncertainty is partly because many of the studies that can potentially explain feeding selectivity are descriptive (e.g. Kaartvedt *et al.*, 2002; Lass *et al.*, 2001; Onsrud and Kaartvedt, 1998) and not manipulative. Although descriptive field

studies provide useful information about what krill may feed upon, they cannot ascertain why krill feed on certain food types. For example, techniques used such as gut content analysis describe food items eaten by krill but do not provide information about why those food items were eaten. Gut content analysis in particular may lead to an over/under estimation of the importance of given food types for example, some food types may remain resident in the gut for longer periods than others, thus those food types with longer gut residence times would accumulate and be interpreted as being more abundant in the gut and therefore eaten more frequently. As a result, whether krill are selective feeders or not is difficult to establish from gut content studies alone. Therefore, there is a need for more experimental studies to provide evidence on whether krill are selective or non – selective feeders.

2.1.3 Potential factors influencing selective feeding

One of the most important potential factors affecting feeding is the feeding apparatus used for handling food. The feeding basket of krill comprises of the thoracic appendages (or pereopods), all of which have setae. These setae overlap creating a mesh or sieve. Hamner (1988) reviewed the biomechanics of filter feeding in *Euphausia superba* and supported the suggestions of Quentin and Ross (1985) that the feeding basket acts like a sieve. That is, the feeding basket expands laterally, drawing water and particles in through the anterior entrance. Water inside the basket is then compressed out laterally leaving the food particles trapped on the setae. Thus, this process of feeding was distinguished from the passive process of filter feeding and called compression filtration by Quentin and Ross (1985). As well as in *E. superba* compression filtration has also been

observed in other euphausiid species for example *Meganyctiphanes norvegica* (see McClatchie, 1985). In fact Hamner (1988) suggests that based on the observations of similarity in morphology of the feeding basket in several species (*Euphausia*, *Thysanoessa*, *Meganyctiphanes* [see Berkes, 1975] and *Meganyctiphanes norvegica*, *Euphausia superba* [see Artiges *et al.*, 1978 and McClatchie, 1985]) and particularly the compression filtration behaviour shown by *M. norvegica* (McClatchie, 1985) it is possible that all the euphausiid species mentioned above all filter feed in essentially the same way.

Given that the feeding basket acts like a sieve and functions by compression filtration it may be expected that certain food types will be retained in the feeding basket more efficiently than others because of their size (cross sectional area, length) and shape and thus give rise to a degree of size selective feeding by krill. For example food types with a longer length and larger cross sectional area than the spaces of the sieve / feeding basket would be expected to be retained as the water is squeezed out of the basket, whereas food types with a smaller length and volume than the spaces of the sieve would be expected to be pass through the sieve as the water is pressed out.

Selection of food types may not only be based on size and morphology but also by the forager making 'decisions.' Optimality models predict that foragers make optimal behavioural decisions (MacArthur and Pianka, 1966; Sibly and Calow, 1986; Cuthill and Huston, 1997). Optimal foraging theory predicts that foragers should select the most profitable food types.

Therefore, it would be expected that krill may exhibit selective feeding in two ways; (a) based on size of food types because of sieving effect of feeding basket (b) based on a 'preference', i.e. actively chooses certain food types for example those food types with greater nutritional value.

The effect of size and shape of food types on feeding by *Euphausia superba* was investigated by Quentin and Ross (1985). Krill were offered uni – algal cultures of 4 phytoplankton species; a flagellate, a pinnate diatom and 2 centric diatoms. They found the maximum clearance rates by krill were directly proportional to spherical radius squared of the phytoplankton cells offered. Consequently Quentin and Ross (1985) suggested that the maximum clearance rates shown by krill were closely related to size, not species and physical dimensions not chemical composition of the food types offered. Meyer and El – Sayad (1983) also investigating feeding in *Euphausia superba* suggested that feeding was probably size dependent.

Haberman *et al.* (2003b) also investigated selectivity for different phytoplankton by Antarctic krill *Euphausia superba* (Dana) but when grazing on mixed phytoplankton assemblages of diatoms, prymnesiophytes and cryptophytes from both the wild and laboratory cultures in contrast to the uni – algal cultures offered to krill by Quentin and Ross (1985). They suggested that *E. superba* actively selected diatoms in phytoplankton mixtures and that selectivity could not be ascribed to particle size alone. Therefore, both these studies suggest that krill are selective feeders, although the basis for selective feeding differs with each study. Quentin and Ross (1985) suggested that feeding was closely related to size and not species whereas Haberman *et al.* (2003b) suggested that the selectivity shown could not be attributed to particle sizes alone and appeared to involve more active mechanisms. Although Haberman *et al.* (2003a) suggest that that there is an optimal size of particle which is grazed better by *E. superba* compared with other sized particles. Therefore, although it seems krill are selective feeders it is unclear whether this selectivity is a 'preference' as Haberman *et al.* (2003b) suggested or due to a handling capability as Quentin and Ross (1985) suggested or most likely

a combination of both preference and handling capability. Most authors (including the studies above) examining selective feeding have only offered krill phytoplankton food types. It is known, however, that many krill species are omnivorous including *E. superba*. Thus, in addition to whether krill select phytoplankton species on a larger scale as a means to understanding how pelagic food webs function, it is important to determine whether krill 'prefer' zooplankton or phytoplankton.

2.1.4 Study design rationale and aims

The main aim of this study was to further elucidate the extent to which *M. norvegica* feeds upon both phytoplankton and zooplankton food types and thus whether they are selective feeders and also whether they are primarily carnivorous or herbivorous. Feeding was quantified by measuring ingestion and clearance rates of *M. norvegica* with various different food types. Clearance and ingestion rates were estimated from end point measurements. End point measurements are taken at the end of an experiment in contrast to time series measurements, which are taken at intervals over the experimental period. The use of end – point measurements to calculate feeding rates has been criticised by McClatchie and Lewis (1986) because it may result in error if feeding ceases or changes during the experiment. Using a time series approach to measurements would, however, make the experiments of this chapter impossible because of the large number of replicates which would be required at each time interval. Therefore even though the use of end point measurements may have limitations but as the main purpose of this study was to determine whether krill are selective feeders the limitations are consistent in each 'feeding treatment.' Also the feeding rates calculated are used as a measure to compare the effect of different food types on feeding by krill and

not to calculate feeding rates *per se*.

In more productive environments, optimal foraging theory predicts that diet should be more specialized whereas a more general diet would be predicted in environments which are less productive. During nocturnal migration to the surface layers of the water column especially during spring bloom periods food is at its most abundant, therefore it may be expected that if krill do select food types they would exhibit most selective feeding in these periods when food is most abundant and thus the environment is at its most productive. Therefore, the choice of food types used in this study was based on food items found in abundance during a nocturnal migration in a spring bloom period (Dawdry and Tiselius, Unpub. Obs). Food items were also chosen to represent a range of different food types, for example, morphologically different food types to examine size and morphological selectivity including both phytoplankton and copepods to compare herbivorous and carnivorous feeding. Therefore, three phytoplankton species were chosen as follows; a large diatom *Coscinodiscus* sp. diameter 100 – 300 µm); a chain forming diatom *Chaetoceros diadema* (diameter 10 – 50 µm) and a small diatom *Thalassiosira weissflogii* (diameter 10 – 30 µm). A natural surface water copepod (length 100 – 500 µm) assemblage was used for carnivorous feeding studies.

2.2 MATERIALS AND METHODS

2.2.1 Collection and maintenance of krill and food types

Meganyctiphanes norvegica were collected from Gullmarsfjord, Southwest Sweden (58°18' N, 11°32' E), using an Isaacs-Kidd midwater trawl (mouth area 0.6 m^2; haul duration = 10 min) on several occasions during Feb and Mar 2002 on the RV *Arne Tiselius*. Krill were transferred (within 10 min of harvest) into sealed thermos containers (Rubbermaid drinking water thermosflask vol. = 80 l) containing ice – cooled (T = 6 °C) filtered sea water (salinity = 34 PSU) and transported to the laboratory at KMRS within 2 h of capture. In the laboratory krill were maintained in fibre – glass aquaria (vol. = 350 l) covered with dark plastic to keep krill in darkness. Aquaria were supplied with flowing natural 'deep' sea water pumped into the station from a depth of 35 m (S = 34 PSU, T = 6 °C). All experiments were carried out within 5 d of capture.

Copepods were collected from surface waters of Gullmarsfjord using a plankton net (200 µm WP–2) and returned to the laboratory < 60 min of capture in sealed thermos containers (Rubbermaid drinking water thermosflask vol. = 16 l) containing filtered sea water. At KMRS copepods were maintained in a single glass aquaria (vol. = 10 l) supplied with natural 'surface' sea water pumped into the station from a depth of 6 m (S = 34 PSU, T = 6 °C). All experiments described below were carried out within 1 – 2 d after copepod collection.

An isolated culture of *Thalassiosira weissflogii* was kindly supplied by Peter Thor at KMRS and *Chaetoceros diadema* by Melissa McQuoid at the University of Gothenburg (Sweden). Batch uni – algal cultures of *Thalassiosira weissflogii* and *Chaetoceros diadema* were maintained in sea water under constant light and temperature (T = 23 – 25 °C) conditions.

Coscinodiscus sp. was, however, not cultured in laboratory due to the time constraints of establishing a culture. *Coscinodiscus* sp. was collected from nearby surface water using a plankton net (90 µm WP–2). A net was used as opposed to collecting a volume of natural sea water in order to concentrate *Coscinodiscus* sp. and also to avoid collecting smaller phytoplankton species. This collected sea water was then filtered through a 200 µm sieve 15 to 20 times in order to separate *Coscinodiscus* sp. from any smaller phytoplankton that may have also been collected.

2.2.2 Feeding experiments

For all feeding experiments a group of similar size krill (body length = 30 – 36 mm) were selected from the stock aquaria and were then transferred to a plastic container with filtered sea water (vol. = 50 l) 24 h previous to any feeding study in order to starve krill. Clearance rates (*F*, volume of water cleared of food by consumer per unit time, equation 2.1) and Ingestion rates (*I*, amount of food consumed per unit time, equation 2.2) were calculated using Frost's (1972) equations modified as suggested by Båmstedt *et al.* (2000) as follows:

$$F = V/(t \times n) \times \ln(C_t'/C_t) \quad (2.1)$$

$$I = F \times [C] \quad (2.2)$$

Where V is the volume of the incubation vessel (ml), t is the time (h) and n is the number of consumers. C_t' is the final food concentration in the control vessels and C_t is the final food concentration in the experimental bottles. The mean food concentration is shown as [C].

A. Herbivorous feeding

The effect of phytoplankton food types on ingestion rate and clearance rate of *M. norvegica* was investigated using the following diatom species: *C. diadema*, *Coscinodiscus* sp. and *T. weissflogii.*

Each of the three phytoplankton food types was offered separately at a concentration similar to that observed in the field during a spring bloom (*Coscinodiscus c.* 200 cells l^{-1} *Thalassiosira c.* 700 cells ml^{-1} *Chaetoceros c.* 350 cells ml^{-1} : Dawdry and Tiselius, Unpubl. Obs). The phytoplankton required was added to filtered 'deep' sea water and mixed thoroughly, in a sufficient volume to achieve the desired final concentration. Control bottles contained the food type only whereas experimental bottles contained an individual krill. Control (n = 6) and experimental (n = 6 – 8) glass bottles (vol. = 2.3 l) were filled with sea water containing the phytoplankton food source in a haphazard order, to account for variation between bottles in food type concentration throughout the 'filling' process. Thorough mixing continued throughout this filling process, to ensure that the food type remained in a homogenous suspension. At the start and end of the filling process two control bottles were taken for quantification of phytoplankton concentration at the start of the experiment. The remaining control bottles at the end (n = 4) compared with control bottles taken at the start of the experiment (n = 2) for phytoplankton quantification, allowed changes in algal concentration not due to grazing to be quantified. An individual krill was placed in each experimental bottle, after which the bottle was then filled until the water overflowed. Plastic film was placed over the mouth of each bottle to exclude air and then the lid gently tightened. In order to maintain phytoplankton in suspension, all bottles were placed on a rotating plankton wheel (2 rev. min^{-1}) and left overnight in a temperature controlled room (T = 6 °C) for 12 – 13 h. At the end of this period,

bottles were removed from the wheel, and the contents analysed for phytoplankton concentration. Different methods were used for quantification of the various food types as the same method could not be used for all food types, the reasons for these different methods are discussed for each food type. Each bottle was rinsed three times to ensure all phytoplankton were removed for quantification. Krill removed from the experimental bottles were also rinsed to remove any phytoplankton adhering to the exoskeleton.

Thalassiosira weissflogii were counted using a particle counter (CIAB chemical instruments AB, Vasavägen 78, S-18141 Lidingo, Elzone 5380 micrometrics) in three 10 ml sub – samples of the bottle content. *Coscinodiscus* sp. were too large to be counted using a particle counter, therefore bottles were emptied and the contents filtered through a 90 µm sieve into a Petri dish with 3 drops of Lugol's solution. *Coscinodiscus* sp. cell counts were then made under low power (x 10) magnification. *Chaetoceros diadema* are chain-forming diatoms, and so like *Coscinodiscus*, could not be counted reliably using a particle counter. Also, as *C. diadema* forms chains of variable lengths, every cell would have to be counted. Consequently, changes in concentration of *C. diadema* were quantified by fluorometric determination of chlorophylls and phaeopigments. Duplicate 100 ml samples from each bottle in *C. diadema* studies were filtered onto Whatman glass micro-fibre filters (GF/F) and extracted in ethanol (90 %) for 12 - 14 h. Chlorophylls and phaeopigments were determined using a fluorometer (10 – AU Turner® designs, Sunnyvale, California) following the procedure suggested by Parsons *et al.* (1984).

B. Carnivorous feeding

Carnivorous feeding experiments used the same apparatus and experimental method as herbivorous feeding experiments with the following modifications:

- Krill were offered a natural copepod assemblage as a food type at a density similar to ambient levels in the field (*ca.* 80 individuals l^{-1}) and also at a 'high' density (*ca.* 340 individuals $l.^{-1}$) to represent 'patches' of copepods, (Dawdry and Tiselius, Unpubl. Obs.).

- Copepods collected were transferred to a plastic container (vol. = 30 l) and left for 24 h to separate copepods from large phytoplankton species by allowing the phytoplankton to settle out thus leaving only the copepods suspended in the water. Surface water from this container was siphoned into another plastic container (vol. = 30 l), where sea water was then added to produce the desired prey density.

- At the end of the experimental period the content of each bottle was emptied and placed in a Petri dish with ethanol (70 %) to fix the remaining copepods. All of the copepods present were counted under low power (x 10) magnification. Copepods were not differentiated with respect to either their species or stage as the main aim of the investigation was to determine whether *M. norvegica* had a preference for food types or to see if food types were limited by a handling capability.

2.3 RESULTS

2.3.1 Herbivorous feeding

Presented in Table 2.1 are data for herbivorous clearance and ingestion rates. Although, the highest mean clearance rate value, 1278 ± 1995 ml individual l^{-1} h^{-1} was shown when krill were offered the chain forming diatom *Chaetoceros diadema* as a food type, the 95 % confidence limits of the mean indicated that the clearance rate was extremely variable leading to a very low, -0.03 ± 0.088 (total pigment individual $^{-1}$ h^{-1}) estimated mean ingestion rate with the food type *C. diadema*.

Table 2.1 Mean clearance and ingestion rates (n = 6 – 8) showing 95 % confidence intervals for *M. norvegica* when offered 3 diatom food types. Ingestion rates refer to number of cells for *Coscinodiscus* sp. *and T. weissflogii* food types and to total pigment for *C. diadema*.

Food type	Mean clearance rate (ml individ. $^{-1}$ h^{-1})	Mean ingestion rate (cells or total pigment ind. $^{-1}$ h^{-1})
Coscinodiscus sp.	40.94 ± 10.14	7.36 ± 1.63
T. weissflogii	11.05 ± 25.0	0.07 ± 0.09
C. diadema	1278.0 ± 1995.0	-0.03 ± 0.09

Mean clearance rate when krill were offered the small diatom food type *Thalassiosira weissflogii* was 11.05 ± 25.01 ml individual $^{-1}$ h^{-1} and was thus also variable but particularly low. Again, an extremely low estimated mean ingestion rate was calculated of 0.07 ± 0.088 cells individual $^{-1}$ h^{-1}.

Mean clearance rate with the relatively large, cylindrical diatom *Coscinodiscus* sp, was 40.94 ± 10.14 ml individual $^{-1}$ h^{-1} and therefore less variable than mean

clearance rates calculated for the other phytoplankton food types above. Mean ingestion rate for the food type *Coscinodiscus* sp. was estimated as 7.36 ± 1.63 cells individual $^{-1}$ h $^{-1}$ and thus was the highest herbivorous ingestion rate observed.

2.3.2 Carnivorous feeding

Figure 2.1 shows mean clearance rates were comparable and not significantly different (one – way ANOVA, $F_{1, 12} = 0.54$, $P > 0.1$) when krill were offered either a high density of copepods or an ambient density of copepods (94.8 ± 36.3 and 92.4 ± 45.5 ml individual $^{-1}$ h $^{-1}$ respectively). Estimated mean ingestion rate increased directly proportionally with an increase in prey density that is 23.6 ± 7.0 copepods individual $^{-1}$ h $^{-1}$ were consumed when krill were offered a high prey density which was four times the ambient density and 5.3 ± 2.2 copepods individual $^{-1}$ h $^{-1}$ with an ambient prey density (see Fig. 2.2).

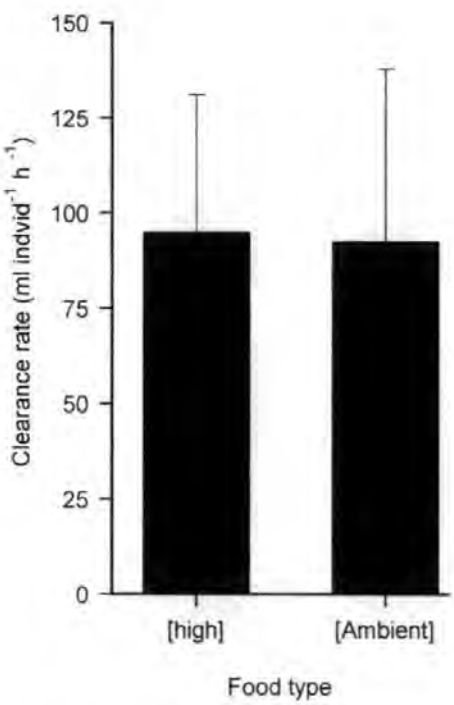


Figure 2.1 Mean clearance rate (n = 6 to 8), showing 95 % confidence intervals for *M. norvegica* when offered an ambient or high density of copepods.

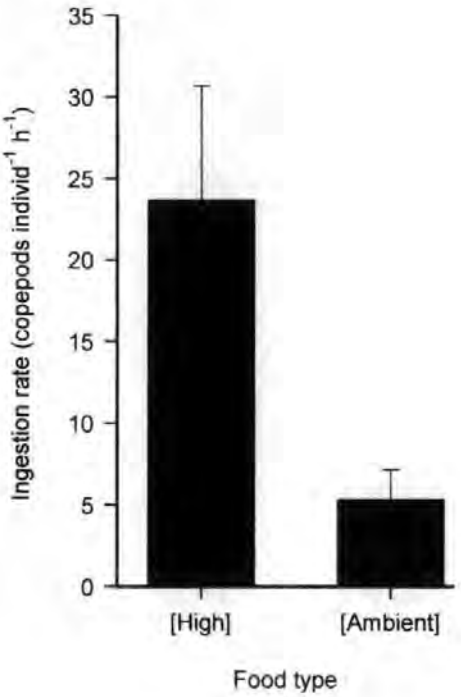


Figure 2.2 Mean ingestion rate (n = 6 to 8), showing 95 % confidence intervals for *M. norvegica* when offered an ambient or high density of copepods.

Therefore, estimated mean ingestion rate was four times higher and significantly greater when krill were offered a high density of copepods than compared with an ambient density of copepods (one – way ANOVA, $F_{1, 12} = 53.55$, $P < 0.0001$).

2.3.3 Carnivorous versus herbivorous feeding

Presented in Figures 2.3 and 2.4 are clearance and ingestion rates for *Meganyctiphanes norvegica* when offered a *Coscinodiscus* sp. or copepod food type.

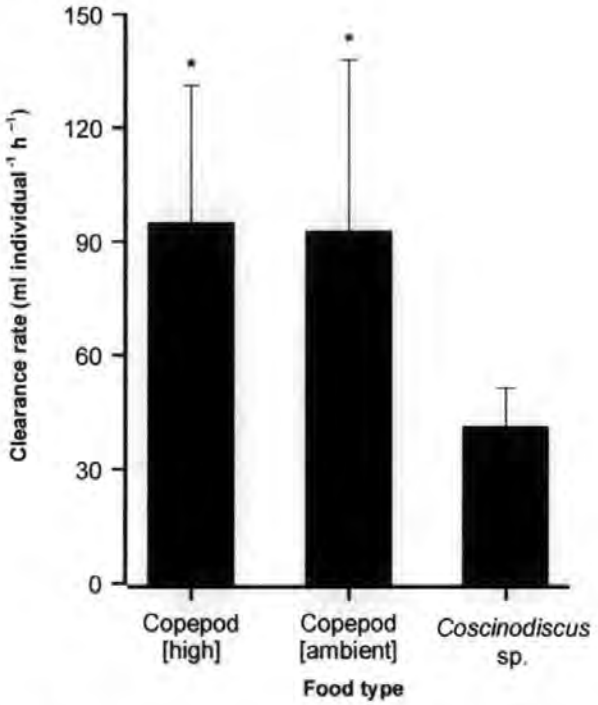


Figure 2.3 Mean clearance rate (n = 6 to 8) showing 95 % confidence intervals for *M. norvegica* when offered phytoplankton or zooplankton food types (* denotes a significant difference, $P < 0.05$).

Mean carnivorous clearance rates for both prey densities were more than twice the amount and significantly greater (one – way ANOVA, $F_{2, 18} = 4.23$, $P < 0.05$) than compared with herbivorous clearance rates with the food type *Coscinodiscus* sp. (see Fig. 2.3).

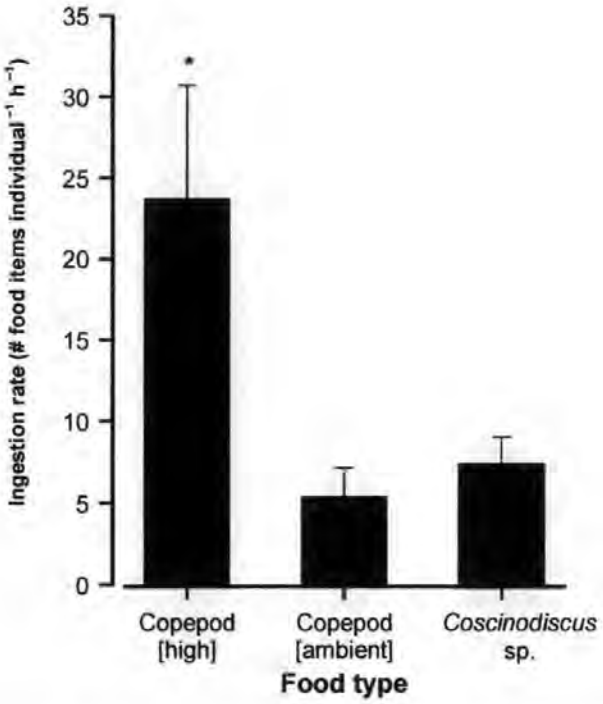


Figure 2.4 Mean ingestion rate (n = 6 to 8), showing 95 % confidence intervals for *M. norvegica* when offered phytoplankton or zooplankton food types (* denotes a significance difference, $P < 0.01$).

Figure 2.4 shows that estimated carnivorous and herbivorous ingestion rates were not significantly different and thus comparable when krill were offered an ambient density of copepods or *Coscinodiscus* sp. as a food type. Krill offered a high density of copepods showed significantly greater estimated ingestion rates were than compared with either an ambient density of copepods or *Coscinodiscus* sp. (one – way ANOVA, $F_{2, 18} = 6.52$, $P < 0.01$).

2.4 DISCUSSION

2.4.1 Herbivorous feeding

Meganyctiphanes norvegica showed extremely variable mean clearance rates with both the smaller diatom food types *Thalassiosira weisflogii* and *Chaetoceros diadema*. Variability in concentration of food between experimental bottles and control bottles will invariably give rise to some variation in calculated clearance rates. The variation is, however, particularly large. As the same method of mixing food types was used for every food type and with *Coscinodiscus* sp. and copepod food types variation was much lower, the large variations in the clearance rate suggests that *M. norvegica* did not feed extensively on these smaller phytoplankton food types. The estimated mean ingestion rates for both *T. weissflogii* and *C. diadema* were extremely low or zero, and thus also indicate that *M. norvegica* did not feed on either of these food types. Although mean clearance rates were lower with the large cylindrical diatom *Coscinodiscus* sp. than with the chain forming diatom *C. diadema* variability of the mean was much lower and therefore suggests that generally *M. norvegica* preferred or could handle *Coscinodiscus* sp. more effectively than the other phytoplankton food types.

2.4.2 Carnivorous feeding

Carnivorous mean clearance rates were comparable at both an ambient prey density and a high prey density, suggesting that *M. norvegica* increased ingestion rate proportionally with an increase in food density, therefore maintaining a similar clearance rate to that observed at lower food densities. An increase in ingestion rate with an increase with food density would be predicted by a functional response, whereas food density increases ingestion rate increases due to a greater encounter with food items. Thus it seems that *M. norvegica* shows a

functional feeding response. In order to determine the type of functional response exhibited by *M. norvegica*, however, further investigation of krill feeding with a range of prey/food densities is needed.

2.4.3 Herbivorous versus carnivorous feeding

Carnivorous mean clearance rates at both prey densities were more than twice those with *Coscinodiscus* sp. Therefore, *M. norvegica* cleared more water of food when offered a carnivorous diet compared with when offered a herbivorous diet. This higher carnivorous clearance rate may suggest a preference for copepod food types compared with phytoplankton food types such as *Coscinodiscus* sp.. The higher clearance rates, however, on these larger food types compared with on the smaller phytoplankton food types suggest that krill prefer or can handle larger food types. Consequently, higher carnivorous clearance rates compared with herbivorous rates with the food type. *Coscinodiscus* sp. may have been due to the slightly longer length of the copepods compared with the diameter of *Coscinodiscus*.

2.4.4 Feeding preference or handling capability?

As *Meganyctiphanes norvegica* showed less variable clearance rates and higher ingestion rates of *Coscinodiscus* sp. than either *Chaetoceros diadema* or *Thalassiosira weissflogii* it may be that *M. norvegica* prefer *Coscinodiscus* sp. as a food type compared with the smaller phytoplankton food types. Although, *C. diadema* is chain forming, the diameter of individual cells (10 – 50 µm) are comparable to that of *T. weissflogii* cells (10 – 30 µm). Therefore, while *C. diadema* may form chains that are similar in length to the diameter of *Coscinodiscus* sp. (100 – 300 µm) when the phytoplankton are filtered through krill

feeding baskets these chains may be broken which would therefore lead to a smaller overall length (i.e. single cell) of *C. diadema*. Therefore, it seems more likely that higher clearance rates are, at least in part, due to a handling capability and not a feeding preference. That is, *M. norvegica* are limited in the food types they can utilize by the constraints of what they can effectively handle. Consequently, it may be that the feeding basket of krill is more effective at retaining food types of a given length/morphology. Hence it seems that krill are more effective at handling larger food types, which are cylindrical in shape. Higher clearance and ingestion rates with copepods would also support that *M. norvegica* are more effective at handling larger food types with a cylindrical shape. Copepods used in the study were around 100 – 500 μm in length compared with the 100 – 300 μm diameter of *Coscinodiscus* sp., therefore higher mean carnivorous clearance rates could also have been due to more effective handling of a longer length food type and not a feeding preference for copepods. These clearance rates suggest that, like *E. superba*, *M. norvegica* cannot effectively handle small food types, therefore supporting the conclusions of Quentin and Ross (1985) that clearance rates are related to the size and shape of food types offered to krill. Therefore, it seems that feeding by *M. norvegica* is a selective process. The basis for this selective feeding seems largely due to more efficient retention of larger food types by the feeding basket. Higher carnivorous clearance rates, however, suggest that some active selection of food types does occur, that is, it appears krill prefer copepods compared with phytoplankton food types supporting the conclusions of Haberman *et al.* (2003b) that some active selection of food types does occur. Haywood and Burns (2003b) suggested that krill *Nyctiphanes australis* can detect and avoid unpalatable food types. Similarly Ikeda and Dixon (1984) found that *E. superba* were reluctant to feed on non – nutritious latex

beads. However, Bargu and Silver (2003) suggested that *Pseudo – nitzschia* was the dominant food in krill when it was the most abundant diatom in field. Also Bargu *et al.* (2003) found that krill species consumed low toxicity and non – toxic *Pseudo – nitzschia* diatoms at similar rates, but that this species *E. pacifica* showed a different pattern of feeding with the low toxicity diatoms compared with the non – toxic diatoms. Therefore it seems that krill can to some extent detect and respond to certain food types. Higher carnivorous clearance rates have also been shown with *E. superba* (Price *et al.*, 1988). Price *et al.* (1988) found clearance rates on copepod food types were on average 3.1 times greater than those on phytoplankton food types. Atkinson *et al.* (1999) recorded crustacean remains in the guts of *E. superba* both during the summer and winter at South Georgia. Yet *E. superba* is widely regarded as an herbivorous feeder whereas *Meganyctiphanes norvegica* is considered another extreme as a carnivore. This categorizing of these species into feeding modes has probably resulted from records of *E. superba* with stomach contents dominated by phytoplankton (e.g. 0.01 – 10 μg chlorophyll a. individ^{-1}, see Perissinotto *et al.*, 1997) and *Meganyctiphanes norvegica* with stomach containing copepod remains (e.g. Bamstedt and Karlson, 1998). Although regarded as mainly carnivorous *M. norvegica* stomach contents have also been frequently recorded with high chlorophyll levels (e.g. up to approx. 1250 ng Tot. pigment stomach $^{-1}$ see Onsrud and Kaartvedt, 1998; up to approx. 1750 ng pigment gut $^{-1}$ see Kaartvedt *et al.*, 2002). It has been proposed that *Euphausia superba* will also take zooplankton prey (Dalley and McClatchie, 1989), and also can resort to carnivory (Cripps and Atkinson, 2000) leading to the interpretation that it prefers phytoplankton compared with zooplankton food types. Consequently, such statements suggest that feeding by *Euphausia superba* is due to a feeding preference and not the

availability of given food types. It has also been suggested that based on the ratio of *Pars molaris* to *Pars incisiva* length in the mandibles of *E. superba* and *M. norvegica* that they have mandibles mainly 'adapted' for filter feeding (herbivorous) and carnivorous feeding respectively (Mauchline, 1980). Although, *E. superba* have been suggested to feed on copepods during non – bloom periods around the island of South Georgia (Atkinson and Snyder, 1997). Also *M. norvegica* is known to feed extensively on phytoplankton during bloom periods (Pers. Obs.). Whether krill switch diets to alternative food sources is debatable (Quentin and Ross, 1991) and it has been suggested that krill seem able to utilise whatever food is available (Schnack, 1985). Felvoden (1982) suggested that high enzyme heterozygosity in *M. norvegica* correlated with a varied diet. Buchholz and Saborowski (2000) that specific induction of chitinases indicated omnivory by both *M. norvegica* and *E. superba* and a capacity to adjust to highly variable trophic environments. Haywood and Burns (2003a) found no significant effects of food type on the body length of sibling *N. australis* reared on different diets. Therefore also suggesting that krill are flexible feeders and able to utilize a variety of food types. Kinsey and Hopkins (1994) suggested that morphological characters partly determined diet, but that behaviour was also important in the euphausiid species they examined in the Gulf of Mexico. According to optimal foraging theory a generalist feeding strategy may be expected in areas like the Gullmarsfjorden, where food abundance may vary greatly during the year and have long periods of low productivity like the Antarctic. Therefore the 'carnivorous' northern krill *M. norvegica* may be more like the 'herbivorous' southern Antarctic krill *E. superba* than has previously suggested by the literature. Similarity of these species may be expected particularly because the design of the feeding basket of both species is functionally similar (McClatchie, 1985). Also both these krill species live in

environments notorious for being low in productivity compared with the rest of the world's oceans. Therefore it may be expected that a generalist opportunistic feeding strategy would be favoured by both these species. Thus it seems that caution should be taken when placing krill into feeding mode groups especially based on gut contents alone because present diet does not indicate the potential ability to feed on food types and therefore feeding mode. Hence it seems that *M. norvegica* mainly show selection by the constraints of what their feeding basket can handle (that is the shape and size of the food types that can be retained) and although they may show feeding preferences for copepods it is more likely that they are generalist opportunistic feeders, feeding on whatever food types are available which can be retained by their feeding basket rather than specialist feeders seeking particular food types.

Chapter 3

Aspects of the functional morphology of the feeding basket of *Meganyctiphanes norvegica*

ABSTRACT

The functional morphology of the feeding basket is a key factor affecting the food types, which can effectively handled by krill. Therefore I examined and described feeding basket morphology in Meganyctiphanes norvegica *in order to understand their feeding behaviour. Feeding basket length and width was examined in relation to krill sex and body length. Setae length and intersetal widths for a range of krill body lengths were determined using light microscopy and image analysis to examine the finer structure of the basket. The finer structure of the feeding basket was also described using light microscopy and scanning electron microscopy. Feeding basket morphology is discussed in relation to krill feeding rates with various food type sizes offered in experimental feeding studies (see Chapter 2). Primary setae length appeared to be more of a potential factor affecting handling capabilities of the basket than intersetal distance or secondary setae length.*

3.1 INTRODUCTION

3.1.1 The structure of the feeding basket

Krill are well known 'filter' feeders, sweeping and filtering the water to collect food. The thoracic appendages and their projecting setae form a mesh – like feeding basket, which has been suggested to be the main apparatus used for capturing prey and collecting food (Mauchline, 1980). Euphausiids are thought to filter feed by a process of compression filtration (Hamner, 1988). Hamner (1988) suggested that water and food are drawn into the basket from the front and once inside the particles (or food) are retained in the basket as the water is squeezed out laterally between the setae. Therefore the setae on the thoracic appendages act like a sieve, retaining the particles within the basket.

Experimental evidence from feeding studies suggests that the size and shape of food types is an important factor affecting feeding rates by krill (see Chapter 2 and Quentin and Ross, 1985). Both *Euphausia superba* (see Quentin and Ross, 1985) and *Meganyctiphanes norvegica* (Chapter 2) appear to handle larger cylindrical phytoplankton food types more efficiently than relatively smaller food types when offered in feeding studies. *Euphausia superba* when offered a large food type (10 times the estimated spherical diameter of a smaller food type) exhibited 3.5 times higher clearance rates than with the smaller food type (Quentin and Ross, 1985). *Meganyctiphanes norvegica* has also shown much higher clearance rates with larger food types. In Chapter 2 feeding studies *M. norvegica* showed greater clearance rates with a large cylindrical diatom (diameter ca. 200 µm) compared with extremely low clearance rates when offered small diatoms (diameter ca. 15 – 50 µm). Given the difference in feeding rates with relatively larger food types compared with smaller food types, it seems likely that the morphology of the

thoracic appendages and their setae is a key factor affecting the food types that can be retained and handled by the feeding basket. Therefore I investigated aspects of the functional morphology of the thoracic appendages and their setae in order to ascertain whether feeding basket morphology was related to the food types handled by krill.

3.1.2 Feeding basket morphology and krill body length and sex

There are many studies investigating various aspects of krill feeding (see Chapter 1 references), however, there are few studies concerning the functional morphology of the feeding basket of species other than Southern Ocean *Euphausia* species. Studies investigating the feeding basket have mostly used intersetal distance in order to calculate filtering efficiency of the basket (e.g. Boyd *et al.*, 1984; Suh and Nemoto, 1987; Suh and Choi, 1998). Therefore, I measured intersetal distance for both primary and secondary setae. I also, however, measured the lengths of both primary and secondary setae in order to further explain the functioning of the basket both throughout the whole feeding basket of a 'typical' length krill and on the first and second thoracic appendage for a range of krill body lengths. In addition to the finer structural measurements (i.e. setae) I also examined feeding basket length and width (large scale measurements) in relation to krill body length. Thus I examined a range of different body length krill in order to investigate the relationship between feeding basket (fine and large scale) morphology and body length.

In addition to investigating the relationship between krill body length and feeding basket morphology I examined the krill sexes separately. Male and female krill have different energetic demands for reproduction and this demand for energy has being suggested as a reason for why female krill may undertake a riskier DVM

than males (Tarling, 2003). Therefore it may be expected that feeding activity differs between male and female krill throughout their DVM. I have recorded such a difference between the feeding activity of male and female krill during their DVM (see Chapter 4). Therefore, I was also interested in whether feeding basket morphology differed with krill sex. I concentrated on comparing the larger scale measurements of male and female feeding baskets to investigate whether the relationship between feeding basket size and body length differed with the sexes.

3.1.3 Describing the feeding basket

In addition to investigating the relationship between krill body length, sex and feeding basket functional morphology I also aimed to describe the feeding basket. Mandible ontogeny in Euphausiacea has been examined by Casonova *et al.* (2002) and the structure and function of feeding appendages in krill larvae has been analysed by Marshall (1985). Although it is important to understand the development of the feeding basket of *M. norvegica* it is beyond the scope of this the investigation as this thesis is focussed on the feeding of adult krill. Most descriptive work on adult krill feeding baskets has been performed on *Euphausia* species (e.g. Hamner, 1988; McClatchie and Boyd, 1983; Suh and Choi, 1998). The morphology of *Euphausia superba* has been particularly well described and reviewed by Hamner (1988). Although the feeding basket of *Meganyctiphanes norvegica* has been also well described by Artiges *et al.* (1978), particularly in their excellent line drawings, I wanted more information on the finer structure of the basket using scanning electron microscopy (SEM). The functional feeding morphology of the euphausiid *Nyctiphanes australis* has also been investigated by Dalley and McClatchie (1989). Using this descriptive information on the finer structure of the basket together with feeding basket and setal measurements I planned to further eludicate the functioning of basket. Moreover, the information

in this chapter aims to compliment the experimental feeding studies of this thesis by investigating whether there is a relationship between krill feeding behaviour and feeding basket morphology.

3.2 MATERIALS AND METHODS

3.2.1 Krill collection

Meganyctiphanes norvegica were collected from Gullmarsfjord, Southwest Sweden (58°18' N, 11°32' E), using an Isaacs – Kidd midwater trawl (mouth area 0.6m^2; haul duration = 20 min) during the day (4th March 2003). Krill were fixed and preserved in a 4 – 5% formaldehyde solution and examined < 6 months after collection.

3.2.2 Whole feeding basket description

The length of the feeding basket was measured from the anterior edge of the first thoracic appendage to the posterior edge of the last thoracic appendage under a low power microscope using callipers (precision 0.02mm) for a range (body length 23 – 40 mm) of female and male krill (total krill examined = 168 individuals). Krill body lengths were measured from the tip of the rostrum to the end of the telson using callipers (precision 0.02mm).

In order to provide detailed images of the feeding basket krill were examined using a scanning electron microscopy (SEM). A number of individuals were examined although printed images presented here in this chapter were taken from the same individual. All of the individuals examined using SEM were a similar body length to those used in other feeding experiments throughout this thesis (i.e. body length 37 mm). The left side of the feeding basket of krill was removed in order to provide

an internal view of the arrangement of setae on the thoracic limbs of the feeding basket. The thoracic appendages of the feeding basket and the mandibles were also removed. All these samples were air dried for 24h after which they were mounted onto stubs and sputter coated with gold (EMITEVH K550 sputter coater). The samples were then examined using a JEOL 56 00 LV Scanning Electron Microscope.

3.2.3 Detailed examination of the feeding basket

For descriptive purposes the lengths of all setae and the distance between primary setae and also between secondary setae was measured on every segment of every thoracic appendage in one krill. The length of each appendage was measured and also the lengths of every segment for each appendage were also measured. The krill used for these measurements was a similar body length (37 mm) to krill used in feeding experiments in other chapters (see Chapters 2, 4 and Appendix A). Although it would, of course, have been good to have examined many more individuals the time involved in measuring each and every setae length throughout the feeding basket was too great in the time scale available to examine more individuals. Therefore, in order to determine whether these lengths and distances were comparable with those in other krill individuals the lengths of setae and secondary setae and intersetule spaces on the carpus of the first (most anterior positioned) and second thoracic appendage in similar length krill were examined. Additionally the lengths and distances between setae on these two appendages were recorded for a range adult krill body lengths (n = 22) from 22 to 40 mm. The carpus was chosen only because it was easy to count and measure setae on this segment of the appendage than any other segment.

The first and second thoracic appendages were dissected from krill under a low power (x 10 magnification) microscope. These appendages were placed on to a microscope slide and using a high power inverted microscope coupled with a digital camera (Nikon E990) attached the carpus of each was photographed at x 40 magnification. The first and second appendages were chosen for no other reason than that they were easier to remove than appendages from the centre of the feeding basket. The carpus was measured primarily because it was easier to photograph than other segments and although it had both primary and secondary setae they were present in fewer numbers than on the first and second segments and therefore was much easier to measure and analyse. For the purposes of this chapter it is assumed that any relationship between krill body length and setae length and/or intersetule spaces on the carpus of the first and second appendage of the feeding basket is allometric and therefore is representative of a relationship throughout the feeding basket.

The images were analysed using an image analysis computer package (Pixera Studio). An image of a 1 mm graticule was also taken at the same magnification as used for each thoracic appendage image in order to calibrate the software. Primary and secondary setae lengths were measured in the centre from their base to tip. Intersetule spaces were measured at the base of setae from the inner margin of adjacent setae.

3.3 RESULTS

3.3.1 Feeding basket description

The feeding basket comprises of seven thoracic appendages. Each of these thoracic appendages consists of an endopodite and an exopodite (see Plate 3.1. A) From each segment of the endopodites setae project, overlapping to create a mesh like basket (see Plate 3.1).

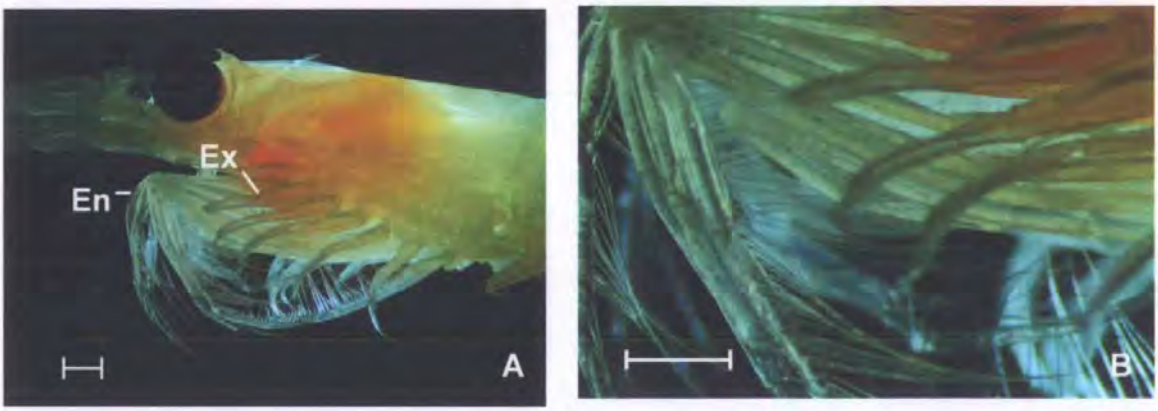


Plate 3.1 A. Thoracic appendages showing endopodites (En) and exopodites (Ex) of the feeding basket of *Meganyctiphanes norvegica*. B. Projecting setae on thoracic limbs overlap creating a mesh like basket. Scale bar = 1 mm in each case.

The primary setae were between 1.43 and 1.86 mm long for krill ca. 37 mm body length krill (using first and second thoracic appendages for measurements). The primary setae appeared to extend from one appendage and overlap with the next thoracic appendage when the feeding basket was laid flat (see Plate 3. 2). The secondary setae were between 0.01 and 0.04 mm long for krill ca. 37 mm body length krill (using first and second thoracic appendages for measurements). The secondary setae protruded from the primary setae creating a more complex mesh structure to the feeding basket.

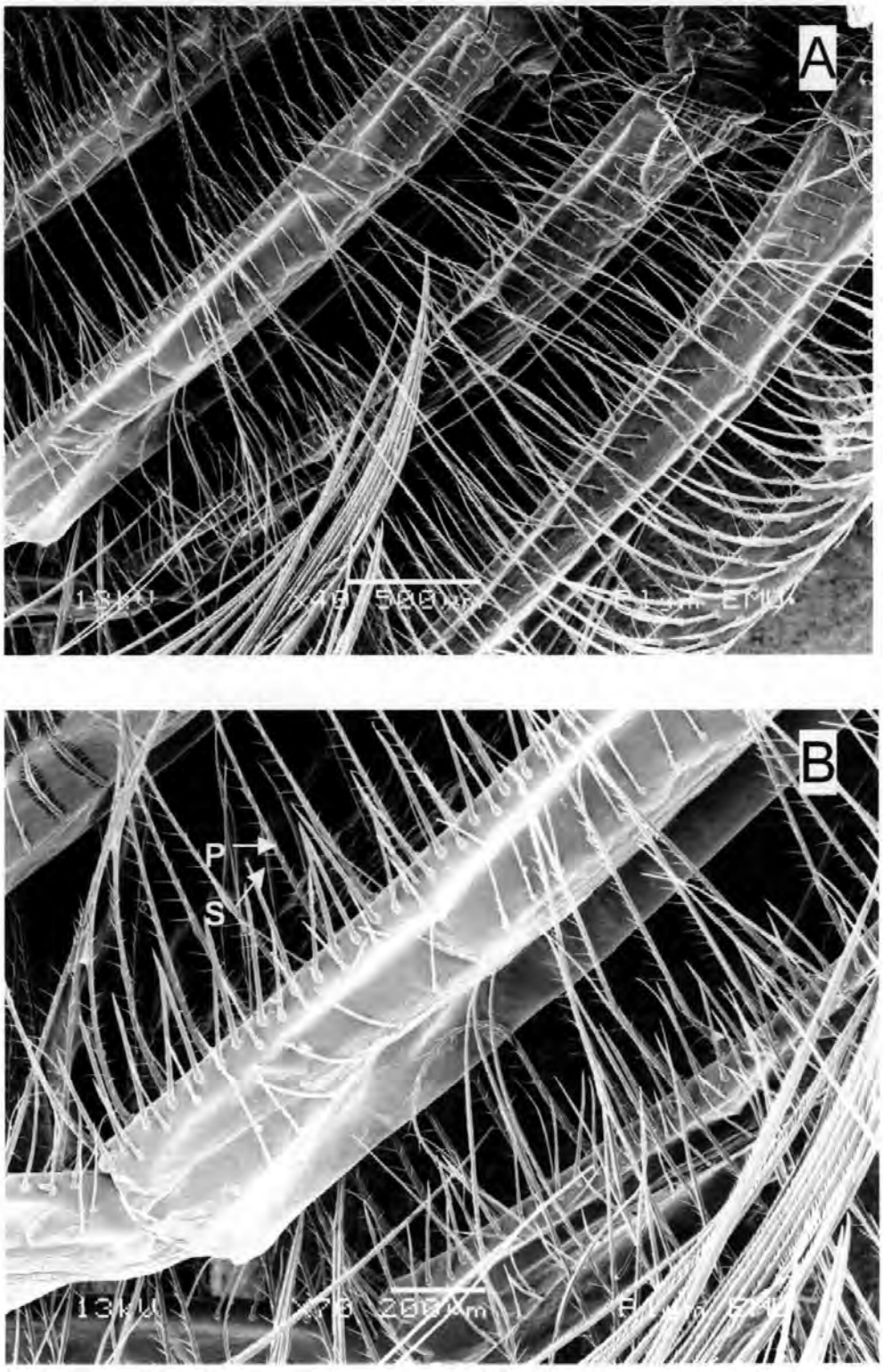


Plate 3.2 A. Structure of feeding basket showing overlapping setae. B. Larger primary setae (P) from which the secondary setae (S) project create the mesh structure of the feeding basket.

Each thoracic appendage from 1 to 6 (anterior to posterior) has 5 segments (see Plates 3.3 A and B) an ischium, merus, carpus, propodus and dactyl. The ischium, merus and carpus had primary and secondary setae. The propodus and dactyl, however, appeared to have primary setae but lack secondary setae. Examples of segments with and without secondary setae are shown by Plate 3.4. The last thoracic appendage differs from the other 6 by being composed of only 2 segments, which has both setae and secondary setae (see Plate 3.3 C).

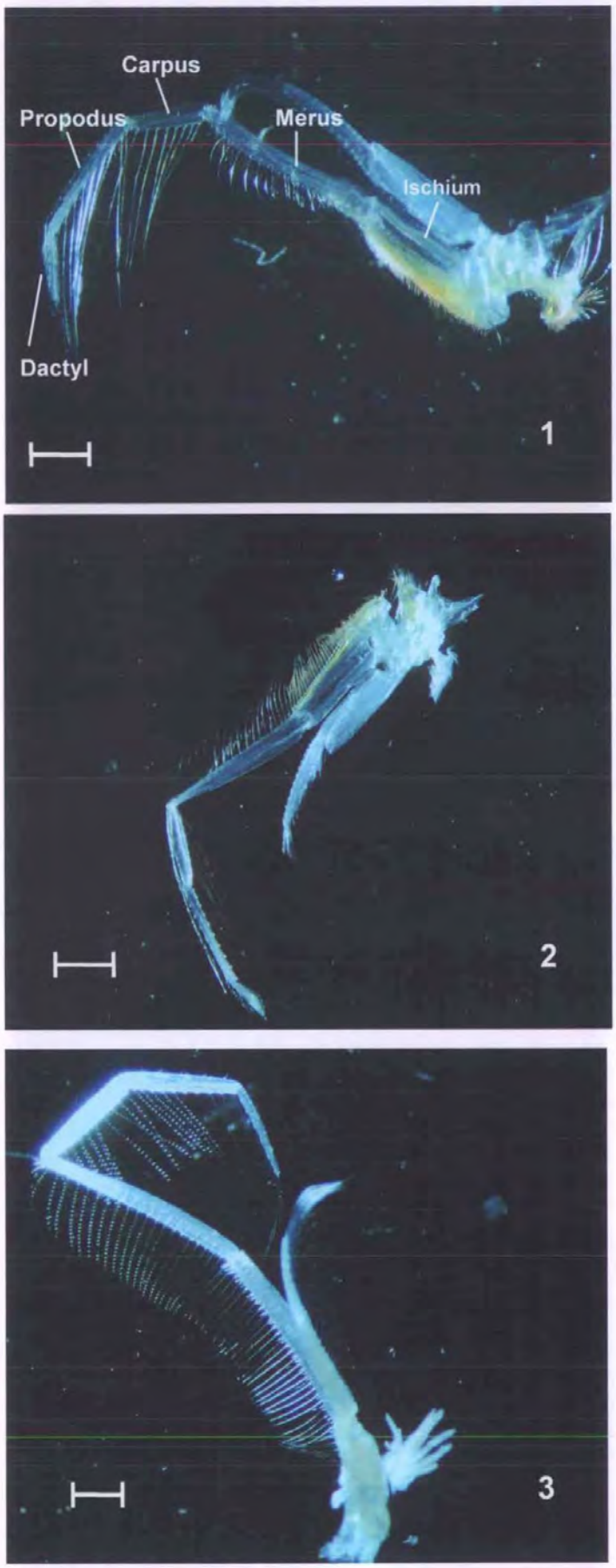


Plate 3.3 A. Thoracic appendages 1 – 3 showing endopodite and exopodite. Ischium, merus, carpus, propodus and dactyl segments labelled on exopodite of appendage 1. Scale bars = 1mm.

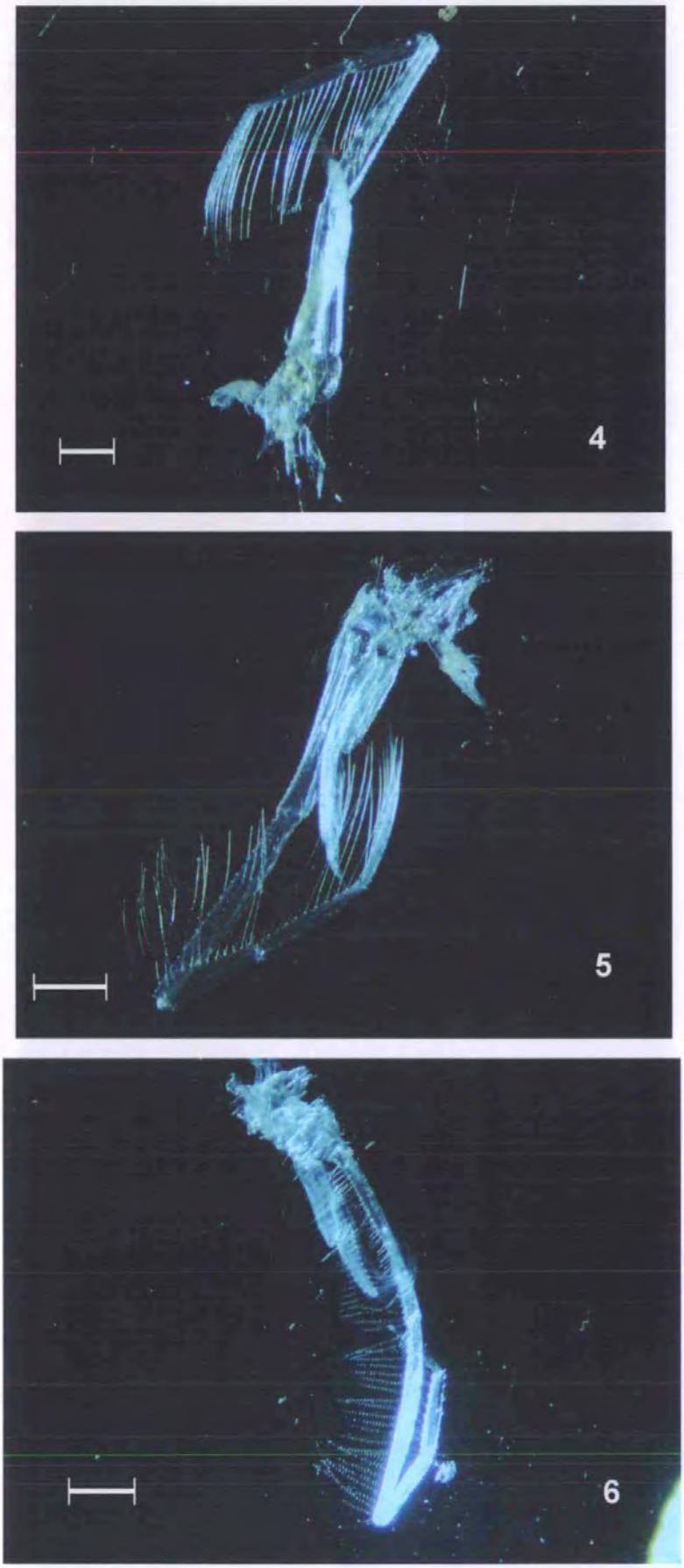


Plate 3.3 B. Thoracic appendages 4 – 6 showing endopodite and exopodite. Scale bars = 1 mm.

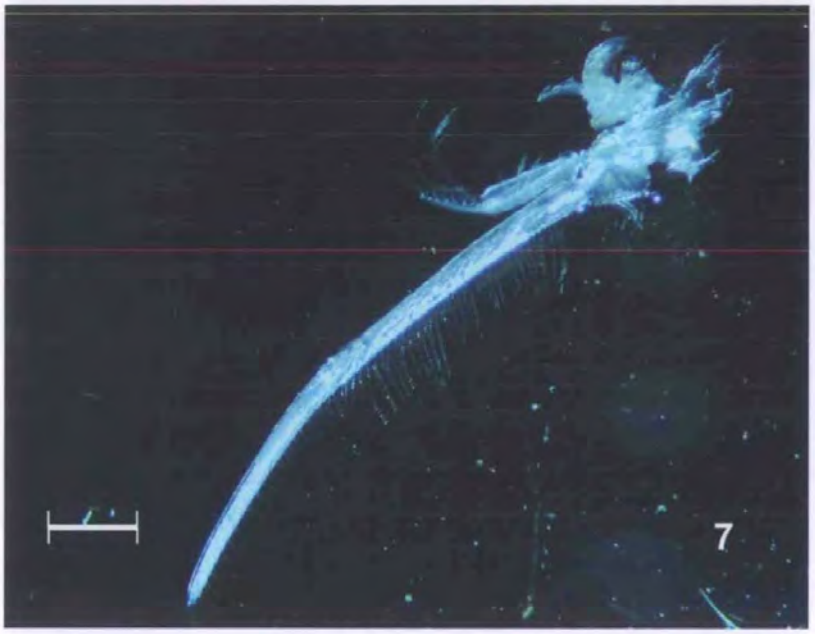


Plate 3.3. C. Thoracic appendages 7 showing endopodite and exopodite. Scale bar = 1 mm.

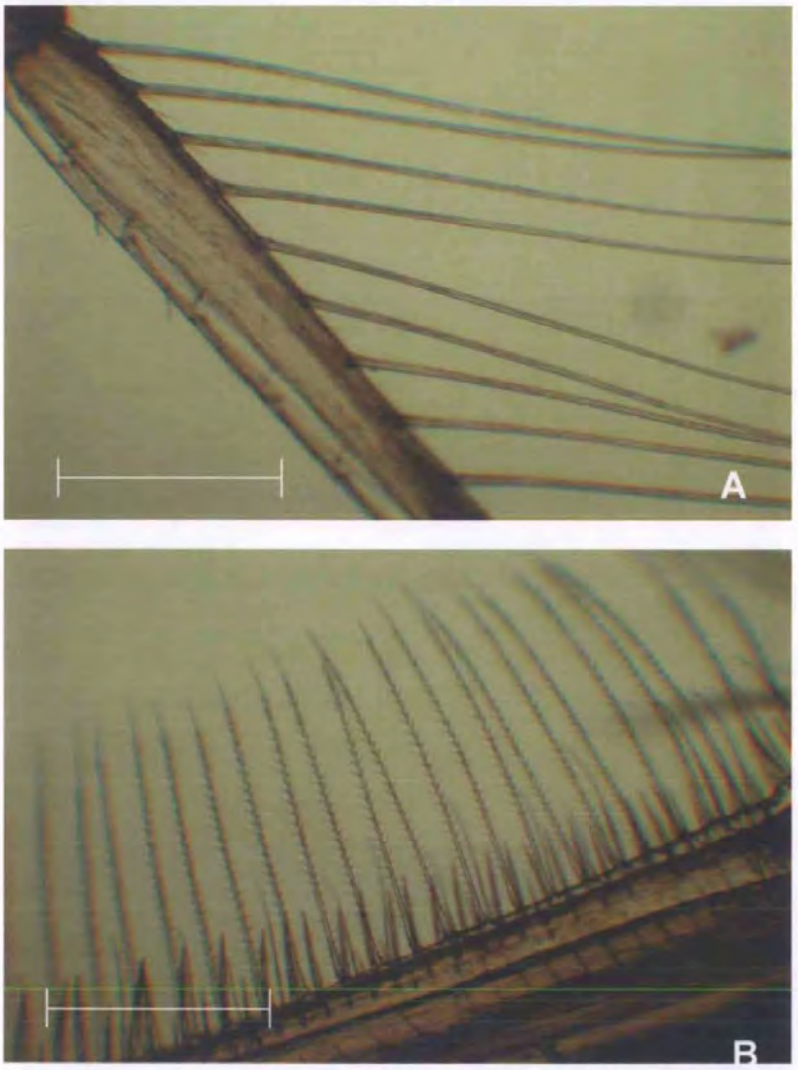


Plate 3.4 Primary setae on segments of thoracic appendages of the feeding basket. A. Primary setae from the propodus with no visible secondary setae. B. Primary setae on the ischium with secondary setae clearly present. Scale bars = 0.5 mm in each case.

Although the first 6 appendages are similar in having the same number of segments they all appear to be different from one another quantitatively and qualitatively in their morphology. For example, the length of the primary setae varied with each appendage of the feeding basket and also with each segments of the same appendage (see Fig. 3.1). Secondary setae were between 0.02 and 0.04 mm in length on all appendages of the feeding basket.

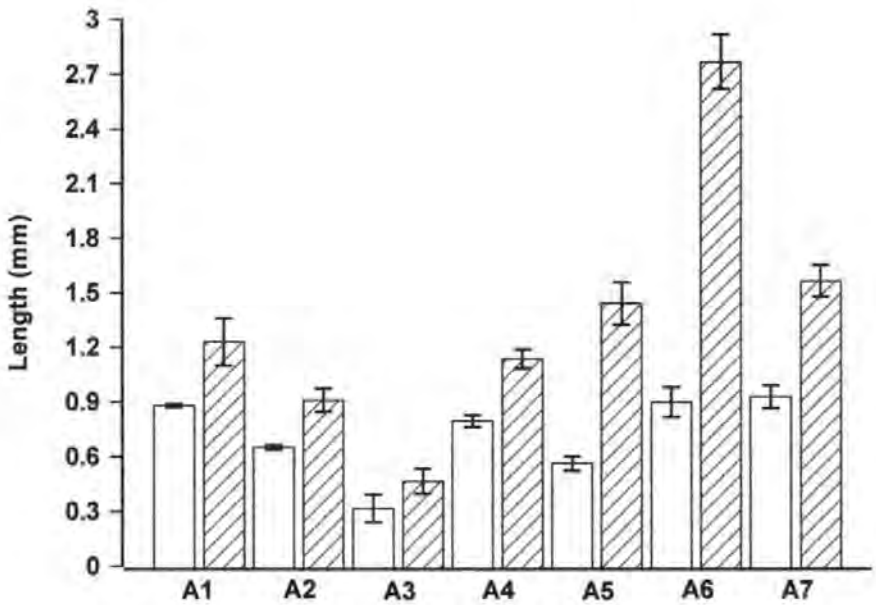
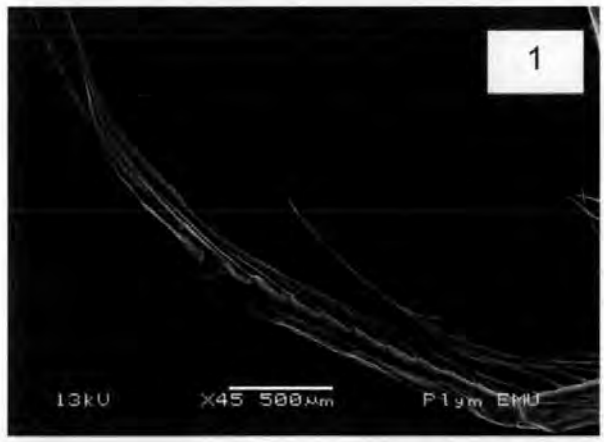
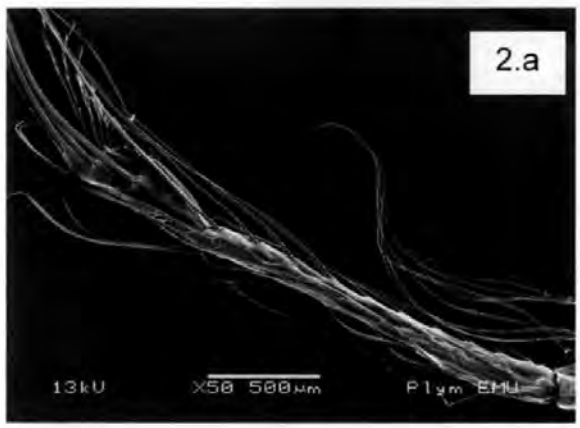


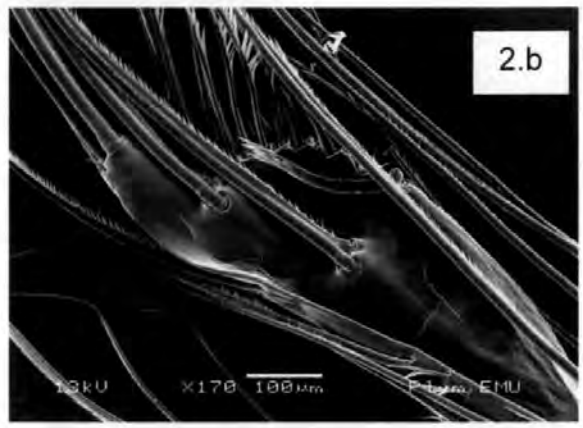
Figure 3.1 Mean length of primary setae (n = 4 – 18) on ischium (open bars) and merus (hatched bars) segments of appendages (A1 – 7) of feeding basket. Values are expressed as means plus or minus 95 % confidence intervals.

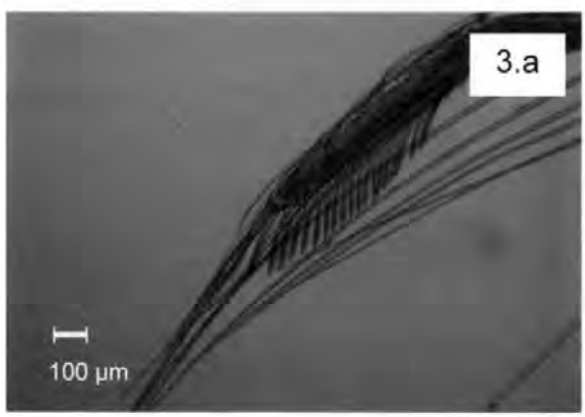
Two lengths, or perhaps layers, of primary setae were evident particularly on the first segments of appendages one and two. These shorter setae were approximately one quarter of the length of the longer primary setae on the 1st appendage whereas they were half the length on the longer primary setae on the 2nd appendage.

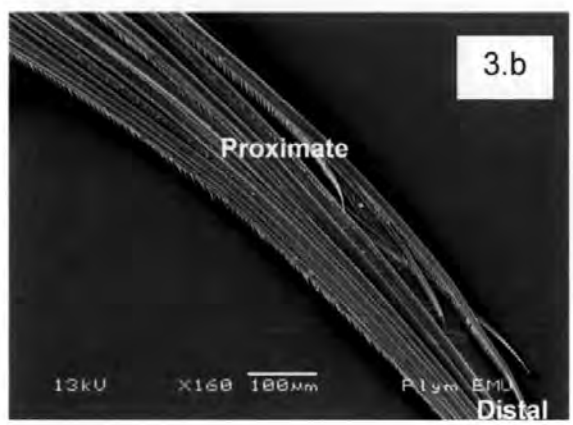
One of the most striking differences between each of the thoracic appendages is the morphology of the dactyl segment of the appendage (Plate 3.5). The dactyl of the first appendage appears to taper into one or possible two primary setae. This tapering of the dactyl is evident in all but one thoracic appendage. Examination of the primary setae using light microscopy (see Plate 3.4) showed they appear to lack secondary setae. SEM images (see Plate 3.5 A), however, showed that there are very short closely spaced setae, upon the primary setae. The second thoracic appendage dactyl shows the greatest contrast in morphology compared with all other appendages of the feeding basket. The dactyl of the second thoracic appendage does not taper to a point as in all other thoracic appendages. Instead it is shorter in length than the dactyl segments of other appendages and in particular is broad and flattened forming a plate – like shape. In particular, primary setae appear to be present not only on the edge of the segment like in other thoracic appendages but also on the ventral flattened surface of the plate. Short closely spaced setae are again present on all primary setae. The dactyl of appendage 3 has a row of shorter spines like setae bearing short comb like secondary setae. The end of segment terminated in a several primary setae. The dactyls of thoracic appendages 4 – 6 all appears to taper to a point ending with two or three primary setae similarly to appendage 1. Again the primary setae had shorter closely spaced setae forming a fine comb on the edge of the primary setae.











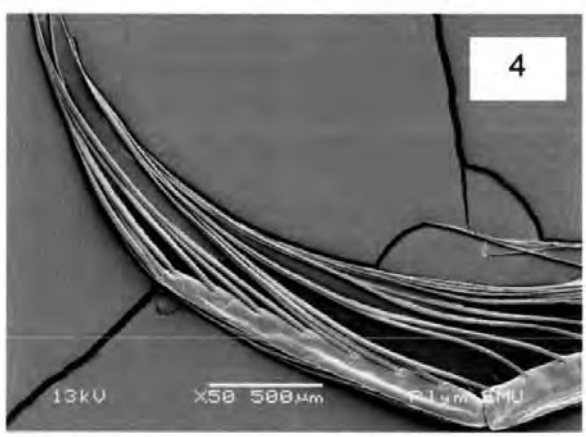


Plate 3.5 A Dactyl segments of thoracic appendages 1 – 4 (as numbered on each image, a and b images show same appendage but at different magnification each given on the micrograph) of the feeding basket

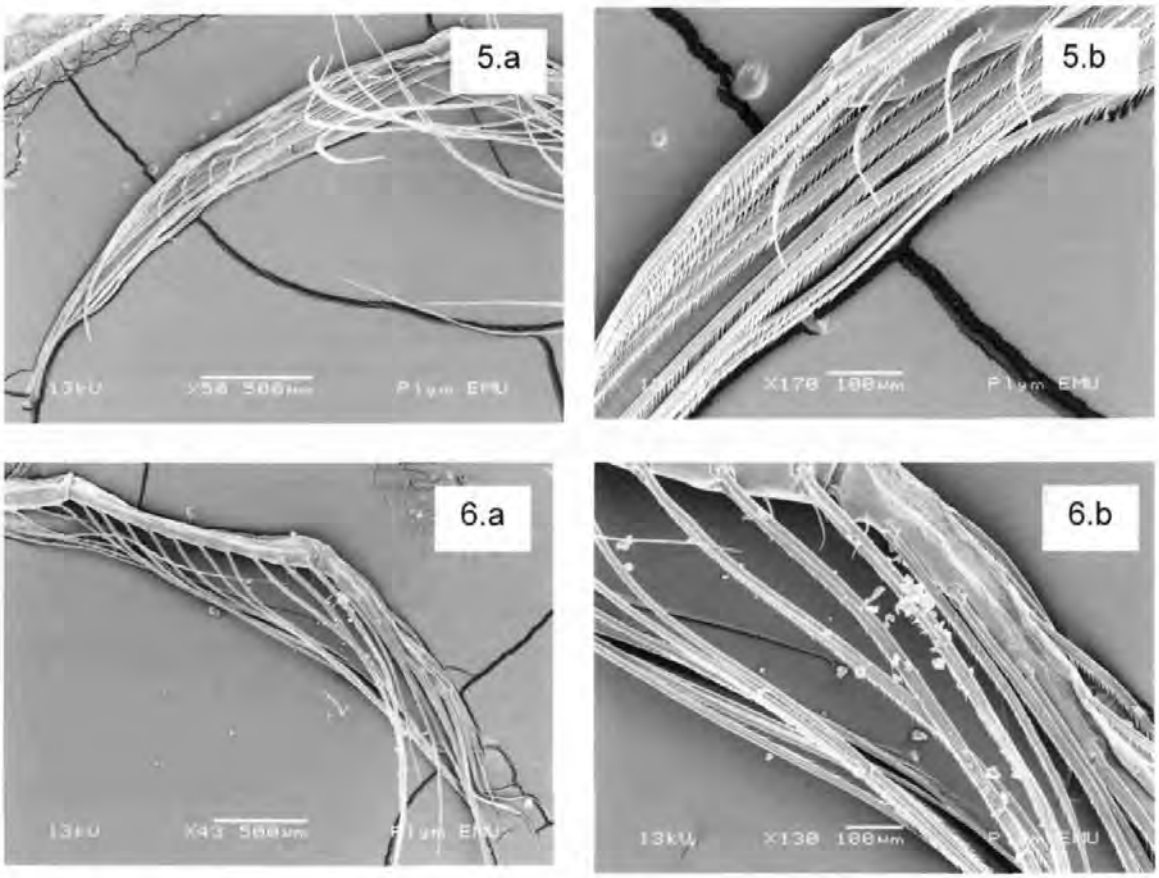


Plate 3.5 B. Dactyls of thoracic appendages 5 and 6 of the feeding basket (as numbered on each image, a and b images show same appendage but at different magnification each given on the micrograph) of the feeding basket.

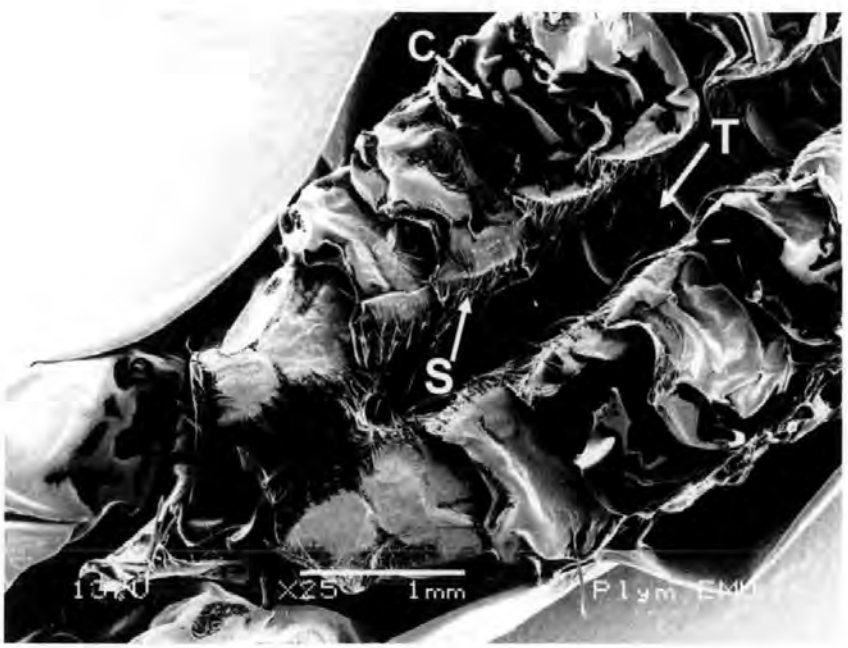


Plate 3.6. Ventral view of the basket of *M. norvegica* with all thoracic appendages removed. C = coxa, S = setae and T = thoracic groove.

Seven coxa were present from which setae projected into the thoracic groove (see Plate 3.6). The mandibular palps are shown overhanging the mandibles (Plate 3.7.A). Towards the end of the mandibular palps secondary setae on the ends of the primary setae formed a comb like structure. The mandibles are shown clasped together underneath the mandibular palps by Plate 3.7.A. Rows of teeth were present on the surface of the mandibles (see Plates 3.7. B and C).

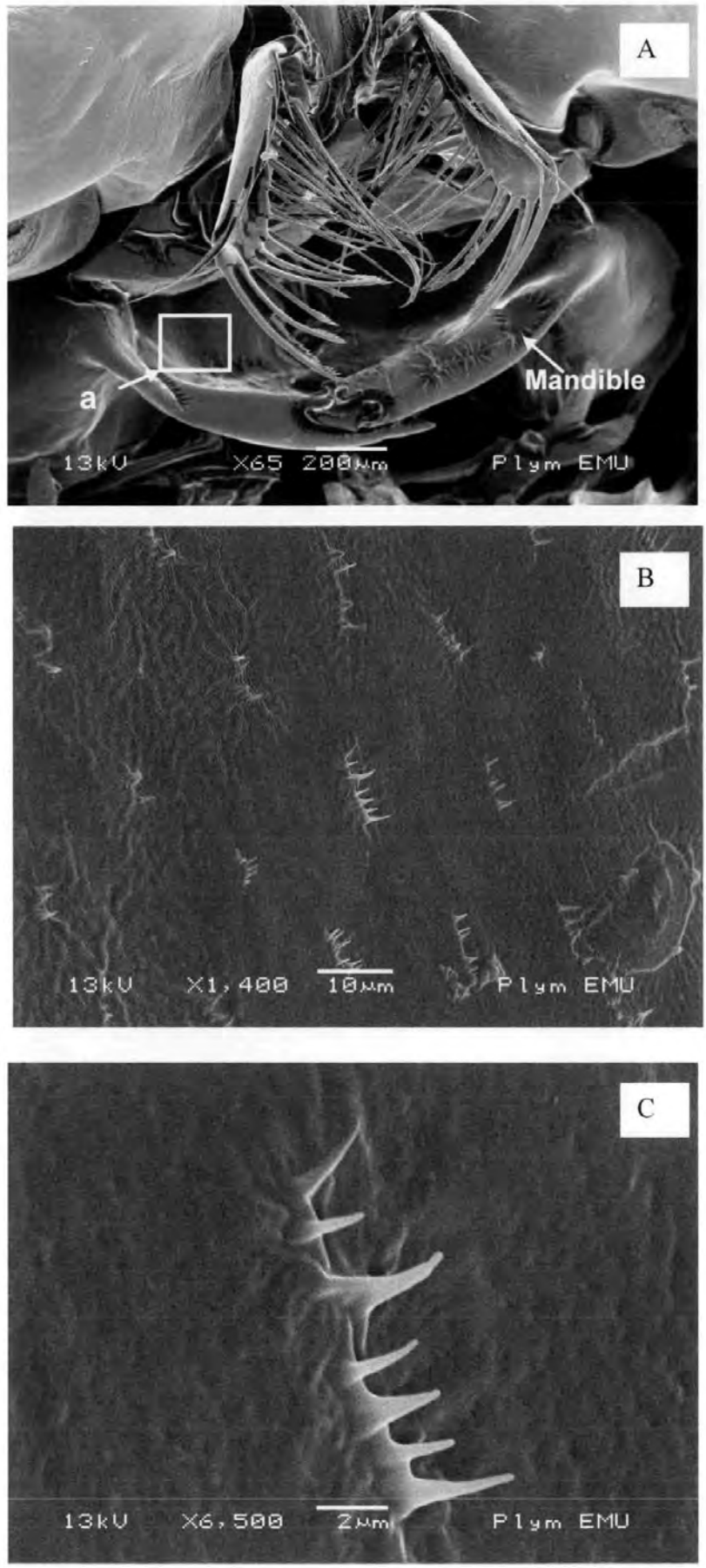


Plate 3.7. Mouthparts of krill. A. Mandibular palps overhanging cutting region. B. Area a. magnified as indicated on micrograph to show rows of spines or 'teeth.' C. Row of spines magnified as indicated on micrograph.

3.3.2 Relationship between individual body length and feeding basket morphology

The feeding basket increased in length and width directly proportionally with an increase in krill body length (see Fig. 3.2).

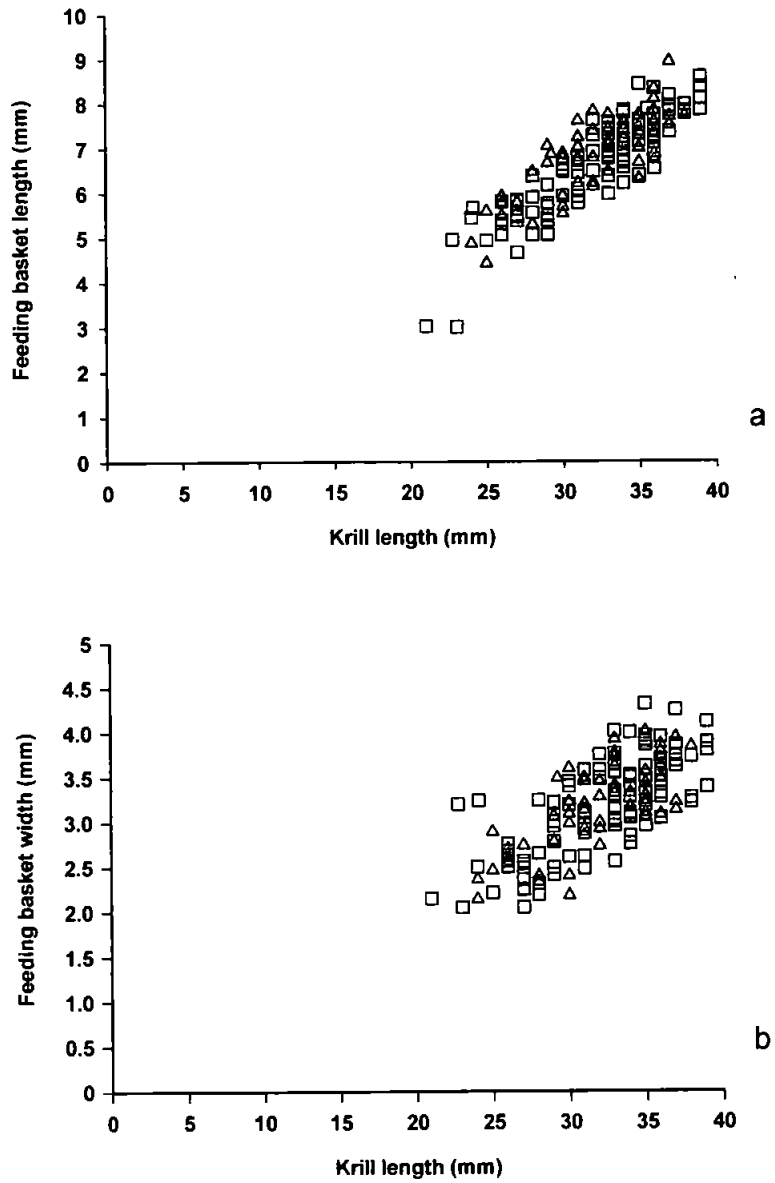


Figure 3.2 Relationship between male (□) and female (Δ) body lengths and feeding basket length (a) and width (b). Each data point represents an individual (male n = 104, female n = 65).

ANCOVA (calculated using a spreadsheet provided by A.J. Underwood Pers. Comm.) detected no significant difference between the slopes of male and female

body length and feeding basket length ($F_{1, 164}$ = 2.72, P > 0.05). There was, however a significant difference between the estimated Y intercepts for male (Y = 0.45) and females (Y = 0.09) feeding basket length versus body length ($F_{1, 164}$ = 9.44, P < 0.01). There was no significant difference between either the slopes ($F_{1, 164}$ = 2.17, P > 0.05) or intercepts ($F_{1, 165}$ = 0.10, P > 0.05) of male and female krill feeding basket width and body length.

This proportional increase of length with body length was also shown with segment length. The length of third segment of appendages 1 and 2 showed a relatively strong relationship with krill body length (see Fig. 3.3). The correlation coefficient for appendage 1 was 0.8804 and for appendage 2 was 0.9178.

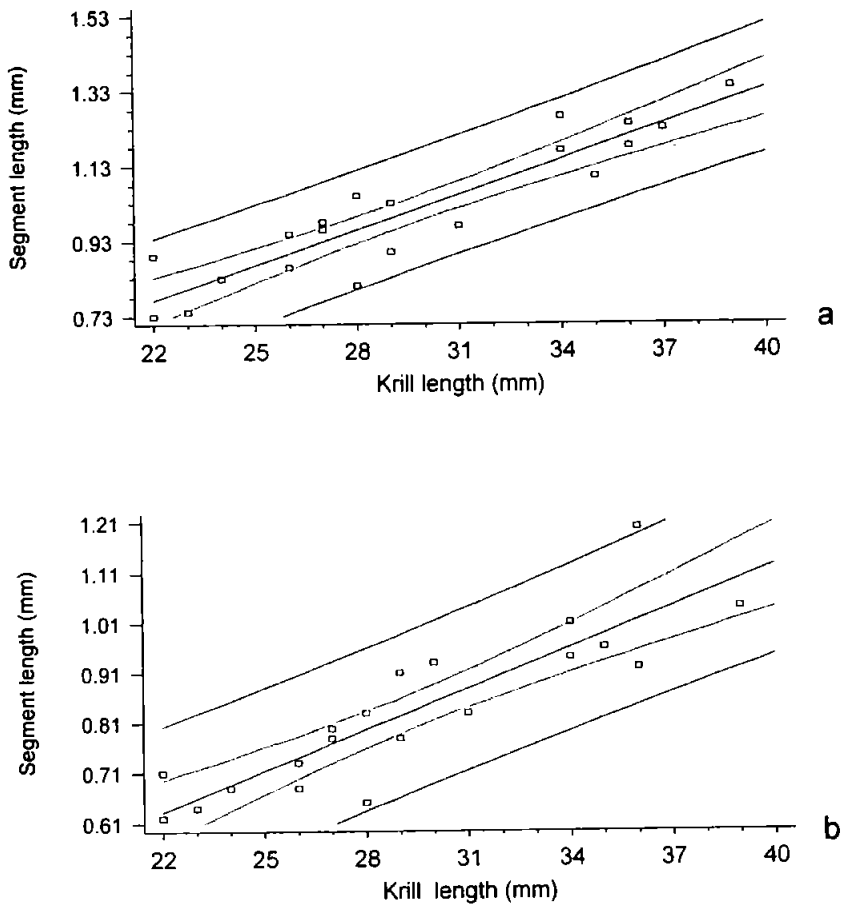


Figure 3.3 Relationship between carpus length of appendages 1 (a) and 2 (b) with krill body length (n = 20). Prediction limits are shown by broken line and 95 % confidence limit by dark grey line.

Again, as shown by Figure 3.4, there was a moderately strong relationship between primary setae length and krill body length with primary setae length increasing with an increase in krill body length in both appendages 1 (correlation coefficient = 0.7012) and 2 (correlation coefficient = 0.6896).

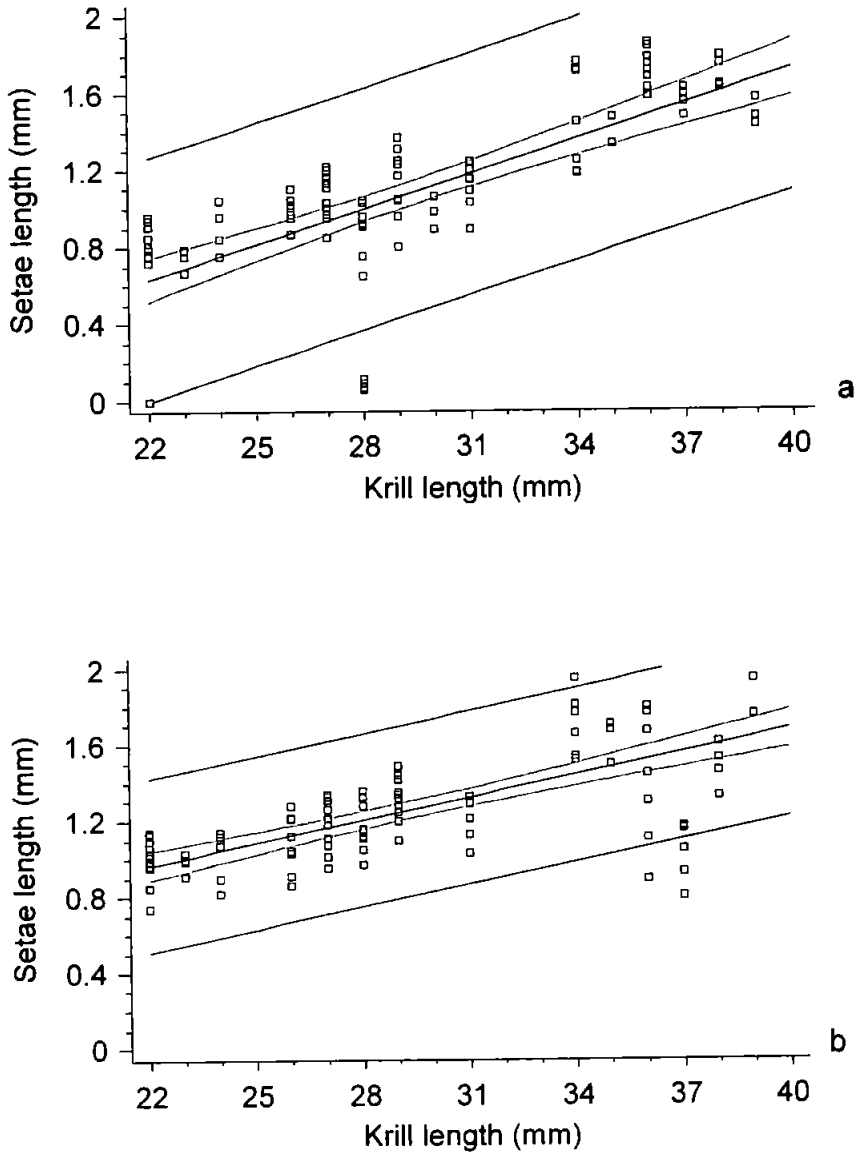


Figure 3.4 Relationship between primary setae length on the carpus of appendages 1 (a) and 2 (b) with krill body length. Prediction limits are shown by broken line and 95 % confidence limit by dark grey line. Values are individuals (a) n = 97, (b) n = 99.

A relatively weak relationship was evident between distance between the bases of the primary setae and krill body length as shown by Figure 3.5. The correlation coefficient for appendage 1 was 0.3978 and for appendage 2 was 0.2072, indicating a weak relationship between the variables.

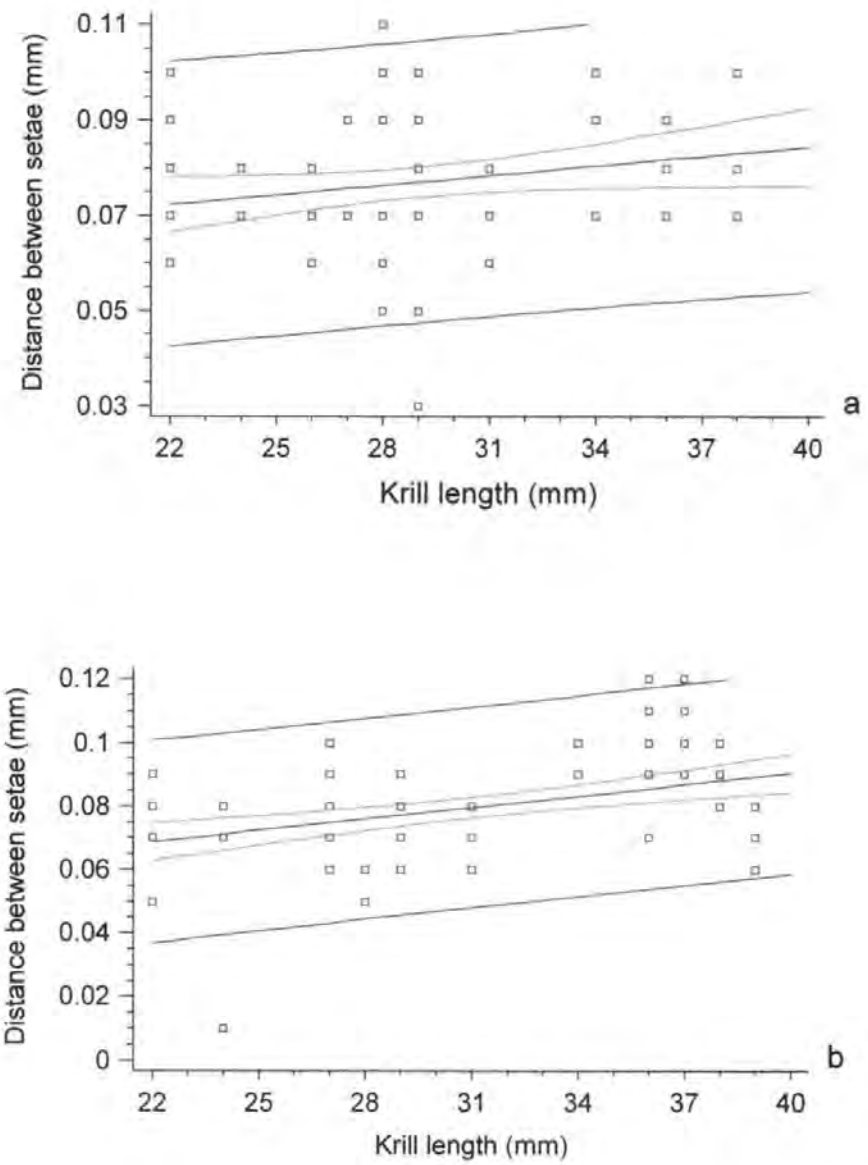


Figure 3.5 Relationship between length of space between primary setae and on the carpus of appendages 1 (a) n = 36 and 2 (b) n = 39 with krill body length. Prediction limits are shown by broken line and 95 % confidence limit by dark grey line.

In contrast to the relationship between primary setae length and krill body length a relatively weak relationship was shown between secondary setae length and krill body length (see Fig. 3.6). A correlation coefficient of 0.1990 for appendage 1 and 0.4527 for appendage 2 indicated this weak relationship.

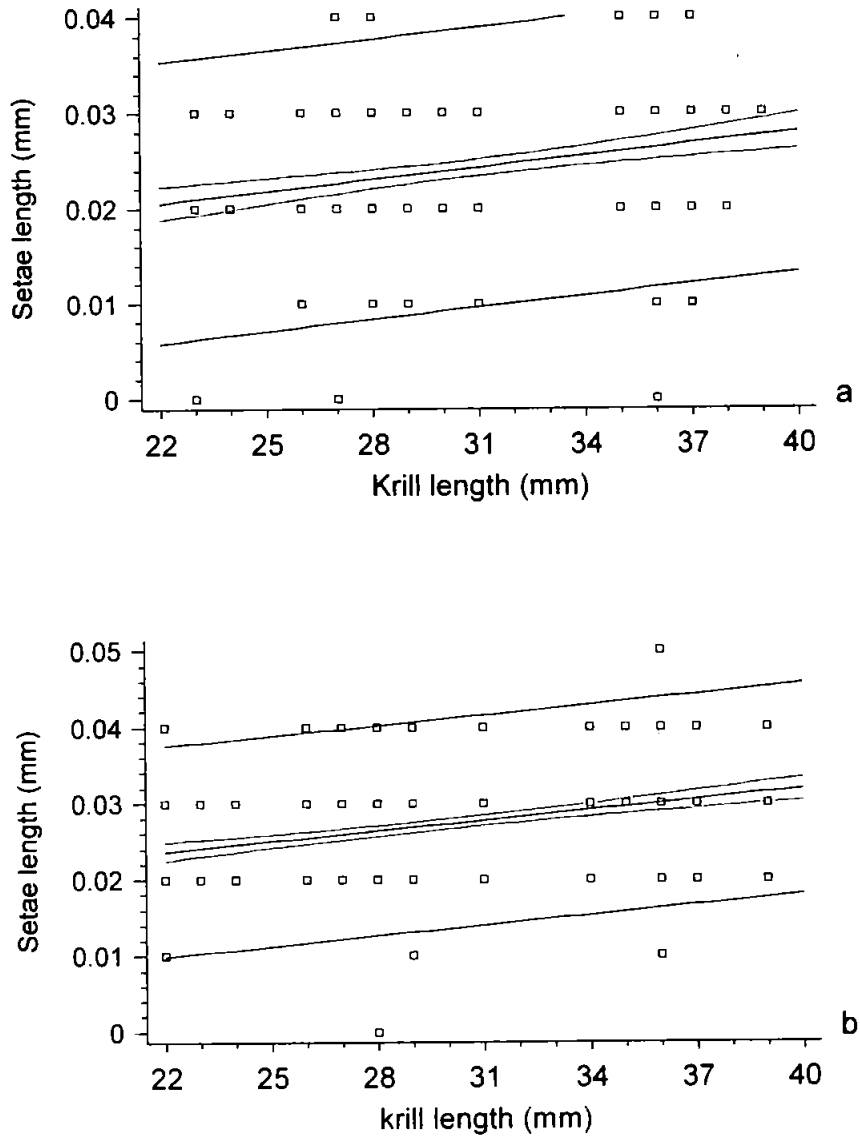


Figure 3.6 Relationship between secondary setae length on the carpus of appendages 1 (a) n = 39 and 2 (b) n = 41 with krill body length. Prediction limits are shown by broken line and 95 % confidence limit by dark grey line.

Again like the spacing between primary setae the length of the spaces between the bases of the secondary setae appeared to show a relatively weak relationship with krill body length. A correlation coefficient of 0.4351 for appendage 2 indicated a weak relationship between the variables. A moderately strong relationship was indicated, however, by correlation coefficient of 0.6049 for appendage 1.

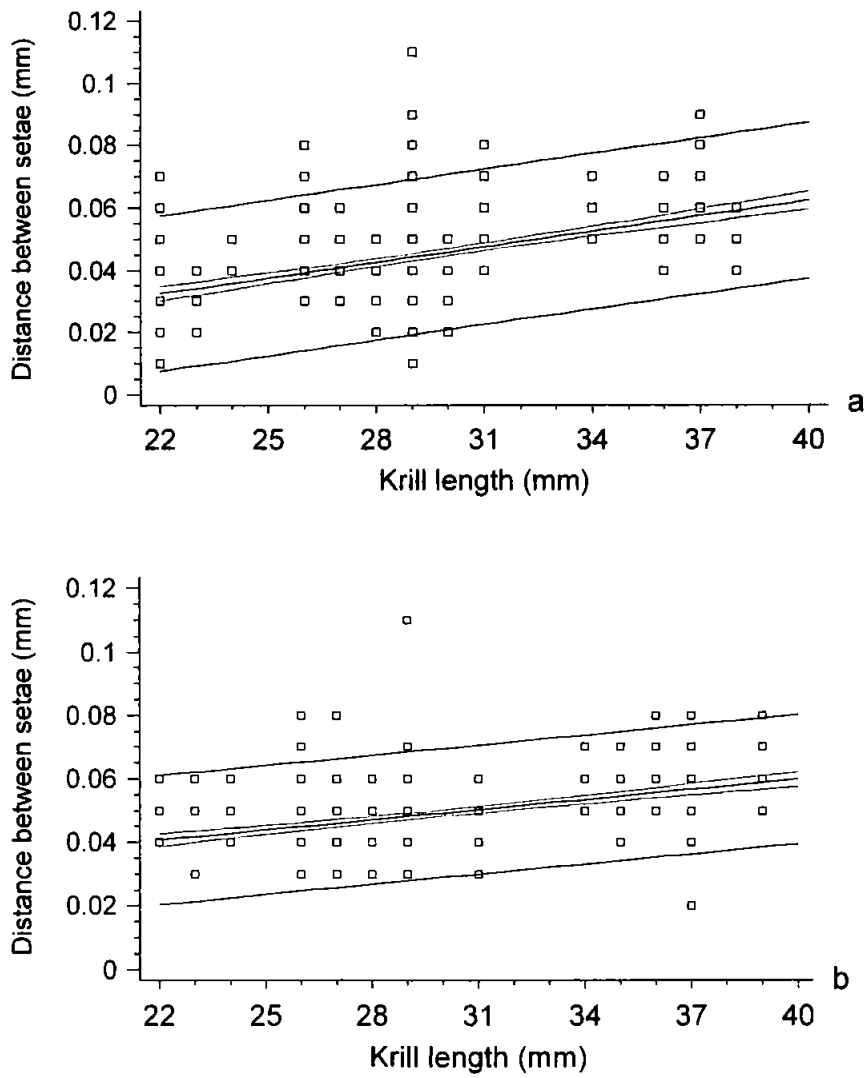


Figure 3.7 Relationship between length of space between secondary setae on the carpus of appendages 1 (a) n = 60 and 2 (b) n = 55 with krill body length. Prediction limits are shown by broken line and 95 % confidence limit by dark grey line.

The relationship between krill body length and various morphological features of the feeding basket is summarized by Table 3.1. The relationship with body length was stronger with the larger scale features of the basket that is feeding basket, length and width and also carpus length. There was also a moderately strong relationship between primary setae length and body length. The relationships between smaller scale features of the basket with body length were, however, weak.

Table 3.1 Summary of relationship between various measures of the feeding basket (variables) with krill body length.

Fig. ref.	Variable	r	r^2 (%)	P value	Intercept	Slope	Relationship of
3.1 a	Feeding basket length (female)	0.789	62	<0.001	0.455	0.230	Moderately strong
3.1 b	Feeding basket length (male)	0.896	80	<0.001	0.095	0.235	Moderately strong
3.2 a	Feeding basket width (female)	0.740	55	<0.001	0.146	0.095	Moderately strong
3.2 b	Feeding basket width (male)	0.637	41	<0.001	0.185	0.094	Moderately strong
3.3 a	Carpus length (appendage 1)	0.880	77	<0.001	0.033	0.027	Moderately strong
3.3 b	Carpus length (appendage 2)	0.918	84	<0.001	0.081	0.031	Strong
3.4 a	Primary setae length (appendage 1)	0.701	49	<0.001	-0.708	0.060	Moderately strong
3.4 b	Primary setae length (appendage 2)	0.690	48	<0.001	0.088	0.039	Moderately strong
3.5 a	Primary setae spacing (appendage 1)	0.207	4	<0.001	0.058	0.001	Weak
3.5 b	Primary setae spacing (appendage 2)	0.398	16	>0.05	0.043	0.001	Weak
3.6 a	Secondary setae length (appendage 1)	0.199	4	<0.001	0.008	0.001	Weak
3.6 b	Secondary setae length (appendage 2)	0.305	9	<0.001	0.014	0.0005	Weak
3.7 a	Secondary setae spacing (appendage 1)	0.489	24	<0.001	-0.001	0.002	Weak
3.7 b	Secondary setae spacing (appendage 2)	0.453	20	<0.001	0.017	0.001	Weak

3.4 DISCUSSION

3.4.1 Description of the thoracic appendages

All the thoracic appendages of the basket were different in their morphology. The primary setae were different lengths on both segments of the same appendage and between appendages therefore suggesting that each thoracic appendage has a different function in the basket. The most noticeable differences were between the dactyl segments of each appendage. The dactyl segments of appendages 1 and 4 – 6 seemed similar and therefore perhaps functionally similar. The dactyl segments of appendages 2 and 3 were extremely different both from the other appendages and from each other. The flattened, dactyl of appendage 2 and the shorter setae with a dense fringe of secondary setae may suggest that these appendages are modified perhaps for grasping or holding onto objects. Although not clear in the images obtained in this study the dactyl of appendage 1 may also be modified in a similar way as the drawings of Artiges *et al.* (1978) show the dactyl with a comb like edge. Determining whether these dactyl segments are modified for grasping would need further investigation. The presence of primary setae with secondary setae on the ischium, merus and carpus may suggest that most filtration occurs in the upper part of the basket. Filtration in the upper part of the basket would seem likely as Hamner (1988) described how the dactyls fold back and overlap with the long setae of the endopodites meaning that it is likely that most of the filtration process occurs through the upper segments of the appendages.

3.4.2 Functional morphology of the basket

The functional morphology of the feeding basket seems to be superficially similar to other krill species with primary and secondary setae on each thoracic appendage creating a mesh like structure to the basket. Dalley and McClatchie (1985) compared filter area with body length and suggested that the design of the feeding baskets of *Nyctiphanes australis*, *Euphausia superba* and *Meganyctiphanes norvegica* were all functionally similar. Hamner (1988) also suggested that species in the genera *Euphausia*, *Thysanoessa*, and *Meganyctiphanes* filter feed in essentially the same way especially because of the observation by McClatchie (1985) which described similarity in anatomy and pumping behaviour of *M. norvegica* and *E.* superba. Although it is likely as Hamner (1988) also suggested that subtle differences in the appendage structure between species may lead to subtle differences in the filtering behaviour of various krill species.

In 4 out of 5 *Euphausia* species examined by Suh and Choi (1998) primary and secondary setae distances increased with growth. By contrast, in this study relationships between intersetal widths and body length were weak suggesting that these features of the basket do not change greatly with growth in *Meganyctiphanes norvegica*. This weak relationship between body length and smaller scale morphological features may suggest, that the food types handled by krill are similar throughout their growth as suggested by Suh and Choi (1998) for *Euphausia pacifica* which had mesh size nearly consistent from juvenile to adult. Marshall (1985) commented when describing the feeding appendages of krill larvae on that it seemed as if there were no apparent differences between the food of larval and adult krill. Therefore if handling capability is based on intersetal distance it is likely, that krill do handle similar sized food types throughout their

growth. Establishing whether different body length krill handle different sized food types more efficiently would, however, require experimental investigation comparing feeding rates with different sized food types against different krill body lengths. Although weak relationships were shown between body length and intersetal distances stronger relationships were evident with larger scale features such as feeding basket length and width and also primary setae length. These increases in feeding basket length, width and primary setae length with increasing body length suggest therefore that some morphological characters of the basket do change with growth. Studies investigating the size of food types retained by the feeding basket tend to have related particle retention efficiency to the finer structure of the basket (e.g. Dalley and McClatchie, 1989; Suh and Choi 1998) i.e. they use the smallest distance between the setae as a means of determining particle retention efficiencies of the basket. Suh and Choi (1998) in particular have examined the finer mesh structure of *Euphausia* species. They measured proportion of projection of secondary setae (PPS) to show whether the secondary setae are longer or shorter than the distance between primary setae and therefore whether the secondary setae complete the filter mesh. They calculated PPS for 5 species and suggested that a higher PPS implies more effective filtration. Calculating PPS for a 37 mm *Meganyctiphanes norvegica* would give a PPS of 53% suggesting that *M. norvegica* is not able to filter as effectively as any of the *Euphausia* species investigated by Suh and Choi (1998). Suh and Choi (1998) did, however find that primary and secondary setae distances increased with growth in 4 *Euphausia* species examined. This increase in primary and secondary setae distances with increasing body length was not evident in *M. norvegica* as the relationship between body length and intersetal width was weak. The results of feeding studies (Chapter 2) suggest that there is minimum size of food type that can be handled by *M. norvegica*. Therefore it may be that particles retained by the

basket of *M. norvegica* are not related to intersetal width but another morphological feature of the basket. These low particle size limits calculated from the finer structure of the basket may not reflect the actual lower size limit that the basket can handle. Suh and Nemoto (1987) suggested the lower limits if filterable particle size were 2 – 7 μm for *E. superba*. Quentin and Ross (1985) reported that *E. superba* retained *Thalassiosira eccentrica* with a calculated spherical diameter (CDS) of 49.7 μm more efficiently (ca. 45 % cells retained) than *Isochrysis galbana* with a CDS of 5.3 μm (ca. 14 % cells retained or *Phaedactylum tricornutum* with a CDS of 7.5 μm (ca. 14 % cells retained). In particular Quetin and Ross (1985) did not find a plateau in retention efficiency for particles at least as large as 50 μm suggesting that retention efficiency may be even higher with larger particle sizes. Suh and Choi (1998) calculated lower filterable particle sizes of 2.3 μm for *Euphausia pacifica*, although admittedly suggest that *E. pacifica* that this species has been found to preferentially feed upon large diatoms (ca. 32 μm) (Parsons *et al.*, 1967). Given that it seems that the food types efficiently retained by krill baskets are much larger than the calculated lower limit of filterable particle sizes estimated using the finer structure of the basket perhaps particles are not directly retained by the finer structural parts of the basket. If the lower limit of filterable particle size were calculated in a similar manner as calculated by Suh and Choi (1998), that is primary setal distance minus secondary setae length for a 37 mm *Meganyctiphanes* in this study it would give an estimated lower particle size limit of 50 μm. In feeding studies (Chapter 2) when food types with a diameter of 50 μm where offered feeding rates were extremely low or zero. Feeding rates were much higher when food types with a larger diameter of 200 – 300 μm upwards were offered. Additionally gut studies of copepod mandible content indicated that the copepods eaten were approximately 1 mm (1000μm) in length (see Chapter 4). Given the low feeding rates with food types of the presumed lower filterable size it

is unlikely that *M. norvegica* effectively feed upon food types at this lower limit of filterable particle size or therefore supporting that the particles retained by the feeding basket are not related to these finer scale structural measurements. Even if only the primary intersetal distance is taken into account (without secondary setae) the intersetal distances were between 60µm and 170µm throughout the whole feeding basket with a median of 90µm, therefore being much smaller than the food types with which *M. norvegica* show highest feeding rates. Given that primary setae lengths were between ca. 0.3 – 1mm (300 – 1000 µm) on the ischium, ca. 0.4 – 2.7 mm (400 – 2700 µm) on the merus and ca. 1 – 1.6 mm (1000 – 1600 µm) on the carpus segments of the appendages comprising the feeding basket it seems that the food types handled by the basket are more related to primary setae length than intersetal distance. The stronger positive relationship between these larger scale features and body length compared with the weaker relationship between smaller scale features may also indicate that the smallest 'mesh' sizes of the basket do not relate to the food types handled by *M. norvegica*. It may also be that *M. norvegica* could take smaller food types if they preferred but that they only take larger food types for whatever reason, perhaps because they are more energetically favourable. Therefore, the stronger relationship with primary setae length and krill body length compared with intersetal distances and that the food type eaten by krill appear to be related to primary setae length suggest that the primary setae length in the case of *M. norvegica* are an important factor in the retention of particles by the basket. It may be that because of the fairly low PPS that the secondary setae in basket of *M. norvegica* do not create the sieve structure like in the baskets of other krill species. Therefore particles may be retained predominantly by the primary setae, with the secondary setae not acting as part of the sieve as such but more as an additional restriction on the longer primary setae helping to retain particles. Determining

whether the primary setae are more related to the food types handles requires more investigation of particle retention by different krill species, which have different mesh sizes.

3.4.3 Female and male krill feeding baskets

The intercept value for basket length versus body length was higher for females than males suggesting that females have a relatively longer feeding basket than males. This is the first time this difference has been recorded for any species of krill. A relatively longer length feeding basket may suggest that females have a larger filter area than males, although this would need further investigation. If the size of the feeding basket and is related to krill sex it may in part explain differences in feeding behaviour between males and females during DVM (see Chapter 4).

Chapter 4

Is nocturnal feeding by krill, *Meganyctiphanes norvegica*, driven by availability of food types during diel vertical migration?

ABSTRACT

Knowing whether krill feed continuously throughout their diel vertical migration or only during certain periods is an essential part of understanding krill feeding biology and therefore understanding pelagic food web function. I investigated feeding by krill during DVM both in situ *and also under laboratory conditions by offering krill food types which would be 'available' during either their nocturnal migration or daytime residence in deeper waters. Krill were offered copepods from depths which they would encounter during either the day or evening/night periods of their DVM in order to measure clearance and ingestion rates. These food types were also offered at several densities (surface water food type densities = 22, 39, 85, 103 and 175 individ.* $^{-1}$*; deep water food type densities = 26, 43 and 63 individ.* $^{-1}$*) in order to investigate functional response of feeding. Gut contents of krill, were examined for both chlorophyll and copepod mandibles from individuals collected during the day, evening and night from 25 m intervals between 0 and 100 m depths. Laboratory feeding experiments suggested that krill did not feed extensively on copepod food types available to them during the day but did show significantly higher ingestion and clearance rates with food types available to them during the evening/night. With evening/night food types offered at several densities krill showed a type III functional feeding response. In situ feeding studies confirmed my laboratory findings as krill appeared to show significantly higher stomach chlorophyll levels during the evening/night compared with during the day. Male and female krill seemed to show both asynchronous DVM and also feeding during their DVM. Therefore it seems that krill feed extensively at night but not during the day and there is a difference in the feeding behaviour together with migratory behaviour of krill sexes during DVM.*

4.1 INTRODUCTION

Given also that most krill are omnivorous feeding upon both phytoplankton and zooplankton and that these food types are abundant at varying depths of the water column, one of the most important potential influential factors affecting krill feeding is their own diel vertical migration (DVM). I am interested in the relationship between krill feeding and DVM for two main reasons (a) krill DVM affects potential food types available for krill utilization (b) what food types are available during DVM in turn also has the potential to influence krill migration to the surface layers of the water column and thus at least be in part a mechanism for DVM.

4.1.1 Krill feeding and diel vertical migration

As noted previously most of the studies that have investigated krill feeding during DVM are descriptive field studies (see Chapter 1 for references). That is, they describe what krill have eaten but cannot explain why or even estimate a time scale for when krill feed on given food types. The absence of a time scale for feeding makes it difficult to establish whether there is a relationship between DVM and krill feeding. Establishing a time scale for feeding is, however, inherently difficult due to the many variables *in situ* potentially affecting gut contents. One method for producing a time scale for feeding could be to estimate gut residence times for food types in controlled conditions in order to give an estimate of the length of time that items remain in the guts of field caught krill. However, this determination under controlled laboratory conditions would in turn give rise to problems when attempting to extrapolate the residence times to field caught krill. These problems would arise because krill can feed upon a variety of food types in the field at differing densities and presumably either singularly and/or in mixtures.

Heyraud (1979) estimated gut transit times in krill, *M. norvegica* to be around 30 min ('large' krill) and 15 min ('small' krill) when given an abundant food supply. He (1979) suggested that this 30 min transit time occurs only when krill are feeding continuously and in the absence of food it is much longer (up to 8 h) before krill empty their guts (La Rosa, 1976 as cited by Heyraud, 1979). This concurs with Perissinotto and Pakhomov (1996) who suggested that feeding activity of krill, *E. superba* strongly correlates with the gut passage time. They suggested that gut evacuation rates can only be estimated when krill are able to continuously ingest particles. In support of the view that transit times are greater in the absence of food, it has been suggested that *Euphausia superba* can retain their gut contents for up to 7 d when starved (Antezana *et al.*, 1982), therefore feeding or starvation status of krill should be an important consideration when estimating gut residence for food types. In particular, factors such as nutritional value of food (Pond *et al.*, 1995) and food type may also affect gut residence times as for example in mackerel prey type has been shown to affect gastric evacuation rate (Temming *et al.*, 2002). Consequently accounting just these factors mentioned above when determining gut residence times would be extremely time consuming. Moreover, fitting various residence times to food items without prerequisite information of all the factors mentioned above would be impossible. Therefore determining when krill feed during DVM relies on understanding the reasons why krill feed on given food types.

Primarily establishing whether krill are selective feeders or more particularly whether they are carnivorous, herbivorous or opportunistic omnivores is fundamentally important for ascertaining whether krill feed throughout DVM because it forms the basis for relationships between krill and their food types. Schnack (1985) suggested that krill utilize whatever food types are available. There is, however, a view implicit in the literature that krill species fit into 'feeding

types'. Part of this suggestion originates from the placing of krill into feeding types based on their mandible type e.g. Mauchline (1980). Using these physical characteristics to predict behaviour may lead to underestimating the flexibility of krill and thus false placing of krill into feeding types. Spicer *et al.* (1999) found that a poor anaerobic capacity of krill *M. norvegica* does not prevent their DVM into hypoxic waters. Therefore krill physiology or anatomy may not always be an accurate predictor of krill behaviour. Again, gut content studies only provide information concerning the food items which have been eaten but not the capability of krill to feed on given food types. Additionally, the suggestion that krill fit into feeding types is intensified by even those authors, which seem to suggest that these feeding types are only tendency. For example, Dalley and McClatchie (1989) used statements such as '*Euphausia superba* was considered an archetypal herbivore but will also take animal food...Another extreme, *Meganyctiphanes norvegica* is predominately carnivorous, but will also feed on small diatoms.' The phrase 'but will also' suggest that they are either herbivorous or carnivorous and other food types not within these categories are taken secondary to their main food types. Although *E. superba* is described as a herbivore and *M. norvegica* a carnivore based on their mouthparts and by gut content studies both species have shown higher clearance rates with copepod food types compared with phytoplankton under controlled conditions (see Price *et al.*, 1988 and Chapter 2 respectively). Cripps and Atkinson (2000) suggested that *E. superba* fed on copepods during non – bloom periods around the island of South Georgia. Kaartvedt *et al.* (2002) suggested that *M. norvegica* shifted between phytoplankton and zooplankton during the day and night and also seasonally. In Chapter 2 it was discussed how both species may be more alike than previously suggested and that are most likely flexible feeders, feeding on whatever food types are available. Thus it seems that krill are flexible and do have

the ability to feed carnivorously and herbivorously depending on availability of food types. Also it seems that on a large spatial (i.e. different seas) and temporal scale (i.e. different seasons) that krill are able to utilize both phytoplankton and zooplankton food types. On a smaller time scale, that is during DVM different food types are available to krill in time and space thus an important part of understanding why krill feed on given food types and thus the basis of trophic relationships in these pelagic food webs depends on when krill feed as this is the primary factor determining the food types are available for krill to utilize. However, exactly when krill feed may also rely on whether krill can feed upon all of the 'available' food types during their DVM. As a result determining if krill can utilize all the food types available during their DVM is key to understanding when krill feed during DVM. Some studies have tried to ascertain why krill feed on given food types by examining whether krill show size selective feeding and/or a preference for given food types (see Haberman *et al.*, 2003b; Quentin and Ross, 1985). Krill appear to seek particular food types (Haberman *et al.*, 2003b) or only handle those of a certain size (diameter, length) and morphology (see Chapter 2; Quentin and Ross, 1985). Therefore krill may only be able to feed during certain periods of DVM because although other food types are available at other times they do not have the capability to handle them or they do not prefer those food types. Consequently why krill feed on given food types may also in part explain why krill migrate to the surface layers of the water column.

4.1.2 Feeding; a basis for DVM?

Diel vertical migration by zooplankton is a well – known phenomenon. The adaptive significance and ecosystem consequences of these migrations by zooplankton have been recently reviewed by Hays (2003). Hays (2003) suggested that there are most likely several ultimate reasons for DVM and also that given

DVM is widespread throughout most taxonomic groups there must be a common underlying cause (see Chapter 1 for discussion). The most common proposed explanations for DVM include predator avoidance, that is descending into deeper depths during the day allows migrants to avoid waters with visual predators where they would be visibly seen and eaten; metabolic advantage, whereby migrants gain an advantage by ascending into warmer surface waters at night; light avoidance, in order to reduce damage from ultraviolet radiation and last but not least feeding, where migrants ascend to the surface waters at night to feed (for review of these explanations see Hays, 2003). Most work has concentrated on predator avoidance as a driving force for DVM. Although krill play a central role in pelagic marine food webs and also the fact that most krill are diel vertical migrators (Mauchline, 1980), the reasons for krill migration and particularly whether they feed throughout DVM or only at night still remains unclear. It has been suggested that krill ascend to the surface waters at night in search of food (Sameoto, 1980) and also that krill distribution and may be linked to predation risk (Alonzo and Mangel, 2001; Tarling, 2000). In particular it has been suggested that *Meganyctiphanes norvegica* only feed at night and that feeding ceases during the day (Lass *et al.*, 2001). Lass *et al.* (2001) suggested that the diel feeding activity rhythms shown by *M. norvegica* in the Kattegat and Clyde Sea is adaptive, minimizing risk from predators and that the species did not seem to feed during the day. Onsrud and Kaartvedt (1998), suggested that *M. norvegica* fed both during the day and night and also carried out DVM regardless of fluctuations in the distribution and abundance of food. Therefore, it is unclear whether krill do feed throughout DVM or only during their nocturnal migration to the upper layers of the water column. Whether krill feed throughout their DVM is essential to understanding food webs in two ways, (a) in order to understand the trophic relationships between krill and their food types as a means to understanding food

web function and (b) as a means to explaining krill DVM behaviour. Whether krill feed throughout DVM is thus an important unresolved question. Consequently I would like to know whether or not krill feed during both the day and night? More specifically I would like to know can krill utilize the food types potentially available to them to both during the day and night?

4.1.3 The model system, study design rationale and aims

Northern krill, *Meganyctiphanes norvegica* provide a useful model system for examining the feeding biology of all krill, i.e. the relationship between DVM and feeding, as they are omnivorous feeding upon both phytoplankton and zooplankton (Lass *et al.*, 2001 Kaartvedt *et al.*, 2002), and also they show a strong DVM behaviour (Liljebladh and Thommason, 2001).

In Gullmarsfjord (Sweden) different food types are concentrated at various depths with phytoplankton and smaller copepod species (e.g. *Acartia* sp., *Oithona* sp., *Psuedocalanus* sp. pers obs, see Appendix A) being found in the surface layers and larger copepod species (such as *Calanus* spp., *Metridia* spp. Pers Obs, see Appendix A) found in the deeper layers with phytoplankton absent (unless sedimentation of phytoplankton has occurred after a bloom period). Therefore smaller copepod food types and phytoplankton are available during the nocturnal ascent by krill to the upper layers of the water column whereas only larger copepod species are available during the day to krill in the deepest 50 m of the water column. As mentioned above it seems that krill are flexible feeders able to utilize both phytoplankton and zooplankton food types providing that these food types can be retained by the feeding basket. Therefore, it may be expected that krill will feed throughout their DVM, providing they can utilize the food types available.

I aimed to combine field investigations with laboratory studies in order to see whether male and female krill feed throughout DVM. That is, field studies provided (a) patterns of krill distribution in relation to the food types available and (b) field information about feeding from gut contents studies. By using this descriptive information together with laboratory studies, which compared feeding rates on food types available either during the day or night I sought to clarify when krill feed during their DVM. The aims of this chapter were achieved by investigating;

- the effect of food types available during the day or night on clearance and ingestion rates by krill and also their effect on the functional response of feeding (how feeding rate changes with food density).
- the pattern of krill feeding during DVM by examination of krill gut content.
- the pattern of male and female migration and feeding during DVM.

4.2 MATERIALS AND METHODS

4.2.1 Laboratory feeding experiments

A. Collection and maintenance of krill and zooplankton

Meganyctiphanes norvegica were collected, transported back to KMRS and maintained as described in chapter 2 methods on several occasions during Jan and Feb 2003. Copepods were collected, from the same location as krill and from depths likely to be encountered by krill during their DVM. Depths likely to be encountered during the day were indicated as 100 – 50 m by previous studies in 2002 (see Appendix A), and also by the back scattering layer at the time of this study shown by the echo – sounder on board the RV whereas at night the back scattering layer indicated krill migrated into the upper 50 m of the water column. Therefore copepods were collected from depths of 100 – 50 m and 50 – 0 m by vertical tows using a plankton net (200 µm, WP2). Copepods from these collection depths will be referred to as 'deep water' and 'surface water' copepods respectively. Copepods were returned to the laboratory < 2 h of capture in sealed thermos containers (Rubbermaid drinking water thermosflask vol. = 20 l) containing filtered sea water. At KMRS copepods were maintained in aerated plastic containers (vol. = 80 l) supplied with natural surface (pumped into station from depth of 6 m, S = 34 PSU, T = 4°C) water or deep water. All experiments were carried out within 5 d of capture.

B. Feeding experiments

The effect of the available food types during DVM on clearance rate and ingestion rate of *M. norvegica* was investigated using deep and surface water copepods. For all feeding experiments a group of similar size krill (body length, i.e. rostrum tip to end of telson = 30 – 36 mm) were selected from the stock aquaria and then

transferred to experimental containers. The distribution of clearance and ingestion rate data was tested for normality (Shapiro – Wilks W statistic test) using STATGRAPHICS Plus 5.0 (1994 – 2000, Statistical Graphics Corp).

Either deep or surface water copepods were offered to krill at several densities to compare both the effect of the food types on clearance/ingestion rates of krill and also the effect of food type density on clearance/ ingestion rate to investigate the functional response of krill feeding. Therefore, deep water copepods were offered at three densities (26, 43 and 63 individual l^{-1}) and surface water copepods at 5 densities (22, 39, 85, 103, 175 individual l^{-1}). Deep water copepods were not offered at the two higher densities (ca. 100 and 175 individual l^{-1}) because copepods could not be collected in sufficient numbers. Although lower food densities for both surface and deep water food types would have represented more realistic environmental food densities, the problems associated with offering a low food density to krill would have confounded the outcomes of the experiment. As the aim of the study was to investigate the functional response of feeding, a range of food types densities were offered to krill. Consequently this meant that experiments had to be run over a long enough period to obtain statistically significant reductions in food by krill at the higher food densities offered. Therefore lower food densities had to be greater than 'realistic' low environmental food densities (estimated from net samples) to ensure that food was not completely consumed by krill during the timescale of the experiment as this would lead to incorrect calculated estimates for clearance and ingestions rates. A volume of stock water/food type was added to filtered, deep sea water and mixed thoroughly to give the desired food type density. Control bottles contained only the food type whereas experimental bottles also contained an individual krill. Control (n = 10) and experimental (n = 10 – 12) glass bottles (vol. = 2.3 l) were filled with sea water containing the copepod food type in a haphazard order, to account for variation

between bottles in food type concentration throughout the 'filling' process. Thorough mixing continued throughout this filling process, to ensure that the food type remained in a homogenous suspension. At the start and end of the filling process two control bottles were taken for verification of copepod concentration at the start of the experiment. An individual krill was placed in each experimental bottle, after which the bottle was then filled until the water overflowed. Plastic film was placed over the mouth of each bottle to exclude air and then the lid gently tightened. In order to maintain zooplankton in suspension, all bottles were placed on a rotating plankton wheel (2 rev. min^{-1}) and left overnight in a temperature-controlled room (T = 6 °C) for 12 – 13 h. Both deep and surface water food type experiments were run over night to ensure that the response shown by krill to each food types was not affecting by a feeding rhythm. At the end of this period, bottles were removed from the wheel, and the contents analysed for copepod density. Each bottle was rinsed three times to ensure all copepods were removed for quantification. Krill removed from the experimental bottles were also rinsed to remove any copepods adhering to the exoskeleton. The contents of each bottle was emptied and placed in a Petri dish with ethanol (70 %) to fix the remaining copepods. All of the copepods present were counted under low power (x 10) magnification. In preliminary experiments separation of copepod species to run single species experiments were attempted. When trying to separate the largest copepod species from the deep water (i.e. *Metridia* spp. and *Calanus* spp.) which was much easier than separating the smaller species of the surface water tows, many of the 'picked' copepods died presumably because of the stress from handling under the microscope. Therefore, as the aim of the investigation was to determine whether krill *M. norvegica* had a preference for deep or surface water food types copepods were not differentiated with respect to either their species or stage. Deep water food types were made up of mainly adult *Calanus* and *Metridia*

and surface water food types comprised of smaller copepod species including *Acartia*, *Oithona*, *Temora*, copepodite *Calanus*, and *Pseudocalanus*. Although, problems of sorting copepod species could have been overcome by culturing copepods, copepod culture was not feasible during the time scale of this study.

4.2.2 Field feeding

Meganyctiphanes norvegica was collected from Gullmarsfjord, southwest Sweden (58°18' N, 11°32' E), using an Isaacs-Kidd midwater trawl (mouth area 0.6m^2; haul duration = 20 min) during the day (4th Mar 2003, sunset = 17.49) proceeding into the night (5th Mar 2003, sunrise = 06.57). Krill were collected from depth ranges of 100 – 75 m, 75 – 50 m and 50 – 0 m during the day (11.00 – 15.00 local time), evening (19.00 – 21.00 local time) and night (03.00 – 06.00 local time) by horizontal oblique tows, except in the evening and night the upper 50 m were split into 2 sampling intervals of 50 – 25 m and 25 – 0 m as the back scattering layer indicated krill were dispersed throughout the water column. A flow meter (General Oceanics, Sweden) was attached to the aperture of the trawl in order to estimate the volume of water filtered and thus in turn krill density per m ^{3} with depth. During 'day' time the back scattering layer on the echo sounder indicated that krill were mainly concentrated at depths between 100 – 50 m therefore one tow was made for krill at both 100 – 75 m and 75 – 50 m. One trawl was made for the range of 50 – 0 m in order to confirm that no krill were indeed present. During the evening and night the backscattering layer indicated that krill were distributed throughout the water column, therefore two tows were made for the following depth ranges 100 – 75 m, 75 – 50 m and 50 – 0 m. Repeated tows for each depth range cannot be regarded as 'true' replicate tows because tows are inevitably temporally different due to the continuous nature of DVM therefore it is impossible to replicate a tow. For this reason that repeated tows are not true replicates and in particular

that the tows from the different depths of the water column needed to be taken as close together as sampling would possibly allow, no tows were replicated in this study.

Zooplankton was collected by a vertical tow using a WP-2 (200 μm) net, from a range of depths encountered by krill during DVM including 100 – 75 m, 75 – 50 m, 50 – 25 m and 25 – 0 m, during the day and night. A flow meter was attached to the aperture of the net in order to estimate the volume of water filtered and thus density of copepods per m^{-3}. Zooplankton were immediately preserved in 4 – 5% formaldehyde solution. Zooplankton tows were not replicated for the same reason as mentioned above for krill tows.

Temperature and salinity were measured throughout the water column using a conductivity temperature depth recorder. Water was collected in Niskin tubes from twelve depths as follows, 100 m, 80 m, 60 m, 50 m, 40 m, 30 m, 20 m, 15 m, 12 m, 7 m, 5 m, and 2 m. A sample of water was also taken to estimate chlorophyll a and thus give a measure of the total phytoplankton at each depth.

Krill were sorted within 10 min of recovering the trawl. For estimation of herbivorous feeding approx. 15 krill were chosen randomly, wrapped in aluminium foil, and frozen (- 20°C) in order to prevent the photo – degradation of chlorophyll pigments present in the gut. Remaining krill were preserved in formaldehyde solution 4 – 5% for enumeration of krill to estimate densities for each depth during day, evening and night periods.

4.2.3 Herbivorous feeding

Herbivorous feeding was estimated by measuring gut content of chlorophyll pigments. Chlorophyll pigments were measured using a fluorescence method (Parsons *et al.* , 1984). Krill were thawed and the stomach/gut dissected out (n =

5 - 8) taking care to avoid damaging the gut and thus causing loss of content. Although, this n value may seem low the time involved in dissecting krill guts and extracting pigment for each individual was high and thus to ensure coverage of all sampling depths/periods in the time available replicates for each respective depth and period of DVM were reduced. Each gut was placed in 90 % ethanol (10 ml) for 12 – 18 h to extract pigments. Chlorophyll a was quantified by fluorometric determination of chlorophylls and phaeopigments using a Turner Systems® fluorometer. Kruskal – Wallis tests were performed followed by a box plot using STATGRAPHICS Plus 5.0 (1994 – 2000, Statistical Graphics Corp) to determine whether there was any significant differences between gut contents at various depths of the water column.

4.2.4 Carnivorous feeding

The guts from krill analyzed for chlorophyll pigments were also used for estimation of carnivorous feeding (n = 5). Although again this n value appears to be small in order to cover the large number of sampling depths and times replicates had to be reduced to achieve this coverage in the time available as examining one krill gut could take up to 3 h. This examination time was prolonged as guts were inspected three times for mandibles to ensure the reliability of results. Stomach/guts were mounted on a glass slide and stained with methylene blue and examined for copepod mandibles using phase contrast microscopy.

4.2.5 Food type availability/abundance

A. Zooplankton

Zooplankton were filtered through a 60 µm sieve, rinsed with fresh water, and replaced in a solution of 70 % alcohol with 3 % glycerol, for ease of working with

sample. Zooplankton samples were sub–sampled using a Flosom plankton splitter and divided into eight equal parts. Three 1/8 subsamples were used for identification and enumeration of zooplankton. All copepods were identified to genus and not to species as the aim of the investigation was to examine feeding on surface and deep water food types not species specific feeding. Small juvenile copepods in surface waters were not differentiated for example Copepodite stages of *Calanus*, therefore all these copepods were placed in category named 'Copepoda J.'

B. Phytoplankton

Immediately upon collection duplicate water samples (vol. = 100 ml) from each depth were filtered onto Whatman glass micro-fibre filters (GF/F) and extracted in 90 % ethanol for 12 – 14 h. Chlorophylls and phaeopigments were determined using the fluorescence method described previously for gut content analysis.

4.3 RESULTS

4.3.1 Krill diel vertical migration

The upward movement of krill to surface layers of the water column during the evening and night from deeper depths is shown by krill abundance at various depths during the course of one day and night by Figure 4.1.

Krill were concentrated in the lower 50 m of the water column and absent from the upper 50 m of the water column during the daytime. In particular krill a higher density of krill were found in the deepest part of their daytime range or distribution, between 100 and 75 m than in the upper part of their range between 75 and 50 m. During the evening krill became diffusely spread throughout all sampling depths (0 – 100 m) of the water column indicating that they had migrated up into the surface layers of the water column with the highest density of krill between 50 and 25 m. In particular krill were most abundant between depths of 50 and 25 m in the evening signifying that the majority of krill had migrated up to the upper layers of the water column. At night, krill were also found throughout the water column, except unlike during the evening most krill were spread over a larger depth range between 75 and 25 m. Also, krill density appeared to be similar over this 50 m mid – water column depth range, compared with the large difference in density observed in the evening between 75 – 50 m with 50 – 25 m.

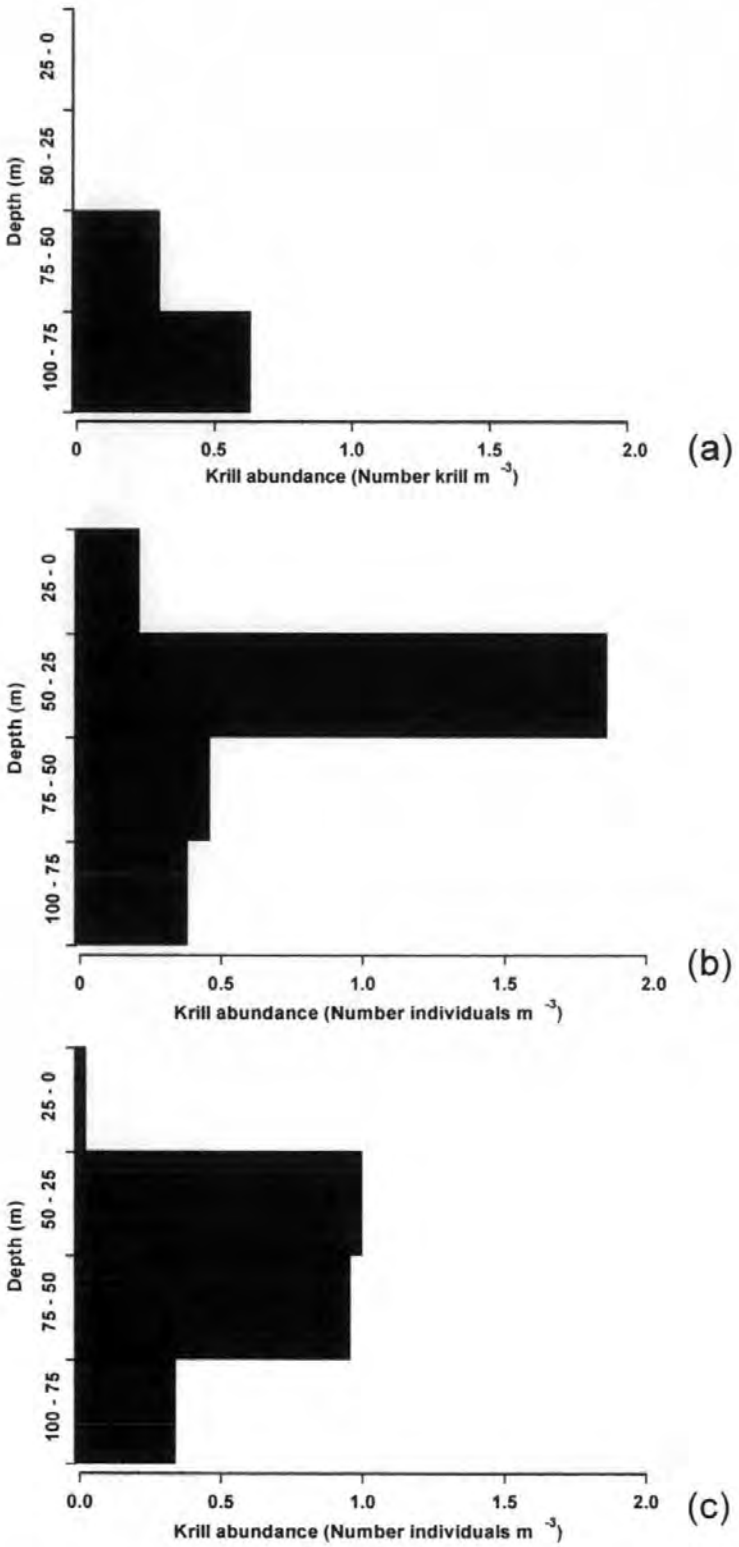


Figure 4.1 Diel vertical migration by krill. Abundance of krill in shallow to deep depths during the day (a), evening (b) and night (c).

Male and female krill showed differing distributions in the water column and asynchronous migration patterns (see Fig. 4.2) during the course of their DVM. Female krill were recorded at shallower depths in the water column than males both during the day and evening. In fact females were recorded at depths where males were completely absent. For example both during the day and evening female krill were found in the shallowest 25 m of the distribution range of krill at the that time, that is during the day females were found between 75 – 50 where males were not found and at night females were found between 25 – 0 m depths where again males were not observed. During the evening krill dispersed throughout the water column but similarly with during the day it seemed that only female krill entered the shallowest depths as again only females were caught from depths between 25 and 0 m. Although some males were caught between 50 – 25 m depths, the ratio of females to males was still much higher in these depths. Again like during the day in deeper depths more males were caught in the deeper range of the krill distribution than females. At night this difference in presence of male and females in deeper and shallower depth ranges seemed to change with males being caught throughout the water column even in the shallower depths, although in the shallower depths of 25 – 0 m mostly females were still caught. More males than females were caught between depths of 50 – 25 m and although males and females were approximately equal between 75 – 50 m mostly female krill were caught in the deepest depths between 100 – 75 m.

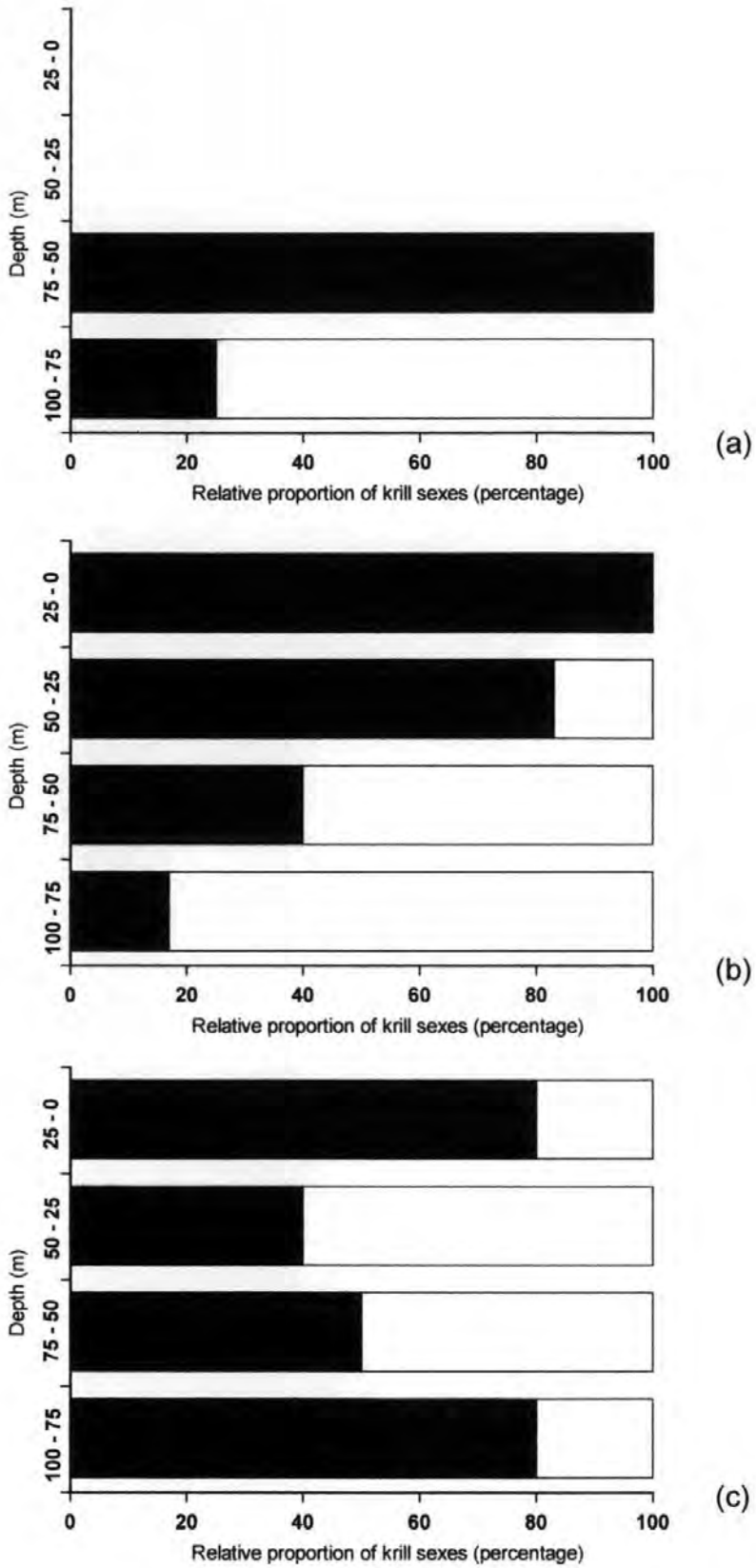


Figure 4.2 Distribution of krill sexes during day (a), evening (b) and night (c) shown by percentage of female (■) and male (□) krill (n = 6 – 16).

4.3.2 Food types in relation to DVM

During the DVM of krill different food types are present and abundant at various depths of the water column. The figures that follow show the abundance of phytoplankton and copepod food types at the depths encountered by krill during their nocturnal migration to the surface layers and sunrise descent to the deeper layers of the water column.

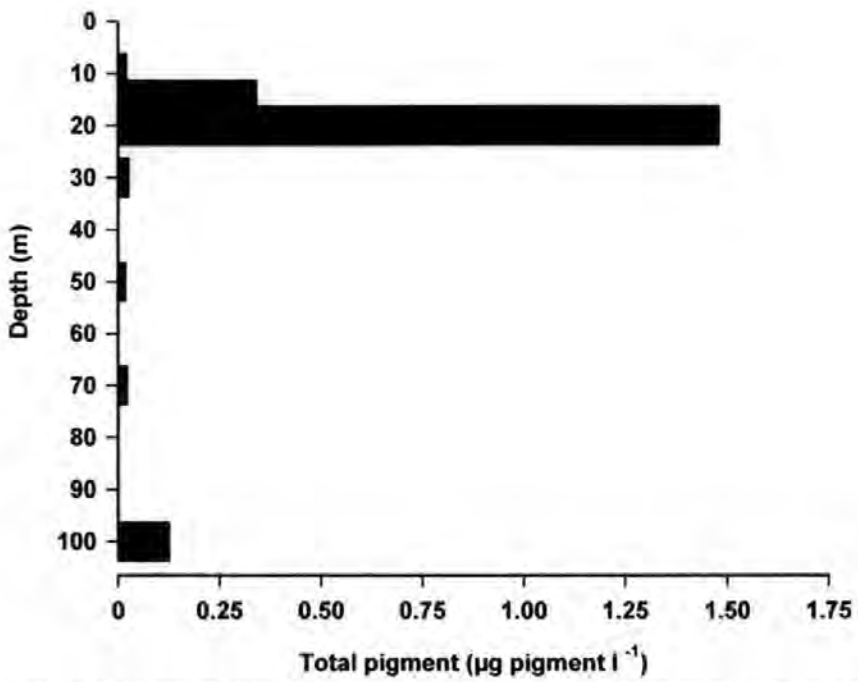


Figure 4.3 Phytoplankton abundance (shown as total pigment) in shallow to deep depths of the water column.

Phytoplankton abundance is shown by Figure 4.3 as total pigment. Phytoplankton was mainly concentrated in the upper layers of the water column between depths of 10 and 30 m (up to 1.48 µg total pigment l^{-1}). Total pigment levels were low throughout the rest of the water column (< 0.03 µg total pigment l^{-1}) until a depth of 100 m where they increased slightly to 0.13 µg total pigment l^{-1}. Therefore the most abundant source of phytoplankton food types would only be available for krill to exploit during their evening and night ascent to the surface layers of the water column.

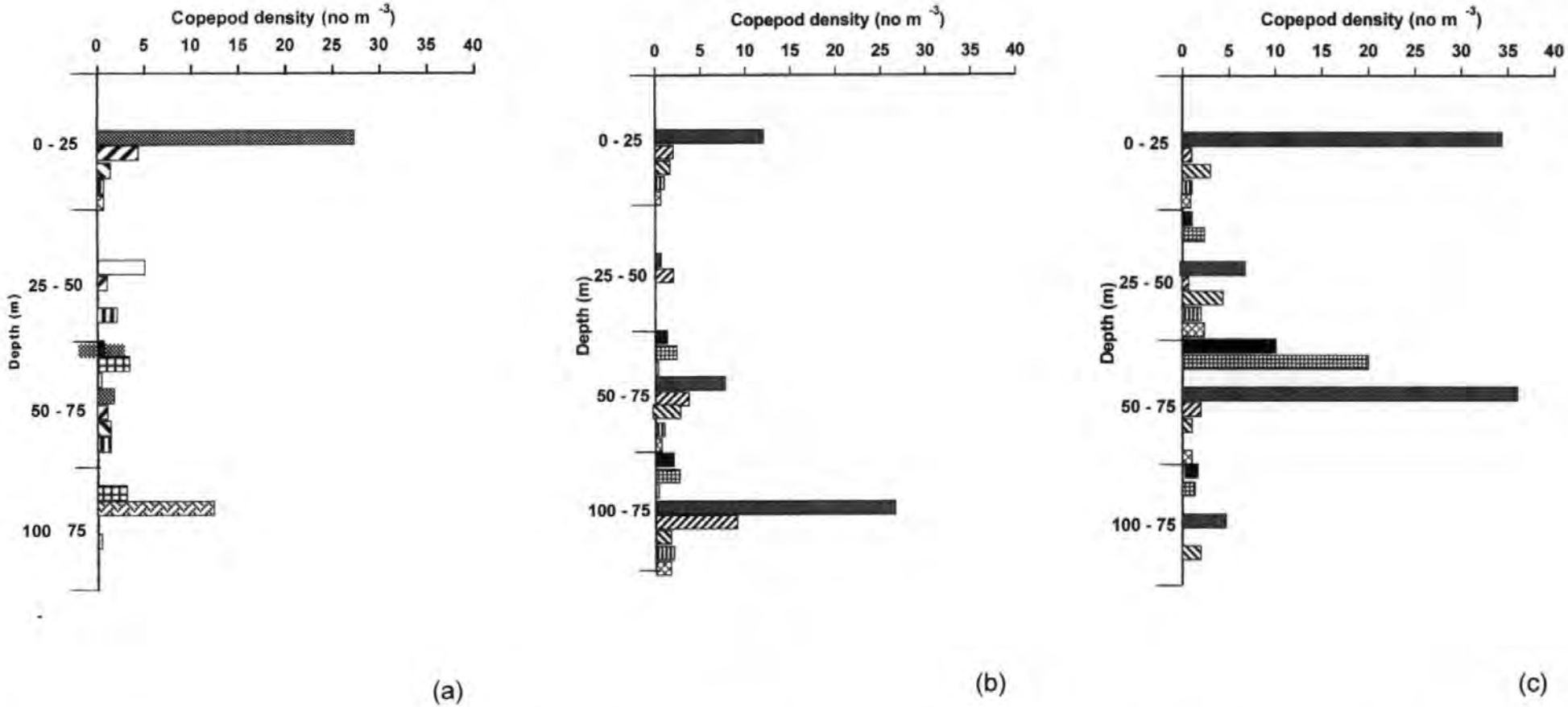


Figure 4.4 Copepod density during DVM. Density of various copepod species (■ = *Calanus* spp. ▦ = *Metridia* spp. ▩ = *Euchaeta* sp. ■ = Copepoda J ▨ *Acartia* sp. ▧ *Pseudocalanus* sp. ▥ *Oithona* sp. ▩ *Temora* sp.) shown in same order at shallow to deep depths during the (a) day, (b) evening and (c) night.

Copepods were found throughout the water column during all sampling periods through DVM (Fig. 4.4). The species composition at various depths did, however, differ between the day, the evening and the night. During the day larger copepod species of *Calanus*, *Metridia* and *Euchaeta* were concentrated in the deep water at a depth of between 100 – 50 m (ca. 1, 3 and 12 individual l^{-1} respectively). Whereas although present at depths from 75 m upwards smaller copepod species such as *Acartia* and Copepoda J were most abundant in the upper 25 m of the water column (ca. 4 and 27 individual m^{-3} respectively). Other small copepods of species such as *Psuedocalanus*, *Oithona* and *Temora* were also found at a density of around 1 individual m^{-3} in this upper 25 m of the water column. Consequently the total density of copepods was much greater in the upper 25 m of the water column (34 individual m^{-3}) and consisted of smaller copepod species compared with the lower total density of larger copepod species found between depths of 100 – 75 m (16 individual m^{-3}) species. Between depths of 25 and 50 m there was a total copepod density of around 8 individuals m^{-3}, which comprised of Copepoda J, *Oithona* sp. and *Acartia* sp. (5, 2 and 1 individual m^{-3} respectively). Between 50 and 75 m the total density of copepods was similar but contained more species at around 10 copepods m^{-3} comprising of *Calanus*, *Metrida*, copepoda J, *Acartia*, *Pseudocalanus* and *Oithona* (1, 3, 2, 1, 1, 1 individual m^{-3} respectively). Therefore in the depths that krill reside in during the day the copepod food types present are in much lower densities than those in the surface waters which the krill swim up to at night. Even when taking into account that krill may swim up to depths up to 50 m (see Appendix A and Fig. 4.1), although small copepod species are present, they are not as abundant as in surface waters.

During the evening, copepods seemed to be distributed throughout the water column with total copepod densities of 17, 3, 20 and 46 individuals m^{-3} for

respective 25 m sampling depth ranges from surface to deep waters. In surface waters the copepod species composition was similar to that observed during the day with small copepod species such as Copepoda J, *Acartia* sp., *Pseudocalanus* sp., *Oithona* sp., and *Temora* sp. present. Densities for the most dense day time surface water species Copepoda J and *Acartia* sp. were about half of those observed (12 and 2 individual m^{-3} respectively). Other small surface water copepod species mentioned above were found in a similar density to that found during the day of about 1 individual m^{-3}. The total copepod density found between 25 and 50 m was, however, much lower than that observed in the day with 3 individual m^{-3} comprising of approximately 66% *Acartia* sp., and 33% Copepoda J. In the deepest 50 m of the water column the total copepod density was much higher than both the upper 50 m of the water column and daytime copepod densities in deeper depths. Densities of 20 and 46 individuals m^{-3} were observed between 50 – 75 m and 75 – 100 m depths respectively. Not only were these densities much higher than those from daytime sampling but they also contained both large and small copepod species. Large copepod species were found at similar densities to daytime densities with between 1 and 2 *Calanus* spp. Individuals m^{-3} and 2 and 3 *Metridia* spp. individuals m^{-3}. Although *Euchaeta* sp. was at much lower densities in these zooplankton samples tows during the evening (< 1 individual m^{-3}) they were observed in high numbers in krill sample tows. Of the small copepod species Copepoda J and *Acartia* sp. were the most abundant with densities of 8 and 26 individuals m^{-3} for Copepoda J and 4 and 9 individuals m^{-3} for *Acartia* sp., for 50 – 75 m and 75 – 100 m depths respectively. *Pseudocalanus* sp. were the next most abundant species with 3 and 2 individuals m^{-3} for 50 – 75 m and 75 – 100 m depths respectively. The *Oithona* sp. and *Temora* sp. were found at similar densities of ca. 1 and 2 individuals m^{-3} for depths of 50 – 75 m and 75 – 100 m.

During the night copepods were again distributed throughout the water column. Total copepod densities at various depths were comparatively much higher than those observed during the day and evening. Around 40, 20, 70 and 9 individuals m^{-3} were observed in each 25 m sampling depth range from shallow to deep. In these surface waters the copepod species composition was similar to that found during the day and evening but density differed with Copepoda J being the most abundant (ca. 36 individuals m^{-3}) then *Pseudocalanus* sp. (ca. 3 individuals m^{-3}) and *Acartia* sp., *Oithona* sp. and *Temora* sp. all the least abundant with a density of around 1 individual m^{-3}. In contrast to during the day and evening larger copepod species *Calanus* spp. and *Metrida* spp. were found higher in water column between 25 and 50 m depth with densities of 1 and 2 individuals m^{-3}. In similarity with day and evening these larger species were found in both 50 – 75 m and 75 – 100 m depths. Densities of *Calanus* spp. and *Metridia* spp. were similar to day and evening densities within 75 – 100 m depths (ca. 1 individual m^{-3}) whereas between 50 – 75 m depths densities were about tenfold higher, than densities observed at any other time (*Calanus* spp. = 10 individuals m^{-3}, *Metridia* spp. = 20 individuals m^{-3}). Small copepod species were low in density or absent from 75 – 100 m depth with Copepoda J and *Pseudocalanus* sp. present at 5 and 2 individuals m^{-3} respectively. At a depth between 50 and 75 m Copepoda J densities were much higher than at any other time (36 individuals m^{-3}) other species were found at densities similar to those observed during the day with *Acartia* sp. at a density of 2 individuals m^{-3}, and *Pseudocalanus* sp. 1 individual m^{-3}. Although *Oithona* sp. were absent from this depth of 50 – 75 m unlike during the day and evening, like during the evening *Temora* sp. were present at a density of 1 individual m^{-3}. Densities of small copepod species like large copepod species were much higher between 25 and 50 m depth than those

observed during the day and evening, and also more species were present compared with day and evening. *Acartia* sp. were present at a density of 2 individuals m^{-3} and both *Pseudocalanus* sp. and *Temora* sp. were present at densities of 1 individual m^{-3}.

4.3.3 Functional feeding response

The figures presented (Figs 4.5, 4.6 and 4.7) show the clearance and ingestion rates of krill when offered various densities of deep and surface water food types.

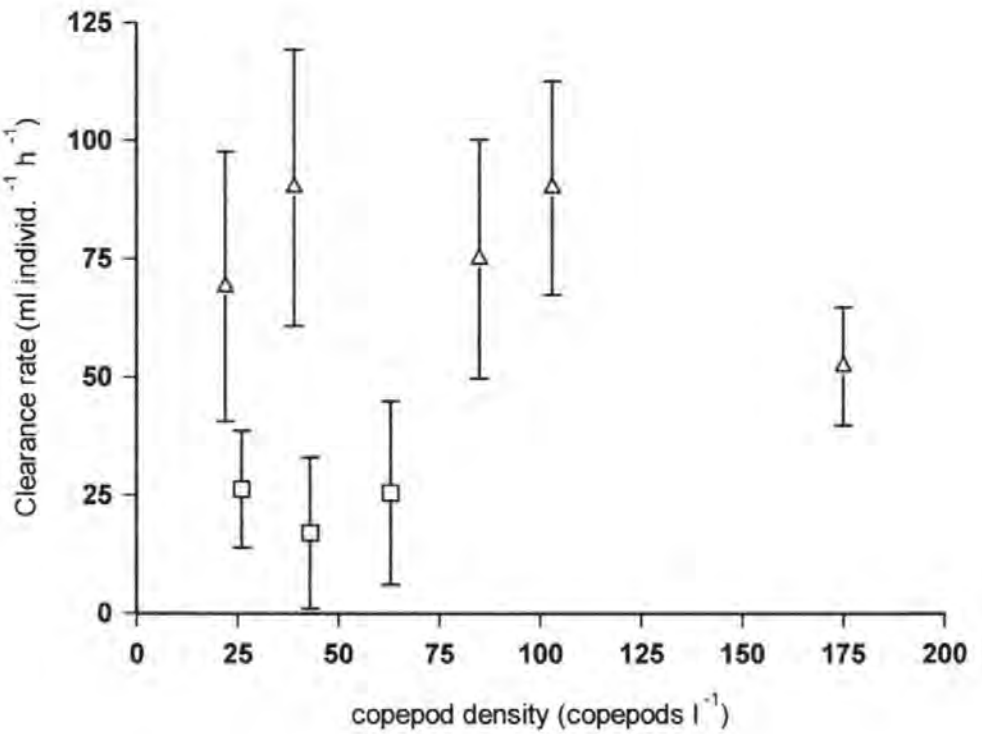


Figure 4.5 Mean clearance rate of krill (n = 10 – 12 for each point) showing 95 % confidence intervals when offered deep (□) and surface (Δ) water copepod food types at several densities.

Krill maintained a reasonably constant clearance rate when offered either surface or deep water food types over a range of densities. Mean clearance rate decreased slightly to 52.3 ml individual $^{-1}$ h $^{-1}$ when krill were offered a high density

(175 copepods l^{-1}) of a surface water food type. Clearance rates with deep water food types were consistently low. ANCOVA indicated that there was a significant difference between the intercepts for clearance rates when krill were offered either deep or surface water ($F_{1, 67} = 30.97$, $P < 0.001$). Additionally, clearance rates were analysed using both comparisons of mean with 95 % confidence intervals and also median clearance rates (using box and whisker plots with median notches). Both these analyses also suggested a significant difference between clearance rates with surface and deep water food types. Significantly greater clearance rates were shown when krill were offered surface water types compared with deep water food types.

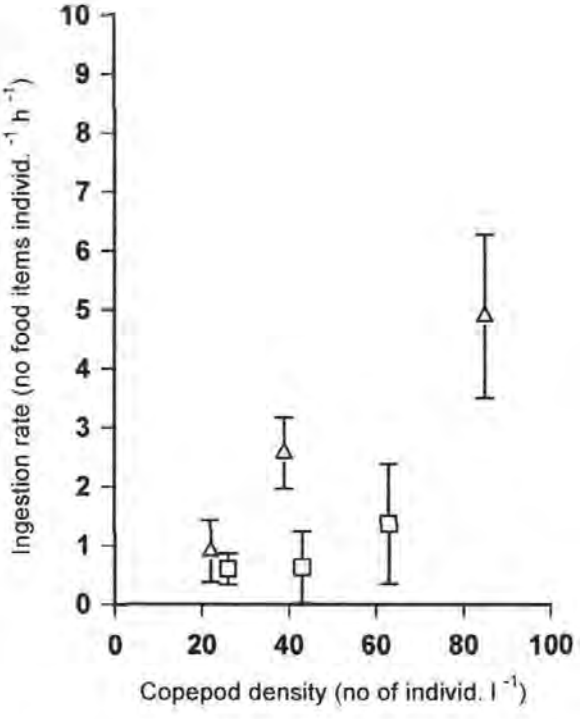


Figure 4.6 Mean ingestion rate of krill (n = 10 – 12 for each point) showing 95 % confidence intervals when offered deep (□) and surface (Δ) water copepod food types at several densities.

As shown in Figure 4.6 ingestion rate increased almost directly proportionally with copepod density when krill were offered a surface water copepod food type. Although mean values are shown in Figure 4.6 for the purpose of illustrating the difference between ingestion rate when krill were offered surface and deep water food types, it should highlighted that the use of means when analysing feeding responses may lead to misinterpreting functional response types for reasons which will be later discussed. Ingestion rates when krill were offered surface water food types approximately doubled with a doubling in food density. In contrast, this proportional increase in ingestion rate with food density was not shown when krill were offered a deep – water food type. When krill were offered a deep – water food type ingestion rate increased slowly with increasing copepod density (ca. $\leq$ 1 individual krill $^{-1}$ h $^{-1}$). Ingestion rates when krill were offered either surface or deep water food types were found to be significantly different (ANCOVA $F_{1, 66} = 6.397$, $P = 0.014$).

Regression curves were fitted to determine the type of functional response when krill were offered surface water food types in a range of densities (see Fig. 4.7).

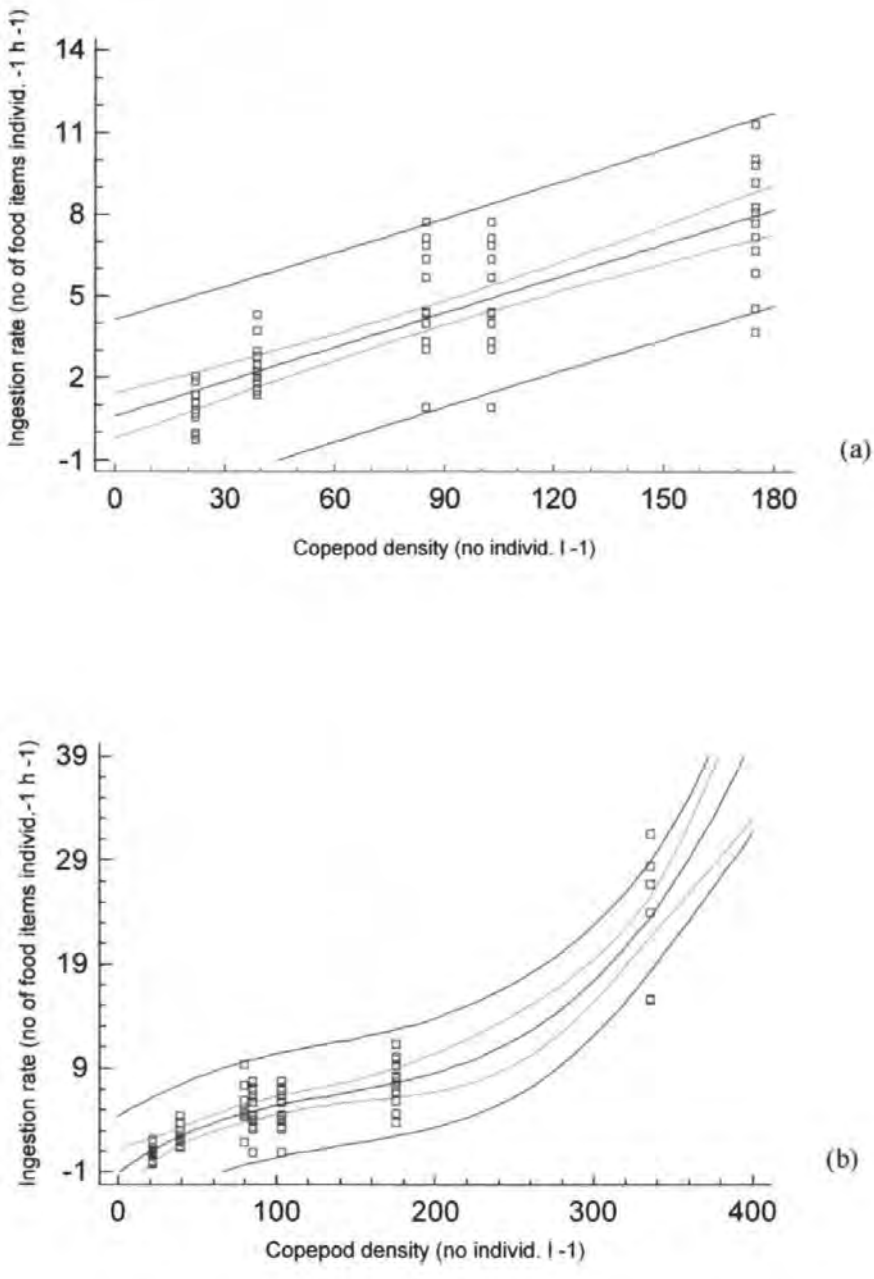


Figure 4.7 Ingestion rates of krill showing confidence limits (dark grey line) and prediction limits (broken line) at the 95 % confidence level for fitted polynomial regression curve (black line) when offered surface water food types at several densities. Data for 2003 only is shown in (a) and data for 2003 plus data from 2002 is shown in (b).

Increasing surface water food density led to an increase in krill ingestion rates which is expected in a functional feeding response (see Fig. 4.7 a). This relationship between density and ingestion rate was significant at the 99 % confidence level (ANOVA, $F_{1, 58}$ = 104. 68, P < 0.0001). A first order polynomial was fitted to data (R^2 = 64.34 %). A lack of fit test (ANOVA, $F_{1, 58}$ = 1.46, P = 0.24) suggested that the model fitted appeared to be adequate for the observed data. This type of polynomial curve suggests a type II response. That is ingestion rate increases with prey density and then slows forming a plateau. This type II response was observed in particular when using mean values for each data point rather than generating a model fitted with all replicates.

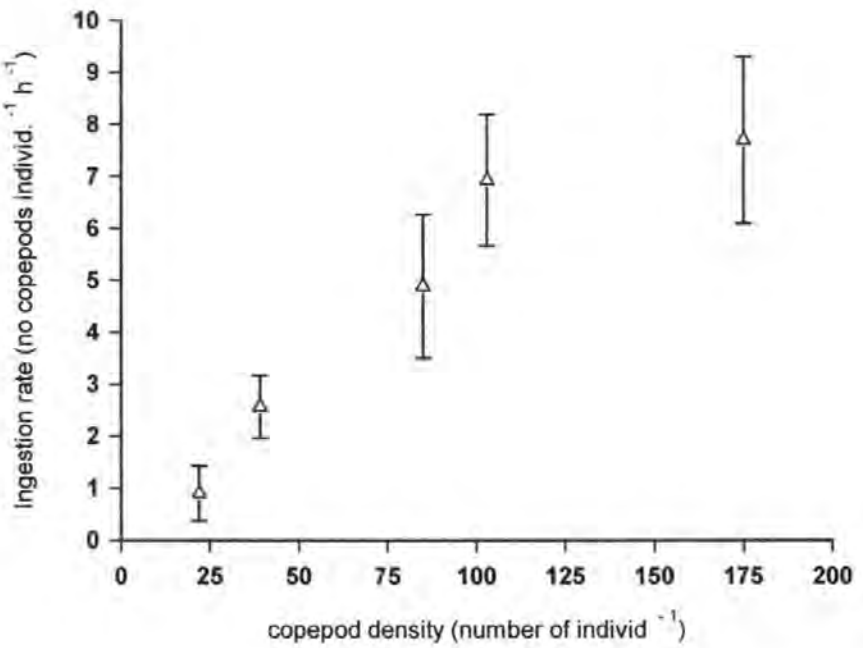


Figure 4.8 Mean ingestion rate of krill (n = 10 – 12 for each point) showing 95 % confidence intervals when surface water copepod food types at several densities.

By using mean values the plotted data appeared to strongly suggest a type II response (see Fig. 4.8). Not all ingestion rate data at various copepod densities showed a normal distribution, therefore as it could not be determined whether this lack of a normal distribution was due to inadequate replication or on the other

hand that the normal distribution shown by some data points was indeed a normal distribution also because of a lack of replicates, it was decided that using all replicates rather than manipulating the data was a better way to determine the functional response. In particular given that using means changed the pattern of the response or type of functional response shown when all replicates are used, using all replicates seems a better approach to determining the type functional response shown by a krill population. The addition of ingestion rates with the higher food density from 2002 studies (see Chapter 2) to the functional response data showed that the response was not the same as that predicted from 2003 data alone (see Fig. 4.7 b). When data from 2002 (see Chapter 2) was added to the 2002 feeding data set, however, a different type of polynomial curve was found to best fit the data. Interestingly ingestion rates from 2002 when krill were offered a similar density of food were found to be remarkably similar. For example when krill were offered a density of ca. 85 or 80 copepods l^{-1} ingestion rates were ca. 5 (2003) and ca. 5.3 (2002) copepods individual $^{-1}$ h^{-1} respectively. The order of the polynomial fitted was changed to third order giving an R^2 value of 84.85 %. Again the relationship between ingestion rate and density was significant at the 99 % confidence level (ANOVA, $F_{3, 70} = 130.69$, $P < 0.0001$). The order of the polynomial was appropriate as the P – value for the order was 0.0059. Therefore, the order was statistically significant at the 99 % confidence level and a lower order was inappropriate. An ANOVA with lack of fit test suggested that a higher order polynomial was also not appropriate and that a third order polynomial was adequate to describe the observed data as the P – value was greater than 0.10 ($F_{3, 67} = 0.50$, $P = 0.68$).

4.3.4 Herbivorous feeding *in situ*

As krill ascended to the shallow depths of the water column during the evening and night a high density of phytoplankton food types became available to utilize compared with the lower phytoplankton density available during the day to krill. The figures that follow in this section (Fig. 4.9, 4.10, 4.11) show how the stomach total pigment levels of krill changed throughout their DVM.

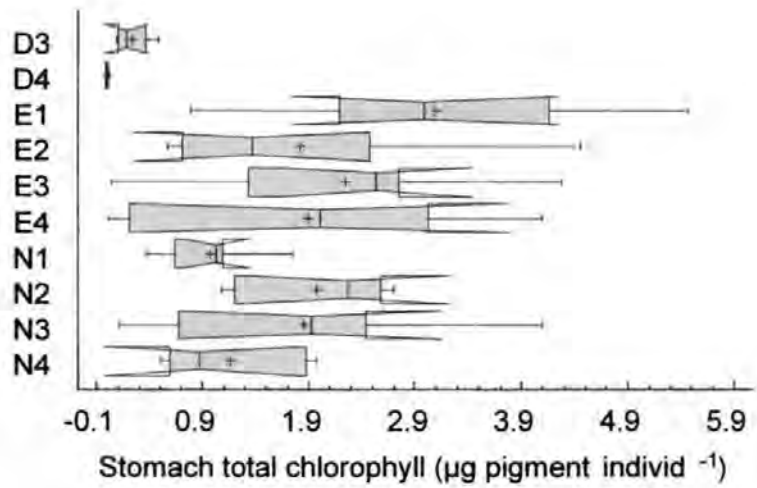


Figure 4.9 Box and whisker plot of krill feeding during day (D), evening (E) and night (N) at shallow to deep depths (1 = 0 – 25 m, 2 = 25 – 50 m, 3 = 50 – 75 m and 4 = 100 – 75 m). Median total pigment (n = 4 – 8) is shown for each depth with confidence intervals. Non – overlapping v – shaped notches indicate significantly different values at the 95 % confidence level.

Krill stomach total pigment was highest in the evening and night periods of DVM compared with during the day as shown by Figure 4.9. There was a statistically significant difference amongst the medians at the 95 % confidence level (T statistic = 30.8605, P = 0.0003).

During the day – time krill had low densities of phytoplankton available to them to feed upon. Total chlorophyll levels in krill stomachs were correspondingly low during the day with mean total pigment (n = 4 – 8) values not exceeding 0 or 0.42

μg chlorophyll individual $^{-1}$ for depths of 100 – 75 m and 75 – 50 m. Interestingly only female krill were caught from depths between 75 – 50 m, therefore comprising 100 % of the mean stomach total chlorophyll value for that depth. Although lower than any mean total chlorophyll values from either the evening or night, stomach chlorophyll content from females caught during the day from 75 – 50 m were higher than in krill caught from 100 – 75 m. In contrast, from depths between 100 – 75 m, mostly male individuals were caught, and the stomach total chlorophyll content from the females caught at this depth was extremely low and similar to that observed in males caught. Stomach chlorophyll levels were much higher during the evening and night than during the day. In fact both daytime mean stomach total chlorophyll values for 25 m intervals between 100 and 50 m were significantly lower than any evening or night – time values. Total chlorophyll levels for krill caught in the evening were up to 5.46 μg pigment individual $^{-1}$ but extremely variable with lower limit values of 0.01 μg chlorophyll individual $^{-1}$. Again at night stomach chlorophyll values were high at 4.10 μg chlorophyll individual $^{-1}$ but displayed huge inter – individual variation being as low as 0.11 μg chlorophyll individual $^{-1}$. Again like during the day the upper distribution limits of krill in the water column were comprised of only female individuals as both during the evening and night only female individuals entered the upper 25 m of the water column. There was no significant difference between the stomach chlorophyll content of krill caught from various depths. Sex was thought to be a possible important factor in feeding as female and male krill were migrating at what seemed like different times and to different depths of the water column. In order to achieve enough replicates to examine whether sex influenced stomach chlorophyll content during the day, evening or night and also the lack of any significant difference between krill caught from various sampling depths, all sampling depths for each time of sampling (i.e. day, evening or night) were analysed collectively.

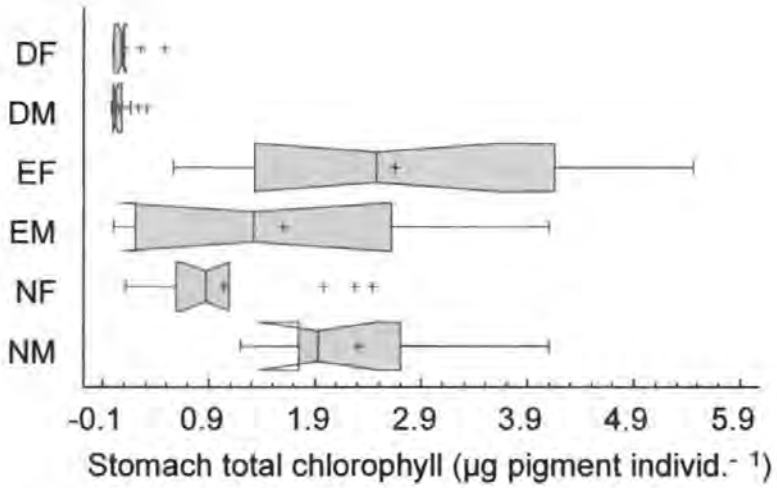


Figure 4.10 Box and whisker plot of herbivorous feeding by krill sexes during day (D), evening (E) and night (N). Median stomach chlorophyll content (n = 7 – 21) for male (shown as M) and female (shown as F) krill during DVM. Non – overlapping V – shaped notches indicate values which are different at the 95 % significance level.

After separation of krill sexes for feeding analysis during DVM, as shown by figure 5.10 it was apparent that there were differences between male and female feeding activity during particularly the evening and night periods of DVM. A Kruskal – Wallis test performed followed by a box plot using STATGRAPHICS Plus 5.0 (1994 – 2000, Statistical Graphics Corp) indicated that there was a statistically significant difference amongst the medians at the 95 % confidence level (T statistic = 54.7307, $P < 0.001$). Median female stomach chlorophyll content was significantly greater in the evening than in the night or during the day. Unlike female krill in male krill there was, however, no significant difference between stomach pigment content during the evening and night, although both these values were significantly greater than day – time values. When comparing sexes directly

at the same sampling times, during the day there was no significant difference between male and female median stomach chlorophyll content. In contrast, female krill showed higher mean stomach chlorophyll content than males during the evening although there was no significant difference but females showed significantly lower stomach chlorophyll during the night than males. At night, feeding by female krill decreased but male feeding increased, although not significantly, compared with evening feeding by males. The highest stomach chlorophyll values for females during the evening and males during the night were not significantly different. Additionally the lower nocturnal feeding values for females during the night and males during the evening were also similar and not significantly different. Therefore asynchronous feeding was shown by krill sexes with the highest feeding activity shown by females during the evening and males during the night.

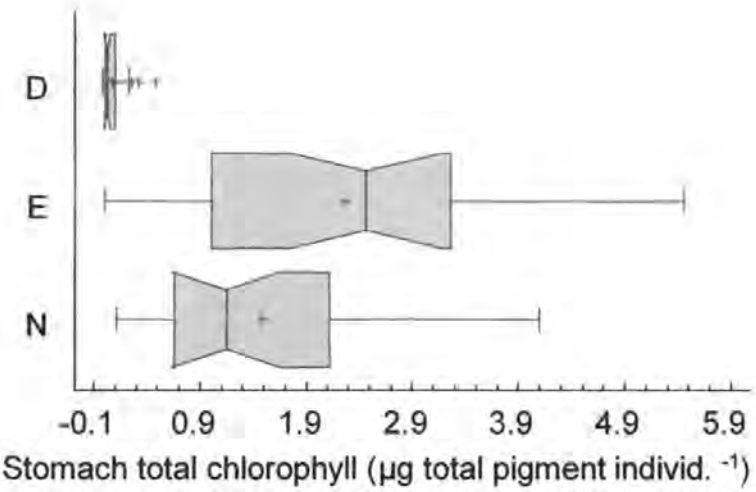


Figure 4.11 Box and whisker plot of herbivorous feeding by krill during the day (D), evening (E) and night (N) shown by median stomach total pigment content (n = 20 – 34) with 95 % confidence intervals. Non – overlapping v – shaped notches indicate at the 95 % significance level that the medians differ.

When both sexes were pooled from all depths at each sampling time in order to achieve enough replicates it was clear that there were differences in feeding activity throughout DVM. Figure 4.11 shows that with more replicates the 95 % confidence intervals were reduced compared with previous analysis with fewer replicates (shown by Figs 4.9 and 4.10).

There feeding was significantly different between the sampling intervals of day, evening and night periods of DVM (Test statistic = 50.3498, $P < 0.001$). During the day feeding was extremely low compared with significantly greater mean stomach chlorophyll contents from evening and night caught krill. With respect to evening and night periods of DVM feeding seemed to be greatest during the evening compared with night as stomach pigment levels were significantly greater during the evening than the night.

4.3.5 Carnivorous feeding *in situ*

Copepod mandibles in krill guts were found haphazardly with respect to sampling depth and sampling time. Furthermore copepod mandibles in krill guts appeared to be either present in large numbers or absent completely. In approximately 200 individual krill examined only 7 guts contained copepod mandibles. One of these guts was from a day caught krill and the other 6 guts were from evening or night caught krill. In three of these guts, only 1 mandible was found, in the other 4 guts 8, 9, 18 and 27 mandibles were counted. In the guts which contained only 1 mandible the mandible widths were 105, 110, 120 and 150 µm. The width of the mandibles found in guts containing more than one mandible in total is shown in Figure 4.12.

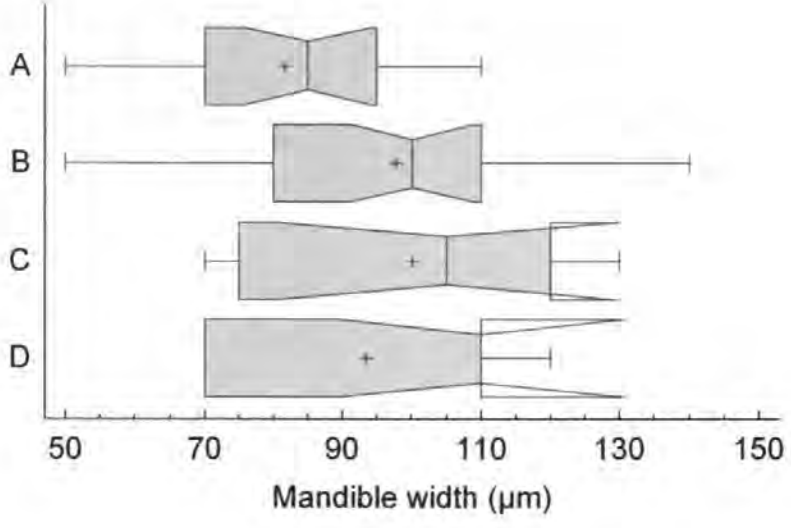


Figure 4.12 Box and whisker plot showing range of widths of mandibles in krill guts (A, B, C and D) which contained more than one mandible. Number of mandibles in gut A = 18, B = 27, C = 8 and D = 9.

The mean values for mandibles widths in Figure 4.12 were similar for example A = 82 ± 18, B = 98 ± 21, C = 100 ± 24, D = 93 ± 22. The shape of the mandibles found in these guts seemed to be that of copepodite stages of *Calanus* spp. and

small copepod species such as *Acartia* and *Pseudocalanus* although it should be highlighted that these mandibles were not identified and quantified because of the difficulty in identifying and separating species using light microscopy. Using the relationship between average carapace length and mandible width from Karlson and Bamstedt (1994) it would seem with mandible widths shown in Figure 4.12 that krill were mostly feeding on copepods of a particular size (0.8 – 1.4 mm in carapace length).

In most of the krill guts examined some copepod remains were found for example copepod furca, antennae together with plant material. In fact, in one krill gut, what appeared to be a krill (species undetermined) mandible was found. These remains were not, however, quantified due to the unreliability of accurately identifying these remains and also because these items in the gut may have been swept into the feeding basket and not actually eaten by krill.

4.4 DISCUSSION

4.4.1 Food type availability during krill DVM

According to the distribution of krill throughout DVM, during the daytime krill would only be able to exploit food types in the deeper 50 m of the water column whereas at night as they migrate and disperse throughout the water column those food types present in the upper 50 m of the water column become 'available' for krill to utilize. The largest difference in distribution and abundance of copepod species between surface and deeper water occurred during the day when krill were concentrated in deeper depths. In 2002 this copepod species difference between surface and deep water was also found (see Appendix A). Although sampling suggests that krill were between 100 and 50 m depths of the water column the lower daytime total abundance may have been due to krill residing even deeper than 100 m. The fjord was around 115 m in depth therefore krill could have been concentrated even deeper in the bottom 15 m than suggested by the net samples because this depth was not sampled as the trawl may have collided with the bottom sediment. In the lowest depths of the water column copepod assemblages mainly consisted of large species such as *Euchaeta* sp., *Calanus* spp. and *Metridia* spp. and although smaller species such as Copepoda J, *Acartia* sp., *Pseudocalanus* sp. and *Oithona* sp. were present between 50 and 75 m depth, krill were less abundant at this depth compared with their abundance between 75 – 100 m where small copepod species were absent. Consequently, during the day mainly large copepod species were available for krill to feed upon. Although small copepod species were present in the upper 50 m of the water column and particularly abundant between 0 – 25 m depth krill would have been unable to exploit this abundant food source as they were concentrated at deep depths. This large contrasting availability of food types between surface and deep waters

diminished during the evening and night. For instance during particularly the evening, but also at night, small copepods became distributed throughout the water column as did krill. Therefore small copepods would be available for krill to feed upon throughout all the depths of the water column. In surface waters the species composition was similar to during the day with small copepods species present except in lower densities than observed during the day. These lower densities could have been due to two factors, (a) these smaller species were present in higher densities at deeper depths than during the day meaning that these copepods had also like krill become diffusely spread throughout the water column and (b) although sampling was carried out in as near a location as day time sampling as possible patchiness in distribution of zooplankton could lead to sampling of varying densities of copepods associated with patches and not with respective depths. Given that observations of DVM in copepods has been well documented, and that there is a difference in species composition with depth in the water column, it is likely that copepods also dispersed throughout the water column during the evening. Therefore during the evening smaller copepod food types were available for krill to utilize as the small copepods were dispersed throughout the water column and also krill migrate into waters where smaller copepod species were present. In addition to copepod availability as krill migrated up to surface waters abundant phytoplankton food types would have also become available for krill to feed upon as they entered the upper 30 m of the water column. Although some chlorophyll was found in the deep parts of the water column this could be due to sinking of phytoplankton from the surface waters. Therefore during the evening more food types were available to krill for two reasons (a) as krill migrated they entered water with different food types available compared with the deep depths they reside in during the day (b) small copepod species had also

become dispersed throughout the water column meaning that these species became available to krill even in the deeper depths.

4.4.2 Krill sex distribution during DVM

As males were completely absent at the upper limit of krill distribution, despite the low number of replicates examined to estimate relative proportions of krill sexes with depth it is likely that there was a difference in the migratory behaviour of males and females. Female krill appeared to migrate to the surface layers earlier than males as mostly female krill found in the upper 50 m of the water column whereas mostly males were found in the deepest 50 m of the water column. Female krill also seemed to stay higher in the water column during the day. Therefore female krill were positioned higher in the water column than males during most of their DVM. The change in distribution of krill sexes throughout the water column between evening and night suggests that females were descending into deeper depths of the water column whereas the males were ascending at night. Consequently male and female krill appeared to exhibit asynchronous DVM, as females stayed higher in the water column during day and evening and also migrated to the surface earlier than males. Differences in male and female DVM where females migrated closer to the surface than males have been shown by *M. norvegica* in the Clyde Sea (Tarling, 2003). Tarling *et al.*, (1999) also found spawning females in shallow waters between 5 and 30 m depths in the Kattegat. Asynchronous migration by krill sexes and particularly the shallower position of females in the water column compared with males suggests that there is some other factor affecting DVM other than predation risk.

Tarling (2003) suggested that females undertook a riskier DVM because of a greater demand for energy in order to fuel reproduction. Energetic demands are a likely explanation for this difference between male and female DVM as migration

by marine copepods has been linked to lipid reserves. Hays *et al.* (2001) found that non – migrating copepods had relatively larger energy reserves than migrants. Hays *et al.* (2001) suggested that copepods with larger energy reserves do not risk imminent starvation and therefore do not need to increase their risk of predation by migrating to surface waters and so stay at deeper depths.

4.4.3 Feeding on surface and deep water food types

Feeding by krill on surface water food types was significantly greater than on deep water food types. *Meganyctiphanes norvegica* exhibited low clearance rates on deep water food types suggesting that they either could not handle (or capture) or did not prefer these larger copepod species. Adult *Metridia* were observed 'jumping' out of the feeding basket of *M. norvegica* in laboratory studies (pers. obs.). These observations of *M. norvegica* with larger copepod food types, such as *Metridia*, suggested that they could not capture or handle these larger copepods efficiently. Even when krill were offered higher densities of deep water copepods which would be available to them during the day part of their DVM they showed consistently low clearance rates. Even these low clearance rates are perhaps artificially high because part of the value could be attributed to the presence of some smaller copepods in the deep water food type therefore meaning that in fact the clearance rates on larger species is lower than suggested.

Clearance rates with surface water food types were similarly consistent but significantly higher than with deepwater food types. These higher clearance rates suggest that krill prefer of can handle smaller copepods present in the surface waters and also available throughout the water column throughout the evening and night more effectively than deep water food types available during the day. Therefore krill show much higher clearance rates with food types available to them during the evening/night part of their DVM than with the deep water food types

available during the day suggesting that they do not feed extensively during the day. Higher clearance rates with evening/night available food types on the other hand suggests that they feed more extensively during their nocturnal migration. Field studies have also suggested that *M. norvegica* feeds extensively at night (Lass *et al.*, 2001).

Lass *et al.* (2001) highlighted the fact that it has been difficult to establish whether the lower ingestion rates observed during the day are due to lower food abundance in the deeper depths which krill reside in during the day. In this study, however, food types available during either the day or night were offered at similar densities therefore the differences in ingestion rates provide evidence that krill do feed more extensively on food types available during the night.

Lower clearance rates of large copepod species compared to smaller copepod species may be explained by an upper size (length/volume) limit of the handling capability of krill. This lower clearance rate on larger copepod species could also be a result of (a) larger copepod species having more efficient escape mechanisms for escaping krill, e.g. they can swim faster than smaller copepod species (b) krill are unable to handle larger copepod species because of the dimension of their feeding basket (c) krill not preferring larger copepod species. It is most likely that it is a combination of all of these factors. In Chapter 2, it was suggested that krill feeding could be limited by the morphology of their feeding basket in what food types they can handle effectively and that they show higher clearance rates with larger, cylindrical food types. Therefore from Chapter 2 it seemed that there was a minimum size of food type that krill could handle. Food types were not, however, as large as those offered in the deep water food type of this study. It would therefore in turn seem likely that there is also an upper limit on the length of copepods that can be handled effectively by the feeding basket of krill. Adults of larger copepod species such as *Metridia* spp. and *Calanus* spp. are

around 5 mm in length. Krill feeding baskets as described in chapter 3 are approximately between 7 and 8 mm in length for krill 33 – 36 mm in length. Therefore it would seem probable that food types nearly the same length as krill feeding baskets would be difficult to retain. The observations of larger species jumping out of krill feeding baskets would suggest that the krill cannot retain these copepods effectively. In addition to the ability of krill being able to retain these larger copepod species in their feeding baskets the escape responses and faster swimming capabilities of larger copepods may make it much more difficult for krill to actually capture a large copepod species. It may also be possible that especially this latter factor of the difficultly to capture larger copepod species that the energy required by krill the capture larger copepods and handle them does not make energetically favourable food types. According to optimal foraging theory krill should select food types, which give them highest net energy gain. Further investigation of energy gain from given food types and the potential energy cost/gain to krill by feeding on these food types would be necessary to determine whether the benefit or cost to feeding on larger copepods. Irrespective of the possible mechanism for why krill do not feed on deep water food types the fact that they show much lower clearance rates on deep water food types than smaller surface water food types suggests that feeding in surface waters may be an part of the mechanisms for why krill migrate to surface waters at night.

4.4.4 Functional response of krill feeding

The functional responses for zooplankton feeding on multiple resources have been reviewed by Gentleman *et al.* (2003). Based on Holling's (1959) explanations for functional response types in *Meganyctiphanes norvegica* showed what seemed to be a Type II response where ingestion rate increased with food density. This was shown in particular when using mean values for ingestion rates at each density. Mean values strongly suggested a Type II response as ingestion rate appeared to plateaux at high food densities. This plateaux was not shown when all replicate values were plotted, therefore suggesting that using means could lead to a misinterpretation of the type of functional response exhibited by krill. Additionally data was added from 2002 (Chapter 3) to the 2003 dataset because in 2002 a much higher food density was used. Interestingly ingestion rates from both 2002 and 2003 with a similar density of copepods were extremely similar, thereby supporting the use of the 2002 data to determine the functional response type. By adding 2002 data which included this higher food density it was clear that the data did not show a typical Type II response by krill. The shape of the curve seemed to suggest a Type III functional response according to Holling's (1959) explanations for the functional response types. A Type III response would suggest that krill can adjust their response with prey density, therefore meaning that at higher food densities their ingestion rate increases meaning they can utilize patches with high food density. It would be expected that eventually krill would be unable to handle any more food and therefore ingestion rate would plateau. McClatchie (1986), however, suggested when investigating the herbivorous feeding of the euphausiid *Thysanoessa raschii* that as clearance rates remained relatively constant that changes in ingestion rate were due to a change in food concentration and not because of an active response of the krill to change their feeding rate according to food density. Although given that krill show significantly greater clearance rates

with copepods compared with phytoplankton (see Chapter 2) it seems likely that krill to a certain extent do actively respond to food types. Price *et al.* (1988) suggested that *E. superba* uses a different feeding mechanism to capture copepods compared with phytoplankton as they showed greater carnivorous clearance rates which were not correlated with herbivorous rates when offered a mixture copepod and phytoplankton. Therefore as krill appear to have a different feeding mechanism for certain food types, it seems likely that if they can actively seek copepods and that they can actively respond to changes in food density or at least prey density.

The change in the type of functional response in particular suggests that data could be could be misinterpreted if only lower or 'realistic' food densities are used to fit the curve and therefore predict the response. Although using high food densities may seem unrealistic, food densities in the pelagic environment are normally estimated by using net samples. By using nets and calculating densities per litre it suggests that organism distribution is uniform in the environment and does not occur in patches. From using high food densities with *M. norvegica* it would suggest that the type of functional response shown is not that which would be predicted by using 'realistic' food densities. If food does occur in high density patches in the pelagic environment then using higher food densities in functional feeding experiments could have great implications about understanding the way pelagic food webs function.

4.4.5 *In situ* feeding of krill during DVM

Krill appeared to feed only during evening and night periods of DVM. Krill showed significantly higher stomach chlorophyll levels during the evening and night compared with extremely low levels in day – caught krill. Therefore it seems that krill feed only in nocturnal migration to surface waters. In fact evening stomach

chlorophyll levels were also significantly greater than night levels suggesting that krill feed most extensively during the evening part of their DVM.

Laboratory investigations in this study and field evidence from several authors (e.g. Sameoto, 1980; Bamstedt and Karlson, 1998; Lass *et al.*, 2001; and Kaartvedt *et al.*, 2002) suggest that copepods are an important food type for *Meganytiphanes norvegica*. However, copepod mandibles where infrequently recorded in krill guts in this study. When mandibles were found, however, they were present in high numbers. This 'all or nothing' presence seems to suggests that either (a) only a few krill in the population feed on copepods or (b) gut transit times are fast and therefore copepod mandibles pass through the guts of krill quickly and are therefore not recorded or (c) copepods have a patchy distribution and therefore the krill that have eaten copepods are also patchy in distribution or (d) the mandibles of copepods are not ingested, although the tissue has been eaten. Given that there is field evidence where most *M. norvegica* sampled in a particular area feed upon copepods, it is unlikely that only a few individuals do feed on copepods. For example Bamstedt and Karlson (1998) found that most *M. norvegica* in the had prey in their stomach content and also that Lass *et al.* (2001) sampled krill from the Clyde Sea and Kattegat and found high levels of fatty alcohols and other lipid markers indicative of a carnivorous diet suggesting that most *M. norvegica* do feed extensively on copepods. Additionally, since both these authors recorded mandibles in stomach contents it suggests that more than just tissue is ingested by krill. The short transit times estimated for *M. norvegica* in abundant food conditions of 15 and 30 min by Heyraud (1979) may mean that mandibles have already passed through the guts of krill when they are captured, this together with a patchy copepod distribution would possibly explain why only some krill were caught with mandibles in their stomach content. Both Bamstedt and Karlson (1998) and Lass *et al.* (2001) suggested that krill showed more

carnivory in some areas than others. Bamstedt and Karlson (1998) suggested that carnivory was less important in the Skagerrak than in North East Atlantic waters. Lass *et al.* (2001) also suggested a difference between sites finding more carnivory associated with a higher ratio of copepod to phytoplankton biomass in the Kattegat compared with the Clyde Sea. Kaartvedt *et al.* (2002) found that algal food was neglected by *M. norvegica* during the late summer when the copepod *Temora longicornis* was eaten during the spring, however food intake from phytoplankton and copepods was comparable. Therefore perhaps less carnivory was shown by krill in this study because it was during a spring bloom and consequently phytoplankton was an abundant food type.

4.4.6 Asynchronous feeding of male and female krill

Similarly to the differences observed between female and male krill migration there was a difference in the herbivorous feeding activity of the sexes during DVM. Females showed greatest consumption of phytoplankton during the evening period of DVM. This together with an earlier ascent to the surface layers of the water column would seem to suggest that females come up to the surface to feed earlier than males. In addition during the day only female krill were found at the upper distribution limit of the krill population. Females in this upper part of the krill distribution showed significantly higher stomach chlorophyll content than krill caught from deeper depths. This also suggests that female krill remain higher in the water column during DVM than males and also show greater consumption of phytoplankton in the day period of DVM. Males appeared to ascend to the surface layers of the water column later than females with males being found in the surface layers at night. Males during the night showed significantly greater phytoplankton consumption than females. However, male stomach pigment did

not reach the high levels shown by females during the evening. Female stomach pigment was significantly lower at night than during the evening. This lower phytoplankton consumption at night together with distribution in deeper water at night suggests that females are descending during the night part of DVM and decreasing their feeding activity. In contrast, males continued to feed at similar levels at night to those during the evening which at night were significantly greater than females. This could be taken as suggesting that males feed over a longer period during DVM than females and particularly with their distribution in shallower waters at night that they also descend later than females to the deeper depths of the water column where they reside during the day. Therefore, it seems that females ascend to the surface layers of the water column earlier than males and show greatest feeding activity in the evening whereas males seemed to feed over a longer period and not to such high levels as female krill. This earlier ascent and greater feeding activity earlier during DVM supports the suggestion that females have a higher energy demand than males. The mechanism for this difference in female and male feeding activity could lead to a possible explanation of DVM by *M. norvegica* as there seems to be a relationship between migration by krill sexes and feeding activity. In particular it raises the question of why do females migrate earlier than males to surface waters and show higher feeding activity in the evening part of DVM than males during any other part of DVM.

4.4.7 Krill DVM and omnivory

Krill appeared to feed only during the nocturnal part of their DVM supporting the conclusions of Lass *et al.* (2001) that *M. norvegica* do not feed extensively during the day. In particular krill showed lower feeding rates with food types which are available during the deep depths they reside in during the day, again supporting the conclusion that krill do not feed during the day.

The fact that krill seem to feed only during the nocturnal part of their DVM is potentially a key factor influencing their feeding strategy. For example if krill fed throughout DVM it may be that they can afford to be more selective of food types as they have more foraging time available. However, given that *M. norvegica* only appear to feed nocturnally it may be that they cannot afford to be a selective feeder as they have less time available for foraging. Being an opportunistic omnivore may therefore enable krill to exploit the food rich surface waters in a short time scale. Additionally, given that female krill show greater feeding activity earlier during their DVM than males, this suggests that some other factor is influencing krill DVM other than just a predation risk. This raises the questions of why do krill not feed during the day and also why do females seem to show an earlier ascent to the surface and greater feeding activity earlier in this ascent than males? Most importantly to understand DVM behaviour it raises the question do krill incur a cost from not being able to feed during the day? Further investigation is therefore necessary to determine whether krill do incur a cost whilst not being able to feed during the day and in turn if there is a cost how do they repay this daytime debt?.

Chapter 5

Feeding and metabolic status of krill: a strategy for diel vertical migration?

ABSTRACT

The strong pattern of krill migrating to the surface to feed and then returning to deeper depths during the day suggests that feeding must form or affect the strategy for DVM. Yet the role of feeding (particularly in relation to metabolism) in the strategy for DVM has largely been neglected. I investigated feeding and metabolism of krill during diel vertical migration in order to further explain their DVM strategy. Krill have been suggested to breakdown haemocyanin for nutrition when starved. Consequently the breakdown of haemocyanin during DVM and the costs of lower haemocyanin levels were investigated in a field experiment by preventing feeding and DVM by krill. The recovery of haemocyanin levels with food types available, either during the day or night periods of DVM, were also investigated under laboratory conditions. Haemocyanin concentrations of day (non – feeding) and caged (prevented from migrating and feeding) were significantly lower than krill that were able to feed during the evening and night, therefore supporting that krill break down their haemocyanin for nutrition. Lactate concentrations were correspondingly high with low Haemocyanin levels suggesting that krill had switched to anaerobic metabolism and therefore incurred an O_2 debt. Krill able to feed during the evening and night recovered their haemocyanin concentrations and consequently reduced their lactate concentrations and therefore their O_2 debt. Glucose concentrations were greater in krill able to feed during the night compared with day – captured krill and krill unable to feed (caged). Laboratory experiments supported field results as krill Haemocyanin concentrations recovered more quickly (and possibly to a higher level) when krill were offered night – time compared with day – time available food types. Male and female showed the same but asynchronous pattern of recovery of haemocyanin content and reduced lactate concentrations with DVM which was identical to their asynchronous feeding pattern. Interestingly female krill showed significantly greater haemocyanin concentrations and also appeared to show a greater magnitude of breakdown and recovery of their haemocyanin and lactate concentrations. Feeding and metabolism seem to be a key part of the strategy for DVM as it appears that krill breakdown their haemocyanin during the day for nutrition whilst not feeding thus incurring an O_2 debt which they then recover from whilst feeding during the night in the surface layers of the water column.

5.1 INTRODUCTION

5.1.1 Krill metabolism and DVM

The primary sources of energy normally considered for metabolism are glucose (stored as glycogen) and lipids. Glucose provides an immediate source of energy. Lipids are extremely concentrated energy reserves, providing relatively more caloric energy than glucose or protein. Utilisation of stored lipids has been suggested as one over wintering strategy in *Euphausia superba* (Hagen *et al.*, 2001). Proteins can also be used as energy sources, although they must first be broken down into amino acids. Consequently, using proteins as an energy source has certain costs to an organism. For instance: proteins are not an immediate source of energy (so they need to be converted into amino acids) and they do not yield as much caloric energy as lipids. Gaining an understanding of how energy reserves in krill are linked with feeding during their DVM is essential to explain the role of feeding in DVM.

As well as understanding energy reserves and feeding it is important to consider the energy costs of DVM. The actual energy costs of DVM are, however, largely unknown. One major cost of DVM may be swimming up to the surface. Swimming speeds have been calculated for krill from acoustic Doppler current profilers between ca. 1 – 3 cm s^{-1} (Liljebladh and Thomasson, 2001; Buchholz *et al.*, 1995). Swimming capacity has also been measured in laboratory conditions by propulsive force in *M. norvegica* by Thomasson *et al.*, (2003). To understand the costs associated with swimming during DVM more empirical evidence is needed on how swimming capacity relates to energetic costs. Although respiration rates have been calculated for *M. norvegica* (e.g. Saborowski *et al.*, 2002) more information is needed on how these rates relate to swimming or krill 'activity' in order to understand the costs of DVM. Examining all the costs associated with

DVM is, however, beyond the scope of this chapter. This chapter focuses on the potential role of Hc together with feeding as a DVM, discussing the potential costs of DVM.

To the best of my knowledge the role of krill metabolism, together with feeding as a mechanism, for DVM has not been considered until recently by Spicer and Strömberg (2002). They investigated krill haemocyanin (Hc) concentrations in relation to environmental factors and found that starved krill showed significant decreases in Hc concentration ([Hc]) compared with fed individuals and suggested that when krill migrate into deeper water during the day they cannot obtain enough energy required for normal metabolic demands and therefore use Hc as energy source. Although, using Hc for nutrition appears an unusual strategy because of the above – mentioned costs of using protein as an energy source. The significantly lower feeding rates on deep – water food types in chapter 4 suggested that krill cannot utilize the food types available during the day in the deeper waters in which they reside to the same extent that they can utilize night time available food types. Therefore, it does seems likely that Hc is broken down during the day for nutrition although there is a need to gain further empirical evidence to support this hypothesis. Although the effect of food availability on crustacean [Hc] has been investigated (Uglow, 1969; Djangmah, 1970; Dall, 1974; Hagerman, 1983) the extremely short timescale for changes in [Hc] found by Spicer and Strömberg (2002) (and not encountered by previous investigators) suggest in the case of krill that the breaking down of Hc may not just be a consequence of starvation but perhaps a purpose such as a strategy for DVM. Again, more investigations are required to examine whether krill [Hc] is related to a strategy for DVM. As a respiratory pigment there must clearly be a cost or debt to oxygen uptake and/or transport (and indeed for re – synthesis) by breaking down Hc for nutrition. It may be expected that as [Hc] decreases krill switch from

aerobic to anaerobic metabolism because of the reduced capacity for transport of oxygen to maintain aerobic metabolism. In common with nearly all other crustaceans (Ellington, 1983; Livingstone, 1983; Greishaber *et al.*, 1994) lactic acid (or *L*-lactate) has been found to be the main end product of anaerobic metabolism in *M. norvegica* (Spicer *et al.*, 1999). Therefore, investigating [Hc] (not only in relation to feeding) but also lactate concentrations is necessary to examine the benefits and costs of breaking down Hc for nutrition and therefore to explain DVM. Glucose may also provide valuable information on the energy gain made by krill during DVM and therefore metabolic state of krill during their DVM. Both not being able to feed on food types and the potential build up of debts and loss of Hc during the day strongly suggests that krill must migrate to the surface to feed and recover their debts.

5.1.2 Study design rationale and aim

The majority of studies investigating feeding in krill are descriptive field studies which do not attempt to examine why and when krill feed upon certain food types (see Chapter 1 for references). More importantly, why krill even migrate to surface waters at night and then return to deeper depths during the day is still largely unknown. Mechanisms for DVM behaviour are mainly speculative and are not based upon empirical evidence. Suggestions for krill DVM have included predator avoidance during day time and then migration to surface layers is in search of food during the night but these suggestions are based on descriptive data and are speculative.

Spicer and Strömberg (2002) found that starved krill had lower [Hc] and suggested that krill may break down [Hc] for nutrition. Field investigations (see Appendix A and Chapter 4) of the feeding of *Meganyctiphanes norvegica* indicated that krill mainly fed during the nocturnal period of DVM. It was also found that clearance

rates were significantly higher with surface water food types than with deep water food types. Therefore work presented in chapter 4 suggested that krill may not feed extensively during the day. If krill do not feed extensively during the day then the question may be posed do krill break down their Hc for nutrition during these non – feeding periods? Furthermore, if krill do break down their Hc for energy then how do they recover their [Hc]? It may be that if metabolic debts are incurred during the day then they are recovered during the nocturnal period of DVM when krill migrate to the food rich surface layers of the water column. This may also help to explain why krill appear to be opportunistic omnivorous feeders, for example, limited time to recover metabolic debts may mean that krill cannot afford the time to be selective feeders.

All of the above assume that feeding is the only factor affecting the metabolic status of krill, however, there may be abiotic factors that also affect krill physiology and thus there metabolic status. Salinity and temperature remain reasonably uniform throughout the depths of the water column which krill are likely to encounter during early spring (i.e. Jan, Feb). Oxygen tension may, however, be low in deeper layers compared with upper layers of the water column. Given that very small variations in oxygen tension have been found to have dramatic effects on the krill physiology (e.g. Childress and Seibel, 1998; van den Thillart *et al.*, 1999; Spicer *et al.*, 1999, Strömberg and Spicer, 2000) even a small differential between surface layers and deep water may be critical. Therefore, oxygen tension may be a major environmental factor influencing both krill physiology and feeding during early spring periods. If oxygen is too low to maintain aerobic respiration and krill have to switch to anaerobic respiration they may incur an oxygen debt and thus high lactate concentrations. Thus, variations in abiotic factors such as temperature, salinity and oxygen throughout the water column (e.g. across a

pycnocline) may affect krill physiology and further intensify any metabolic debts they may have.

Therefore the aim of this chapter was to determine

- is there a metabolic cost of not being able to feed and thus do krill have metabolic debts incurred during the day which they have to recover at night when feeding in surface layers? Thus, what happens to the metabolic status of krill when they are prevented from migrating to surface layers and or feeding at night?
- is the metabolic status of krill affected by the food types upon which they feed and do krill have to be opportunistic feeders in order to maintain energy levels i.e. they feed on whatever food types are available in order to meet their energy demand. And thus, even if some food types are more energetically favourable than others do krill continue to not discriminate between food types because their strategy is feed as much as possible and be a generalist, non – selective opportunistic feeder?

These objectives were investigated by determining the following;

- Feeding *in situ* during the day and night of krill performing DVM and krill prevented from performing DVM (i.e. placed in cages).
- Haemocyanin, glucose and lactate concentrations of krill during the day and night of krill performing DVM and krill prevented from performing DVM (i.e. placed in cages).
- Haemocyanin concentrations of krill fed on diets of phytoplankton only, copepods from either surface of deep waters and a mixture of surface water copepods and phytoplankton.

5.2 MATERIALS AND METHODS

5.2.1 Collection of krill and food types

Meganyctiphanes norvegica were collected from Gullmarsfjord, Southwest Sweden (58°18' N, 11°32' E), using an Isaacs-Kidd midwater trawl (mouth area 0.6m^2; haul duration = 20 min) during the day (4th Mar 2003, sunset = 17.49) proceeding into the night (5th Mar 2003, sunrise = 06.57). Krill were collected from depths between 100 – 75 m during the day and evening to stock cages. Cages deployed during the day were then retrieved during the evening; those cages deployed in the evening were retrieved during night sampling. Free swimming krill were collected from depth ranges of 100 – 75 m, 75 – 50 m and 50 – 0 m during the day (11.00 – 15.00 local time), evening (19.00 – 21.00 local time) and night (03.00 – 06.00 local time) by horizontal oblique tows, except in the evening and night the upper 50 m were split into 2 sampling intervals of 50 – 25 m and 25 – 0 m as the back scattering layer indicated krill were dispersed throughout the water column. A flow meter (General Oceanics, Sweden) was attached to the aperture of the trawl in order to estimate the volume of water filtered and thus in turn krill density per m ^{3} with depth. During 'day' time the back scattering layer on the echo sounder indicated that krill were mainly concentrated at depths between 100 – 50 m therefore one tow was made for krill at both 100 – 75 m and 75 – 50 m. One trawl was made for the range of 50 – 0 m in order to confirm that no krill were indeed present. During the evening and night the backscattering layer indicated that krill were distributed throughout the water column, therefore two tows were made for the following depth ranges 100 – 75 m, 75 – 50 m and 50 – 0 m. Repeated tows for each depth range cannot be regarded as 'true' replicate tows because tows are inevitably temporally different due to the continuous nature of DVM therefore it is impossible to replicate a tow. For this reason that repeated

tows are not true replicates and in particular that the tows from the different depths of the water column needed to be taken as close together as sampling would possibly allow, no tows were replicated in this study.

Zooplankton was collected by a vertical tow using a WP-2 (200 µm) net, from a range of depths encountered by krill during DVM including 100 – 75 m, 75 – 50 m, 50 – 25 m and 25 – 0 m, during the day and night. A flow meter was attached to the aperture of the net in order to estimate the volume of water filtered and thus density of copepods per m^{-3}. Zooplankton were immediately preserved in 4 – 5% formaldehyde solution. Zooplankton tows were not replicated for the same reason as mentioned above for krill tows.

Temperature and salinity were measured throughout the water column using a conductivity temperature depth recorder (CTD). Water was collected in Niskin tubes from twelve depths as follows, 100 m, 80 m, 60 m, 50 m, 40 m, 30 m, 20 m, 15 m, 12 m, 7 m, 5 m, and 2 m. A sample of water was also taken to estimate chlorophyll a and thus give a measure of the total phytoplankton at each depth.

5.2.2 Summary of field experiment design

As the course of sampling during the experiment was fairly involved the course of sampling and methods employed are described in some detail below but also in simplified form in Figure 5.0. Field sampling consisted of sampling caged and 'free swimming' krill. Therefore 'caged krill' refers to any krill sampled (i.e. for gut content or haemolymph) from cages (vol. ≅ 30 l, mesh size = 1mm) and 'free swimming' krill refers to krill sampled from the water column by means of nets.

Two cages were placed on a line at 80 – 90 m (deep water) and another two cages placed on the same line at 20 – 30 m (surface water). A total of three lines were used in the study, with all lines placed at the same location (58°18' N, 11°32'

E), as sampling more than one location in the fjord was impossible because of the time involved in placing and retrieving lines with cages and also sampling of free swimming krill and zooplankton. Therefore, three lines with two cages in deep and two in surface waters gave a total of six replicate surface cages and six replicate deep cages. Cages were stocked with ca. 30 krill per cage allowing between 12 and 20 individuals to be sampled at any one sampling time. Cages were stocked with krill from tows taken between 100 and 75 m depths. Free swimming krill were also collected at the same location as the cages from four 25 m depth intervals and sampled, like krill from cages, for gut content (n = 5 – 8) and metabolic status i.e. haemocyanin, lactate and glucose (n = 6 – 10). These krill were then immediately frozen at $^{-}$20 °C until their sex and moult stage could be determined back at the laboratory. Although these n values seem low the time involved in dissecting krill guts, extracting pigments, performing biochemical analysis and sexing and moult staging individuals was extremely high thus to ensure coverage of the large number of depth intervals, caged and non caged 'treatments' and sampling times during DVM in the time available replicates were reduced.

DAY **04/03/03** Start of sampling (Time = 09.00)	**Sampling** CTD measurements and collection of water from 10 depths **Caged krill** - Krill collected from main back scattering layer of 100 – 75 m to place in cages (deep and surface waters). Some of these krill also sampled for gut content and Hc, lactate and glucose. **'Free swimming'** - krill collected from 100 – 75 m, 75 – 50 m, 50 – 0 m for gut content and Hc, lactate and glucose. **Zooplankton** – vertical hauls made from 100 – 75 m, 75 – 50 m, 50 – 25 m and 25 – 0 m to collect zooplankton.
EVENING **04/03/03** (Time = 17.00)	**Sampling** **Cage sampling** - cages placed in water during the day brought to surface and sampled for gut content and Hc, lactate and glucose. **Caged krill** - Krill collected from main back scattering layer of 50 – 75 m to place in cages (deep and surface waters). Some of these krill also sampled for gut content and Hc, lactate and glucose. **'Free swimming'** - krill collected from 100 – 75 m, 75 – 50 m, 50 – 25 m and 25 – 0 m for gut content and Hc, lactate and glucose. **Zooplankton** – vertical hauls made from 100 – 75 m, 75 – 50 m, 50 – 25 m and 25 – 0 m to collect zooplankton.
NIGHT **05/03/03** (Time = 01.00)	**Sampling** **Cage sampling** - cages placed in water during the day brought to surface and sampled for gut content and Hc, lactate and glucose. **'Free swimming'** - krill collected from 100 – 75 m, 75 – 50 m, 50 – 25 m and 25 – 0 m for gut content and Hc, lactate and glucose. **Zooplankton** – vertical hauls made from 100 – 75 m, 75 – 50 m, 50 – 25 m and 25 – 0 m to collect zooplankton.

Figure 5.0 Schedule of field sampling. All times shown were local times. Arrow indicates order of sampling.

5.2.3 Sampling of caged and free swimming krill

Krill were sorted within 10 min of landing on deck. During the day only free swimming krill were sampled (as no cages were in place). During the evening and night both free swimming and caged krill were sampled. For all sampling of free swimming krill and caged krill for estimation of herbivorous feeding approx. 15 krill were chosen randomly, wrapped in aluminium foil, and frozen (- 20°C) in order to prevent the photo – degradation of chlorophyll pigments present in the gut from light. From another approximately 10 – 15 krill haemolymph was removed for haemocyanin analysis and biochemical analysis (see haemolymph removal methods below, Section 5.2.6). Remaining free – swimming krill were preserved in 4 – 5% formaldehyde solution for enumeration of krill to estimate densities for each depth during day, evening and night periods.

5.2.4 Herbivorous feeding

Herbivorous feeding was estimated by measuring gut content of chlorophyll pigments. Chlorophyll pigments were measured using a fluorescence method suggested of Parsons *et al.* (1984). Krill were thawed and the stomach/gut dissected out (n = 5 – 8) taking care to avoid damaging the gut and thus causing loss of content. Each gut was placed in 90 % ethanol (10 ml) for 12 – 18 h to extract pigments. Chlorophyll a was quantified by fluorometric determination of chlorophylls and phaeopigments using a Turner Systems® fluorometer.

5.2.5 Carnivorous feeding

The guts from krill analyzed for chlorophyll pigments were also used for estimation of carnivorous feeding (n = 5). Although again this n value appears to be small in order to cover the large number of sampling depths and times replicates had to be

reduced to achieve this coverage in the time available as examining one krill gut could take up to 3 h. This examination time was prolonged as guts were inspected three times for mandibles to ensure the reliability of results. Stomach/guts were mounted on a glass slide and stained with methylene blue and examined for copepod mandibles using phase contrast microscopy.

5.2.6 Haemolymph removal and moult stage determination

Haemolymph was collected immediately (within 5 to 20 min after capture) from individual krill sampled either from caged or 'free swimming' conditions. Krill were gently blotted dry using tissue paper in order to avoid diluting the haemolymph sample. Haemolymph was removed from individuals using a microsyringe (Hamilton, 50 µl capacity). The needle of the microsyringe was inserted dorsally under the carapace by puncturing the arthrodial membrane linking the carapace with the abdomen. Haemolymph was collected mainly from the heart but also the internal spaces within the thorax. Haemolymph was then directly transferred to a microcentrifuge tube kept on ice. Haemolymph samples were then kept at 0 – 4 °C until they were analysed. Krill were kept on ice after haemolymph sampling and then stored at ¯ 20 °C for moult stage and sex determination.

Moult stage of krill was determined using the methods of Buchholz (1982) and Cuzin – Roudy and Buchholz, (1999) by examination of the antennal scale under a microscope (x 10 – 40 magnification).

5.2.7 Haemocyanin, glucose and lactate determinations

The concentration of *L*-lactate in haemolymph samples (vol. = 10 µl) was estimated using an enzymatic method described by Gutmann and Wahlefeld (1974) but modified according to Engel and Jones (1978), in which pyruvate is converted to lactic acid in the presence of lactate dehydrogenase and the

proportional conversion of NAD to NADH at this time is followed spectrophotometrically at 340 nm. Haemolymph samples were deproteinised by the addition of cold perchloric acid (10 µl, 600 mmol.l^{-1}). After centrifugation (15 min, 2°C, 9000 x *g*) the resultant solution was neutralised by the addition of K_2CO_3 (2.5 M) and centrifuged once more. The resultant supernatant was analyzed for its [lactate].

The glucose concentration of untreated haemolymph was assayed using an enzymatic method (Slein, 1965). Here hexokinase catalyses the phosphorylation of glucose by ATP. The resultant glucose-6-phosphate is oxidized in the presence of NADP by glucose-6-phosphate dehydrogenase. The amount of NADPH formed in this last step is proportional to the glucose-6 phosphate formed from the glucose and is determined spectrophotometrically at 340 nm.

The [Hc] in the haemolymph of individual krill was estimated using an established spectrophotometric method (Nickerson and van Holde 1971, Hagerman and Weber, 1981; Hagerman 1983, Spicer and Baden, 2000). Twenty microlitres of haemolymph were made up to a final volume of 600 µl with an appropriate saline solution (Schlieper 1972, p.335). The absorbance of the resultant mixture (λ = 335 nm) was measured using a spectrophotometer (Hitachi U2000). Matched quartz cuvettes (Hel, pathlength 1 cm, max. capacity = 1.5 ml) were used throughout the study. Haemocyanin concentration was calculated using the extinction coefficient given by Nickerson and van Holde (1971) (E_{mmol} = 17.26), assuming M_r = 75 kDa for krill subunits (Bridges *et al.* 1983).

5.2.8 Food type availability/abundance

Zooplankton were filtered through a 60 µm sieve, rinsed with fresh water, and replaced in a solution of 70 % ethanol with 3 % glycerol, for ease of working with sample. Zooplankton samples were sub–sampled using a Flosom plankton splitter

and divided into eight equal parts. Three 1/8 subsamples were used for identification and enumeration of zooplankton. All copepods were identified to genus and not to species as the aim of the investigation was to examine feeding on surface and deep water food types not species specific feeding. Small juvenile copepods in surface waters were not differentiated for example copepodite stages of *Calanus*, therefore all these copepods were placed in category named 'Copepoda J.'

Immediately upon collection duplicate water samples (volume = 100 ml) from each depth were filtered onto Whatman glass micro-fibre filters (GF/F) and extracted in 90% ethanol for 12 – 14 h. Chlorophylls and phaeopigments were determined using the fluorescence method described previously for gut content analysis.

5.2.9 Haemocyanin recovery (laboratory) experiment

Meganyctiphanes norvegica were collected from Gullmarsfjord, southwest Sweden (58°18' N, 11°32' E), using an Issacs – Kidd midwater trawl (Mouth area 0.6 m^2; haul duration = 10 min) on several occasions during Jan and Feb 2003 using the RV '*Arne Tiselius*.' Krill were transferred (within 5 min of harvest) into sealed thermos containers (Rubbermaid drinking water thermosflask, vol. = 80 l) containing filtered sea water (salinity = 34 PSU) and transported to KMRS within 2 h of capture. In the laboratory krill were maintained in fibre – glass aquaria (vol. = 350 l) covered with dark plastic to keep krill in darkness. Aquaria were supplied with natural 'deep' sea water pumped into the station from a depth of 35 m (salinity = 34 PSU, T = 6 °C). All experiments were carried out within 5 d of capture.

Copepods were collected, from the same location as krill and from depths likely to be encountered by krill during their DVM. Depths likely to be encountered during the day were indicated as 100 – 50 m by previous studies in 2002 (see Appendix A and Chapter 4), and also by the back scattering layer at the time of this study

shown by the echo – sounder on board the RV Arne Tiselius, whereas at night the back scattering layer indicated krill migrated into the upper 50 m of the water column. Therefore food types copepods and phytoplankton were collected from depths of 100 – 50 m and 50 – 0 m by vertical tows using a plankton net (200 µm WP – 2). Copepods from these collection depths will be referred to as 'deep water' and 'surface water' copepods respectively. Copepods were returned to the laboratory within 2 h of capture in sealed thermos containers (Rubbermaid drinking water thermosflask vol. = 20 l) containing filtered sea water. At KMRS copepods were maintained in aerated plastic containers (vol. = 80 l) supplied with natural surface (pumped into station from depth of 6 m S = 34 PSU, T = 4°C) water or deep water. All experiments were carried out within 5 d of capture.

For all experiments a group of similar size krill (body length, i.e. rostrum tip to end of telson = 30 – 36 mm) were selected from the stock aquaria and then transferred to experimental containers. Krill (n = 10) were placed in glass aquaria containing 18 litres of filtered sea water (total of 12 aquaria). Krill were starved for a 12 h period (T- 0). Haemolymph was then sampled from krill individuals in each aquaria using identical removal methods as described for field sampled krill (see Section 5.2.6). Remaining krill were then subjected to various 'feeding' conditions, that is they were either starved for a further period of 12 h or given a food type treatments were as follows;

- Filtered sea water (3 aquaria)
- Filtered sea water plus deep water copepods (ca. 40 individ. l^{-1}) (3 aquaria)
- Filtered sea water plus surface water copepods (ca. 40 individ. l^{-1}) (3 aquaria)
- Filtered sea water plus mixed phytoplankton/copepod food type from surface waters (3 aquaria).

After this 12 h period haemolymph was sampled from krill (n = 5). in each aquaria. Krill were kept on ice after haemolymph sampling and then stored at ¯ 20 °C for moult stage and sex determination. An identical experiment was also performed except with the modification that after the 12 h starvation period krill were either starved or fed for a reduced period of 6 h and then sampled.

Krill moult stage was determined as described previously (Sect. 5.2.6).

5.3 RESULTS

5.3.1 Food availability and physico – chemical charcteristics

Phytoplankton was mainly concentrated in the upper layers of the water column between depths of 10 and 30 m (up to 1.48 µg total pigment l^{-1}). Total pigment levels were low throughout the rest of the water column (< 0.03 µg total pigment l^{-1}), until a depth of 100 m where they increased slightly to 0.13 µg total pigment l^{-1}. Therefore the most abundant source of phytoplankton food types would only be available for krill to exploit during their evening and night ascent to the surface layers of the water column.

A summary of copepod abundance at various water column depths during the day, evening and night is shown in Table 5.1. A more extensive description of copepod distribution data throughout the water column is found in Chapter 4. In summary, during the total copepod density was greatest in the surface layer of the water column comprising of copepod species whereas larger copepod species were found in deeper depths of the water column. During the evening and night all copepod species became distributed throughout the water column. During the evening total copepod density appeared to be greatest in the deeper 50 m of the water column, whereas at night copepods were most abundant between 50 and 75 m depth and also in the surface layer of the water column.

Table 5.1 Summary of total copepod abundance and species compoistion with water column depth during the day and night. Species compostion ranked with most abundant species at that depth first.

Depth (m)	Day		Evening		Night	
	Total copepod abundance (No. individ. m^{-3})	Species composition	Total copepod abundance (No. individ. m^{-3})	Species composition	Total copepod abundance (No. individ. m^{-3})	Species composition
0 – 25	284	Copepoda J *Acartia* *Pseudocalanus* *Oithona, Temora*	270	Copepoda J *Metridia* *Acartia* *Pseudocalanus* *Oithona* *Temora*	451	Copepoda J *Metridia* *Pseudocalanus* *Acartia, Oithona* *Temora*
25 – 50	42	Copepoda J *Oithona* *Acartia*	24	*Acartia* Copepoda J	181	Copepoda J *Pseudocalanus* *Metridia, Temora* *Oithona* *Calanus* *Acartia*
50 – 75	78	*Metridia* Copepoda J *Pseudocalanus* *Oithona* *Calanus* *Acartia* *Euchaeta*	609	Copepoda J *Acartia* *Pseudocalanus* *Metridia* *Calanus* *Oithona* *Temora* *Euchaeta*	450	Copepoda J *Metridia* *Calanus* *Acartia* *Pseudocalanus* *Temora*
75 – 100	13	*Euchaeta* *Metridia*	1320	Copepoda J *Acartia* *Metridia* *Calanus, Oithona* *Temora* *Euchaeta*	287	Copepoda J *Pseudocalanus* *Calanus, Metridia*

As krill were concentrated in the depths of the water column between 50 and 100 m during the day only larger copepod species and low phytoplankton densities were available for krill to utilize. During the evening and night, as krill ascended to the surface layers of the water column both smaller copepod species and higher densities of phytoplankton became available. The change in copepod distribution throughout the water column nocturnally compared with during the day also meant that smaller copepod species became available to krill even at deeper depths of the water column.

Physico – chemical characteristics were relatively uniform throughout the water column. Temperature ranged from ca. 4 °C to ca. 8 °C in shallow to deeper depths of the water column. Salinity also remained fairly constant from depths of 110 m to 20 m in the water column ranging from a salinity of ca. 30 to ca. 34. From depths of 20 m up to the surface the salinity was lower ranging from 30 to 17 respectively. Oxygen content of surface and deeper depths of the water column was relatively uniform with ca. 60 % oxygen at depths of 100 m and ca. 70 % oxygen at depths of 50 m.

5.3.2 Moult stage and Hc

The relative composition of krill moult stages during DVM is shown by Figure 5.1. During the day the proportion of both male and female krill was approximately split between moult stages A/B and B/C, whereas during the evening slightly more females appeared to be in B/C stages compared with A/B moult stages. Similarly during the evening the proportion of males in B/C moult stages compared with A/B seemed to increase. During the night, nearly all free – swimming female krill sampled were in moult stage B/C. The proportion of male krill in moult stage B/C during the night in contrast with females was less with a higher proportion of males

in moult stage A/B. Both male and female krill in d stage moult appeared to be low throughout DVM varying between 0 and ca. 20 %.

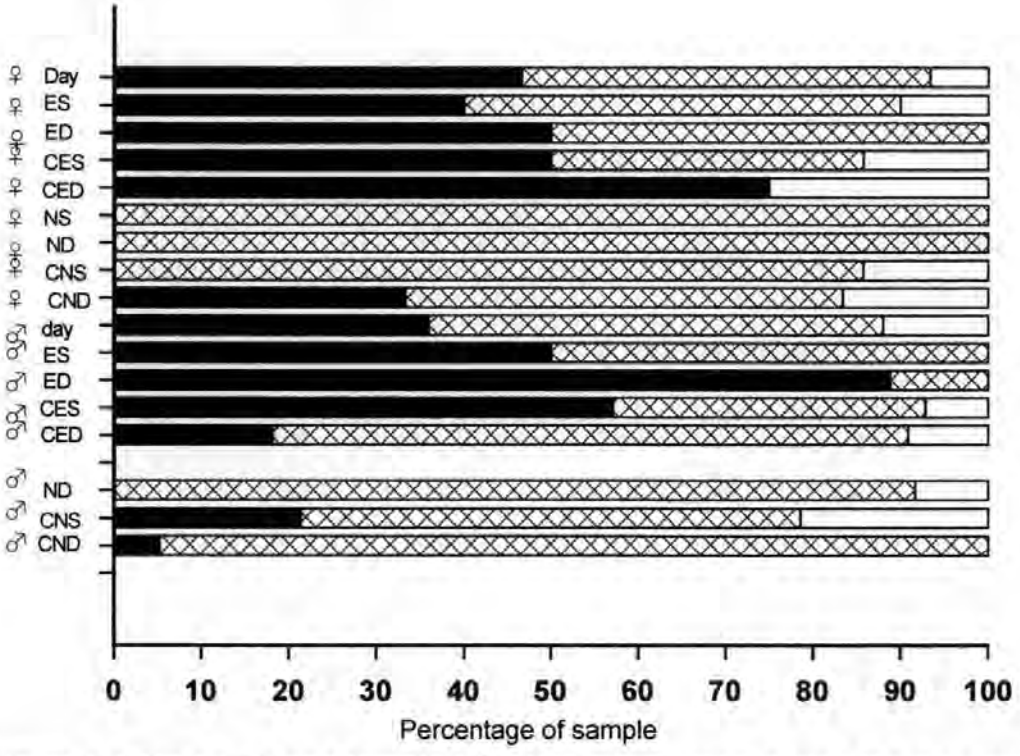


Figure 5.1 Male (♂) and female (♀) krill moult stage during the day, evening (E), and night (N) from surface (S) depths of 0 – 50 m and deep (D) depths of 50 – 100 m. Caged krill indicated by C. Moult stage A/B = solid bars, B/C = crosshatched bars and D = open bars. (n = 14 – 25).

When comparing krill [Hc] with moult stage and sex the most noticeable difference in [Hc] were related to sex and not moult stage (see Figs 5.2, 5.3 and 5.4). Female krill appeared to approximately twice the [Hc] compared with males. During DVM median female [Hc] ranged from 1.84 to 2.40 mmol l^{-1} whereas male [Hc] ranged from 0.90 to 1.22 mmol l^{-1}.

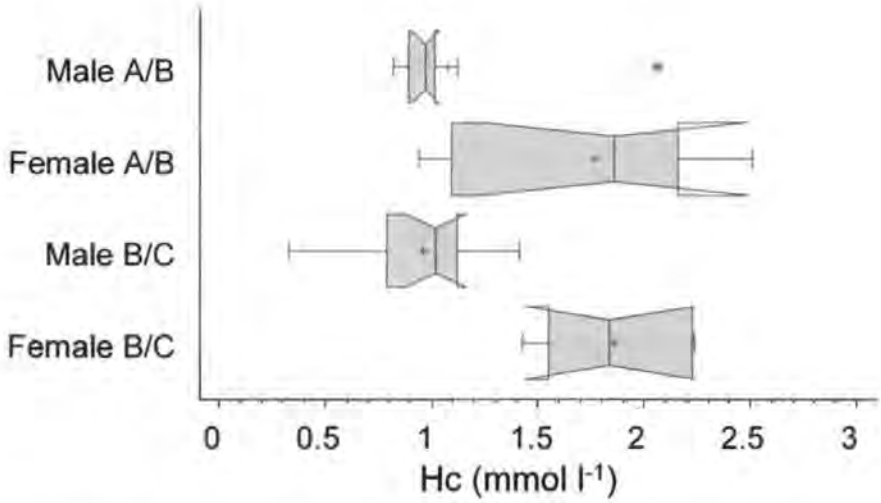


Figure 5.2 Median [Hc] of krill (n = 9 – 13) shown with 95 % confidence intervals in different moult stages during the day from depths between 50 and 100 m the water column. Non – overlapping v – shaped notches indicate significantly different values at the 95 % confidence level.

During the day there was no significant difference between [Hc] in moult stages A/B or B/C in the same sex krill (Fig. 5.2). There were, however, significant differences in the [Hc] of each moult stage between male and female krill.

Again, as shown by Figure 5.3 similarly with during the day there were no significant differences between Hc levels in moult stages A/B or B/C in the same sex krill at any depth during the night. There were significant differences between both male moult stages at any depth and all female moult stages and any depth.

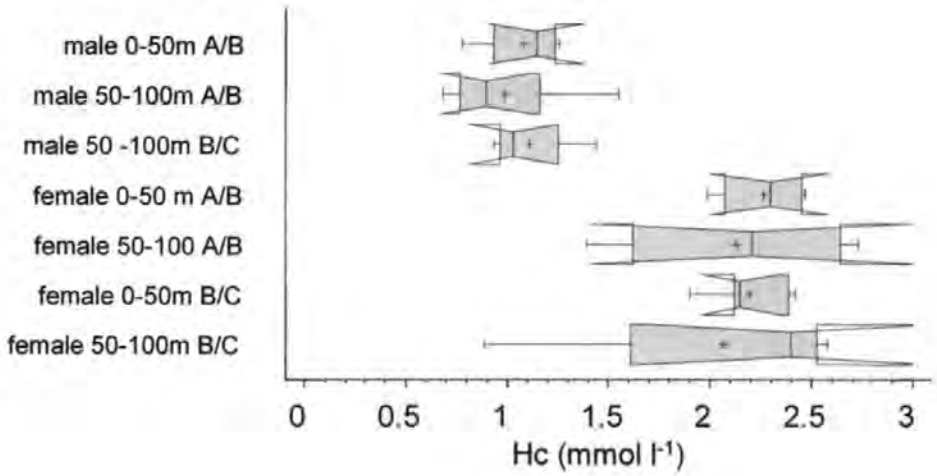


Figure 5.3 Median [Hc] of krill (n = 4 – 8) shown with 95 % confidence intervals in different moult stages during the evening from depths between 50 to 100 m and 0 – 50 m the water column. Non – overlapping v – shaped notches indicate significantly different values at the 95 % confidence level.

Again these differences in [Hc] between male and females were also evident during the night with female [Hc] significantly greater than male [Hc] (see Fig. 5.4).

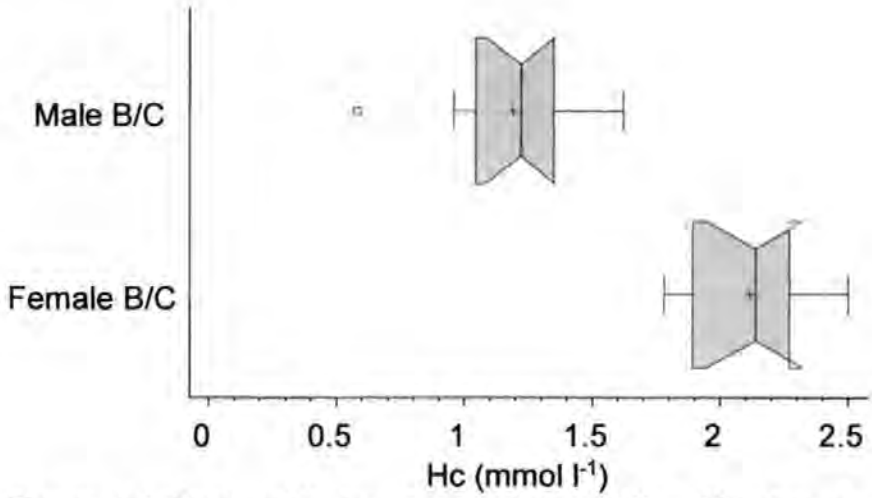


Figure 5.4 Median Hc levels of krill (n = 10 – 12) shown with 95 % confidence intervals in different moult stages during the night from depths between 25 to 100 m in the water column. Non – overlapping v – shaped notches indicate significantly different values at the 95 % confidence level.

5.3.3 Feeding, [Hc], lactate and glucose during DVM

Feeding by male and female caged krill was low during the evening and similar to day captured krill stomach total pigment levels (Fig. 5.5). There were, however, significant differences amongst the median krill stomach total pigment values during DVM and caged krill and non –caged krill as indicated by a Kruskal – Wallis test performed followed by a box plot (T statistic = 100.98, $P < 0.01$). There were no significant differences between surface and deep caged male krill either during the evening or night. Caged male krill with the exception of night – time retrieved surface caged krill did not have significantly greater stomach total pigment content than day captured krill. All male caged krill had significantly lower stomach total pigment levels (like day – captured krill) compared with evening or night captured krill. Caged females similarly with caged males had similar and not significantly different stomach total pigment contents during the evening compared with day captured. Evening sampled female caged krill, as male caged krill had significantly lower stomach total pigment content than compared with evening or night captured female krill. Night caged females in contrast with males did have

significantly greater stomach total pigment values compared with day captured krill. Female krill caged in surface waters during the night in particular were not significantly different to night captured female krill, although those females caged in deeper water at night did have significantly lower stomach total pigment content than night captured female krill.

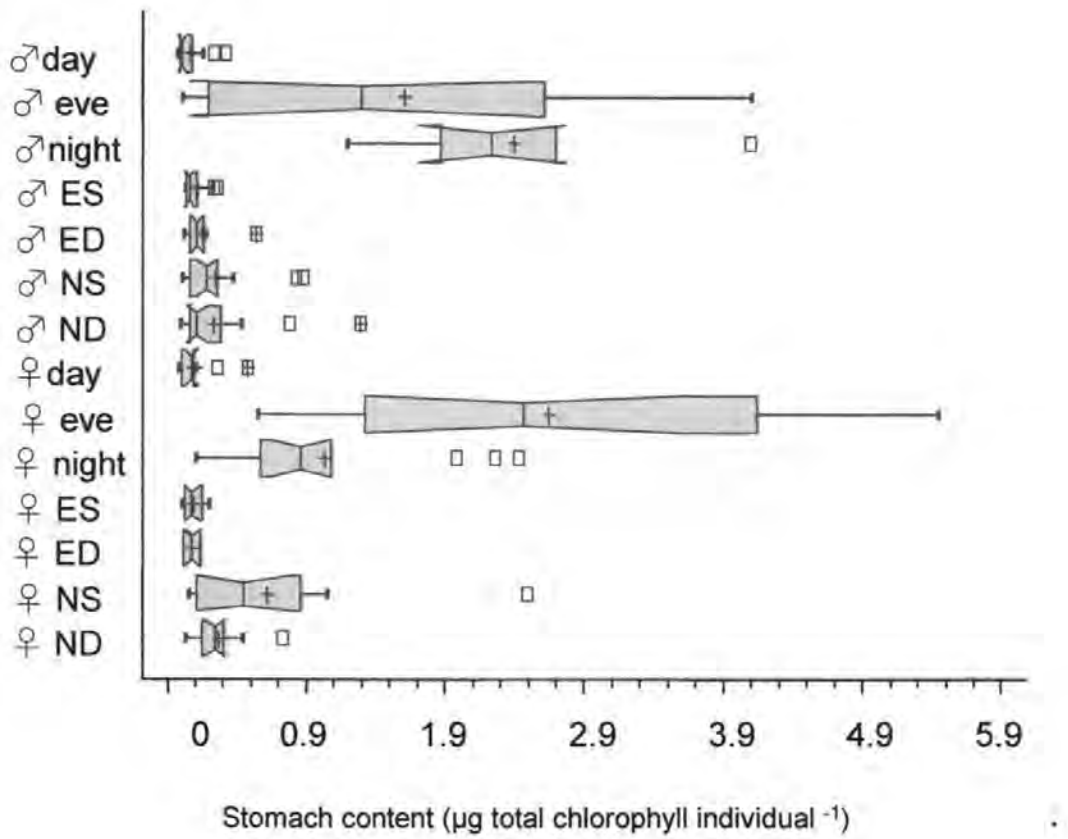


Figure 5.5. Median herbivorous feeding by male and female free – swimming krill (n = 6 – 21) during the day, evening and night and by male (♂) and female (♀) caged krill during the evening (E) and night (N) in surface depths (S) and deep (D) depths. Non – overlapping v – shaped notches indicate significantly different values at the 95 % confidence level.

Haemocyanin concentration in male and female krill also appeared to be related to their DVM as shown in Figure 5.6. A Kruskal – Wallis test performed followed by a box plot indicated that there were significant differences between the median [Hc] at the 95 % confidence level (T statistic = 107.746, $P < 0.01$).

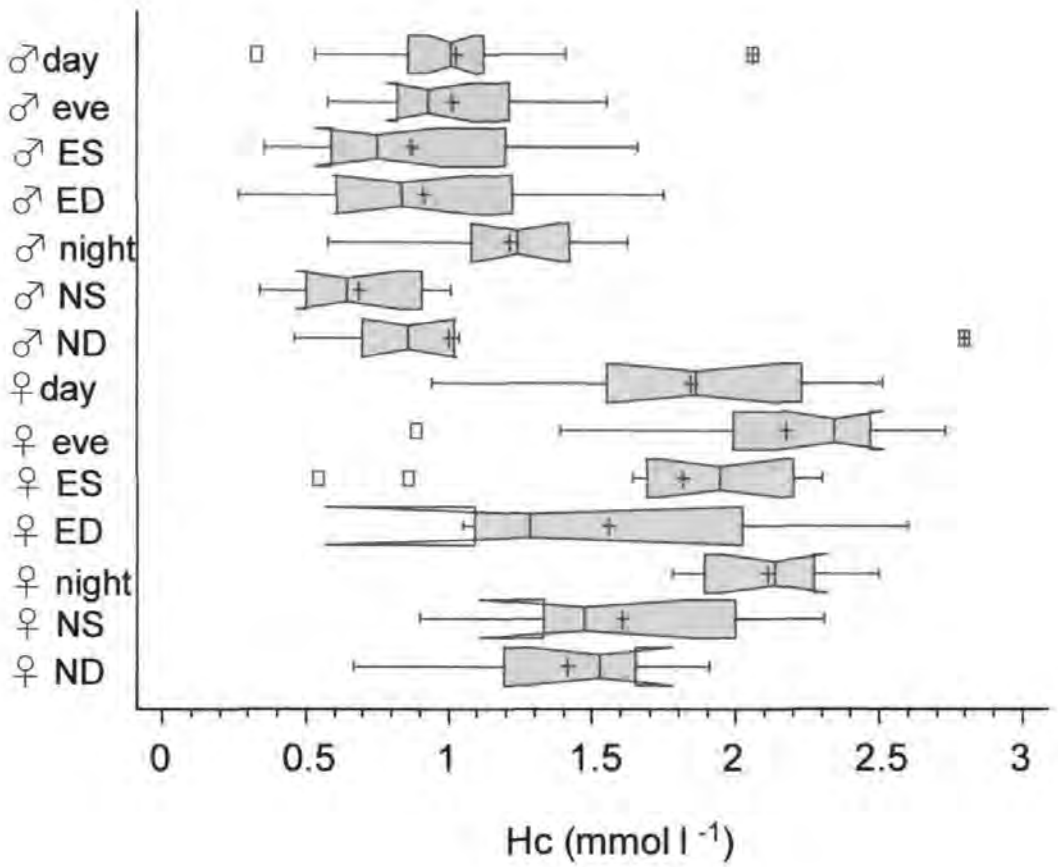


Figure 5.6 Median [Hc] of male and female free – swimming krill (n = 4 – 25) during the day, evening and night and by male (♂) and female (♀) caged krill during the evening (E) and night (N) in surface depths (S) and deep (D) depths. Non – overlapping v – shaped notches indicate significantly different values at the 95 % confidence level.

Male [Hc] were significantly greater during the night than during the day or evening. There was also a significant difference between male krill caged in surface water during the night and males captured during the evening. There

were, however, no significant differences between day captured, evening captured, evening caged or night deep water caged male krill. In contrast with males, female [Hc] increased significantly during the evening part of their DVM. Female [Hc] from evening captured krill were not significantly different to night captured female levels. Both evening and night captured female krill [Hc] were significantly greater than female krill retrieved from cages during those periods. In particular evening and night caged krill [Hc] were not significantly different to day captured krill [Hc].

Again as with herbivorous feeding rates and [Hc], haemolymph lactate concentrations appeared to be related to krill DVM (Fig. 5.7).

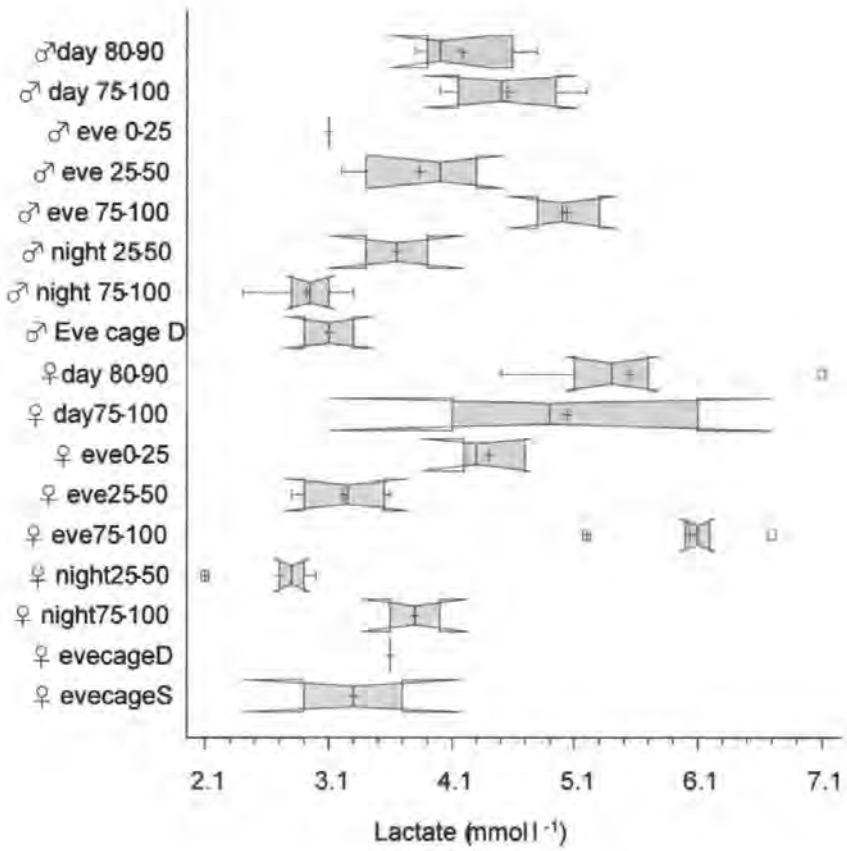


Figure 5.7. Median lactate concentrations of male (♂) and female (♀) free – swimming krill (n = 2 – 7) from various depths as indicated during the day, evening (eve) and night and by male and female caged krill (cage) in surface depths (S) and deep (D) depths. Non – overlapping v – shaped notches indicate significantly different values at the 95 % confidence level.

A Kruskal – Wallis test performed followed by a box plot indicated that there were significant differences amongst the medians at the 95 % confidence level (T statistic = 56.351, $P < 0.001$). Male lactate concentrations were significantly less during the night than compared with during the day. Indeed male lactate concentrations during the night were significantly different with water column depth. That is to say lactate concentrations were lower in krill captured from deeper water than compared with surface waters. In contrast during the evening male lactate concentrations were significantly greater in krill captured from deeper

water than compared with those captured from surface waters. In fact those lactate concentrations from male krill caught in deeper waters were higher but not significantly different to day captured krill from 100 – 75 m although they were significantly greater than compared with day captured krill from 80 – 90 m. Similarly female krill showed this same pattern of significantly greater lactate concentations in krill captured during the night from 100 –75 m depths than compared with day captured krill from 80 –90 m but no significant difference compared with day captured krill from 80 –90 m. Female lactate concentrations were significantly lower in krill captured during the night from surface waters between 0 – 50 m than compared with deeper depths between 50 and 100 m. Interestingly, female krill captured during the night from surface water had even lower lactate concentrations, although from deeper depths there was no significant difference between evening and night captured individuals.

A similar pattern to lactate was shown for krill glucose concentrations during DVM with the difference that glucose concentrations seemed to increase during the evening and night rather than the decrease shown by lactate concentrations. The pattern of krill glucose concentrations with DVM is shown in Figure 5.8.

A Kruskal – Wallis test performed followed by a box plot indicated that there were significant differences amongst the medians at the 95 % confidence level (T statistic = 56.198, $P < 0.001$).

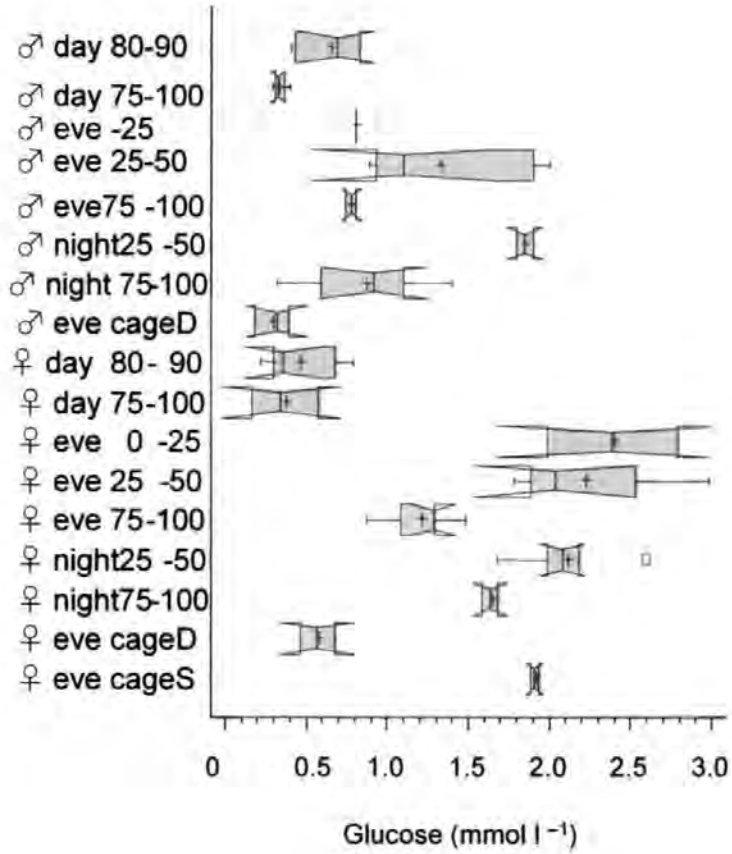


Figure 5.8. Median glucose concentrations of male (♂) and female (♀) free – swimming krill (n = 2 – 7) from various depths as indicated during the day, evening (eve) and night and by male and female caged krill (cage) in surface depths (S) and deep (D) depths. Non – overlapping v – shaped notches indicate significantly different values at the 95 % confidence level.

Male glucose concentrations were significantly greater during the evening in surface waters than compared with either during the day and from deeper depths during the evening. Moreover male glucose concentrations were significantly greater during the night in surface waters than compared with during the evening in surface waters. Glucose concentrations from krill captured from deeper water (100 – 75 m depths) were similar with those of evening captured krill from similar depths of between 75 and 100 m. Male krill retrieved from cages during the evening had similar glucose concentrations with day captured male krill. Female

krill glucose concentrations increased significantly during the evening compared with during the day. During the evening female glucose concentrations were significantly greater in shallower depths between 0 and 50 m than compared with 75 – 100 m depths. Female glucose concentrations from 75 – 100 m depths during the night rose significantly above evening levels although they were still significantly lower than glucose concentration of krill captured from shallower depths between 25 and 50 m during the night. Female krill retrieved from surface waters in the evening had significantly greater [Hc] than those retrieved from deeper depths

5.3.4 Recovery of [Hc]

The recovery of [Hc] after a 12 h starvation period with various food types available at different points during krill DVM is shown by Figure 5.9. The proportion of male and female krill pooled in the samples for each each treatment is presented in Table 5.2

Table 5.2 Proportion of male and female krill in pooled haemolymph samples for each feeding treatment. Values are presented as means +/- SD.

	Pooled sample sex composition (%)	
Treatment	Female	Male
No food type – starvation prior to feeding	48 ± 6	52 ± 6
Deep water food type	54 ± 12	46 ± 12
Surface water food type	43 ± 8	57 ± 8
No food type starved	56 ± 9	44 ± 9

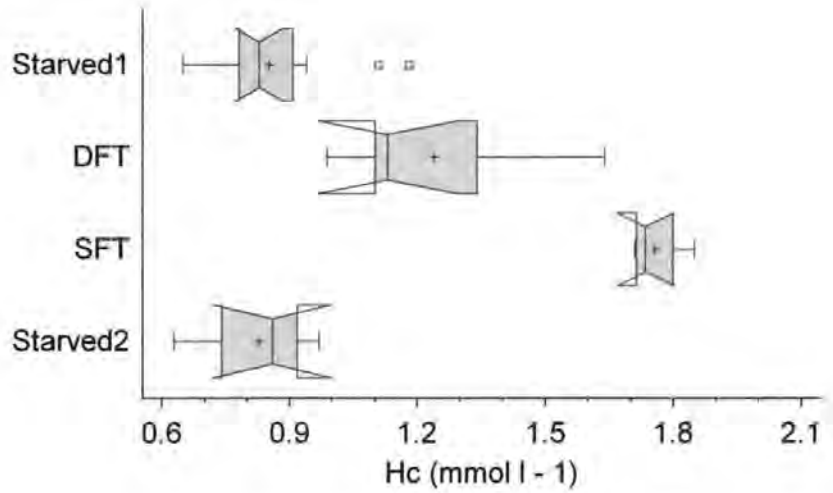


Figure 5.9 Recovery of haemocyanin after a 12 h period starvation period (Starved1) followed when subsequently offered a deep water food type (DFT) or surface water food type (SFT) or no food type (Starved2). Median Hc level (n = 2 –6) shown with 95 % confidence intervals. Non – overlapping v – shaped notches indicate significantly different values at the 95 % confidence level.

A Kruskal – Wallis test performed followed by a box indicated that there was a significant difference amongst the medians at the 95 % confidence level (T statistic = 10.52, $P < 0.0014$). There were no significant differences between krill starved in the 12 h period prior to feeding and those krill that continued to starve for a further 12 h period. [Hc] was, however, significantly greater when krill were offered either food type. In particular krill [Hc] was significantly greater when offered a surface water food type than compared to a deep water food type.

5.4 DISCUSSION

5.4.1 Moult stage, sex and [Hc]

The difference in moult stages observed progressively through DVM suggests that krill change from moult stages A/B to B/C fairly quickly during DVM. The higher proportion relative to during the day of females in B/C stages in the evening compared with males suggests again asynchronous behaviour in the form of changing of moults stages by males and females. This earlier change to B/C moults stages by females may be a consequence of their earlier ascent to the surface waters to feed, although further investigations with many more replicate krill would be required to determine whether this is the case.

Values for [Hc] of *Meganyciphanes norvegica* presented here are some of the highest recorded (1 – 2 $mmol.l^{-1}$) for any crustacean (Mangum, 1983; Truchot, 1992). This has been noted before by Spicer and Strömberg (2002). However, what was not noted by these authors is the fact that there is such a massive sex difference in [Hc]. This is because that the individuals used by Spicer and Strömberg (2002) were predominantly males, most of the females were gravid and so were not used in experiments. It does mean that on the rare occasions where they pool males and females for analysis (Spicer and Strömberg, Pers. Comm.) the derived data should now be considered suspect. Fortunately this does not change the overall story they present. Sex differences are present but rare in crustaceans (e.g. Horn and Kerr, 1963; Baden *et al.*, 1990; Chen and Cheng, 1993; Spicer and Baden, 2000) but even then the difference I found is unprecedented.

5.4.2 The cost of not feeding

Male and female [Hc] appeared to significantly increase in the night and evening periods of DVM respectively. Recorded in Chapter 4 is the pattern for krill feeding and sex where females seemingly feeding earlier during the evening than males which fed most extensively at night. Therefore this increase in [Hc] occurs at the same time as increased feeding occurs for both male and female krill. In support of krill [Hc] appeared to be associated with feeding during DVM, those krill prevented from feeding during DVM (by placing in cages) did not show the same increases in [Hc] as krill which had been able to feed. Therefore not being able to feed during their DVM means that they cannot restock their [Hc]. Lower [Hc] associated with unfed individuals compared with fed individuals agrees with the conclusions by Spicer and Strömberg (2002) that Hc is broken down by krill for nutrition. Moreover, the laboratory experiments investigating the subsequent recovery of [Hc] after krill had being starved and then offered a food types also support the idea that krill break down their Hc when starved. These laboratory experiments also suggested that krill can then recover their [Hc] more quickly (and perhaps to a higher level) with certain food types. [Hc] of krill offered a surface food type compared with a deep water food type were significantly greater after 12 h. These [Hc] associated with deep water food types suggests and supports the conclusions of Chapter 4 that krill do not feed extensively during the day period of their DVM when only these deep water food types are available.

Lower lactate concentrations and higher glucose levels also appeared like [Hc] to be associated with feeding by krill during DVM. Significant variation in lactate and glucose levels with different depths for each of the sampling times during DVM is likely to be associated with the asynchronous feeding pattern of krill as discussed in Chapter 4. That is krill at various depths in the water are likely to have fed to different extents due to different timing of their ascent to the surface layers of the

water column, therefore possibly explaining the variation between lactate and glucose levels between water column depths at any one sampling time. For lactate concentrations asynchronous feeding is a likely explanation as during the day when krill do not feed extensively lactate levels were similar. Although, during the day lactate concentrations were significantly different between sampling depths both lactate concentrations were low and significantly lower for krill feeding during the evening. Lactate levels during the nights were lower (< 4 mmol l^{-1}) and nearer to the values obtained by Spicer *et al.* (1999) under normoxic laboratory conditions (2.03 mmol l^{-1}) than compared with during the day (> 4 mmol l^{-1}). Female glucose reached significantly greater concentrations during the evening period of their DVM than for males at any time during their DVM. Lactate concentrations were also much higher and significantly greater in depths of 80 – 90 m during the day for females than for males during the day. Therefore it appears that female krill show a more extreme pattern of metabolism during their DVM. That is females show greater lactate debts but also higher stomach total pigment content and greater glucose levels during their DVM than males. [Hc] were also significantly greater for females than males.

5.4.3 Feeding – part of the strategy for DVM?

The need to migrate to the surface to recover from debts incurred during the day has been suggested by De Robertis *et al.* (2001). When investigating the anoxic feeding and subsequent recovery of the amphipod *Orchomene obtusus*, they suggested that these amphipod must migrate from the anoxic bottom waters where they feed at least once a day to the oxygenated regions of the water column to recover from the anaerobic respiration debts they incur whilst in the anoxic waters. Therefore, whether the cause of the oxygen debt is from switching to anaerobic metabolism because of anoxia/hypoxia or from switching to anaerobic

metabolism because of a reduced capacity to transport oxygen because of reduced [Hc] from starvation it seems that a mechanism of recovering from these depths is part of the strategy for migration. Therefore in the case of *M. norvegica* it would seem that an important part of their DVM strategy must be to recover from the debts they incurred during the day that is lower [Hc] and consequently an oxygen debt from anaerobic metabolism.

Using Hc for nutrition may incur difficulties for krill. As Hc is a very large protein it must be broken down into amino acids before it can be used for energy. Additionally, as it is a large protein there must be a substantial energy cost to re-synthesizing Hc if it is to be used as a respiratory pigment. Consequently there are two costs to using Hc for nutrition i.e. it must be broken down first and secondly it must then be re – synthesised to use a respiratory pigment. There is also the question of what happens to the copper from Hc once the respiratory pigment has been broken down. To fully understand the role of Hc (for nutrition) in DVM these factors need further consideration. Investigating blood ammonia in relation to [Hc] during DVM may provide more evidence concerning whether Hc is being broken down during DVM. Examining the energy content of Hc together with the energy costs of re - synthesis would provide more necessary information to explain the costs and/or benefits of using Hc for nutrition.

Although there are 'problems' associated with using Hc for nutrition it does seem clear from the laboratory experiment investigating the recovery of Hc with feeding that krill [Hc] can recover to higher levels in a shorter timescale with some food types compared with others. Indeed krill recovered their Hc to a greater concentration when offered surface water food types compared with deep water food types. Therefore supporting the hypothesis that krill recover their [Hc] when they migrate to the surface to feed at night. Males and females seem to follow the same pattern of Hc, lactate and glucose concentrations although asynchronously

during their DVM, thus supporting the idea that the metabolic state of krill drives the feeding pattern of DVM. As suggested by Spicer *et al.* (1999) there must be driving force such as predator avoidance to enter hypoxic waters. As the krill appear to break down their Hc during the day without hypoxia I would further suggest that there must be a driving force such as predation for krill to show a strategy involving the breaking down and rebuilding of their [Hc] during DVM. Furthermore given that female krill show a more extreme change in metabolic state during their DVM together with that they have [Hc] approximately twice those of males there must be another factor than just predation influencing their DVM strategy. It would seem that female krill have a greater need to feed and gain energy and greater costs of residing in deeper depths during the day. It may be, as Tarling (2003) suggested, that greater demand for energy for reproduction by females may be driving their riskier DVM than males. Cuzin – Roudy *et al.* (2004) suggested that food quality and quantity are extremely important in growth moult and egg development. Therefore its seems likely that the energy required by females for these processes in reproductive development are greater than for males and therefore mean females have a greater need for energy.

Spicer and Strömberg (2002) found that there was an increase in [Hc] with increased temperature in fed animals whereas in contrast with an increase in temperature [Hc] were lower for starved individuals. It may be advantageous to krill if during for example during the summer months when temperatures are much warmer in the surface layers than compared with deeper layers if when they migrate to the surface to feed in these warmer waters that they can rebuild their [Hc] more quickly. Spicer *et al.* (1999) found that *M. norvegica* under hypoxic conditions produced more *L*–lactate. Therefore even though krill seem to switch to anaerobic metabolism and incur an oxygen debt under normoxic conditions in deeper waters as they break down their Hc it seems that if these deeper waters

are hypoxic the oxygen debts are far greater. Consequently, environmental conditions may intensify the oxygen debts that krill already have from breaking down their Hc during DVM. On the other hand environmental conditions such as temperature as mentioned above may actually facilitate the recovery of [Hc] when krill go up to the surface and feed.

Chapter 6

General Discussion:

The role of feeding in krill diel vertical migration

6.1 Feeding and metabolism, a strategy for DVM?

The strong pattern of krill migrating to the surface, feeding and then returning to deeper depths of the water column (e.g. Appendix A, Chapter 4, Lass *et al.*, 2001, Onsrud and Kaartvedt, 1998; Stuart and Pillar, 1990; Simard *et al.*, 1986) suggests that feeding must form, or affect, part of the strategy for DVM. Therefore, although modelling studies have suggested that patterns of predation risk may be key to understanding krill DVM (e.g. Alzono and Mangel, 2001) it seems surprising that there so few studies consider the possible role of feeding in DVM. Although feeding *during* DVM has been documented it is difficult to establish the role of feeding *in* DVM because most of the studies which have examined feeding in relation to DVM have described patterns but have not investigated mechanisms (e.g. Sameoto, 1980; Onsrud and Kaartvedt, 1998; Lass *et al.*, 2001). Although it is important to establish patterns of feeding during DVM it is also true that it is difficult to interpret from field observations the mechanisms for DVM. Tarling and his co – workers (2000) suggested that in an optimisation model they developed that both feeding and predation risk are important driving factors during DVM. The results of this thesis suggest that the feeding strategy of krill and their DVM strategy seem to be very interlinked and thus highlight the importance of considering feeding as part the mechanism for DVM.

A summary of the DVM strategy and feeding strategy of *M. norvegica* proposed from the results of this thesis is presented below (see Fig. 6.1). The model presented is discussed in relation to the results of this thesis in the text that follows

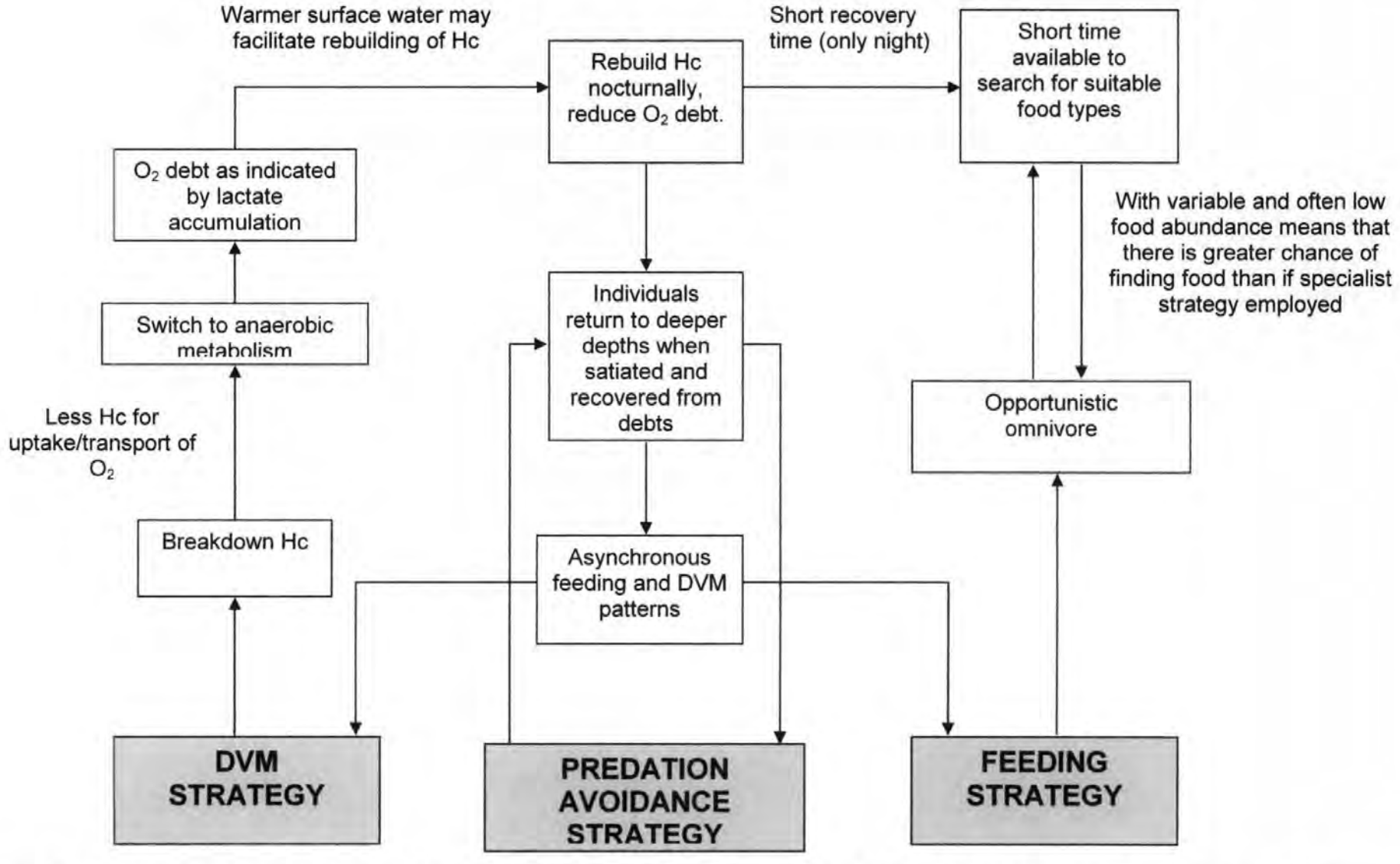


Figure 6.1 Tentative model of the mechanisms involved in DVM illustrating the inter- linked nature of DVM, feeding and predation avoidance.

The timing of feeding during DVM is a key part of determining the relationship between DVM and feeding and therefore whether a NDVM to the surface layers is to feed. Even though there have been studies suggesting a timescale for feeding (e.g. Sameoto, 1980; Onsrud and Kaartvedt, 1998) their suggestions are based upon field observations. They are also primarily based upon gut content analysis and therefore cannot suggest when food types have been eaten as they may have remained in gut content. The laboratory experiments presented in Chapter 4 could be taken to suggest that krill do not feed on deep – water food types available to them during the day but on surface water food types available to them during the night, therefore supporting the idea that krill do not feed during the day but only nocturnally. The observed concentration of krill in deeper layers during the day and low pigment content of day caught krill (see Chapter 4) also supports the suggestion that krill do not feed during the day. Consequently, the low feeding rates on deep water food types (Chapter 4) and higher feeding rates on surface water food types means that krill must ascend to the surface to feed. Nakagawa *et al.* (2003) also found a similar a pattern of decreased daytime feeding and increased feeding in the night for the krill *Euphausia pacifica*. Increased feeding at night has also been recorded in both *Euphausia vallentini* and *Euphausia longirostris* (Gurney *et al.*, 2002.) Additionally it would appear that as copepods also became dispersed and therefore available throughout the water column at night whereas phytoplankton remained only available in higher densities in the surface waters that migration to feed in the surface layers of the water column must be to feed on both copepods and phytoplankton. Therefore if krill do not (or cannot) feed during the day on the food types available then it lead to the question, is there a cost of not being able to feed? Spicer and Strömberg (2002) suggested that krill break down their Hc when starved for nutrition in a higher

magnitude and shorter timescale than has been recorded for other crustacean species. Therefore, over a short timescale under conditions of starvation krill can break down their Hc. Krill appeared to break down their Hc during the day whilst not feeding (Chapter 5). Reduced [Hc] could in turn mean a reduced capacity for the uptake and transport of oxygen and consequently a reduced capacity for aerobic metabolism (while the very low O_2 affinity of *M. norvegica* Hc (Spicer and Strömberg, 2002) makes the role of this pigment in gas transport questionable, we really need values oxygen tensions for arterial and venous haemolymph before we can make meaningful interpretations). Therefore, as expected krill switched to anaerobic metabolism and this was indicated by an increase in lactic acid in the haemolymph. Therefore [Hc] was being broken down at the same time as an oxygen debt was being generated. This could be interpreted as meaning there was indeed a cost from not being able to feed during the day. Therefore with a clear cost to not feeding during the day krill must have some means for recovering from the debts they incurred during the day. Laboratory experiments investigating the recovery of [Hc] in starved krill when offered food types available either during the day or night periods of krill DVM indicated that [Hc] were recovered more quickly (and possibly to greater concentrations) on surface water food types compared with deep water food types (see Chapter 5). Field data suggested a similar pattern of Hc breakdown and recovery, as [Hc]s were significantly greater at night in female and males than compared with during the day. Females [Hc] increased earlier during DVM with an earlier evening ascent to the surface water than compared with later ascent to the surface at night by males (see Chapters 4 and 5). Indeed this increase in both males and females followed the same pattern as their feeding again suggesting that [Hc] were recovered nocturnally when feeding in surface waters. Consequently the breakdown and subsequent recovery of Hc was completed in the course of DVM. The large variance in stomach

pigment content with depth in both male and female krill during day, evening and night periods of DVM (see Chapter 4) suggests that krill are feeding asynchronously. The asynchronous feeding activity of both male and female krill strongly supports the hunger – satiation hypothesis elegantly presented in the recent review by Pearre (2003) whereby ascent to the surface layers is driven by hunger with individuals then descending to deeper depths when satiated. Hays *et al.* (2001) suggested that in the copepod *Metridia pacifica* individuals with larger lipid stores remained at depths and did not migrate to the surface layers as their risk of starvation was not imminent and therefore there was no reason for exposure to the increased predation risk in the surface layers. Therefore, perhaps not only do krill migrate at different times during their DVM but also maybe not all krill in each DVM cycle, depending on their energy status, need to migrate. Large variation between individuals was also shown in their lactate and glucose levels at each sampling period during DVM, again supporting the interpretation that krill did not migrate, feed and recover synchronously. The hunger – satiation hypothesis as discussed extensively by Pearre (2003) fits this pattern of krill feeding and metabolism especially if satiation is modified to include the recovery of metabolic debts incurred during the day. Such asynchronous patterns for feeding and recovery of debts suggest that DVM is an individual based strategy. That is, it is the recovery and feeding of the individual that determines their ascent and descent in DVM and not the movement of a group or swarm of individuals, which determines their movement. Although, Burrows and Tarling (2004) suggested from their model of DVM that krill may make decisions based on the behaviour of their conspecifics as well as their own internal state because there is a high premium to occupying the most profitable depth zone at night. Perhaps asynchronous behaviour of individuals actually facilitates the use of resources by krill as not all krill occupy the same depth at any time. Consequently,

as it seems that krill migrate individually, whether krill actually swarm or whether these groups of individuals observed in the field are coinciding DVMs of individuals must be questioned. Therefore it seems that krill suffer costs during the day but with only a limited time for nocturnal ascent to the surface layers of the water column to feed the recovery of Hc must be quick and therefore their energy acquisition from feeding must be equally as short in order to rebuild Hc concentrations.

6.2 DVM and its influence on krill feeding

A limited time to feed means that krill must have an appropriate feeding strategy if they are to recover from the debts that they incurred during the day. Krill were suggested to be opportunistic omnivores in Chapter 2 with the food types eaten related to the morphology of the feeding basket (see Chapter 3 for discussion). A short time available for searching for food (i.e. only at night) and often a variable abundance of food such an opportunistic feeding strategy would facilitate the recovery of debts incurred during the day. According to optimal foraging theory as proposed by MacArthur and Pianka (1966), a generalist feeding strategy would be favoured in variable environments particularly when krill have such a limited time to recover their debts from during the day. That is, by including more food types (possibly less profitable) in their diet, generalists have to spend less time searching for food. In addition to observations that krill are flexible feeding on a variety of food types (see Chapter 2 for discussion) krill also seem to be equipped to deal with a varied diet. Fevolden (1982) suggested that the higher degree of enzyme heterozygocity in *M. norvegica* compared with *Thyansoessa rashii* was related to a more varied diet. Buchholz and Saborowski (2000) also suggest that induction of enzymes including chitinases indicates ominvory in both *E. superba*

and *M. norvegica* and emphasizes their ability to respond to highly variable trophic environments. Krill may not only have to deal with a variety of food types but also differing densities of food types both during their DVM (i.e. in surface and deeper depths) and seasonally (i.e. with spring blooms). When krill were offered various densities of surface water food types they showed a steeper increase in ingestion rates at higher food densities suggesting that they are able to utilize patches of high food density (see Chapter 4). Buchholz and Saborowski (2000) suggested that a basal level of digestive enzyme activity ensures immediate utilization of patchy food sources. An ability to quickly respond to patches of food would be advantageous to krill when trying to recover in a short time from their daytime incurred debts. Meyer *et al.* (2002) suggested that enzyme activity in *E. superba* was significantly higher in lower food conditions and suggested that therefore food assimilation was more efficient at low food levels. Therefore, the ability of krill to respond to food patches and also to efficiently assimilate food at low food levels is advantageous in a variable food environment, as it would facilitate the recovery of debts incurred during the day even under low food conditions. Therefore above all krill seem flexible generalist feeders able to utilize both phytoplankton and zooplankton.

6.3 Female and male DVM

Tarling (2003) predicted that female krill undertake a riskier DVM (in terms of predation risk) than male in order to fuel reproduction by ascending higher in the water column. Female krill in this study also appeared to ascend to shallower depths than males (see Chapter 4) however, not only did females appear to undertake a riskier DVM in terms of predation risk they also seemed that to be undertaking a more extreme pattern of DVM than males in terms of their feeding and metabolism (see Chapter 5). Tarling (2003) also suggested that female and

male krill have different energetic demands as a result of their reproduction based on the findings of Cuzin – Roudy (2000) that by the time female *M. norvegica* are ready to spawn they have accumulated more than 1 000 eggs with a lipid content of more than 3 mg ash – free dry weight. It is difficult to comment, however, on any possible differences between female and male metabolism. Although there have been numerous studies investigating krill metabolism in relation to feeding/energy demands (e.g. Holm – Hansen and Huntley, 1984; Ikeda, 1984; Ikeda and Dixon, 1984; Frazer *et al.*, 2002; Saborowski *et al.*, 2002 and also see review by Quentin *et al.*, 1994) no distinction has been made between males and females. Virtue *et al.* (1996) found that mortality was significantly greater in reproductive male *Euphausia superba* than in females and suggested that this was because of low lipid levels in males. Therefore perhaps the apparent riskier DVM strategy of females is not riskier in the long term as they have a lower mortality because they have higher lipid stores. Therefore, determining (a) whether female krill do have higher metabolic demands compared with males, (b) how females assimilate energy to fuel their reproductive demands and (c) whether the pattern of female DVM is indeed riskier should be the focus of future work considering the mechanism for the differences in the DVM of krill sexes.

6.4 Wider implications for proposed DVM model

Most krill are omnivorous feeding upon both phytoplankton and zooplankton (Mauchline and Fisher, 1969; Mauchline, 1980). *Meganyctiphanes norvegica* is normally described in the literature as predominately carnivorous. Herbivorous feeding by *M. norvegica* is clear from the results of this thesis (see Chapters 2 and 4) and has been frequently documented (e.g. Sameoto, 1980; Simard *et al.*, 1986; Onsrud and Kaartvedt, 1998; Kaartvedt, *et al.*, 2002 Lass *et al.*, 2001) and suggested from fatty acids as dietary indicators (Virtue *et al.*, 2000). Other

species (*Thysanoessa macura*) considered as mainly carnivorous have been suggested to feed on phytoplankton based on the presence of enzymes such as laminarinase and galactosidase (Mayzaud *et al.*, 1985). Species which have been described predominately as herbivorous such as, *E. superba* actually appear to be flexible feeders and omnivorous because feeding on animal prey has been frequently documented (e.g. Price *et al.*, 1988; Pakhomov *et al.*, 1997; Atkinson and Snÿder, 1997; Atkinson *et al.*, 1999; Cripps *et al.*, 1999). Enzyme activities characteristic of an omnivore have been recorded (e.g. Mayzaud *et al.*, 1985; Buchholz and Saborowski, 2000). *Nyctiphanes australis* has also been suggested to feed upon both phytoplankton and zooplankton (Dalley and McClatchie, 1989; Ritz, *et al.*, 1990). *Euphausia pacifica* has been suggested to feed upon both diatoms and carnivorously on copepods in laboratory conditions (Ohman, 1984; Stuart, 1986) and even ciliates (Nakagawa *et al.*, 2004). *Euphausia lucens* has also been suggested to feed upon both phytoplankton and zooplankton (Stuart and Huggett, 1992; Gibbons, *et al.*, 1991). Therefore, all of the above mentioned species seem flexible, omnivorous feeders perhaps suggesting that they have similar feeding strategies. If most krill are omnivorous and flexible feeders then it raises the question is their feeding strategy driven by the same need to acquire energy quickly in a short time? Consequently it may be hypothesised that perhaps the model of DVM proposed in this thesis where krill have to recover from debts in a short time scale during the night may be common to all euphausiids, although further investigations would be required examining more krill species to determine whether this is the case.

The pattern of ascending to feed at night and then returning to deeper depths has been recorded in *M. norvegica* (Chapter 4, Appendix A, Simard *et al.*, 1986; Onsrud and Kaartvedt, 1998) *E. pacifica* (Nakagawa *et al.*, 2003) *E. lucens*

(Gibbons, 1993). This similar ascent to surface layers in other krill species further suggest that maybe krill have common strategy for DVM. It may be expected if other species of krill also do not feed during the day but only nocturnally that they have similar costs which they must recover from like *M. norvegica*. Furthermore nocturnal feeding has also been suggested in copepods (Simard *et al.*, 1985; Atkinson *et al.*, 1996; Hays *et al.*, 2001; Urban – Rich *et al.*, 2001; Tarling *et al.*, 2002; Kibirige and Perissinotto, 2003) suggesting that maybe the proposed model of DVM could be a more general strategy found in other zooplankton such copepods. Moreover, (Kibirige and Perissinotto, 2003) found higher plant pigment concentrations in the guts of all the species (including mysids and shrimp *Palaemon* spp.) they examined during the night than compared with during the day. Again this diel cycle of feeding by other crustacean species may suggest that they may follow a similar strategy of feeding and DVM with *M. norvegica*. Particularly if DVM is driven by the need to obtain energy or recover from debts. As Hays *et al.* (2001) suggested that copepods *Metridia pacifica* did not migrate if they had larger lipid stores perhaps DVM in copepods is indeed driven by the same motivations to obtain energy. Sekino and Yamamura (1999) from an optimisation model demonstrated that zooplankton individuals change their migrating behaviour depending on the amount of accumulated energy. It seems extremely likely that part of the drive for DVM is driven by the need to obtain energy. Studies such as Tarling *et al.* (2002) have dismissed the hunger – satiation hypothesis (Tarling *et al.*, 2002 was criticised by Pearre, 2003 although Pearre's criticism was then refuted by Tarling *et al.*, 2003). They have suggested that DVM is driven by predator evasion as Pearre (2003) pointed out copepods in this study actually descended before the arrival of krill suggesting that copepods were perhaps indeed satiated and not descending because of predation. The fact that both copepods and krill co occur during DVM in the surface water suggests

that they are both driven by a common need to obtain energy for example. The need to migrate may also be to recover from debts in some way. The need to migrate to the surface to recover from debts incurred during the day has been suggested by De Robertis *et al.* (2001). When investigating the anoxic feeding and subsequent recovery of the amphipod *Orchomene obtusus*, they suggested that these amphipods must migrate from the anoxic bottom waters where they feed at least once a day to the oxygenated regions of the water column to recover from the anaerobic respiration debts they incur whilst in the anoxic waters. Therefore the need to migrate may not be to feed but also to recover from debts that have been incurred. It is also likely that predation is a factor in DVM as in particularly the case of *M. norvegica* why would they descend to depths especially when hypoxic and suffer costs such as the breakdown of their Hc when they are so poorly equipped for anaerobic metabolism (Spicer *et al.*, 1999). The effect of predators on the DVM of *Daphnia* has been documented. Von elert and Pohnert (2000) suggested that *Daphnia* showed a stronger DVM response with increasing concentration of fish karimone. Brewer *et al.* (1999) also found a similar effect of the presence of fish karimone on *Daphnia*. They found that *Daphnia pulicaria* clones were about twice as sensitive to fluid disturbances in the presence of fish karimone in light conditions. Loose and Dawidowicz (1994) has also demonstrated that *Daphnia magna* shows increasing strength of migration with increased concentration of fish karimones. Therefore, its seems that, in common with the view that recurs through all DVM literature, predator evasion is likely to be part of the DVM strategy for zooplankton. There is, however, no reason assume that it must be *either* predation avoidance or feeding that drive DVM. It is most likely that as suggested by several authors (e.g. Gabriel and Thomas, 1988; Lampert, 1989; Tarling *et al.*, 2000) that DVM is driven by a need to obtain food *and* to avoid visual predators. Houston *et al.* (1993) suggest that often

maximizing energy intake is associated with an increase in predation risk and that optimal fitness is a trade – off between balancing these two factors. Lui *et al.* (2003) modelled the DVM of zooplankton and showed that they can balance optimal food intake against predation risk. Sekino and Yamamura (1999) suggested that migrating behaviour of *Daphnia* changes depending on the amount of accumulated energy and concluded that this implies that the nutritional status of zooplankton is important in determining their migrating behaviour. Burrows and Tarling (2004) also predicted that by reducing metabolic cost in their model that animals would spend less time in the surface ascending later and descending earlier than if metabolic costs were greater. Therefore, it is likely that the driving force of DVM is a balance between obtaining sufficient energy and avoiding predators and with perhaps the risks that are taken in terms of predation dependent upon the energy state of a particular individual.

In summary it seems that as *M. norvegica* appears to break down its respiratory pigment (Hc) for nutrition and when it has such a poor capacity for anaerobic metabolism as suggested by Spicer *et al.* (1999) the need to reside in the deeper layers of the water column must indeed be great (for example from predators). Consequently it would seem that the drive to migrate to the surface to feed and therefore recover from daytime incurred debts must be equally as great. Consequently, I suggest that DVM is driven by the internal state of individual krill, *M. norvegica*. Furthermore, the model of DVM proposed may be common to all krill that are diel vertical migrators and it may also be applicable other zooplankton such as copepods which show DVM with increased nocturnal feeding.

6.5 Future considerations and research

The results of this thesis strongly suggest that feeding should be considered as an important component and indeed included as at least a part of the mechanism for DVM. Further investigations are required to investigate whether the model DVM strategy proposed is common to both other krill species and other zooplankton. Further investigation is also required to examine the difference in the DVM of female and male krill, again not only in *M. norvegica* but also in other krill species.

Appendix A

Preliminary investigations of associations between feeding by krill and DVM

A.1 INTRODUCTION

A.1.1 Krill DVM and feeding

Krill are well known diel vertical migrators (Mauchline, 1980). The pattern of feeding during diel vertical migration (DVM) of northern krill, *Meganyctiphanes norvegica* has been documented (e.g. Sameoto, 1980; Simard *et al.*, 1986; Kaartvedt *et al.*, 2002; Onsrud and Kaartvedt, 2002) although, there is still much uncertainty surrounding the feeding behaviour of this species during its DVM and also why it shows this DVM behaviour. For example, some studies have suggested that *M. norvegica* are mainly carnivorous (Bamstedt and Karlson, 1998) whereas other studies suggest an opportunistic omnivorous feeding habit (Lass *et al.*, 2001; Kaartvedt *et al.*, 2002). Whether *M. norvegica* feeds throughout its DVM or only at night is also still unresolved (see Chapter 1, General Introduction). The questions of whether krill feed throughout DVM, why they feed on given food types and also the mechanism underlying their DVM are central to the purpose of this thesis. Consequently establishing a pattern for feeding during DVM is of critical importance to the questions I will address. Therefore although not a novel study, the pattern of *M. norvegica* feeding during their DVM in Gullmarsfjorden was examined to provide information to design the experiments of the thesis. Thus the objectives of this field study were to observe the pattern of krill feeding in relation to the available food types (i.e. phytoplankton and zooplankton) by measuring herbivorous and carnivorous feeding *in situ* on these food types during the day and night of their diel vertical migration. This field study of DVM is based on observing patterns and, also, is not entirely novel. Therefore it has not been included as a chapter in the thesis.

A.1.2 Physico – chemical water column characteristics and food type availability

The effect of the physical environment on feeding by *M. norvegica* was not the focus of this thesis. Evidence concerning whether the hydrography affects the diel vertical migration of *M. norvegica* is contradictory. Onsrud and Kaartvedt (1998) examined the DVM of *M. norvegica* in relation to the physical environment, food and predators and suggested that the pycnocline did not represent an impenetrable barrier (c.f. Bergstrom and Strömberg, 1997). They also highlighted that in studies suggesting that the ascending migration of krill at night was inhibited by a pycnocline that the difference in krill behaviour may have not been due to the physicals gradients involved but due to alternative explanations not examined by those studies. To inform the designing of the experiments presented in this thesis, however, salinity and temperature were measured in the studies of this appendix.

Investigating the effect of food type availability on the diel vertical migration by krill was, however, a key focus of this thesis. Therefore, to inform future experiments on krill feeding was investigated in relation to the food items available during its DVM.

A.1.3 Estimating *in situ* feeding rates

Most feeding rates are calculated in laboratory – controlled conditions. These controlled conditions, however, can be problematic if the aim is to extrapolate from laboratory – calculated, feeding rates to krill in field conditions. Morris (1984) suggested filtration data for *E. superba* must be extrapolated with care, as the filtration mechanism may be different under various environmental and energetic constraints. Price *et al.* (1988) suggested that laboratory feeding rates

underestimate *in situ* feeding rates because of the small experimental vessels used in laboratory studies. They found that the clearance rates of Antarctic krill, *Euphausia superba* feeding on copepods were seven to ten times greater and on algae were around two times higher when krill were allowed to feed in large 50 litre tubs compared with smaller 5 litre bottles. Similarly, Morris (1984) found that filtration rates for *E. superba* in flow through conditions compared with closed or constant volume experiments were much higher. Therefore being able to estimate ingestion rates in field conditions overcomes the problems associated with laboratory experiments such as small unrealistic experimental containers. A method of overcoming these problems associated with calculating feeding rate under laboratory conditions is to try and estimate feeding rates from field caught individuals. Carnivorous *in situ* feeding rates have been determined for *M. norvegica* by Bamstedt and Karslon (1998). They calculated ingestion rates using laboratory derived digestion times together with field caught krill stomach contents. Kiørboe *et al.* (1982) found that gut content from field caught copepods *Centropages hamatus*, multiplied by gut clearance rates gave similar ingestion rates to those calculated in their laboratory studies. Perissinotto and Pakhomov (1996) highlighted that *in situ* techniques such as gut fluorescence used to estimate herbivorous feeding by krill have shown ingestion rates are higher than estimated under laboratory conditions. Wang and Concover (1986), however, found that using the gut fluorescence method underestimated ingestion rates in the copepod *Temora longicornis* and suggested this may have been because plant pigments were broken down into non fluorescent residues during their residence in the gut. Concover *et al.* (1986) supported these ideas as they found that chlorophyll a. and its derivatives can be destroyed or absorbed during the passage through the gut. The use of gut fluorescence does however provide a means of tracing individuals during their DVM and give an idea of feeding activity. Thus

although using the gut fluorescence method may underestimate the amount of pigment in the gut it does provide a means of quantifying pigments in order to compare feeding activity of krill during their DVM. Another problem with using these methods of calculating *in situ* ingestion rates is, however, that they are essentially based upon laboratory derived digestion times or gut evacuation rates. Heyraud (1979) suggested that transit times are shorter when krill are feeding continuously and in the absence of food it is much longer (up to 8 h) before krill empty their guts (La Rosa,1976 as cited by Heyraud, 1979). Indeed Antezana *et al.* (1982) suggested that krill *Euphausia superba* can retain their gut contents for up to 7d when starved. Perissinotto and Pakhomov (1996) when investigating gut evacuation rates of phytoplankton by *E. superba* suggest that gut evacuation rates can only be realistically estimated when krill are allowed to continue ingesting particles uninterruptedly. Bamstedt and Karlson (1998) also highlight the need for determining digestion times based on krill feeding continuously.

A.1.4 Krill density and feeding

Krill are often found in layers or aggregations in the water column (Mauchline, 1980; Siegel and Kalinowski, 1994). Studies manipulating feeding conditions for krill are, however, mostly performed in the laboratory using isolated krill. Ritz (2000) suggested that higher estimated field ingestion rates compared with laboratory estimated rates may not be attributed to the confinement of experimental containers but may be due to a higher feeding efficiency within aggregations compared with the isolated krill used in laboratory studies. Antezana and Ray (1984) thought that the large chlorophyll gut content of *E. superba* could not be attributed to phytoplankton alone and therefore suggested that coprophagy may occur in a swarm. Therefore although in this study it was only possible to examine the effects of small groups of krill on feeding I was

interested in whether krill group size affected the clearance and ingestions rates of each experimental container.

A.1.5 'Active' or 'passive' feeders?

Whether krill can detect and stay with patches of high food density is an important consideration when trying to establish a pattern and also mechanism for their feeding behaviour. McClatchie (1985) suggested that *M. norvegica* feeds where its prey is in high density or patch. Hofmann *et al.* (2004) examined the spatial distribution of *E. superba* and suggested that krill swim slower and turn more frequently in areas of high food concentration. Price (1989) found that *Thysanoessa raschii* kept themselves in algal patches by turning back at the boundary. Establishing whether krill actively swim searching for food or whether they passively filter water and collect food is therefore an important part of determining feeding behaviour during DVM. Examining feeding rates of tethered krill against freely swimming krill in laboratory conditions could in part explain whether krill are active or passive feeders.

A.1.6 Rationale of appendix and aims

Although not included as part of the main body of the thesis the studies presented here (Appendix A) were important in forming the basis for particularly the early chapters of the thesis. In particular it was important to examine and understand the pattern of krill feeding with DVM in the model system to be used before designing experiments to manipulate the model system. Also although some of these studies were 'unsuccessful' they were influential in forming the ideas, direction and methods for the experiments in the chapters of this thesis.

The main aim of the appendix was to provide information about the pattern of krill feeding with DVM in the model system to inform the experimental design for each

of the chapters of this thesis. As describing the pattern of feeding by *M. norvegica* during DVM has already been documented (e.g. Simard *et al.*, 1986; Onsrud and Kaartvedt, 1998; Lass *et al.*, 2001), this study has not been included in the main body of the thesis. In particular the description of krill DVM presented in this appendix has not been included in the thesis for the reason as pointed out by Hays (2003) when reviewing the adaptive significance and ecosystem consequences of zooplankton DVM 'there is probably little more to be gained by more simple descriptions of day and night vertical distributions of zooplankton.' Other studies presented in this appendix, although 'unsuccessful' they were influential in forming the ideas, direction and methods for the experiments in the chapters of this thesis and thus have been included in this appendix.

Therefore the aims of the work presented in this appendix were to:

- determine a pattern for krill feeding during DVM to provide information to design the studies of this thesis;
- and to explore potential experiments that could be useful in determining the feeding strategy of krill.

A.2 MATERIALS AND METHODS

A.2.1 Collection of Plankton

Meganyctiphanes norvegica were collected as described in chapter 4 methods during the day (11th Mar 2002) proceeding into the night (12th Mar 2002). Krill were collected from depth ranges of 100 – 75 m, 75 – 5 0m and 50 – 0 m by horizontal oblique tows. During 'day' time the back scattering layer on the echo sounder indicated that krill were mainly concentrated at depths between 100 – 50 m therefore three replicate tows were made for krill at 100 – 75 m and 75 – 50 m. One trawl was made for the range of 50 – 0m in order to confirm that no krill were present. During the night the back scattering layer indicated that krill were distributed throughout the water column, therefore two tows were made for the following depth ranges 100 – 75 m, 75 – 50 m and 50 – 0 m. Repeated tows for each depth range cannot be regarded as 'true' replicate tows because tows are inevitably temporally different due to the continuous nature of DVM. Therefore technically it is impossible to replicate a tow. Consequently, although even if repeated tows were taken they cannot be regarded as replicates but only as individuals tows. I did, however, on this first sampling occasion take 3 (during the day) and 2 (during the night) tows at the same depth to examine the variance between tows.

Zooplankton were collected by a vertical tow using a WP – 2 (200μm) net, from a range of depths encountered by krill during DVM (100 – 75 m, 75 – 50 m, 50 – 25 m and 25 – 0 m), during the day and night. A flow meter was attached to the aperture of the net in order to estimate the volume of water filtered and thus density of copepods per m^{-3}. Zooplankton were immediately preserved in 4 – 5% formaldehyde solution.

Temperature and salinity were measured at various points throughout the water column using a conductivity temperature depth recorder. Water was collected in Niskin tubes from twelve depths as follows, 100 m, 80 m, 60 m, 50 m, 40 m, 30 m, 20 m, 15 m, 12 m, 7 m, 5 m, and 2 m. A 200 ml sample was taken from each of these tubes and preserved with Lugol's solution for phytoplankton identification. A sample of water was also taken to estimate chlorophyll a and thus give a measure of the total phytoplankton at each depth. Although it would have been more informative to have more than one zooplankton tow for each depth and also more than one water collection for each depth for phytoplankton analysis, time constraints made this impossible. Also, as stated above repeated tows would be temporally different and thus not replicates.

Krill were sorted within 10 min of landing on deck. For estimation of herbivorous feeding approx. 50 krill were chosen randomly, placed in aluminium foil, and frozen (˜ 20°C) in order to prevent the degradation of chlorophyll pigments present in the gut from light. Remaining krill were preserved in 4 – 5% formaldehyde solution for analysis of gut content and enumeration of krill.

A.2.2 Herbivorous feeding

Herbivorous feeding was estimated by measuring gut content of chlorophyll pigments. Chlorophyll pigments were measured using a fluorescence method suggested by Parsons *et al.* (1984). Krill were thawed and the stomach/gut dissected out (n = 15 – 30) taking care to avoid damaging the gut and thus causing loss of content. Each gut was placed in 90 % ethanol (10 ml) for 12 – 18 h to extract pigments. Chlorophyll a was quantified by fluorometric determination of chlorophylls and phaeopigments using a Turner Systems® fluorometer. Kruskal – Wallis tests followed by box plots using STATGRAPHICS plus 5.0 (1994 – 2000,

statistical Graphics Corp) were performed to analyze difference between feeding at different depths.

A.2.3 Carnivorous feeding

The guts from krill analyzed for chlorophyll pigments were used for estimation of carnivorous feeding (n = 15). Stomach/guts were stained with methylene blue and examined for copepod mandibles.

A.2.4 Food type availability/abundance

A. Zooplankton

Zooplankton were filtered through a 60 µm sieve, rinsed with fresh water, and replaced in a solution of 70 % ethanol with 3 % glycerol. Zooplankton samples were sub-sampled using a Flosom plankton splitter and divided into eight parts. Three 1/8 subsamples were used for identification and enumeration of zooplankton. Copepods were identified to genus. All other zooplankton was identified to class or genus.

B. Phytoplankton

Immediately after collection duplicate water samples (100 ml) from each depth were filtered onto Whatman glass micro-fibre filters (GF/F) and extracted in 90% ethanol for 12 – 14 h. Chlorophylls and phaeopigments were determined using the fluorescence method described previously for gut content analysis. A 50 ml water sample (preserved in Lugol's solution) was also placed in a settlement chamber for 24 h, after which phytoplankton were identified to genus and counted.

A.2.5 Laboratory feeding experiments

Meganyctiphanes norvegica were collected as described in methods of chapter 2 during Jan and Feb 2002. Krill were transferred (within 5 min of harvest) into sealed thermos containers (Rubbermaid drinking water thermosflask, vol. = 80 l) containing filtered sea water (salinity = 34 PSU) and transported to KMRS within 2 h of capture. In the laboratory krill were maintained in fibre – glass aquaria (vol. = 350 l) covered with dark plastic to keep krill in darkness. Aquaria were supplied with natural 'deep' sea water pumped into the station from a depth of 35 m (salinity = 34 PSU, T = 6 °C). All experiments were carried out within 5 days of capture.

Copepods were collected, from the same location as krill. Copepods were returned to the laboratory within 2 h of capture in sealed thermos containers (Rubbermaid drinking water thermosflask vol. = 20 l) containing filtered sea water. At KMRS copepods were maintained in aerated plastic containers (vol. = 80 l) supplied with natural surface (pumped into station from depth of 6 m S = 34 PSU, T = 4°C) water or deep water. All experiments were carried out within 5 d of capture.

An isolated culture of the algae *Thalassiosira weissflogii* was kindly supplied by Peter Thor at KMR. Batch uni – algal cultures of *Thalassiosira weissflogii* were maintained in sea water under constant light and temperature (T = 15 °C) conditions.

A.2.6 Krill density and feeding rates

The effect of the krill density on clearance rate and ingestion rate of *M. norvegica* was investigated using various densities of krill. ANOVA was used to test for significant differences in feeding rates using STATGRAPHICS plus 5.0 (1994 – 2000, statistical Graphics Corp). For all feeding experiments a group of similar size

krill (body length, i.e. rostrum tip to end of telson = 30 – 36 mm) were selected from the stock aquaria and then transferred to experimental containers.

A surface water assemblage of copepods (density = ca. 120 copepods l^{-1}) was offered to several densities of krill (1, 3, 5 and 7 individual krill per 2.3 l bottle) to examine the effect of krill density on clearance/ingestion rates per bottle. A volume of stock water/food type was added to filtered surface sea water and mixed thoroughly to give the desired food type density. Control bottles contained only the food type whereas experimental bottles also contained the required krill density. Control (n = 6) and experimental (n = 4) glass bottles (vol. = 2.3 l) were filled with sea water containing the copepod food type in a haphazard order, to account for variation between bottles in food type concentration throughout the 'filling' process. Thorough mixing continued throughout this filling process, to ensure that the food type remained in a homogenous suspension. At the start and end of the filling process two control bottles were taken for verification of copepod concentration at the start of the experiment. A number of krill to give the required density were placed in each experimental bottle, after which the bottle was then filled until the water overflowed. Plastic film was placed over the mouth of each bottle to exclude air and then the lid gently tightened. In order to maintain zooplankton in suspension, all bottles were placed on a rotating plankton wheel (2 rev. min^{-1}) and left overnight in a temperature controlled room (T = 6 °C) for 12 – 13 h. At the end of this period, bottles were removed from the wheel, and the contents analysed for copepod density. Each bottle was rinsed three times to ensure all copepods were removed for quantification. Krill removed from the experimental bottles were also rinsed to remove any copepods adhering to the exoskeleton. The contents of each bottle was emptied and placed in a Petri dish with ethanol (70 %) to fix the remaining copepods. All of the copepods present were counted under low power (x 10) magnification.

A.2.7 Gut evacuation studies

The diatom *Thalassiosira weissflogii* was added to 50 l of filtered sea water to produce a concentration of 0.3 mg carbon l^{-1}. Approximately 50 to 70 similar sized krill (body length, i.e. rostrum tip to end of telson = 30 – 36 mm) were selected from the stock aquaria and then transferred to the experimental container. Krill were left for 3 h to feed. After this 3 h time krill were transferred to 50 l of filtered sea water and then at time intervals (0, 5, 10, 20, 40, 60, 90, 180, 240 min) 5 krill were removed for stomach chlorophyll content analysis. These krill were frozen immediately at ⁻80 °C. Stomach chlorophyll content was determined using exactly the same method as for field caught krill (see herbivorous feeding section above). This gut evacuation study was also repeated with the modification that when krill were transferred after 3 h to filtered seawater, the filtered seawater contained 6 mg carbon l^{-1}. Carbon was added in an attempt to maintain ingestion and in turn gut evacuation. Perissinotto and Pakhomov (1996) suggested that gut evacuation rates could only be realistically determined when krill are allowed to continue ingesting particles uninterruptedly. They also suggest that gut evacuation rates and passage times were not constant in *Euphausia superba* and slow down when feeding ceases or slows.

A.2.8 Tethered krill experiment

The effect of the ability of krill to swim and search for food on clearance rate (volume of water cleared of food by consumer per unit time) and ingestion rate (amount of food consumed per unit time) of *M. norvegica* was investigated using tethered krill. For all feeding experiments a group of similar size krill (body length, i.e. rostrum tip to end of telson = 30 – 36 mm) were selected from the stock aquaria and then transferred to experimental containers.

A surface water assemblage of copepods (density = ca. 90 copepods l^{-1}) was offered to tethered and non – tethered krill to examine the effect the ability of krill to swim and search for food clearance/ingestion rates per bottle. A volume of stock water/food type was added to filtered surface sea water and mixed thoroughly to give the desired food type density. Control bottles contained only the food type whereas experimental bottles contained either a 'freely swimming' individual krill or a tethered krill. Krill were tethered to a wire as follows; gently blotted krill (to remove excess water) were fixed by their carapace to wire using adhesive. Cold seawater was poured over the adhesive to speed fixing time and therefore reduce the time krill were out of water. Control with food type only (n = 4); control with food type only with wire in bottle (n = 4) and experimental krill tethered (n = 8) and non – tethered krill (n = 8) glass bottles (vol. = 2.3 l) were filled with sea water containing the copepod food type in a haphazard order, to account for variation between bottles in food type concentration throughout the 'filling' process. Thorough mixing continued throughout this filling process, to ensure that the food type remained in a homogenous suspension. At the start and end of the filling process two control bottles were taken for verification of copepod concentration at the start of the experiment. Tethered or non – tethered krill were placed in each experimental bottle, after which the bottle was then filled until the water overflowed. In order to maintain zooplankton in suspension, all bottles were placed on a rotating plankton wheel (2 rev. min^{-1}) and left overnight in a temperature controlled room (T = 6 °C) for 12 – 13 h. At the end of this period, bottles were removed from the wheel, and the contents analysed for copepod density. Each bottle was rinsed three times to ensure all copepods were removed for quantification. Krill removed from the experimental bottles were also rinsed to remove any copepods adhering to the exoskeleton. The contents of each bottle was emptied and placed in a Petri dish with ethanol (70 %) to fix the remaining

copepods. All of the copepods present were counted under low power (x 10) magnification.

Preliminary studies investigating the functioning of the feeding basket involved removal of appendages from the feeding basket. However, this investigation of feeding basket function was not pursued because most krill did not survive 12 h after removal of appendages from the basket.

A.3 RESULTS

A.3.1 Krill and their DVM

The ascent of krill into the surface layers at night from deeper depths is shown by krill abundance at various depths during the course of one day and night by Figure A.1.

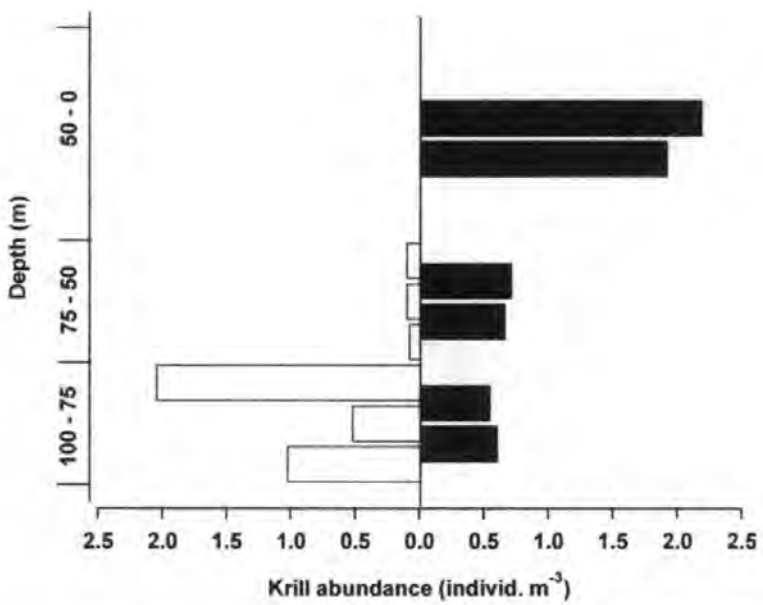


Figure A.1 Diel vertical migration by krill. Abundance of krill in shallow to deep depths during the day (open bars) and night (solid bars). Each bar represents one 'replicate' tow.

Krill were concentrated in the lower 50 m of the water column and absent from the upper 50 m of the water column during the day. In particular the greatest densities of krill were recorded in the deepest part of their daytime distribution between 100 and 75 m. During the night krill had become spread throughout the water column and were recorded in all sampling depths. At night krill were most abundant in the

upper 50 m of the water column signifying that most krill had migrated up into the upper layers of the water column.

Difference between tows taken at the same depth during the night were slight, with the largest difference in abundance being 0.27 individ. m^{-3} for tows taken between 50 and 75 m. The differences between tows during the day were, however, greater with a difference of as much as 1.52 individ. m^{-3} between tows taken for 100 – 75 m. Although between 50 and 75 m abundance estimates were similar with the greatest difference between tows only being 0.02 individ. m^{-3}.

The distribution of krill body lengths in the water column during the day and night are shown by Figures A.2 and A.3. During the day smaller body length krill appeared to reside higher in the water column than longer length krill.

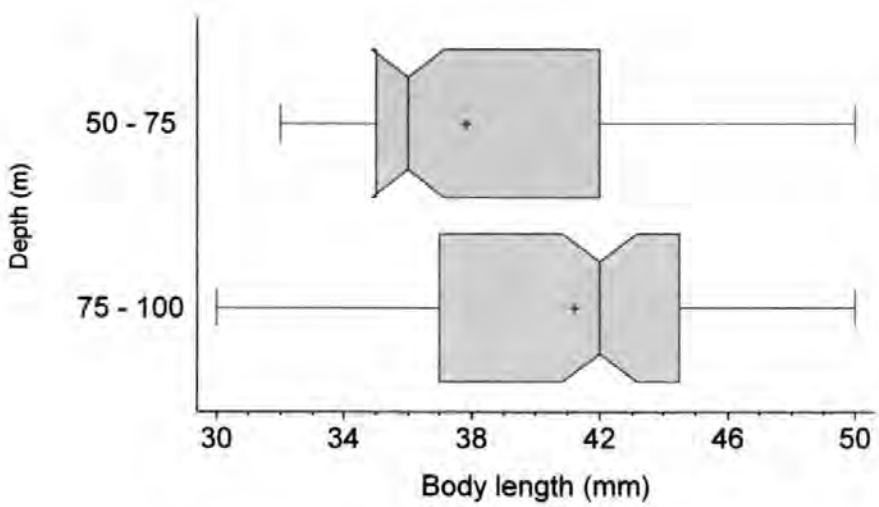


Figure A.2 Day – time distribution of krill sizes. Median krill body length shown with 95 confidence intervals at sampling depths of 50 – 75 m (krill measured = 90) and 75 – 100 m (krill measured =100). Non – overlapping v – shaped notches indicate significantly different values at the 95 % confidence level.

At night the median body length of krill in the deepest depths between 75 and 100 m of the water column was less slightly higher in the water column between 50 and 75 m. In depths between 50 and 75 m krill median body length was significantly longer than in the surface waters above between 0 and 50 m. There was no significant difference between median body lengths of krill caught in the deepest depths between 75 and 100 and surface waters between 0 and 50 m.

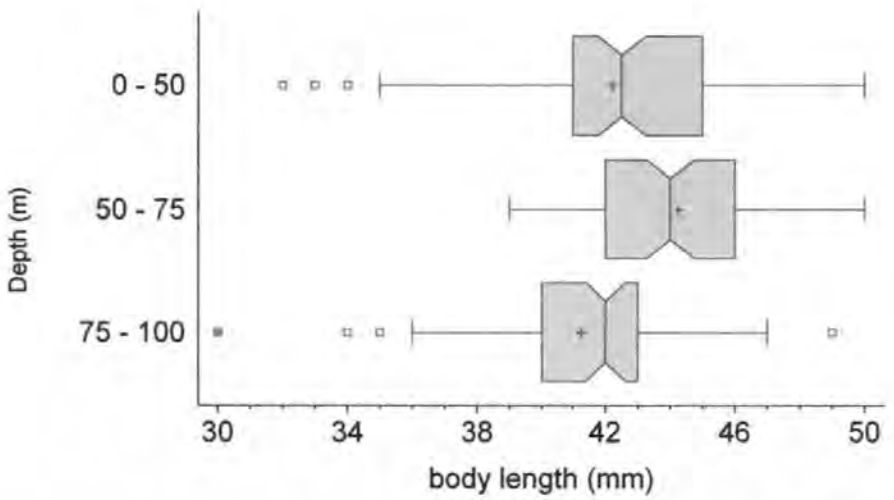


Figure A.3 Night – time distribution of krill sizes. Median krill body length shown with 95 confidence intervals at sampling depths of 0 – 50 m (krill measured = 70), 50 – 75 m (krill measured =70) and 75 – 100 (krill measured = 60). Non – overlapping v – shaped notches indicate significantly different values at the 95 % confidence level.

Body length showed a proportional relationship with wet body mass see Figure A.4.

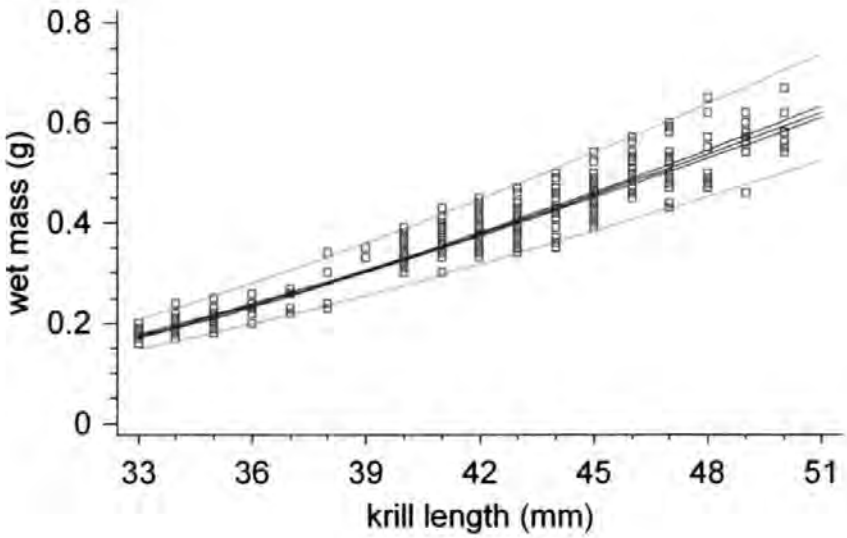


Figure A.4 Relationship between krill body length and krill wet mass. Confidence limits (dark grey lines) and prediction limits (broken line) at the 95 % confidence level for fitted s – curve regression model. (n = 389).

The relationship between krill body length and wet mass was relatively strong fitting a s – curve regression model ($r^2_{1, 386}$ = 93.13, P < 0.0001) were wet mass = exp (1.85816 – 119.04/length). Therefore as krill body length increased wet mass increased proportionally.

Although there was a difference in the body lengths of krill found at different depths of the water column there did not seem to be any relationship between krill body length and gut content (Fig. A.5).

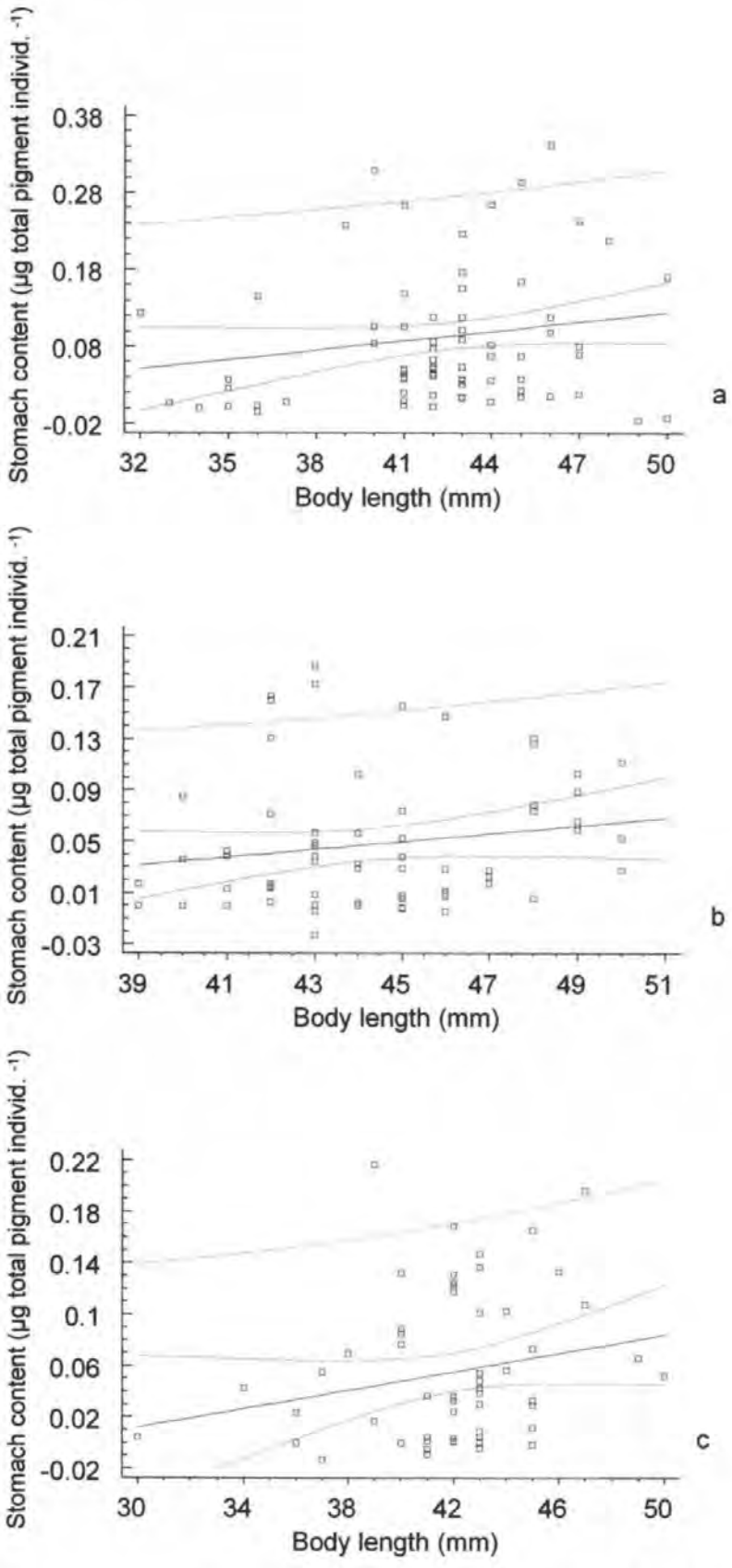


Figure A.5 Stomach pigment content shown against krill body length at different depths of the water column (a = 0 – 50 m, b = 50 –75 m, c = 75 – 100 m) during the night. Fitted with linear regression model (black line) shown with confidence limits (dark grey line) and prediction limits (broken line).

During the night in depths between 0 and 50 m there was no significant relationship at the 95 % confidence level ($r^2_{1, 106} = 2.59$, $P > 0.05$) between krill body length and stomach pigment content. Between depths of 50 and 75 m, again there was no statistically significant relationship at the 95 % confidence level ($r^2_{1, 66} = 2.75$, $P > 0.05$) between krill body length and stomach pigment content. In the deepest depths between 75 and 100 m there was also no statistically significant relationship at the 95 % confidence level ($r^2_{1, 57} = 4.35$, $P > 0.05$) between krill body length and stomach pigment content.

A.3.2 Physico – chemical water column characteristics and food type availability

Differences in the physico – chemical characteristics were observed throughout the water column (see Figs A.6, A.7 and A.8). Both temperature and salinity generally increased with increasing depth of the water column on all three sampling occasions during February and March. In contrast chlorophyll a. increased with decreasing water column depth. Therefore as krill migrated to the surface layers of the water column they would have experienced a decrease in temperature and salinity but an increase in phytoplankton abundance. This increase in chlorophyll concentration became more marked at the start of March up to ca. 0.4 kg chlorophyll a. m^{-3}, see Fig. A.7) and slightly later in March (up to ca. 0.6 kg chlorophyll a. m^{-3}, see Fig A.8) with the onset of the spring bloom compared with early February (ca. 0.2 kg chlorophyll a. m^{-3}, see Fig. A.6). There did not, however to be a large change in the profile of temperature and salinity in the water column on the three sampling occasions.

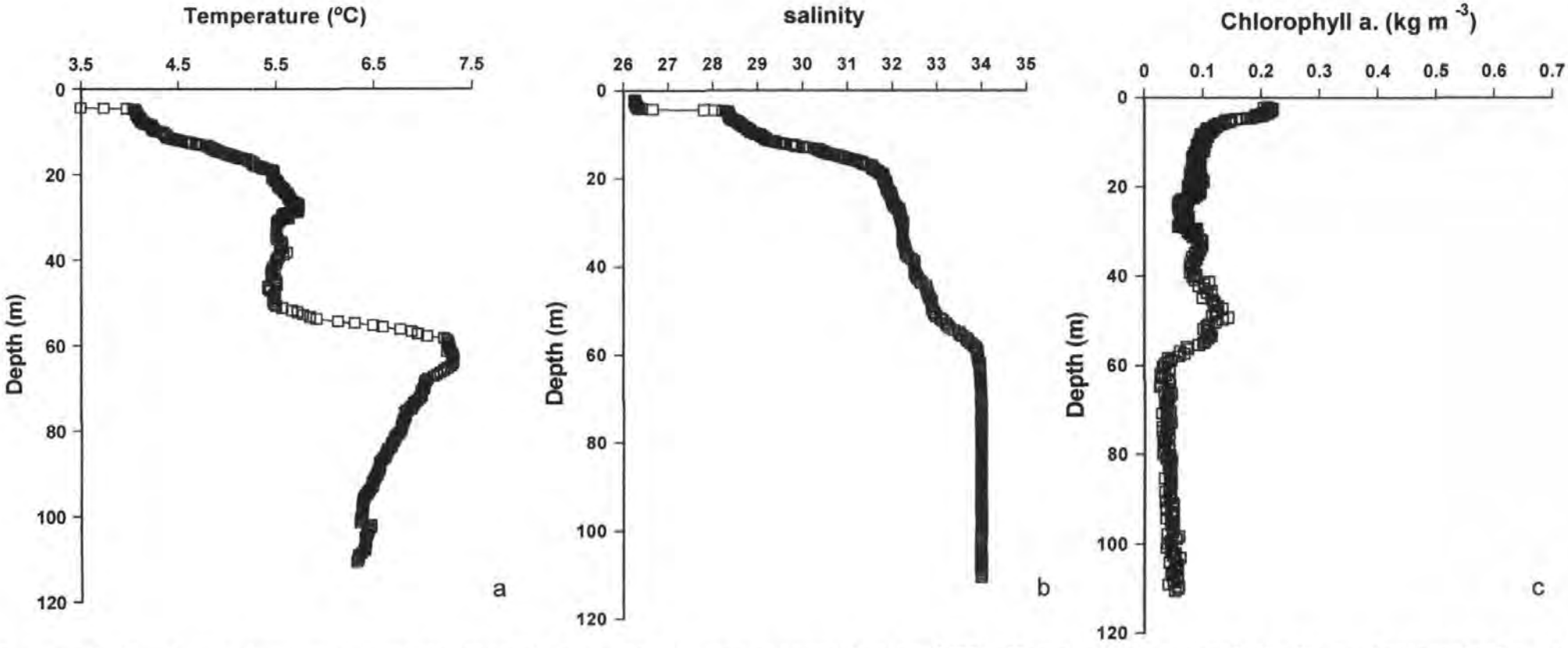


Figure A.6 Physico – chemical characteristics of the water column recorded using CTD. Temperature (a), salinity (b) and chlorophyll a. (c) shown with depth of the water column during the day of the 26th February 2002.

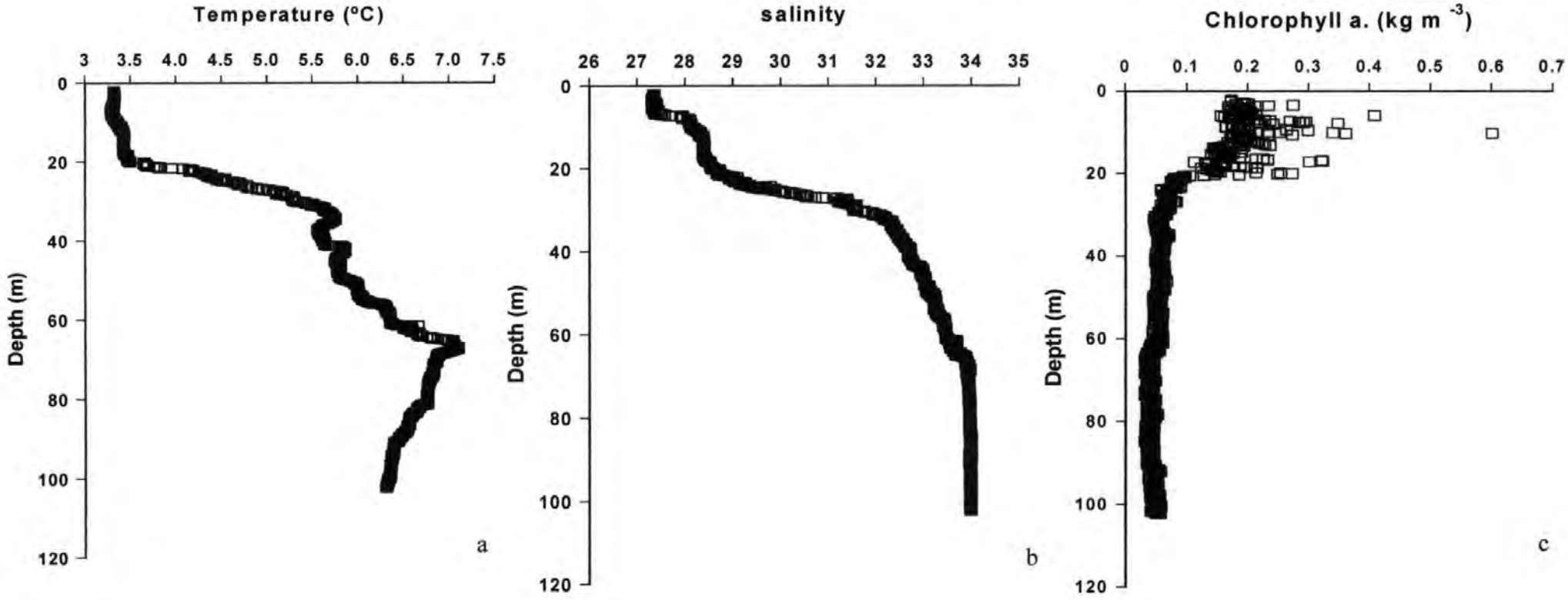


Figure A.7 Physico – chemical characteristics of the water column recorded using CTD. Temperature (a), salinity (b) and chlorophyll a. (c) shown with depth of the water column during the day of the 5th March 2002.

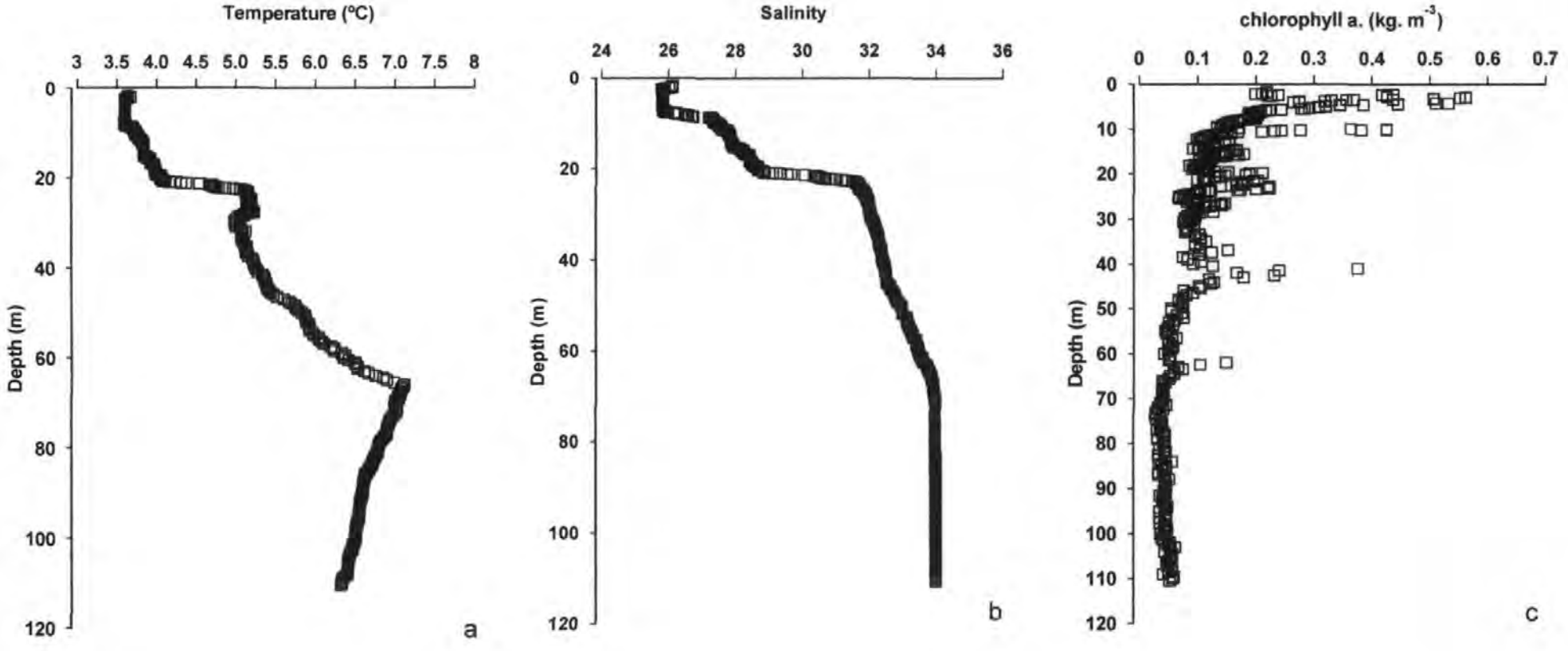


Figure A.8 Physico – chemical characteristics of the water column recorded using CTD. Temperature (a), salinity (b) and chlorophyll a. (c) shown with depth of the water column during the day of the 11th March 2002.

Food type availability changes during the course of krill DVM due to differing abundance of food types at various depths of the water column. The figures that follow indicate the availability of phytoplankton and copepod food types at the depths encountered by krill during the day and during their nocturnal ascent to the surface layers of the water column.

The increase in phytoplankton abundance with decreasing water column depth as shown by chlorophyll a. levels estimated from water samples taken from various depths throughout the water column as shown by Figure A.9 confirms the chlorophyll profile recorded by the CTD (see Figs A.6 – A.8). Phytoplankton was mainly concentrated in the uppermost 20 m of the water column (up to ca. 2.5 µg chlorophyll a. l^{-1}). Thus, as krill migrated into the surface layers of the water column a greater abundance of phytoplankton would have been available compared with at the deeper depths that they reside in during the day.

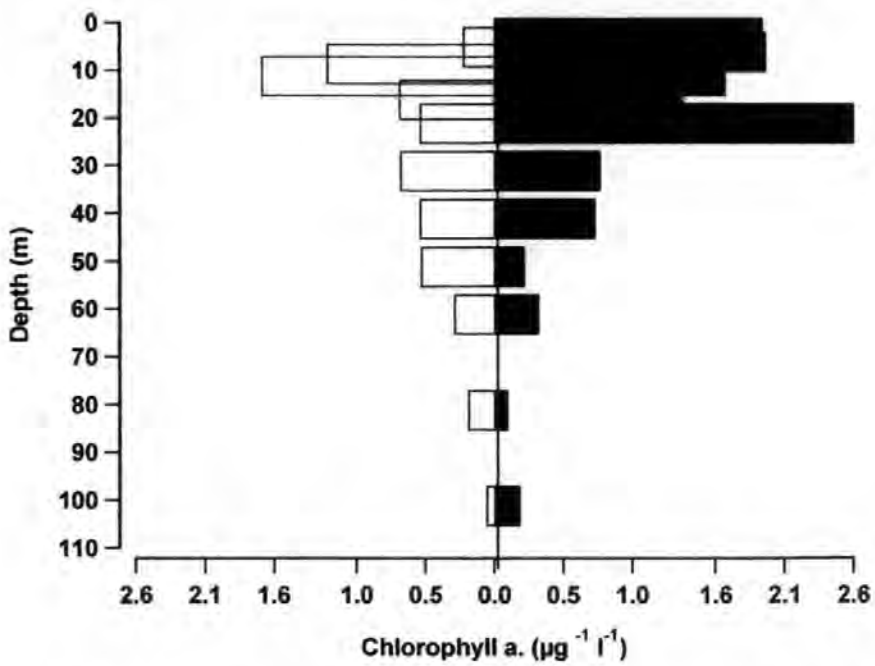


Figure A.9 Phytoplankton abundance (shown as chlorophyll a.) in shallow to deep depths of the water column during the day (open bars) and night (solid bars).

Copepods were found throughout the water column during both during the day and night, however, copepod species composition and density differed between day and night between and between sampling depths (Fig. A.10).

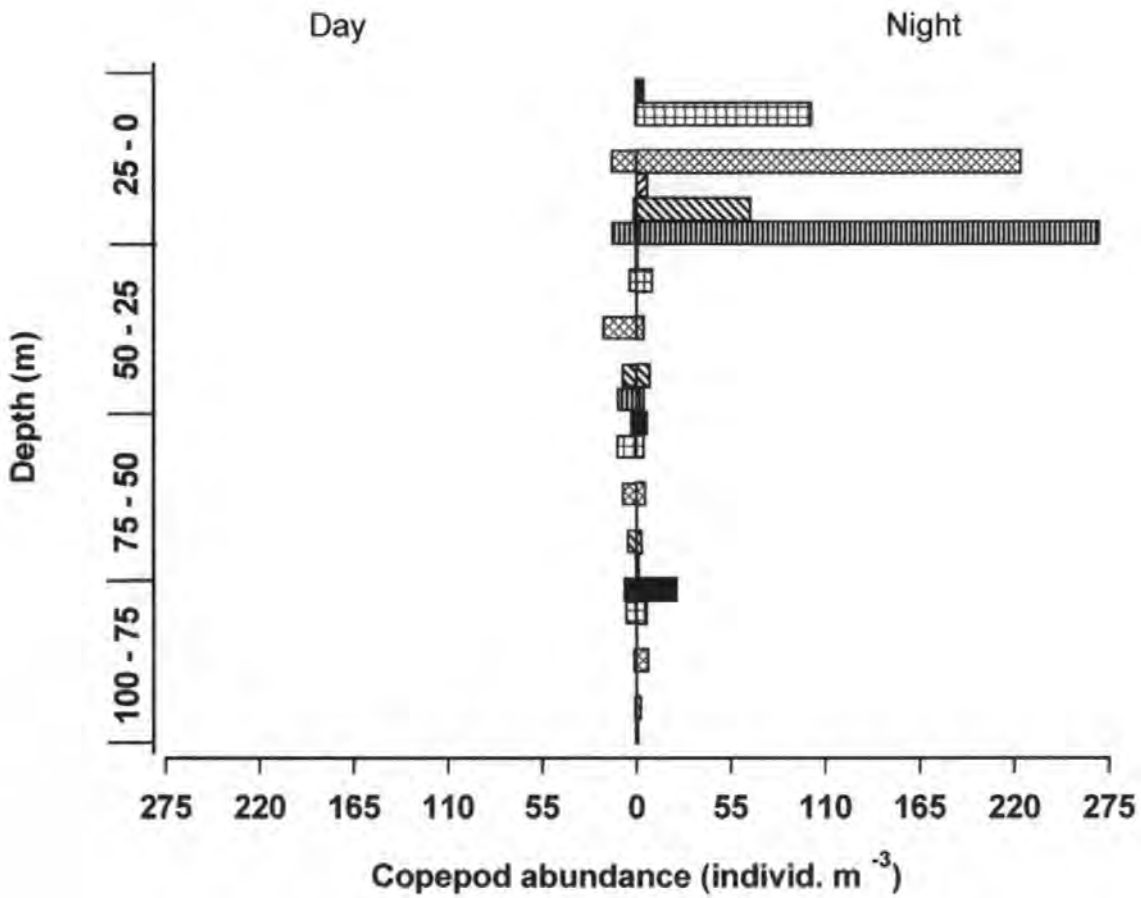


Figure A.10 Copepod density during DVM. Density of various copepod species (■ = *Calanus* spp. ▦ = *Metridia* spp. ⊠ = Copepoda J ▨ = *Acartia* sp. ▨ = *Pseudocalanus* sp. ▥ = *Oithona* sp) shown in the same order at each depth during the day (shown left of central axis) and night (shown right of central axis).

The greatest density of coepods was observed in the mid to upper parts of the water column both during the day and night (see Table A.1). Therefore copepods would have become more abundant as krill migrated to the upper parts of the water column at night.

Table A.1 Total copepod abundance and species compoistion with water column depth during the day and night. Species compostion ranked with most abundant species at that depth first.

Depth (m)	Day		Night	
	Total copepod abundance (No. individ. m^{-3})	Species compostion	Total copepod abundance (No. individ. m^{-3})	Species compostion
0 – 25	52	*Oithona* Copepoda J *Pseudocalanus*	1119	*Oithona* Copepoda J *Metridia* *Pseudocalanus* *Acartia* *Calanus*
25 – 50	71	Copepoda J *Oithona* *Pseudocalanus* *Metridia*	43	*Metridia* *Pseudocalanus* Copepoda J *Oithona* *Calanus*
50 – 75	45	*Metridia* Copepoda J *Pseudocalanus* *Calanus*	31	*Calanus* Copepoda J *Metridia* *Pseudocalanus* *Oithona*
75 – 100	25	*Calanus* *Metridia* Copepoda J	66	*Calanus* Copepoda J *Metridia* *Pseudocalanus* *Oithona*

There was also a difference, both during the day and night, between the species compostion of the upper parts of the water column (0 – 50 m depth) and deeper parts of the water column (50 – 100 m depth). This difference between species compostion between deeper parts the water column and surface waters was mainly associated with coepod size, for example larger copepod species were found in deeper depths and smaller copepod species found at shallower depths. During the day small copepod species such as *Oithona*, and copepoda J were most abundant in shallower depths of 0 – 50 m (between 0 – 25 m *Oithona* = 24

individ. m^{-3}, Copepoda J = 24 individ. m^{-3} and between 25 – 50 m *Oithona* = 17 individ. m^{-3}, Copepoda J = 32 individ. m^{-3}) compared with deepr depths 50 – 100 m where *Oithona* appeared to be absent and the density of copepoda J became less with increasing depth (copepoda J density = 13 individ. m^{-3} between 50 – 75 m and only 2 individ. m^{-3} 75 – 100 m). *Pseudocalanus* appeared to be most abundant int the middle of the water column during the day with densities between 13 and 8 individ. m^{-3} between depths of 75 and 25 m. Larger copepod species such as *Calanus* spp. and *Metridia* spp. were most abundant between 100 and 50 m in the water column with densities of between 5 and 11 individ. m^{-3} for *Calanus* spp. and 10 and 18 individ. m^{-3} for *Metridia* spp. compared with densities of < 1 individ. m^{-3} (*Calanus* spp) and < 7 individ. m^{-3} (*Metridia* spp.). Similarly with during the day at night smaller copepod species dominated the copepod assemblage of the surface waters with *Oithona* (449 individ. m^{-3}), *Pseudocalanus* (110 individ. m^{-3}), *Acartia* (10 individ. m^{-3}) and Copepoda J (373 individ. m^{-3}) all found in their greatest densities between 0 and 25 m depth. *Metridia* were also present in high numbers (169 individ. m^{-3}) between 0 and 25 m. All species at night seemed to be distributed throughout the water column with even larger copepod species such as *Calanus* recorded in shallow depths and smaller species such as *Oithona* found in deeper depths (see Table A.1). Therefore at night there was not such as great contrast in species composition between shallow and deeper depths although there was a great difference between copeopd abundance between shallow and deeper depths with copepod densities between 25 and 0 m more than ca. 20 times those between 50 and 100 m.

A.3.3 Herbivorous feeding

As krill migrated to the shallow depth of the water column a higher density of phytoplankton food types became available to utilize compared with the low density of phytoplankton in the deeper depths that krill resided in during the day. The Figure A.11 shows how stomach chlorophyll a. content changed throughout krill DVM.

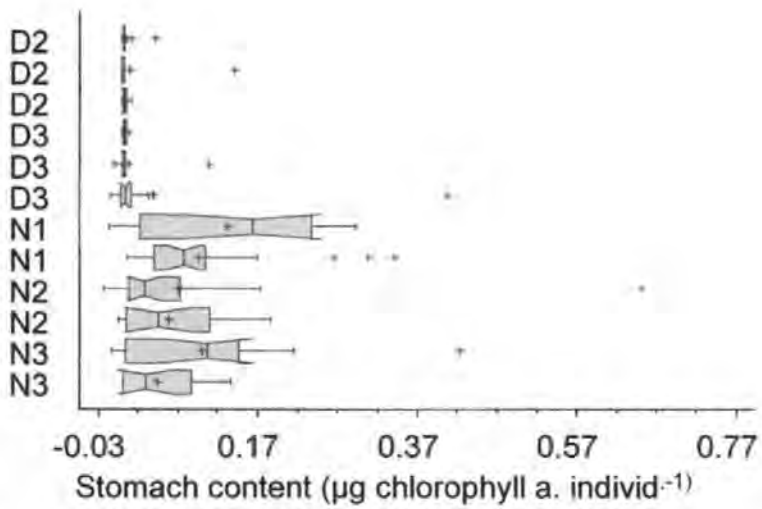


Figure A.11 Krill feeding during the day (D) and proceeding night (N) of the 11/12th March 2002 at shallow to deep depths (1 = 0 – 50 m, 2 = 50 – 75 m, 3 = 75 – 100m). Median chlorophyll a. shown for each depth with 95 % confidence intervals (n = 29 to 40 for each sample). Non – overlapping v – shaped notches indicate significantly different values at the 95 % confidence level.

Krill stomach chlorophyll a. content was greatest during the night than compared with during the day (see Fig. A.11). A Kruskal – Wallis test performed followed by a box plot indicated that there was a significant difference amongst the medians at the 95 % confidence level (T – statistic = 174.832, $P < 0.05$). During the day krill had low densities of phytoplankton available to utilize and a correspondingly low stomach chlorophyll a. content. At night as krill migrated to the surface layers a greater abundance of phytoplankton would have become available and stomach chlorophyll a. content was much greater than during the day. Stomach chlorophyll

a. content at night was, however, very variable with values ranging from as low as 0.004 up to 2.82 µg chlorophyll a. individ. $^{-1}$.

Leading up to the spring bloom krill were sampled only during the day from the deepest 50 m of the water column. No krill were caught in the upper 50 m of the water column on any of the sampling occasions during February and March 2002. Stomach chlorophyll contents from krill caught in tows taken from the same depth were variable, and in some cases significantly different from those obtained for tows taken at the same depth. For example, a Kruskal – Wallis tests indicated that there was a significant difference amongst median stomach content for krill sampled during the day on February the 26th (T – statistic = 16.5068, $P < 0.001$) and also on the 5th March (T – statistic = 25.2448, $P < 0.001$). Although stomach chlorophyll content was variable between tows, all daytime stomach chlorophyll contents values obtained were extremely low (Figs A.11, A.12 and A.13). In particular all median stomach chlorophyll values on the night of the 11th March were greater than 0.03 and up to 0.16 µg chlorophyll a. individ. $^{-1}$ whereas daytime stomach chlorophyll content values on the 11th of March did not exceed 0.0035 µg chlorophyll a. individ. $^{-1}$.

Median stomach content values at any depth during the day of the 26th February did not exceed 0.003 µg chlorophyll a. individ. $^{-1}$. Similarly during the day of 5th March median stomach content values did not exceed 0.003 µg chlorophyll a. individ. $^{-1}$. These low stomach chlorophyll contents were similar to daytime stomach content values during the spring bloom period sampled on the 11th of March when daytime stomach pigment content values did not exceed 0.0035 µg chlorophyll a. individ. $^{-1}$. Therefore even when chlorophyll a. values and therefore

phytoplankton abundance was greater in the surface layers daytime stomach chlorophyll levels were still extremely low

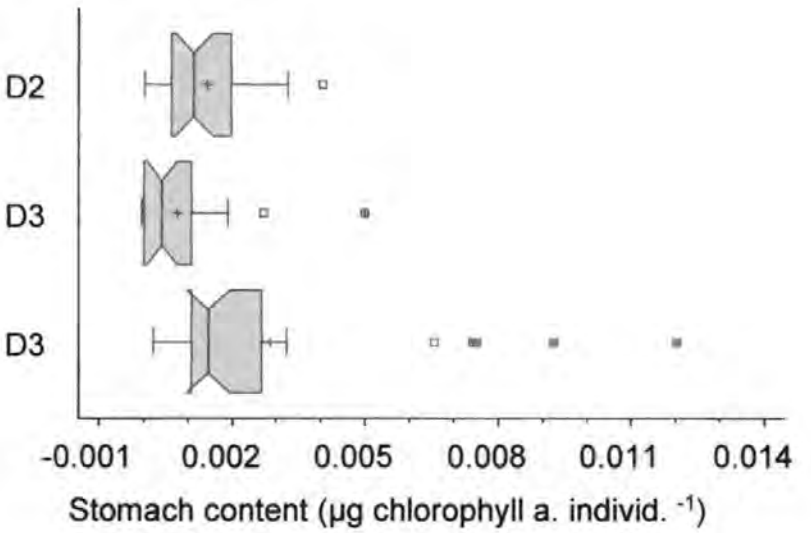


Figure A.12 Krill stomach content (n = 25 for each sample) during the day (D) on the 26th February 2002 at deeper depths of the water column (2 = 50 – 75 m, 3 = 75 – 100m). Median chlorophyll a. shown for each depth with 95 % confidence intervals. Non – overlapping v – shaped notches indicate significantly different values at the 95 % confidence level.

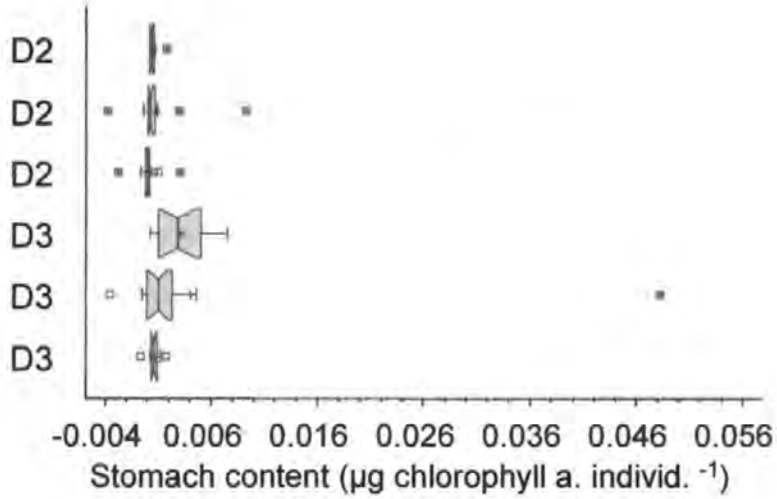


Figure A.13 Krill stomach content (n = 10 –15 for each sample) during the day (D) on the 5th March 2002 at deeper depths of the water column (2 = 50 – 75 m, 3 = 75 – 100m). Median chlorophyll a. shown for each depth with 95 % confidence intervals. Non – overlapping v – shaped notches indicate significantly different values at the 95 % confidence level.

Krill which were starved for 18 h in filtered sea water under laboratory conditions had lower stomach chlorophyll levels than daytime caught krill. The median stomach chlorophyll value for starved krill (n = 10) was 0.0002 µg chlorophyll a. individ. $^{-1}$.

A.3.4 Krill density and feeding rates

The density of krill in this particular study appeared to have an affect on the clearance and ingestion rates per bottle. As shown by Figures A.14 and A.15 clearance and ingestion rates did not increase directly proportionally with and increase in krill density. In fact although as krill density increased clearance and ingestion rates per bottle increased as the krill density reached 5 or 7 individuals per bottle the increase was less marked than at lower krill densities.

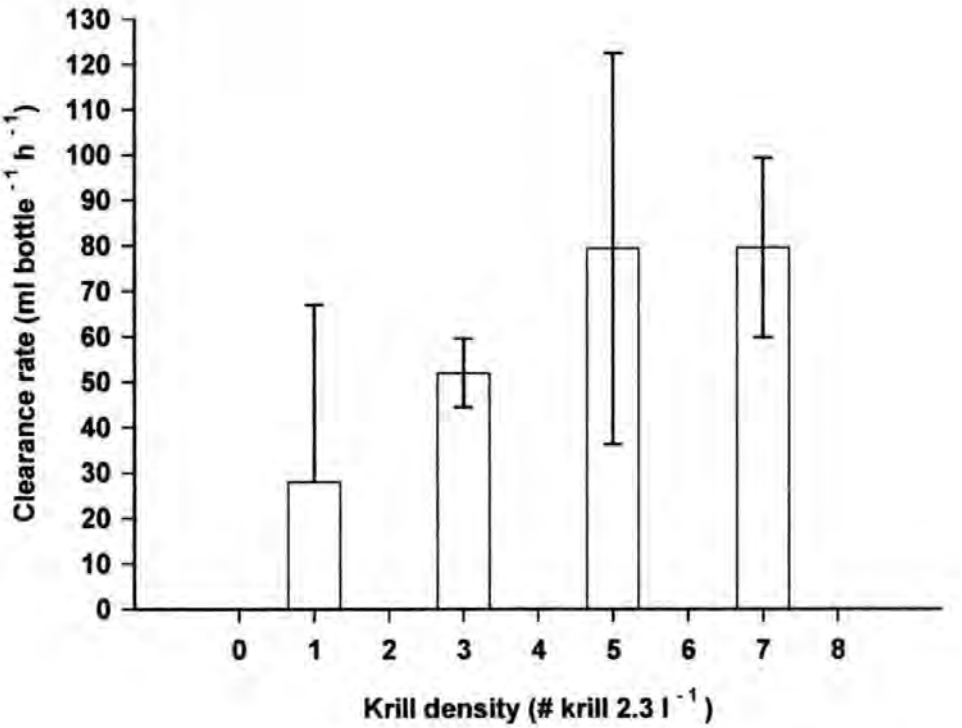


Figure A.14 The effect of krill density (n = 4) on mean clearance rate for each krill density showing 95 % confidence intervals.

The effect on individual krill clearance rates was not extrapolated from the data because comparisons between krill density data would then be confounded because it cannot be assumed that clearance rates of individuals in each bottle are similar. As can be seen from clearance and ingestion rates where one krill is present the 95 % confidence intervals indicate that the mean clearance rate is variable due to the variation between individuals. Therefore clearance and ingestion rates per bottle and not individual krill rates were used to examine the effect of krill density clearance or ingestion rate.

ANOVA performed indicated that there was a significant difference amongst mean clearance rates at the 95 % confidence level ($F_{3,11} = 5.63$, $P < 0.02$). Fishers least significant difference test suggested that the difference was between krill densities of 1 and 5 and 1 and 7.

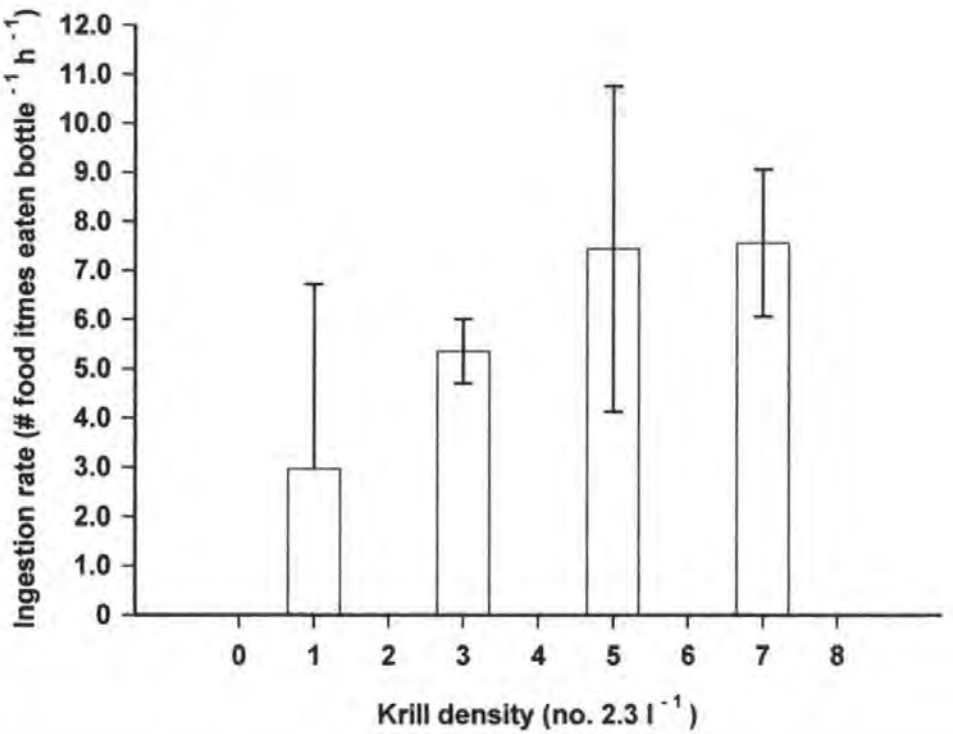


Figure A.15 The effect of krill density (n = 4) on mean ingestion rates for each krill density showing 95 % confidence intervals.

ANOVA performed using STATGRAPHICS plus 5.0 (1994 – 2000, statistical Graphics Corp) indicated that there was a significant difference amongst mean ingestion rates at the 95 % confidence level ($F_{3, 11} = 5.98$, $P < 0.02$). Fishers least significant difference test suggested that the difference was between krill densities of 1 and 5 and 1 and 7.

A.3.5 Gut evacuation rates

Gut evacuation studies were characterised by extremely variable results. The relationship between stomach chlorophyll content and time was negatively correlated (intercept = 0.011, slope = - 0.000074) but weak ($r^2_{1, 48} = 9.21$, $P < 0.05$) when krill were given *T. weissflogii* as a phytoplankton food type (Fig. A.16).

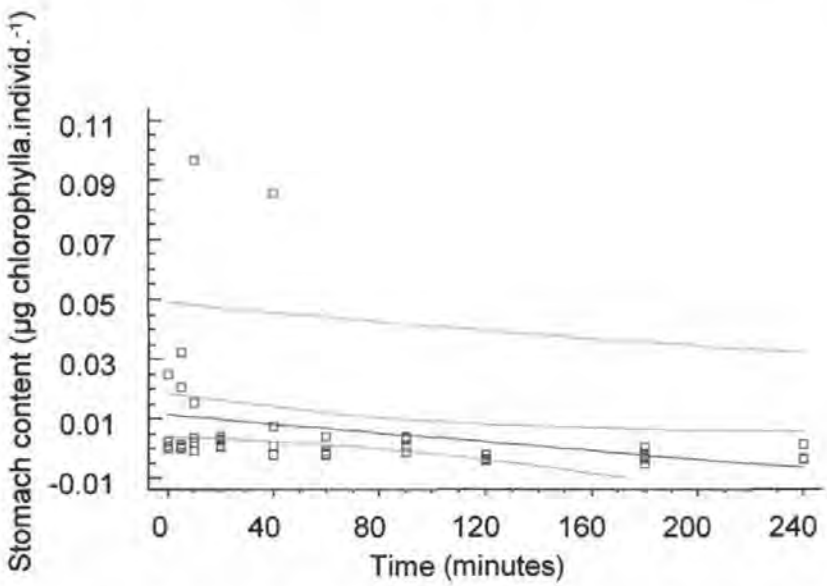


Figure A.16 Gut evacuation of *M. norvegica* (n = 5 for each time point) when given *T. weissflogii* phytoplankton food type. Line of best fit shown (stomach content = 0.0111992 – 0.0000740214 *time).

When charcoal was added to try and maintain passage of gut content and prevent the retention of material in the gut there was no relationship ($r^2_{1, 43} = 0.24$, $P > 0.05$) between gut chlorophyll content and time (Fig. A.17).

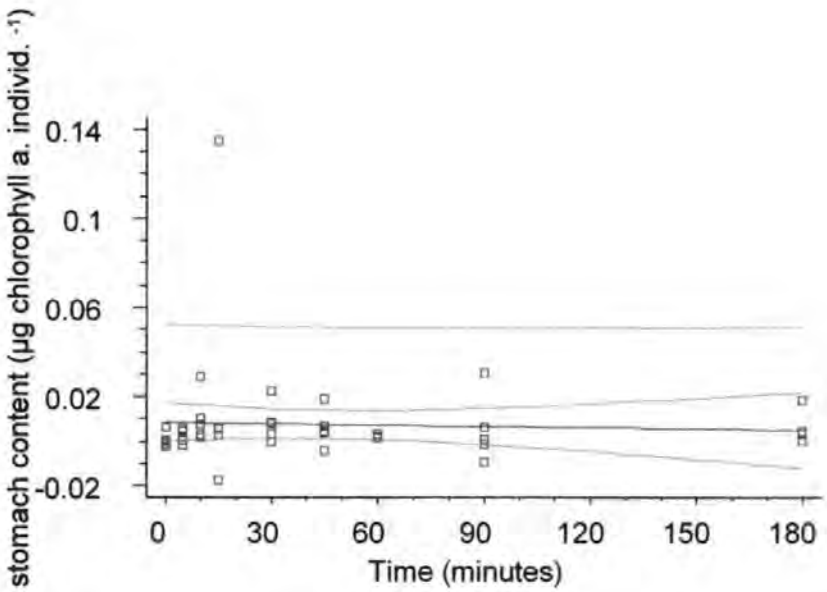


Figure A.17 Gut evacuation of *M. norvegica* (n = 5 for each time point) when given *T. weissflogii* phytoplankton food type and then charcoal to maintain gut evacuation.

Gut evacuation studies using field caught krill were also unsuccessful due to the variability of individual krill gut content at time zero.

A.3.6 Passive or active feeders?

Studies which prevented the movement of krill by tethering krill were unsuccessful as most of the krill did not survive the duration of the experiment. Again, krill did not survive the removal of parts of the feeding basket therefore investigating feeding basket function by removal of parts of the basket was not successful.

A.4 DISCUSSION

A.4.1 Krill DVM and food type availability

Krill were concentrated in deeper depths of the water column during the day and distributed throughout the water column and night therefore exhibiting a strong diel vertical migration, similar to that recorded by Liljebladh and Thommason (2001) and Tarling *et al.* (1998). Indeed, krill may have been concentrated at even deeper depths during the day and this deeper daytime residence may explain the variability between repeated tows taken at the deeper depth during the day and also the overall lower daytime density of krill compared with at night. The fjord was ca. 115 m deep therefore krill may have been residing at depths greater than 100 m. It was, however, not possible to sample this deepest 15 of the water column because of the possibility that the trawl may have collided with the sediment.

Significantly smaller body length krill appeared to reside higher in the water column during the day than longer body length individuals. Again at night significantly different body length krill were found at different depths of the water column for example significantly longer body length krill were found between 50 and 75 m depths compared with between 0 and 50 m and 100 an 75 m depths. This difference in body length with depth suggests an asynchronous migration pattern for krill of various body lengths. Whether this difference in distribution is also related to krill sex requires further investigation.

Krill appeared to reside in deeper water column depths during the day on all sampling occasions. The low gut chlorophyll content of individuals collected during the day on these sampling occasions suggests that krill did not go into surface depths to feed during the day even as the spring bloom became more prominent and phytoplankton levels became greater in the surface layers of the

water column. When sampled at night krill appeared to have migrated into colder, shallow waters. During this ascent to the surface layers of the water column krill would have experienced a slight decrease in salinity. Although both this decrease in salinity and temperature would have become much greater compared with the conditions in deeper water if krill did migrate into the upper 25 m of the water column. Due to the depths sampled it is impossible to determine whether krill did migrate into the upper 25 m of the water column as one trawl was taken for the depths between 0 and 50 m. In order to determine whether krill were present in the upper 25 m of the water column at night krill trawls would need to be taken both from smaller depth intervals e.g. 0 – 25 m. As copepods and phytoplankton were most abundant in this upper 25 m of the water column krill would have to experience greater changes in temperature and salinity compared with those they experience during the day at deeper depths in order to utilize the more abundant food sources of the surface layers of the water column. The effect of temperature and salinity on krill were not the focus of this thesis and therefore although interesting it was more important to pursue whether krill do enter the surface water than the effect of these physico – chemical characteristics on the krill.

As krill appeared to remain in the deepest 50 m of the water column during the day the higher phytoplankton abundance in the surface layer of the water column would have been unavailable to krill to utilize during the day. Also, mainly larger copepod species such as *Calanus* spp. and *Metridia* spp. would have been available to krill during the day and relatively low densities of smaller copepod species compared with the higher densities of smaller copepods in the surface water. Therefore there was a difference in the 'size' of copepod species available for krill to utilize during the day compared with at night. Consequently investigations concentrated on the effect of food types available during the day compared with those available at night on feeding rates.

A.4.2 Herbivorous feeding

Night – time caught krill had significantly higher stomach chlorophyll levels than compared with the extremely low values obtained for day – caught krill on any sampling occasion. Therefore krill appeared to feed only herbivorously during their nocturnal ascent to the surface layer of the water column. These lower stomach chlorophyll values during the day support the conclusions of Lass *et al.* (2001) that krill do not feed during the day. Although this change in herbivorous feeding activity may, as Simard (1980) suggested, be that krill switch to herbivorous feeding at night, it is unlikely as Lass *et al.* (2001) have found that krill also have mandibles in their stomach content at night. Krill starved for 18 hours had lower stomach chlorophyll levels than daytime caught krill. Lower values of starved krill does not necessarily suggest that krill were feeding during the day on phytoplankton. The 18 h period that krill were starved for in the laboratory is a longer time period than krill caught in the day would have potentially been starved for from the previous night feeding. Additionally, the krill starved in the laboratory probably had lower stomach chlorophyll values at the start of the 18 h period than krill which had been feeding during a 'night' period in the field. On no occasion even when krill were fed a natural surface water food type in the laboratory were stomach chlorophyll values recorded as high as those from field caught krill. Therefore for the same reasons as discussed for gut evacuation experiments it was not possible to gain a realistic stomach chlorophyll content value for starved krill either in the laboratory or from field caught krill. For this reason feeding rate studies in the laboratory in this study are not used to extrapolate to krill in field conditions but as a means to explaining feeding feeding behaviour under different feeding conditions. Variability in median stomach chlorophyll content between tows during the day may have also been due to different sampling times during

DVM and therefore differing length 'starvation' periods. For example krill captured in tows taken later in the day may have had a longer 'starvation' period and therefore lower stomach chlorophyll content.

As krill appeared to feed only during their nocturnal ascent to the surface layers large variability within tows of stomach chlorophyll content suggests asynchronous migration by krill. As there was a significant difference in krill body length at different depths of the water column again further investigation considering krill sex as a possible variable in DVM need to be considered in future studies.

There was either no relationship or a weak relationship between krill body length and stomach chlorophyll content of krill caught at various depths of the water column. This lack of relationship may be explained by the great variation between individuals and therefore difficulty in teasing out a relationship between body length and stomach pigment content form the background noise. That is, asynchronous migrations would lead to variability between individual stomach chlorophyll content. Therefore, establishing whether there is a relationship between krill body size needs to be investigated under controlled conditions i.e. in the laboratory. Performing feeding studies in the laboratory with different body length krill may further explain this relationship between krill body length and feeding.

A.4.3 *In situ* ingestion rates

Gut evacuation studies were on the whole unsuccessful. Many of the gut evacuation studies attempted were based on using a food type which *Meganyctiphanes norvegica* from Gullmarsfjorden was thought to consume (J. I. Spicer, Pers. Comm). Feeding experiments (see Chapter 2), however, suggested that *M. norvegica* did not feed upon the phytoplankton *T. weissflogii* . These gut evacuation experiments confirmed the results of feeding experiments of Chapter 2

many experimental modifications such as changing the density of *T. weissflogii*, changing the incubation time of krill with the food type all suggested that *M. norvegica* did not feed upon this food type. The main problem concerning these gut evacuation studies therefore appeared to be offering krill a food type which not only would they consume but that they would feed upon in a sufficient density so that there would be a great change in stomach pigment levels with gut evacuation. Therefore gut evacuation studies were performed on night – time field caught krill. Using field caught krill, however, also created problems as the variability between individual krill was too great to observe any relationship between gut content and time and therefore estimate gut clearance rates. Although this variability may have been overcome by using greater replicates, it was concluded that with the great amount of variables potentially affecting gut clearance rates of krill in the field, e.g. food density that in the limited time available carrying out more gut clearance studies was not pertinent to the investigations of this thesis. That for the gut evacuation studies to be used to calculate ingestion rates of field caught krill information would be required on the effect of food density on gut evacuation rates and the effect of mixed 'natural' diets on gut evacuation rates. Given that it is evident that krill can retain gut contents for up to 7 d (Antezana *et al.*, 1982) when starved it is likely that variables such as food density affect gut passage time of given food types. Therefore gut evacuation rate studies were not pursued as investigating the effect of food density of various food types on gut evacuation rates was not feasible in the time scale of this thesis and not of direct consequence to addressing the questions of this thesis.

A.4.4 Krill density

The effect of increasing krill density on clearance and ingestion rates was not directly proportional. Increasing the number of krill did not lead to a directly

proportional increase in clearance and ingestion rates. Although at higher krill densities clearance rate was significantly greater than compared with lower krill if individual krill rates in these larger group sizes could be determined they would be lower than for individual krill. Although it has been suggested that krill swarming actually increases feeding efficiency the numbers of krill used in this study are low compared with swarms of krill and therefore as Ritz (2000) suggested krill may not behave the same way in small groups in the laboratory as they do in large swarms in the field. Consequently, the problems of creating 'swarm' behaviour in the laboratory or at least using large groups of krill in the laboratory was not possible therefore because of the lack of relevance these krill density studies were not pursued any further.

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