Feeding biology of three euphausiid species in the vicinity of the Prince Edward Archipelago (Southern Ocean)

This thesis is submitted in fulfillment of the requirements for the degree of Master of Science at Rhodes University.

Leigh Josephine Gurney

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Supervised by Dr. P.W. Froneman and Prof. C.D. McQuaid

Dedicated to my father,

John Joseph Gurney.

Abstract

The feeding biology of three euphausiid species, *Euphausia vallentini* (adults and juveniles), *E. longirostris* and *Nematoscelis megalops* was investigated during austral autumn (April/May) of 1998 and 1999, in the vicinity of the Prince Edward Islands (Southern Ocean). Data on the abundance and biomass of these species, estimated from bongo net tows, were investigated. Trophic position was assessed using gut contents and stable nitrogen isotope measurements. Feeding rate and daily carbon ration were estimated using the gut fluorescence and the gut fullness techniques. Vertical migrations into the surface waters at night were found to be strong for *Euphausia vallentini* adults and juveniles. Associated with these migrations were clear diel feeding patterns. Insufficient data during daylight hours for *E. longirostris* made it impossible to determine diel feeding patterns, but high feeding activity did occur during dark hours. *Nematoscelis megalops* did not show any distinct diel feeding pattern, but slightly higher gut fullness indices in the late afternoon suggested that feeding activity may have been highest during this period. For both *Euphausia* spp. high gut pigment levels were recorded in 1999, which corresponded to higher ambient chlorophyll *a* concentrations for that year. Highest initial gut pigment levels and highest ingestion rates were found for *Euphausia longirostris* in both years and lowest values were observed for *N. megalops*. High phytoplankton and low metazoan contributions to the diet of *Euphausia vallentini* juveniles, as shown in the gut content analysis, and low stable nitrogen isotope ratios ($\delta^{15}N = 1.39 \pm 0.31$), both indicated that this group was principally herbivorous. The results of gut content analysis of the adults of *E. vallentini* were similar to those of the juveniles, however, stable nitrogen isotope results showed that there was a higher degree of omnivory ($\delta^{15}N = 3.81 \pm 0.66$). Daily ration estimates from the gut fluorescence and fullness techniques showed that between 3.3 and 25.7 % of *E. vallentini* adults total daily carbon ration was derived from autotrophic sources. Although the contribution of carnivory to the diet was difficult to determine, the adults of this species may be considered omnivorous. Irrespective of the degree of carnivory, a dietry shift with an increase in size was evident for this species. Gut content analysis for *Euphausia longirostris* showed that this species consumed large amounts of both phytoplankton and metazoan prey and this was reflected in the stable nitrogen isotope results ($\delta^{15}N =$

6.88±0.60). These findings were supported by the results of the daily carbon ration estimates which showed that autotrophic carbon contributed between 6.9 and 20.3 % of the daily carbon consumption. The gut content analysis suggested that *N. megalops* was omnivorous, and the stable nitrogen isotope results place it in a trophic position equivalent to that of *E. longirostris* ($\delta^{15}N$ = 6.83±0.78). Calculations from daily ration estimates suggested that only 3.1 % in 1998, and 3.2 % in 1999, of the carbon ingested was of autotrophic origin. This species may therefore be considered carnivorous. Implications of the findings of this study are discussed in terms of carbon cycling in the Southern Ocean.

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Introduction

General Introduction

The Southern Ocean is generally characterised by relatively low biological productivity (Le Jehan and Tréguer 1985). Exceptions to this include enhanced primary productivity at the oceanic fronts (Lutjeharms *et al*. 1985) and in the waters surrounding the oceanic islands (Allanson *et al*. 1985; Perissinotto 1992; Pakhomov and Froneman 1999b). Many of the subantarctic islands are breeding grounds for seabirds and seals and the waters surrounding these islands support large populations of these predators (Ridoux 1988). Euphausiids have been identified as important components in the diets of many of these top predators and are the principal prey item for many fish, squid, birds, seals and whales (e.g. Kawamura 1974; Serebriakova 1989; Croxall *et al.* 1985; Hunter 1985; Kock 1985; Miller *et al.* 1985; Nemoto *et al.* 1985; Williams 1985; Brown and Klages 1987; Ridoux 1988; Cooper and Brown 1990; Bost *et al.* 1994a, b; Yatsu 1995). Euphausiids often constitute a large proportion of zooplankton biomass (Holm-Hansen and Huntley 1984) and their ability to feed on relatively small particles in comparison to their size makes them an important link between microscale production and large predators.

The Prince Edward, Kerguelen and Crozet Islands are all found in subantarctic waters in the south-west Indian Ocean. Euphausiids form an important part of the diet of many of the top predators in the vicinity of these islands. Although identification to species level is not always available (e.g. Duhamel and Hureau 1985; Steele and Klages 1986), *Euphausia vallentini* has been shown to be a principal prey item for Gentoo Penguins at all three archipelagos (Brown *et al.* 1990; Bost *et al.* 1994 a, b), and has also been found in the stomachs of Macaroni Penguins and Rockhopper Penguins at both Crozet and Marion Island (Brown and Klages 1987; Ridoux 1988). Euphausiids are an important component of the diets of other seabirds, including the Whitechinned Petrel, Blue Petrel and Salvins Prion (Cooper and Brown 1990; Cooper *et al.* 1992) and have been found in the stomachs of mesopelagic and nototheneid fish (Perissinotto and McQuaid 1992; Pakhomov *et al*. 1996).

The Prince Edward Archipelago comprises two islands, Marion and Prince Edward, which are located in the south Indian Ocean at 46° 50' S; 37° 50' E (Fig 1.1). Prince Edward Island lies 19 km north-east of Marion Island and the two are separated by a shallow inter-island shelf of approximately 200m depth (Pakhomov and Froneman 1999b). The islands lie in the path of the Antarctic Circumpolar Current (ACC) (Deacon 1983; Lutjeharms *et al.* 1988), between the Sub-Antarctic Front (SAF) to the north and the Antarctic Polar Front (APF) to the south (Lutjeharms and Valentine 1984). The ACC is easterly flowing and has an average speed of ≈35 $cm.s^{-1}$ with sea surface temperatures ranging from 3°C to 7°C. (Pakhomov and Froneman 1999b). The positions of the two fronts, which border the water mass surrounding the islands, are highly variable (Valentine and Lutjeharms 1983; Duncombe Rae 1989). The Sub-Antarctic Front (SAF) is usually situated north of the islands but has been recorded to the south (Lutjeharms 1990), and latitudinal shifts of up to 2° have been observed in a period of one month (Duncombe Rae 1989; Lutjeharms 1990). Warmer subtropical water which originates north of the SAF, and cold water from the APF have both been observed in the vicinity of the islands (Miller *et al.* 1984). It is proposed that the intrusions of water masses may be from vagrant eddies, frontal meanders or cross frontal mixing (Allanson *et al.* 1985; Ansorge *et al.* 1999; Froneman *et al.* 1999). The zooplankton community in the vicinity of the islands is not endemic (Pakhomov and Froneman 1999b), but comprises a combination of species with Antarctic, subantarctic and subtropical origins (Boden and Parker 1986; Perissinotto 1989; Pakhomov *et al.* 1998a). The oceanographic environment is highly variable on both short and long term scales, and the distribution of euphausiids in the vicinity of the islands is subsequently also highly variable.

The three euphausiids selected for this study are *Euphausia vallentini* Stebbing 1900, *Euphausia longirostris* Hansen 1908 and *Nematoscelis megalops* GO Sars 1883. Considering the importance of euphausiids as a prey item, little is known of the biology of these species. Limited data are available on the feeding biology of subantarctic euphausiids in general, and this study aims to provide preliminary findings on the feeding biology of the larger euphausiid species found in the vicinity of the Prince Edward Islands.

Euphausia vallentini is a circumpolar species which is restricted to the subantarctic zone (Mauchline and Fisher 1969). The northern limit of its distribution is the Subtropical Convergence (STC) and its southern limit, the Antarctic Polar Front (APF). Its distribution, according to Baker (1965), is between 50° and 60° S, while Mauchline and Fisher (1969) summarise its distribution as occurring between 45° S and 60° S. *Euphausia longirostris* is also a subantarctic species occurring between 40° S and 55° S (Baker 1965; Mauchline and Fisher 1969). It does not occur south of the APF but has been recorded north of the STC (Mauchline and Fisher 1969). *Nematoscelis megalops* has a wide distribution and is found in the northern and southern regions of the Atlantic, but only occurs in the southern regions of the Pacific and Indian Oceans (Mauchline and Fisher 1969). Mauchline (1980) delineated the southern limit of *N. megalops* in the Indian Ocean as being between latitudes 40° and 45°S. This species is, therefore, at its southern most boundary in the vicinity of the Prince Edward Islands.

In the surface waters of the Indian Ocean, between 40° and 50° S, Baker (1965) found *E. vallentini* to be the dominant euphausiid of the genus *Euphausia* (51%) with *E. longirostris* and *E. similis* each constituting approximately one quarter of the catch. Abundance of *E. vallentini* in the vicinity of the Prince Edward Islands has been found to range between 7 and 1412 ind.1000 m^{-3} (Boden and Parker 1986). *Euphausia longirostris* has been found in the vicinity of the islands with average abundance estimates ranging between 0.3 and 201.4 ind.1000 m^{-3} (Pakhomov and Froneman, in press). Abundance estimates for *Nematoscelis megalops* from previous studies in the vicinity of the islands have been found to range from 35 to 141 ind.1 000 m^{-3} (Boden and Parker 1986).

Feeding biology

The feeding appendages of euphausiids are the labrum, the paired mandibles, labia, maxillules, maxillae and the first and second thoracic limbs (Fig 1.2) (Mauchline 1967). In some euphausiids a barrier of tissue and setae is formed by the thoracic appendages when they are pressed close together and this is commonly termed the “feeding basket” (Mauchline 1967). Euphausiids are limited in what they can consume by the structure of the mandible and the thoracic limbs (Mauchline 1967). The jaw of the mandible, the *corpus mandibulae,* has cusps (the *pars incisiva*) in the ventral inner region, and a

grinding surface (the *pars molaris*) in the dorsal inner region (Mauchline 1967). The *pars molaris* is responsible for crushing diatoms, particularly chain-forming species with hard frustules, foraminiferans, and silicoflagellates (Nemoto 1967 in Mauchline and Fisher 1969). Relative to the mandible as a whole, the grinding surface is largest in the herbivorous species, smaller in more omnivorous species and smallest in carnivorous species (Mauchline and Fisher 1969). Thus, carnivorous species may have difficulty in ingesting large diatoms or chain-forming species as they are unable to crush them.

Euphausia vallentini and *E. longirostris* have setose feeding baskets and are both believed to obtain their food by filter feeding (Mauchline and Fisher 1969; Mauchline 1980). According to Suh and Nemoto (1987), the size of particle which can be consumed by euphausiids is determined by the spacing between the setae. These authors classified *E. vallentini* as a medium mesh filter feeder with a lower limit of particle size retention of between 8 and 12 μm in diameter. In Mauchline (1967) euphausiids are grouped into four groups based on their feeding appendages. *Euphausia longirostris, E. triacantha, E. spinifera* and *E. hanseni* are grouped together. All of the limbs of *E. triacantha* are more heavily setose than those of the other three species, with the overall shapes of the appendages in the four species otherwise being similar (Mauchline 1967). *Euphausia triacantha* was classified as a "macrofiltrator" by Suh and Nemoto (1987), as it has a coarse mesh filter (27 μm). They proposed that this species would be unable to feed on nanoplankton (ranging between 2-20 μm). If this is the case, then *E. longirostris* may also be unable to feed on particles smaller than 20μm. Mauchline (1980) does point out that the feeding appendages may limit the size of food particles but not the type of particle and, therefore, although one may classify an organism as a filter feeder, some degree of omnivory will always occur.

Mouthparts in species of the genus *Nematoscelis* are generally less setose than the two *Euphausia* spp. and therefore are not well adapted for filter feeding (Mauchline 1967; Mauchline and Fisher 1969). The second pair of thoracic limbs in *Nematoscelis megalops* are greatly elongated and associated with this modification is the division of the eyes into two lobes (Fig 1.3)(Mauchline 1980). These two modifications are thought to be for carnivorous feeding, but this is as yet unproven (Roger 1973; Mauchline 1980).

Euphausiids are generally considered to be opportunistic omnivores (e.g. Mauchline and Fisher 1969; Mauchline 1980; Ohman 1984; Price *et al.* 1988; Pilditch and McClatchie 1994). However, the literature suggests that *E. vallentini* is principally herbivorous; *Euphausia longirostris,* because of its larger size, may display a higher degree of carnivory and consequently may be considered omnivorous; and *N. megalops* is believed to be carnivorous by virtue of its morphology (Mauchline and Fisher 1969; Mauchline 1980).

Aims

This study aims to:

1) make observations on the diet
2) assess the trophic positions
3) estimate feeding rate and daily carbon ration

of *Euphausia vallentini*, *E. longirostris* and *Nematoscelis megalops* in the vicinity of the Prince Edward Islands during April / May of 1998 and 1999.

There is, as yet, no single technique which adequately investigates the feeding biology of crustacean zooplankton. For this study four techniques have been chosen, namely gut content analysis, stable nitrogen isotope analysis, gut fluorescence and the gut fullness techniques. The gut content and stable isotope measures complement each other. Results from the gut content analysis provide an instantaneous measure of what has recently been consumed while the stable nitrogen isotope analysis gives insight into what food has been assimilated into the tissue over a longer period of time. The fluorescence data provide information on the intake of phytoplankton and allow an estimate of the quantity of autotrophic carbon consumed to be made. The gut fullness technique provides a daily ration which is based on total food consumed and, therefore, includes both autotrophic and heterotrophic carbon. In this way each technique provides information on a different aspect of the feeding biology of these organisms.

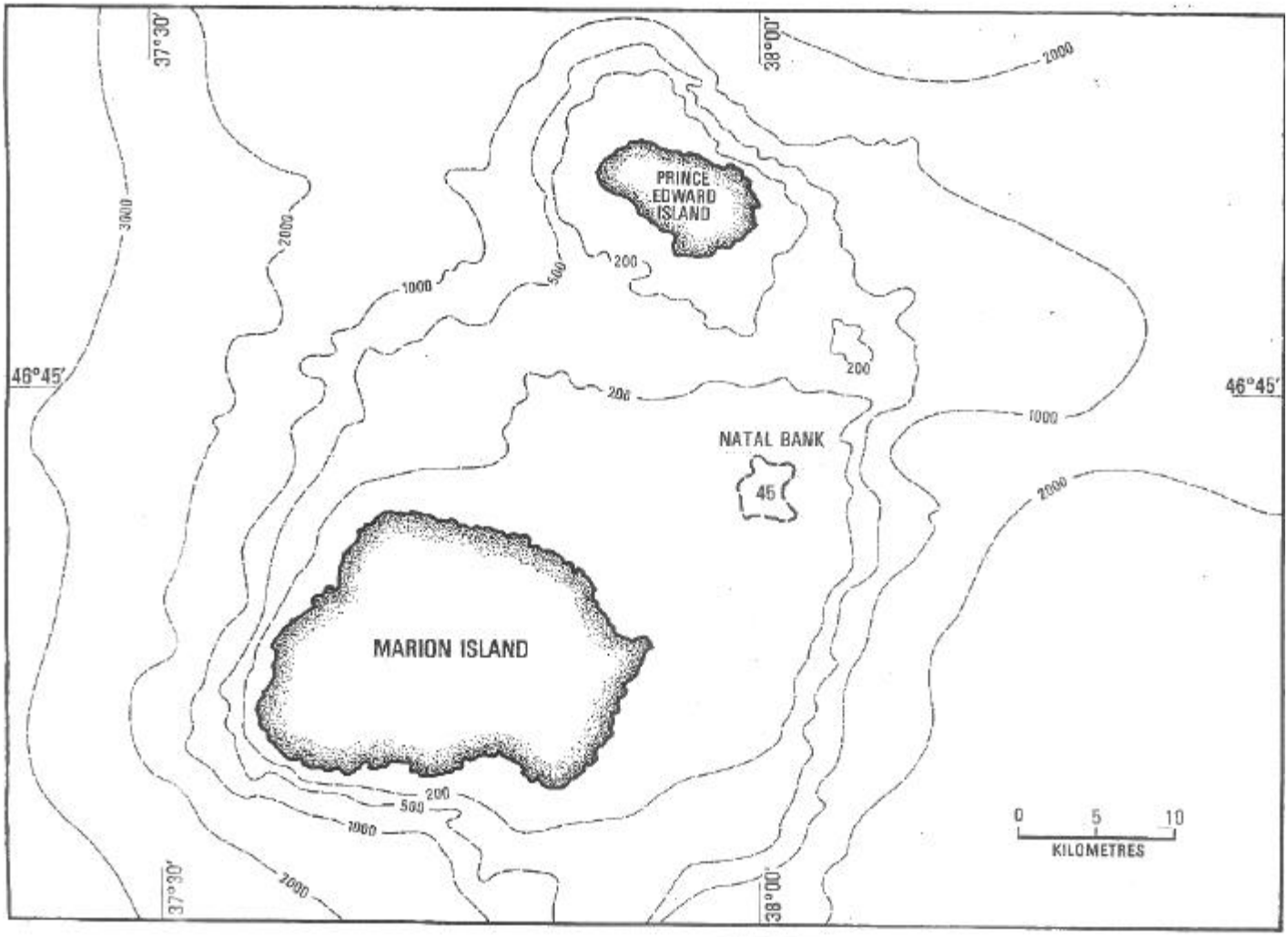


Figure 1.1 Map of the Prince Edward Islands.

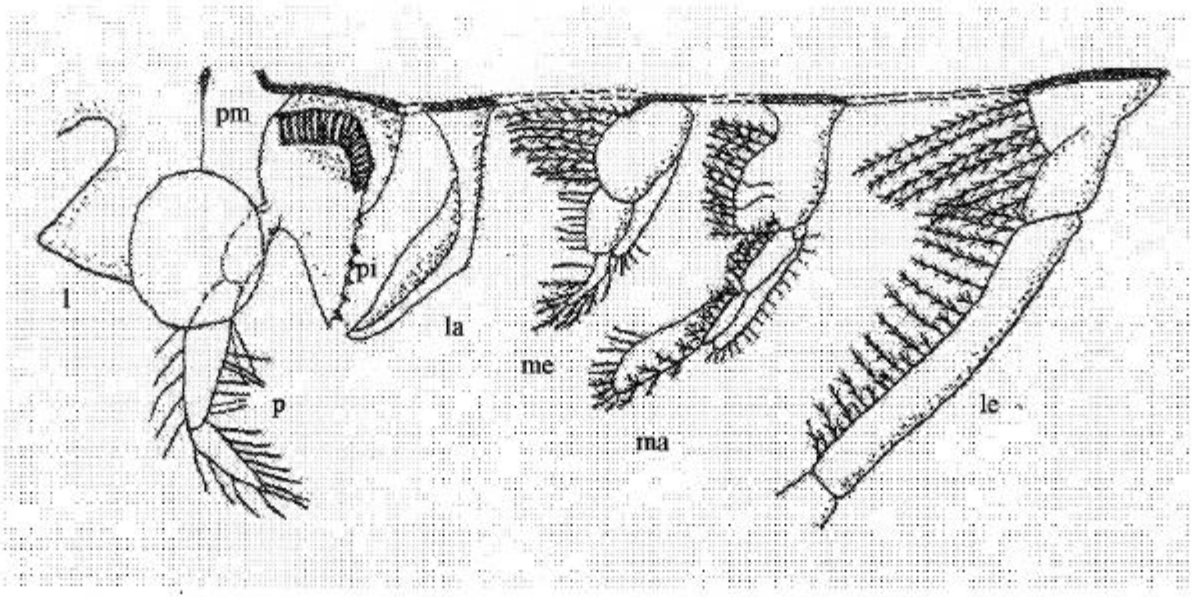
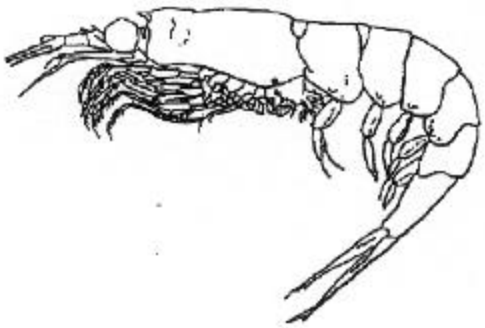


Figure 1.2 Generalised figure of euphausiid feeding appendages adapted from Mauchline (1967). These right hand appendages are drawn from the left side and separated for clarity; l = labrum; la = labium; le = first thoracic limb; m = mandible; ma = maxilla; me = maxillule; p = mandibular palp; pi = *pars incisiva*; pm = *pars molaris*.

(a)



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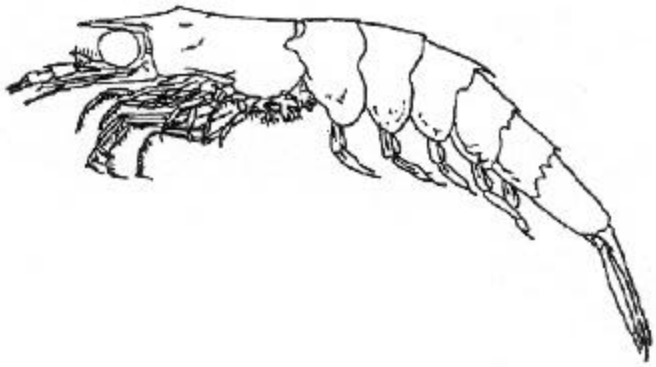

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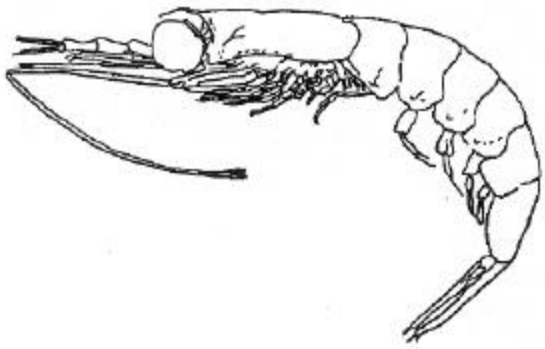


Figure 1.3 Illustration of *Euphausia vallentini* (a), *Euphausia longirostris* (b) and *Nematoscelis megalops* (c) (Adapted from Baker *et al.* 1990).

Chapter 2
Distribution, abundance, biomass and size structure

Introduction

The opportunity to carry out this research forms part of a five year Marion Island Oceanographic Survey (MIOS) programme which was initiated in 1996 and is sponsored by the Department of Environmental Affairs and Tourism, South Africa. The station numbers and associated physical data are presented in Appendix 1, together with the corresponding maps of stations sampled. This chapter serves to introduce the distribution, abundance, biomass and size structure of *Euphausia vallentini*, *E. longirostris* and *Nematoscelis megalops* in the vicinity of the islands for the MIOS 3 and MIOS 4 cruises.

Materials and methods

All samples were taken during austral autumn (April/May) of 1998 and 1999 aboard the research and supply vessel, the mv *SA Agulhas* (Voyages 87 and 90) in the vicinity of the Prince Edward Archipelago. Specimens for this study were collected from the fifty-seven net tows from the MIOS 3 survey in 1998, and 39 tows from the MIOS 4 survey in 1999. Specimens were collected using a Bongo net (0.5 m^{-2} mouth area), equipped with one 300 μm and one 500 μm mesh net. The net was fitted with a universal underwater unit (U^3) for the continuous monitoring of depth and temperature (Robertson *et al*. 1981). The volume filtered by the net was calculated using an electronic flowmeter and ranged from 43 to 803 m^3. The net was towed obliquely and the towing speed varied between 1.5 and 3 knots. In 1998, daytime tows were conducted to a maximum depth of 300 m while nighttime tows were to a maximum depth of 200 m. In 1999, all tows were to a depth of 300 m. Where sounding was less than the maximum towing depth, the net was taken to within 5 m of the bottom.

Specimens captured in the 300 μm mesh were preserved in 4-6% buffered formalin for abundance and length-weight analysis. In the laboratory, samples were sorted,

euphausiids identified, counted, individually measured from the tip of the rostrum to the end of the telson with a precision of ≈ 0.5 mm, and oven dried at 60°C for 36 hours. Identifications were made using a dissecting microscope operated at 25x magnification using the works of Baker *et al*. (1990) and Kirkwood (1982). Whole samples or random subsamples of between $^1/_2$ and $^1/_{32}$ were taken, depending on the number of euphausiids in the sample. Abundance of each species was expressed as number of individuals per 1 000 m^{-3}. Individual specimens were weighed using a Sartorius microbalance from which length-weight relationships were assessed. Biomass estimates were made using the dry weights of all euphausiids in the subsample and were expressed as mg dry weight per 1000 m^{-3}.

Results

Abundance and biomass

Abundance and biomass data at each station are presented in Tables 2.1 and 2.2. *Euphausia vallentini* was by far the most abundant euphausiid and was found at almost all stations in both years (Fig. 2.1 and 2.2). The mean abundance of this species was higher in 1998 (2154 ind.1000 m^{-3}) than in 1999 (515 ind.1000 m^{-3}), although there was a high degree of variability during both years (Tables 2.1 and 2.2). The mean abundances of *E. longirostris* and *Nematoscelis megalops* were generally an order of magnitude lower than those of *E. vallentini*. *Euphausia longirostris* abundance was lower in 1998 when compared to 1999 with a mean of 8 and 38 ind.1000 m^{-3} being found in each year respectively. Similar abundances were observed for *N. megalops* in both years, with a mean of 25 ind.1000m^{-3} in 1998 and 35 ind.1000 m^{-3} in 1999. A similar pattern was observed for the biomass of these species in each year (Tables 2.1 and 2.2).

Diel vertical migrations

Figures 2.3 and 2.4 illustrate diel vertical migrations of the three euphausiid species. The time intervals were divided into 4 categories: 1 - dawn (5h00 to 8h00), 2 - daylight (8h00 to 17h00), 3 - dusk (17h00 to 20h00) and 4 - night (20h00 to 5h00). Only samples

collected at oceanic sites (sounding > 300 m) were considered for this analysis. There were no significant differences between abundance or biomass for any species when comparing these time intervals (ANOVA). In 1998, however, there was evidence that *E. vallentini* exhibited diel vertical migrations. Lowest abundances were observed during daylight hours (Fig. 2.3 a) and highest abundances were observed at night. This pattern was also observed for *E. longirostris* in 1998 (Fig. 2.3 c). Abundance estimates for *N. megalops* increased from dusk through until dawn and were low during the day (Fig. 2.3 e). Biomass data reflected the abundance data patterns for both *E. vallentini* and *E. longirostris* (Fig. 2.3 b, d), however, for *N. megalops* highest biomass was observed during the day (Fig. 2.3 f). In 1999, *E. vallentini* again exhibited a diel pattern with highest abundance values occurring at early evening and during the dark hours (Fig. 2.4 a). Lowest abundances for *E. longirostris* were observed at dusk with highest values observed during the dark hours and early morning (Fig. 2.4 c). Highest abundance and biomass estimates were observed during the night for *N. megalops* (Fig. 2.4 e, f). High biomass values were recorded for *E. vallentini* at night, and at dawn for *E. longirostris* (Fig. 2.4 b, d).

Comparisons of abundance and biomass data from those samples which were collected over the inter-island shelf compared to the oceanic sites are presented in Figures 2.5 and 2.6. High abundance and biomass were recorded for *E. vallentini* at both the inter-island shelf and oceanic sites. No *E. longirostris* specimens were caught over the shelf in 1998 and lower abundance and biomass values were recorded at inter-island sites in 1999 for this species. Slightly higher abundance and biomass values were recorded at oceanic sites for *N. megalops* in 1998 and 1999.

Size structure

Euphausia vallentini become sexually mature between 15 and 25 mm, and reach a maximum size of 28 mm (Mauchline and Fisher 1969). As no attempt was made to assess the sex or maturity of the euphausiids for this study, all *E. vallentini* individuals less than 15 mm in length were considered juveniles, and any greater than this size were considered adults. *Euphausia longirostris* adults range in length from 21-34 mm and *Nematoscelis megalops* from 20-26 mm (Mauchline and Fisher 1969).

From the analysis of the length weight regressions (Fig 2.7), the power equation for each species was calculated to be as follows:

E. vallentini $y = 0.0005 L^{3.2296}$ ($r^2 = 0.986$, n = 295)

E. longirostris $y = 0.0006 L^{3.1664}$ ($r^2 = 0.989$, n = 71)

N. megalops $y = 0.0034 L^{2.5852}$ ($r^2 = 0.746$, n = 74)

Figure 2.8 illustrates the size frequencies of the three species (data combined from both surveys). *Euphausia vallentini* juveniles and adults were caught in large numbers, however for *E. longirostris* and *N. megalops* predominantly adults were caught in both years. For this reason in most of this study, four euphausiid groupings were considered, namely *E. vallentini* adults, *E. vallentini* juveniles, *E. longirostris* adults and *N. megalops* adults.

Discussion

Euphausiids, although not important in terms of their contribution to abundance, often constitute a large proportion of the biomass of the zooplankton community in the waters surrounding the Prince Edward Islands (Perissinotto 1989; Ansorge *et al*. 1999, Froneman *et al*. 1999; Pakhomov and Froneman 1999a). Net avoidance by euphausiids is a well known phenomenon and this may lead to underestimates of both abundance and biomass of these species (Hempel 1985). Bongo nets are generally used to sample mesozooplankton, therefore the estimates from this study should be regarded with caution and may only provide lower limits on the abundance and biomass (Miller and Hampton 1989). Perissinotto *et al.* (1997) found that acoustic data estimated biomass/abundance to be up to two orders of magnitude higher than estimates based on bongo net tows. Unfortunately no acoustic data were available for either cruise in this study.

Many zooplankton undergo vertical migrations, usually descending to dimly lit areas during daylight hours to avoid visual predators and then returning to the surface layer at night to feed (Dagg 1997). Euphausiids have been observed to undergo vertical

migrations (Mauchline and Fisher 1969), and the three euphausiids under investigation have all been observed to undergo vertical migrations to some extent in the vicinity of the islands (Perissinotto 1989, Pakhomov and Froneman 1999a).

According to Mauchline and Fisher (1969), *Euphausia vallentini* probably occurs mainly between 250 and 100 m depth during the day and rises to the surface at night. This supports the findings of this study and those of Boden and Parker (1986) who found *E. vallentini* abundances greatest between 0 and 100 m at night and between 500 and 300 m during the day. In Mauchline and Fisher (1969) there is no reference to the diurnal patterns of *E. longirostris*, although they state that *N. megalops* probably occurs below 300 m during the day, migrating to the surface at night. The vertical migration patterns of *N. megalops* have been found to be variable, in some cases vertical migrations have been observed (Lindley 1982) while in others they are uncertain (Barange *et al.* 1991). Boden and Parker (1986) only recorded the presence of *N. megalops* during night time tows and abundances were similar across all depths from 500m to the surface. In this study there was no distinct diel pattern. Higher abundances were observed at dawn in 1998 (Fig.2.3), and during the night in 1999 (Fig. 2.4).

Comparison between the diel patterns of abundance and biomass data allows an indirect assessment of the size of individuals captured with a high biomass corresponding to a low abundance suggesting a predominance of larger individuals, and *vice versa*. Diel variation in biomass showed a similar pattern for both *E. vallentini* and *E. longirostris* in 1998 (Fig 2.3 b, d). In the same year *N. megalops* abundance was low while biomass was high during the day (Fig 2.3 e, f) suggesting that individuals caught during the day were comparitively large.

The abundance of *E. vallentini* were similar at both dusk and night, however biomass only increased substantially during the dark hours. This suggests that smaller individuals migrate to the surface at dusk and the larger individuals only migrate to the surface once the sun has set. The results of the biomass data for diel patterns for *E. longirostris* suggest that those specimens found in the water column during the day were relatively small in size (Fig 2.4 b, d). Net avoidance during light hours may confound the results of an

investigation into diel vertical migrations. Larger euphausiids like *E. longirostris* adults may be able to avoid net capture to a greater extent during the day and the lower abundance/biomass or absence of this species from day time tows may be partly due to this.

The islands are separated by an inter-island shelf, and zooplankton are at times advected onto this shallow shelf (Perissinotto and McQuaid 1992). Of the three euphausiids, *E. vallentini* was found in highest densities in the inter-island shelf region in both 1998 and 1999 (Figures 2.5 and 2.6; note log scale of y-axis). In 1998, *E. longirostris* was only captured at oceanic sites. In 1999, although *E. longirostris* individuals were caught over the shelf, the biomass data suggested that the average size of individuals over the shelf was smaller than individuals captured in deeper water (Fig 2.6 b). Higher abundance and biomass were observed for *N. megalops* at oceanic sites, particularly in 1998 (Fig 2.5 a and b) but this species was also found on the inter-island shelf. The biomass data do not suggest that there was any difference in size of individuals of this species found either on or off the shelf.

It has been proposed that many of the near shore top predators benefit from the advection of zooplankton onto the inter-island shelf at night (Perissinotto 1989, Perissinotto and McQuaid 1992). As zooplankton are unable to migrate to deeper waters once day breaks, they may be more susceptible to capture by visual predators (Perissinotto 1989, Perissinotto and McQuaid 1992). This could be one factor contributing to the patchy distribution of these crustaceans (Genin *et al*. 1994), or it may reflect the oceanographic variability of the environment in which the euphausiids were found (Froneman *et al.* 1999a). The absence or relatively low abundance and biomass data found for the two larger species suggests that either these species have deep vertical migrations and are not in the surface layers and therefore do not get advected onto the shelf (Pakhomov and Froneman 1999a); or once advected onto the shelf they may, because of their larger size be more heavily preyed upon. Evidence from previous studies and from this survey illustrate that the distribution of these organisms is not uniform, but that there is a high degree of variability in both the abundance and biomass.

The chapters which follow focus on the feeding biology of these organisms.

Table 2.1 Abundance and biomass values for the three euphausiid species in the vicinity of the Prince Edward Islands for April/May 1998.

Station	*E. vallentini*		*E. longirostris*		*N. megalops*	
1998	**abundance (ind.1000m^{-3})**	**biomass (mg.1000 m^{-3})**	**abundance (ind.1000 m^{-3})**	**Biomass (mg.1000 m^{-3})**	**abundance (ind.1000 m^{-3})**	**biomass) (mg.1000 m^{-3})**
MS3-1	465.9	881.9				
MS3-3	59.7	78.2			64.0	14830.5
MS3-4	353.8	152.7	39.3	768.3	19.7	
MS3-5	1116.7	5787.0	5.6	228.9	178.7	1898.2
MS3-6	1695.3	5092.2	32.8	1178.0	80.7	375.2
MS3-7	16.6	17.1	16.6	233.7	299.2	3101.2
MS3-8	265.1	1047.1				
MS3-9	52.3	176.3				
MS3-10	845.2	1732.8			30.2	302.0
MS3-11	488.8	27.2	122.2	1474.5	244.4	3269.8
MS3-12	1225.5	2018.5	2.3	72.1	13.5	243.3
MS3-13	396.3	1034.6	5.2	140.8	5.2	93.9
MS3-15	245.5	91.7			3.4	46.3
MS3-16	192.9	4.6			8.0	56.8
MS3-17	127.6					
MS3-18	10.9	19.1				
MS3-19	787.4	2628.6				
MS3-20	312.8	114.9			3.6	28.3
MS3-21	154.7	246.0	4.6	244.7	43.9	798.8
MS3-22	587.6	880.5				
MS3-23	473.0	676.5				
MS3-24	138.1	145.2			39.4	258.8
MS3-25	79.6	109.9				
MS3-26					3.7	29.7
MS3-27	48.0	33.5				
MS3-29	15.4	16.0				
MS3-31	55.9	228.2				
MS3-32	110.1	80.6			5.2	103.2
MS3-33	228.4	457.8			105.4	1198.7
MS3-34	1981.0	5904.0			28.1	258.9
MS3-36	183.6	25.9	61.2		61.2	665.8
MS3-38						
MS3-46	27.9	68.5				
MS3-48	12.4	23.7	12.4			
MS3-49	169.2	153.2	31.7	7.4		
MS3-51	347.2	1765.7	36.5	828.8	18.3	192.2
MS3-52	534.8	587.1	97.2	14	6.1	61.5
MS3-53	840.4	4964.5	44.2	493.0	9.7	135.5
MS3-54	61.2	148.9			5.1	54.5
MS3-55						
MS3-56	47.0	5.7				
MS3-57	47.7	2.2				
MS3-58	1243.7	4248.0	6.5	259.1	103.6	1050.6
MS3-59	920.9	3807.4			2	365.4
MS3-60	108.5	311.6				
MS3-61	203.4	220.6				
MS3-62	788.7	2794.4			49.3	603.6

Table 2.1 continued…

Station	*E. vallentini*		*E. longirostris*		*N. megalops*	
1998	**abundance (ind.1000m^{-3})**	**biomass (mg.1000 m^{-3})**	**abundance (ind.1000 m^{-3})**	**biomass (mg.1000 m^{-3})**	**abundance (ind.1000 m^{-3})**	**biomass) (mg.1000 m^{-3})**
TR1	128.9	22.1				
TR2	256.9	819.8				
TR3	364.9	17.3				
BOX 1	369.8	421.9				
BOX 2	92.6	128.3				
BOX 3	90956.1	41866.5				
BOX 4	134.9					
Station 1	2226.9	15352.6				
Station 2	1644.8	1124.4			8.6	154.2
Station 3	8512.3	20588.1				
Mean	**2153.6**	**2265.8**	**9.1**	**106.5**	**25.6**	**529.4**
SD	**12031.2**	**6412.6**	**22.5**	**290.2**	**58.2**	**2037.8**

Table 2.2 Abundance and biomass values for the three euphausiid species in the vicinity of the Prince Edward Islands for April/May 1999.

Station	***E. vallentini***		***E. longirostris***		***N. megalops***	
1999	**abundance (ind.1000m^{-3})**	**biomass (mg.1000 m^{-3})**	**abundance (ind.1000 m^{-3})**	**biomass (mg.1000 m^{-3})**	**abundance (ind.1000 m^{-3})**	**biomass) (mg.1000 m^{-3})**
MS4-1	378.8	790.9	59.8	382.2	139.6	963.2
MS4-3	100.8	176.1	37.8	168.3	88.2	885.7
MS4-4	26.7	2.5	53.4	664.7	6.7	
MS4-5	361.6	1027.4	51.7	53.5	38.7	
MS4-7	539.0	4054.9			5.9	
MS4-8	106.5	77.9	21.3	26.9	106.5	865.3
MS4-8A	114.3	42.9				
MS4-9	407.4	2382.6	63.7	877.6	18.6	211.3
MS4-10	310.3	4927.2	19.3	820.8	50.1	232.7
MS4-11	392.0	397.9	65.3	13.9		
MS4-12	146.1	70.2			58.5	633.1
MS4-13	2217.1	17347.3				
MS4-14	563.8	2380.1	1.3	44.4	47.1	
MS4-15	144.4	656.9	72.2	39.7	72.2	666.3
MS4-16	1445.8	2838.0				
MS4-17	18.8	27.8			37.5	351.5
NT7	122.5	835.6		14.3	10.2	148.6
NT91	37.3	416.3	186.4	3040.9	11.7	194.6
NT92						
NT93			94.9	363.9	94.9	834.5
NT94	445.5	762.0			501.2	4696.6
NT96	245.1	243.6	637.3	1440.7	24.5	476.0
NT97						
NT99	483.7	183.1	3.0	108.8	12.1	154.5
NT101	97.2	22.9	6.1	371.4	4.6	94.7
MS4-33	247.1	33.6				
MS4-34	660.1	425.4				
MS4-36	214.1	57.3	42.8	788.1		955.9
MS4-38	1705.1	1801.0	12.5	450.4	6.3	133.9
MS4-40	166.0	222.4			15.6	322.3
MS4-42	249.2	125.3				
MS4-43	275.4	225.1			12.9	246.5
MS4-44	247.6	765.7				
MS4-45	2915.5	10967.9			4.3	82.6
MS4-50	520.1	970.9				
MS4-51	68.8	141.4	8.6	138.1		
MS4-52	561.1	1160.3	56.1	1118.3	1.8	37.5
MS4-53	543.6	2939.4		62.7	1.7	46.1
MS4-54	3033.7	1768.6			5.6	97.5
Mean	**515.7**	**1571.0**	**38.3**	**281.8**	**35.3**	**341.8**
SD	**739.8**	**3273.3**	**105.3**	**577.5**	**84.2**	**776.3**

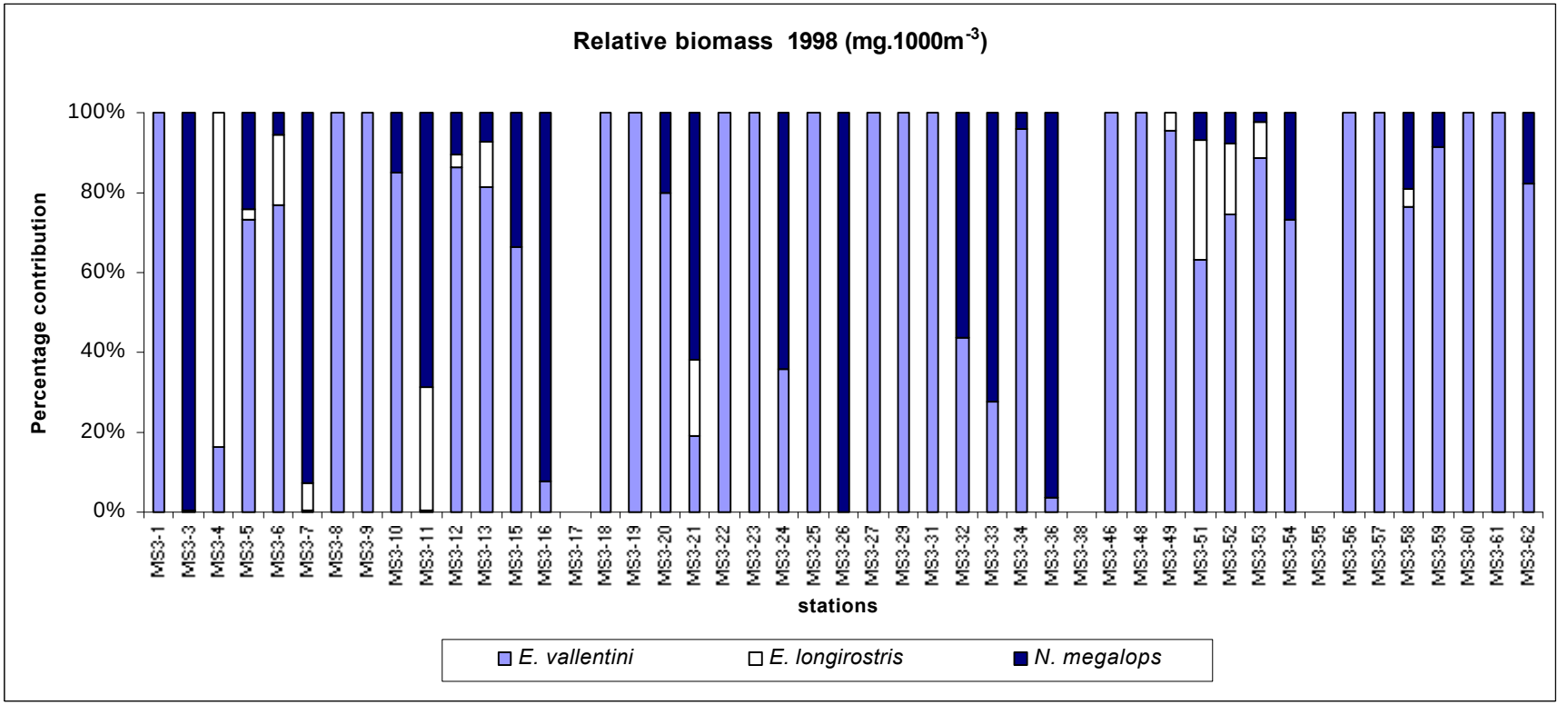


Figure 2.1 Relative abundance of *E. vallentini*, *E. longirostris* and *N. megalops* during MIOS 3, 1998.

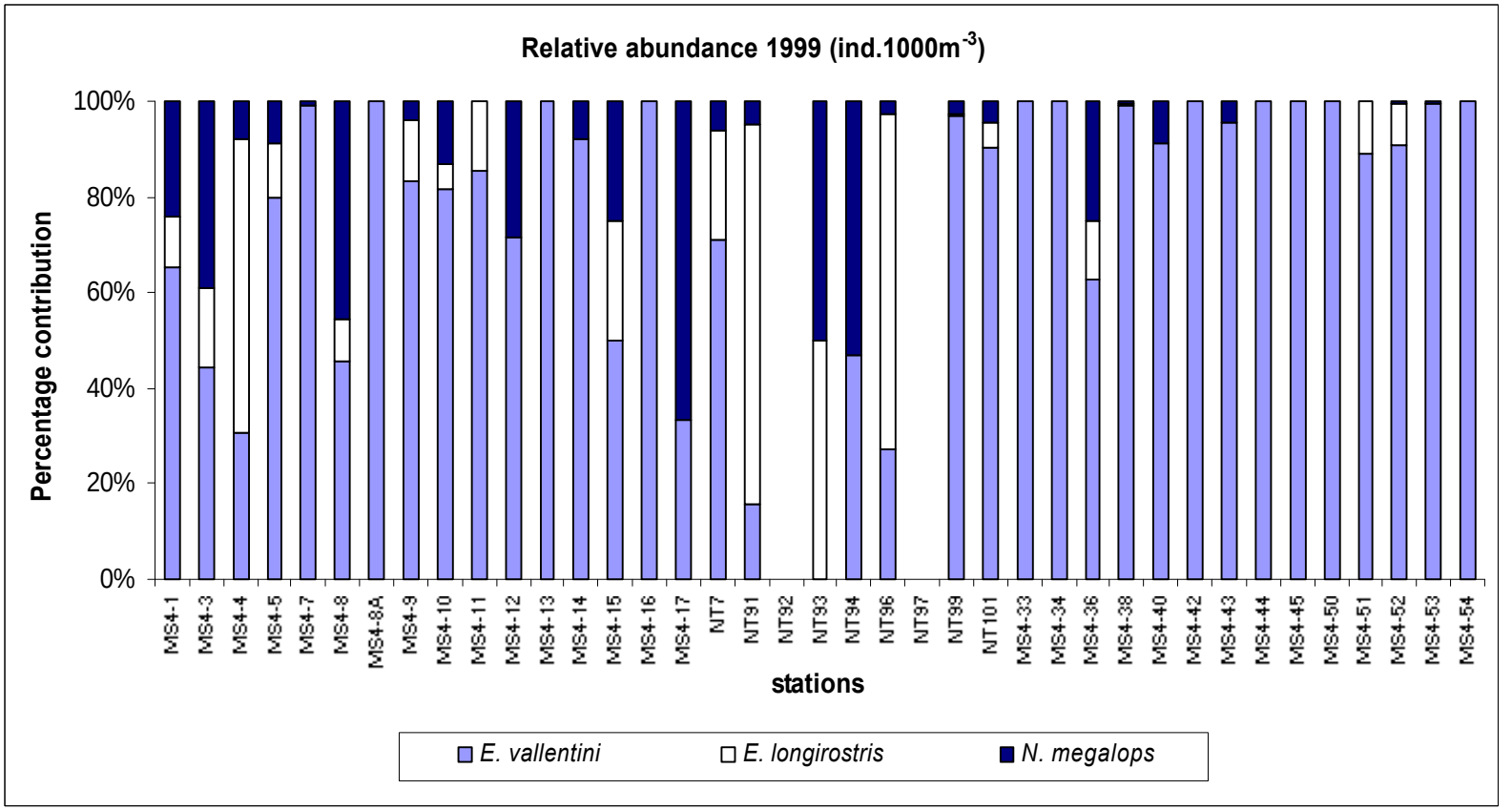


Figure 2.2 Relative abundance of *E. vallentini*, *E. longirostris* and *N. megalops* during MIOS 4,

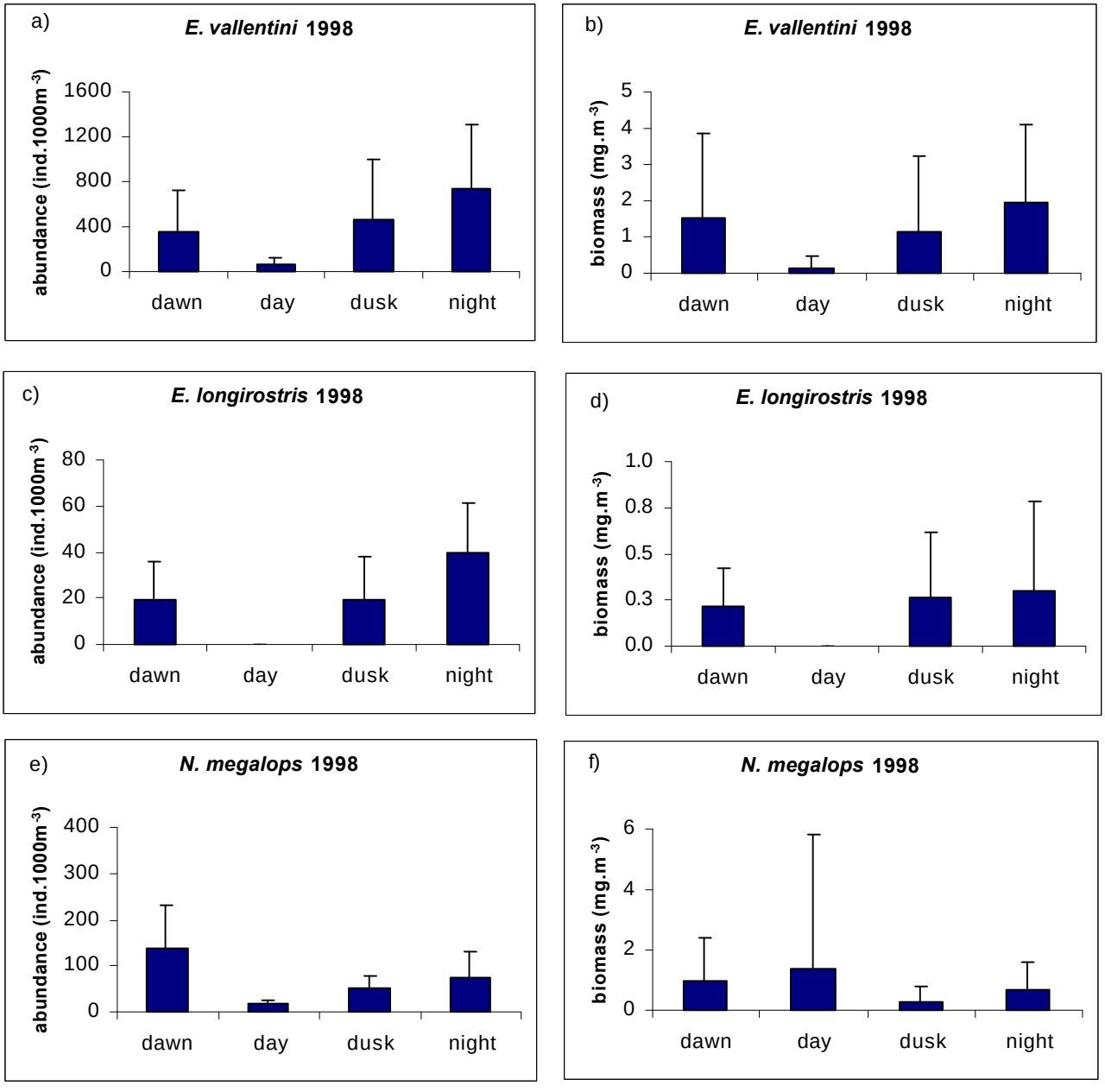


Figure 2.3 Diel patterns using abundance (ind.1000 m^{-3}) and biomass ($mg.m^{-3}$), of *E. vallentini* (a and b), *E. longirostris* (c and d) and *N. megalops* (e and f) in 1999 for four categories; Dawn: 5h00 to 8h00; Daylight: 8h00 to 17h00; Dusk: 17h00 to 20h00; Night: 20h00 to 5h00 (Only oceanic sites were considered for the analysis).

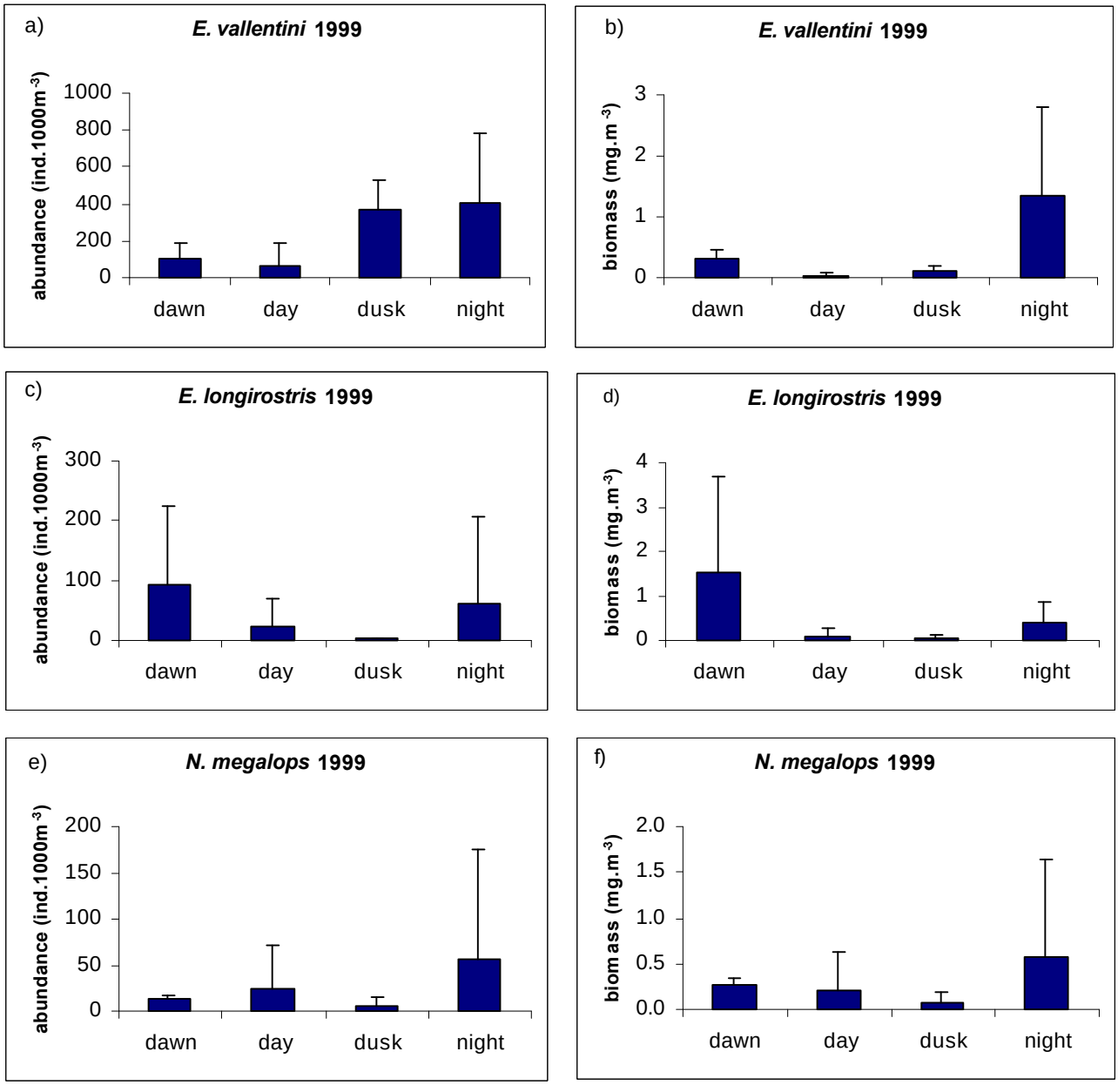


Figure 2.4 Diel patterns using abundance (ind.1000 m^{-3}) and biomass ($mg.m^{-3}$), of *E. vallentini* (a and b), *E. longirostris* (c and d) and *N. megalops* (e and f) in 1999 for four categories; Dawn: 5h00 to 8h00; Daylight: 8h00 to 17h00; Dusk: 17h00 to 20h00; Night: 20h00 to 5h00 (Only oceanic sites were considered for the analysis).

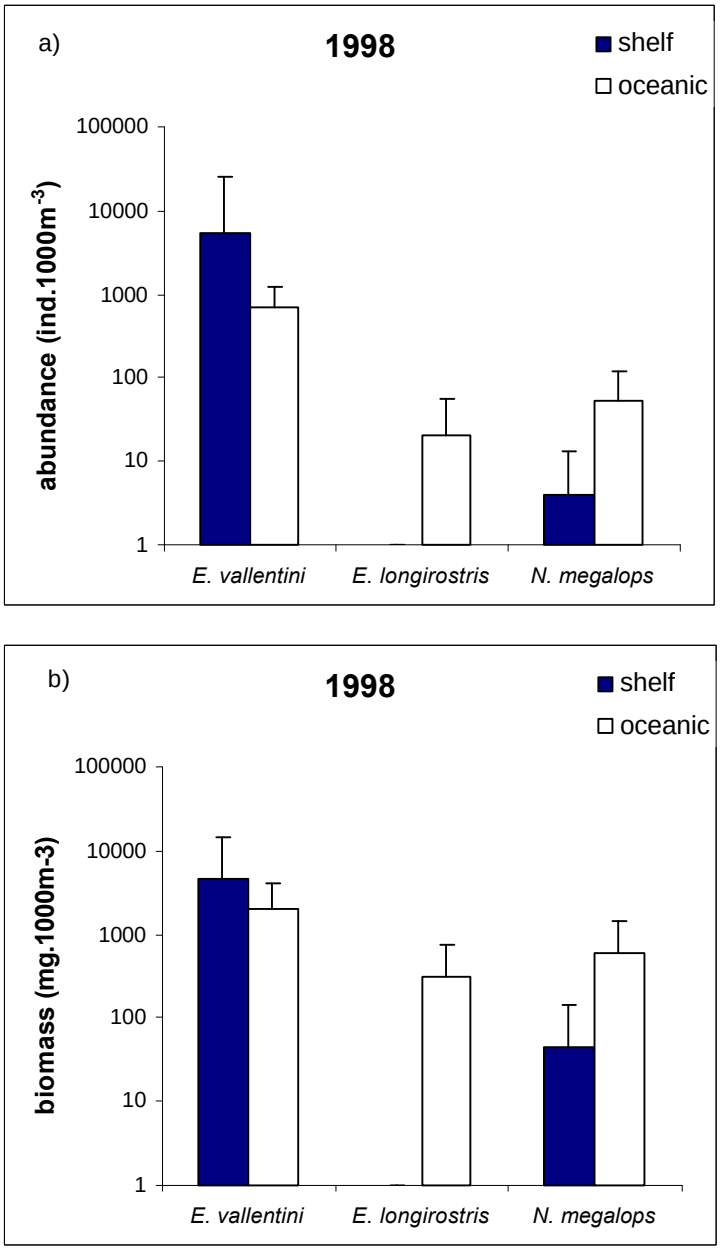


Figure 2.5 Comparison of abundance (a) and biomass (b) of *E. vallentini*, *E. longirostris* and *N. megalops* in shelf and oceanic sites in 1998 (Only night tows were considered for the analysis).

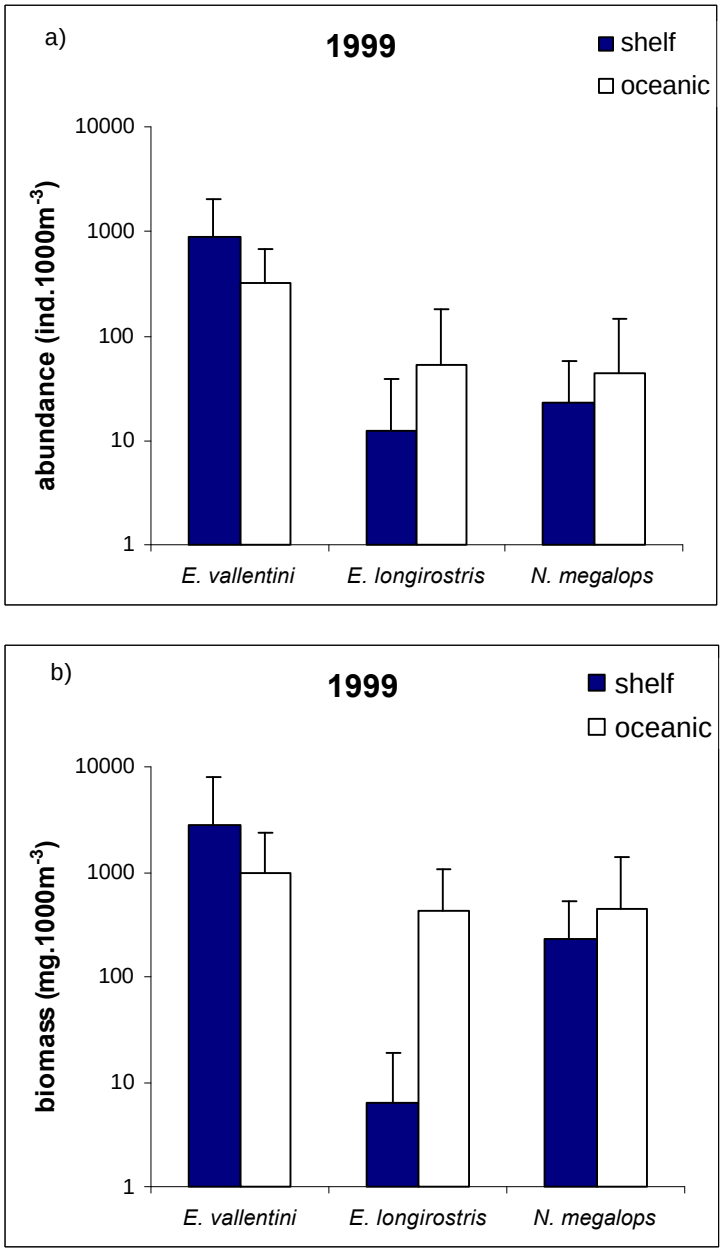


Figure 2.6 Comparison of abundance (a) and biomass (b) of *E. vallentini*, *E. longirostris* and *N. megalops* in shelf and oceanic sites in 1999 (Only night tows were considered for the analysis).

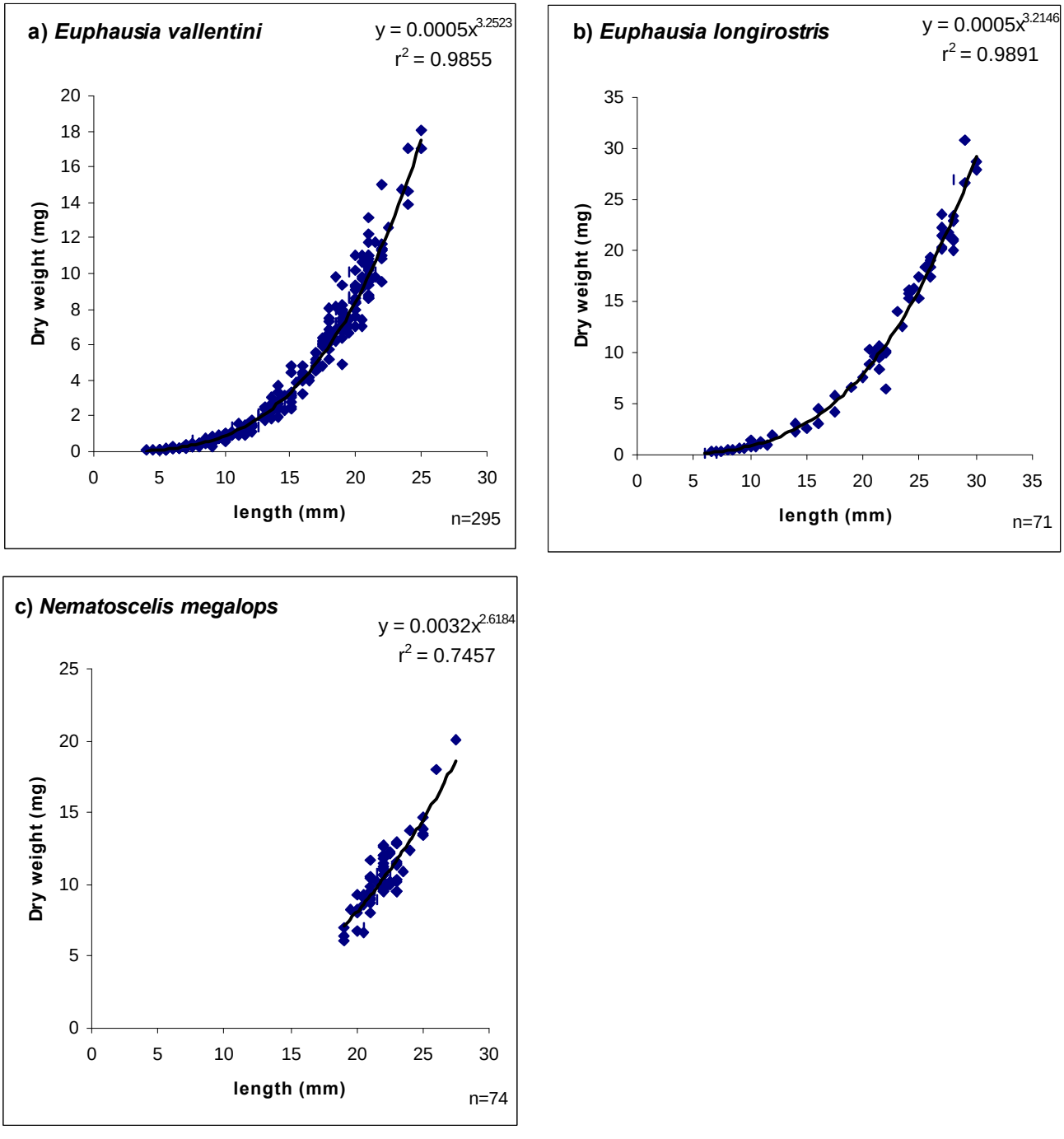
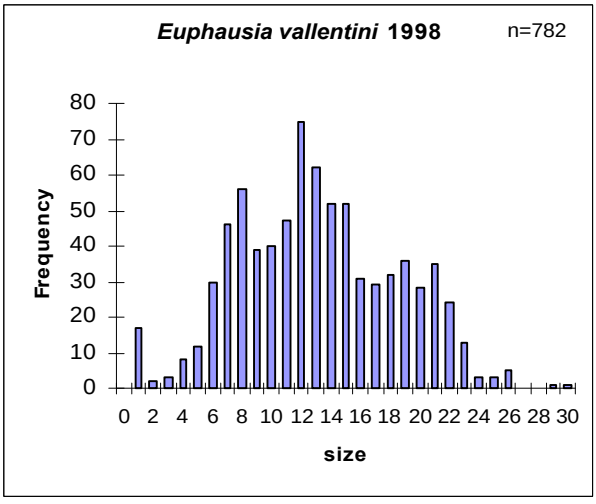
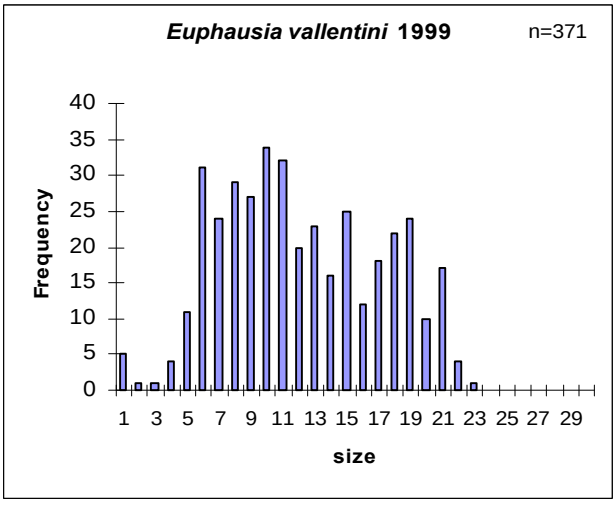


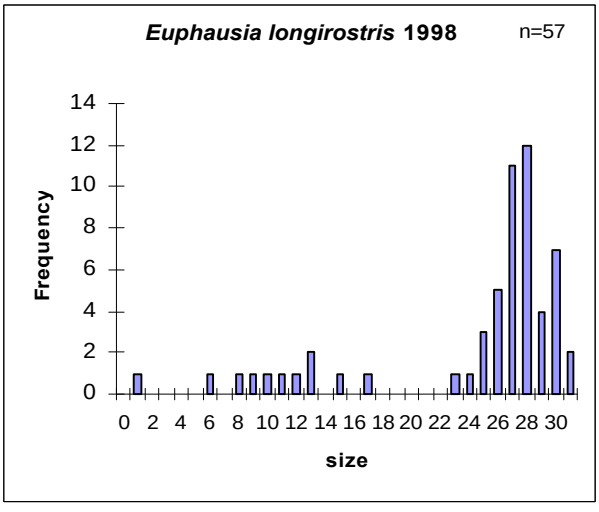
Figure 2.7 Length weight relationships for a) *E. vallentini*, b) *E. longirostris* and c) *N. megalops.*

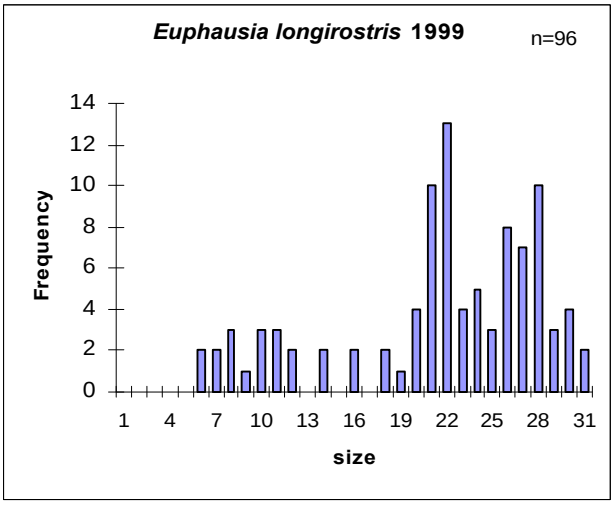
a)



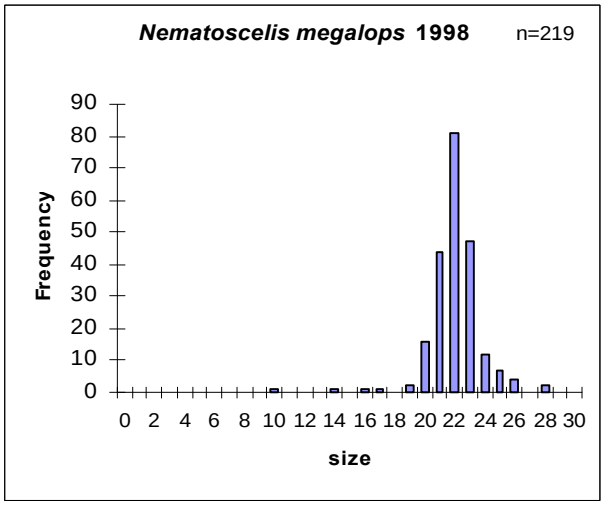


b)





c)



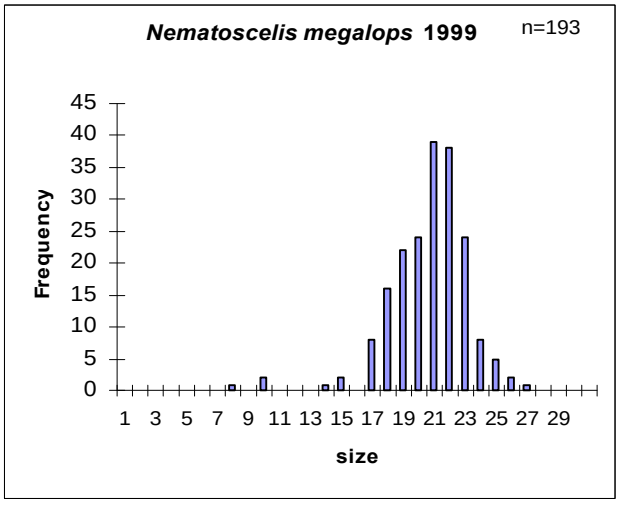


Figure 2.8 Size frequency histograms of a) *E. vallentini*, b) *E. longirostris* and c) *N. megalops* in 1998 and 1999.

Chapter 3
Gut content analysis

Introduction

Gut content analysis is one of the techniques traditionally used when considering the feeding biology of an organism (Hopkins 1985; Wassenberg and Hill 1989; Pasternak 1995; Maynou and Cartes 1997). Ideally, the analysis of stomach contents allows one to assess the relative proportions of articles found in the gut and provide quantitative information on the diet of the organism. However, in the case of euphausiids, accurately quantifying articles represented in the gut is particularly difficult.

Although *Euphausia* spp. have a well developed stomatogastric system in the foregut (Suh and Nemoto 1988), articles with hard frustules (e.g. diatoms) can be resistant to maceration by the mouthparts and the armature of the stomach wall and are often over represented in gut content analysis (Mauchline and Fisher 1969; Roger 1973; Mauchline 1980). The converse is also true for soft bodied organisms, or those lacking any form of armature (for example athecate dinoflagellates and gelatinous zooplankton), which become crushed and unrecognisable in the stomach (Mauchline 1980). Consequently, these components may be underestimated in the diet. The amount of time that has elapsed since an animal's last meal, and the amount of food ingested during that meal, will also affect what is found in the stomach (Sardà and Valladares 1990).

Quantitatively assessing the relative contribution of carnivory is also difficult. Indigestible parts of prey items, for example copepod mandibles, are often observed in euphausiid stomach contents (Sullivan *et al.* 1975). One way of quantifying the contribution of copepod fragments is by making estimates of the dimensions of the mandibles found in the stomach (Barange *et al.* 1991; Karlson and Båmstedt 1994). These fragments can often be identified to species level which can be very valuable. However, even these carefully made estimates may provide inaccurate results. Some euphausiids do not ingest their prey entirely but have been observed to pierce the body

wall of their copepod prey and suck out the body contents (Ponomareva 1955 in Mauchline and Fisher 1969). In these instances where the exoskeleton is discarded, the origin of the material which enters the stomach is often unidentifiable (Mauchline and Fisher 1969; Mauchline 1980). Ohman (1984) found that when *E. pacifica* preyed on copepods there was incomplete consumption of 22% of prey items, large portions of the prey body were not removed. In contrast, *Euphausia superba* has been observed in the laboratory to consume the entire animal in almost all cases (<1 % of copepods captured were only partly consumed) indicating that this species either ingests its prey whole or retains and ingests all pieces of it (Price *et al*. 1988). Therefore, laboratory observations need to be undertaken for each species if any quantitative assessment of the fragments represented in the stomach contents is to be made. These factors highlight why it is impossible to get quantitative data from such analyses and illustrate why a list of dietary components may often be misleading (Mauchline 1980; Ohman 1984).

In order to make quantifiable estimates of stomach contents, percentage composition by number (%N), percentage composition by volume (%V) or weight (%W), and percentage composition occurrence (%O) would be necessary (Tirasin and Jørgensen 1999). Because of the complications of misrepresentation of articles in the gut, I have chosen for this study to limit the data to percentage composition by number and percentage occurrence. Percentage composition by number represents the proportion of a particular item relative to the total number of all items counted in the stomach, and provides information on each individual's feeding behaviour. Percentage occurrence provides information on the proportion of stomachs containing a particular prey item, irrespective of the amount, and reflects the uniformity with which a particular prey item is selected by a group of specimens (Tirasin and Jørgensen 1999).

For this study, the stomach contents of *Euphausia vallentini* adults and juveniles, *E. longirostris* and *Nematoscelis megalops* were analysed and the relative trophic positions of these species assessed.

Materials and methods

Ten adult specimens of each species were randomly selected from samples collected during a 24 hour station (MS3-33, MS3-34 and MS3-36) in 1998 (Table 3.1). In addition, ten *Euphausia vallentini* adults and ten *E. vallentini* juveniles were selected from an *ad hoc* tow, Box 3, from the same year. Length measurements were made of each individual from the tip of the rostrum to the tip of the telson with 0.5 mm precision. Stomachs were carefully dissected out and each individual was weighed using a Sartorius microbalance. Stomach fullness was estimated visually and the contents were then emptied into a cavity slide, mixed as evenly as possible and allowed to settle (Hopkins and Torres 1989; Hopkins *et al.* 1993). Individual food items were identified and counted using an inverted microscope operated at 200 to 400 x magnification. Items were identified to species level, where possible, using the works of Priddle and Fryxell (1985), Boden and Reid (1989) and Thomas (1996). In samples where there was a high density of articles, all objects in every 5^{th} field of view were counted until 500 or more objects had been counted. In samples where subsampling occurred, the whole sample was inspected for large items (e.g. whole copepod carapaces). In many cases the entire sample was considered (29/50 of the samples). An ANOVA, followed by a Newman-Keuls multiple range test, was employed compare the dry weights of specimens.

Results

Mean lengths and weights of the specimens analysed are shown in Table 3.1. Results from the analysis of dry weight showed that *Euphausia vallentini* adults from the *ad hoc* tow were slightly, but not significantly, larger (in terms of length and weight) than those taken from the downstream station ($p>0.05$). Both groups of adult *E. vallentini* were significantly larger than the juveniles (ANOVA, $p<0.05$; Newman-Keuls multiple range analysis, $p<0.05$). *Nematoscelis megalops* were of a similar size to *E. vallentini* adults while *E. longirostris* individuals were significantly larger than all other groups ($p<0.05$).

Results from the gut content analyses are presented in Table 3.2. Detailed results are included in Appendix 2. *Euphausia vallentini* adults and juveniles had similar phytoplankton contributions of between 37 and 42 %. *Euphausia longirostris* and *N.*

megalops had similar phytoplankton contributions of 30 and 32 % respectively. Of the diatoms identified in all stomachs analysed, *Fragillariopsis* spp. accounted for the highest percentage by number (over 85 %). The percentage occurrence was 100 % for all groups, with the exception of *N. megalops*. Although the percentage contribution to all groups of *Thallasiosira* spp. was relatively low, species from this genus were observed in all but four individuals. Other diatoms, including *Dactyliosolen* spp., *Nitszchia* spp., *Navicula* spp., *Asteromphalus* spp., *Thalassionema* spp., and *Amphisolena* spp., were observed but constituted less than 9 % of diatoms represented in the stomachs of all groups combined. Similar amounts of protoplankton were found in all groups, ranging from 7.0 % in *E. vallentini* juveniles to 12.7 % in *N. megalops*. *Nematoscelis megalops* had the lowest percentage occurrence of all groups for tintinnids and silicoflagellates. Ciliates were particularly difficult to identify and may have been underestimated because of this. 'Round bodies' were unidentifiable objects that were most likely protozooplankton, but may also have included some crustacean eggs.

Euphausia vallentini adults (from both stations) and juveniles had low mesozooplankton contributions, ranging between 1.2 and 2.2 %. These low percentages did, however, include carapace fragments and appendages of crustaceans for all individuals. Ommatidia (usually the crystalline cones of crustacean compound eyes) were observed in 19 of the 30 specimens and *Limacina* shells were only observed in one adult. *Euphausia longirostris* had the highest mesozooplankton contribution of 23.4 %, but this was largely attributed to the high contribution of *Limacina* shells. When shell fragments are excluded from the analysis, the mesozooplankton contribution dropped to 5.6 % for this species. All individuals of this species had carapace fragments, and all but one had crustacean appendages. Mesozooplankton fragments comprised 12.7 % of the articles counted in *N. megalops*. A high percentage occurrence was observed for both carapace fragments and appendages. Only one individual was found to have ommatidia and no *Limacina* shells were found. The contribution of setae ranged from 18 to 35 %. The origin of this material could not be identified and contribution to diet was therefore difficult to determine as the ingestion of, for example, one polychaete could produce hundreds of setae. The contribution of foraminiferans was low for all groups considered. The remaining groups

consisted of unidentifiable material and boluses of amorphous material found in all specimens and ranged from 15.0 to 24.4 % in number for all groups considered.

Discussion

Cod-end feeding must be considered when assessing the gut contents of organisms. Hopkins (1985) stated that, although cod-end feeding could not be discounted, the environment in the cod-end was turbulent during the time of capture and euphausiids were not observed consuming prey once brought up from depth. In this study, the effect of cod-end feeding for *N. megalops* was probably negligible as many individuals (approximately 70 %) showed evidence of barotrauma with the eyes often damaged. In contrast, *E. longirostris* individuals were generally in good condition and had various articles in their feeding baskets.

A considerable contribution of phytoplankton, in the form of diatoms, was found for each group considered. *Fragillariopsis* spp. were identified by Pavlov (1971a and b in Mauchline 1980) as having robust frustules that are often over-represented in gut content analysis. He stated that the less robust diatoms are easily destroyed and will not be found, particularly if stomachs are not dissected out soon after capture. *Euphausia vallentini* adults (from both stations) and juveniles had the highest contribution of diatoms numerically (≈ 40 %), and the percentage occurrence (%O) for these three groups was high. The contribution of diatoms to the diet of *E. longirostris* was lower than for *E. vallentini,* but the percentage occurrence was also high. As both euphausiid species have feeding appendages which are adapted to filter feeding this result was to be expected. Although the contribution of phytoplankton to *N. megalops* was similar to that of *E. longirostris*, the percentage occurrence of diatoms was lower than for all other groups (between 30 and 70 %). As with previous studies (Mauchline 1980) it was not possible to assess whether the phytoplankton had been grazed on directly by this species or whether it was secondary in origin and had entered the gut through feeding on herbivores or on faecal pellets.

Although the contribution of protoplankton to the diet of all groups of euphausiids was not high, it may be a nutritionally important component (Hitchcock 1982). Biochemical studies have shown that many diatoms do not represent a food source of high nutritional value relative to other planktonic groups (Ben-Amotz *et al.* 1987) and that the nutritional value of ciliates and dinoflagellates may be as much as 3 – 5 times higher than for diatoms (Hitchcock 1982). This has been shown experimentally where zooplankton selected against diatoms when offered both diatoms and dinoflagellates (*E. lucens*; Stuart 1989) or ciliates (copepod, Fessenden and Cowles 1994; *E. superba*; Granéli *et al.* 1993; Atkinson 1996). The absence of tintinnids and silicoflagellates in the diet of *N. megalops* may be related to the nature of the mandibles of this species which are unable to crush these food items.

Mesozooplankton, although in some cases a relatively minor component of the diet (e.g. *E. vallentini* adults and juveniles), may also be of high nutritional value. Experimental results from euphausiid incubation experiments have shown that copepod prey are preferentially select for over diatoms (Granéli *et al.* 1993). Furthermore, feeding rates on copepods are higher in comparison to diatom diets (Stuart 1986; Price *et al.* 1988; Pilditch and McClatchie 1994; Atkinson 1996). The highest contribution of mesozooplankton fragments was found in the largest euphausiid in this study, *E. longirostris*, and it was the only group for which whole copepod carapace fragments were recorded. In one instance the carapace of the head region of a *N. megalops* individual, including its elongated second appendage, was found. When the contribution of *Limacina* shells is excluded from the results for *E. longirostris*, mesozooplankton fragments contributed less than 6 % of all articles counted. The shell of one *Limacina* individual may result in hundreds of fragments in the stomach and so its exclusion from the analysis may give a more accurate estimation of the diet. When the *Limacina* shell fragments were excluded from the analysis, *E. longirostris* was shown to have an omnivorous feeding habit, with the percentage contribution of mesozooplankton fragments half of that observed for *N. megalops.* The differences in the mode of capture may be an important factor in interpreting these results. If *E. longirostris* consumes its prey entirely (as is the case for *E. superba*) and *N. megalops* does not, the identifiable fragments observed in the stomach will be different for each species.

Species that have elongated thoracic appendages, such as *N. megalops*, have a more carnivorous diet than species which lack them (Roger 1973), and a relatively high contribution of mesozooplankton fragments was found for this species. If *N. megalops* pierces the prey (suggested by the lack of whole carapaces in the stomach contents) this estimate may be conservative. It should be noted that the visual estimates of gut fullness for all specimens *of N. megalops* were particularly low (10 % or less). This may be due to the species being found in sub-optimal conditions (southern limit of its distribution) or because digestion is generally quicker for carnivorous species (Sarda and Valladares 1990).

Included among the mesozooplankton fragments were ommatidia. Only one specimen of *N. megalops* contained ommatidia, but all *E. vallentini* adults from Box 3 were found to contain these fragments. Ommatidia have been recorded in the stomachs of many euphausiids (Mauchline 1980). Ponomareva and Kuznetsova (1989) proposed that during periods when the availability of phytoplankton is limited, euphausiids may consume ommatidia to supplement their diet, particularly with Vitamin A as this nutrient is concentrated in euphausiid eyes.

Finally, large boluses of material which could not be separated out and identified were observed in all stomachs examined. Mauchline (1980) states that this is inevitable as soft bodied prey items, amorphous material and other ingested articles at various stages of digestion may not be recognisable.

Conclusions

It is difficult to gain an accurate estimate of the true diet of organisms using gut content analysis alone. However, the results from this analysis showed that all euphausiid groups examined exhibited some degree of omnivory. *Euphausia vallentini* adults and juveniles were both predominantly herbivorous, while *E. longirostris* and *N. megalops* both exhibited a relatively high degree of carnivory.

Table 3.1 Station numbers, average dry weight, length and gut fullness for each of the five groups of specimens on which stomach content analysis was performed, n = 10 for each group (J = juveniles; A = adults).

Species	**Station**	**Mean length** ±SD (mm)	**mean dry wt** ±SD (mg)	**Mean gut fullness** (%)
***E. vallentini* (J)**	Box 3	11.50 ±0.47	1.39 ±0.31	23
***E. vallentini* (A)**	Box 3	22.10 ±0.74	14.00 ±2.90	53
***E. vallentini* (A)**	MS3-36	20.15 ±2.16	9.01 ±2.86	17
E. longirostris	MS3-33/-34/-36	29.15 ±1.23	24.90 ±3.36	78
N. megalops	MS3-36	21.60 ±1.05	10.85 ±1.83	6

Table 3.2 Stomach contents of the five groups of euphausiid analysed from samples collected in 1998, n = 10 for each group. (%N = percentage of articles counted within a group; %O = percentage occurrence within a group; J = juveniles; A = adults).

	***E. vallentini* (J) Box 3**		***E. vallentini* (A) Box 3**		***E. vallentini* (A) MS3-36**		***E. longirostris* MS3-33/34/36**		***N. megalops* MS3-36**	
	%N	%O	%N	%O	%N	%O	%N	%O	%N	%O
Phytoplankton	**42.0**		**37.4**		**41.7**		**30.5**		**32.6**	
Fragillariopsis spp	38.8	100	34.4	100	35.2	100	23.7	100	26.4	60
Thalassiosira spp	1.9	90	1.3	100	3.4	100	2.4	100	3.1	70
Diatoms *	1.3	80	1.8	100	3.2	90	4.4	90	3.1	30
Protoplankton	**7.0**		**11.8**		**9.5**		**10.8**		**12.7**	
Dinoflagellates	0.4	50	1.2	90	0.6	70	0.7	80	0.4	30
Dinoflagellate cysts	0.3	40	1.5	100	1.2	50	1.5	100	1.0	20
Tintinnids	0.6	60	1.2	90	3.0	90	2.4	100	3.5	30
Silica flagellates	3.1	90	3.0	100	0.8	60	2.5	100	2.4	10
Ciliates	0.0	0	0.0	0	0.0	0	0.3	40	0.0	0
Round bodies	2.5	100	4.9	100	3.9	80	3.3	80	5.4	80
Mesozooplankton fragments	**1.3**		**1.2**		**2.2**		**23.4**		**12.7**	
Carapace fragments	0.4	100	0.2	100	1.2	100	1.5	100	5.4	90
Appendages	0.4	100	0.4	100	0.6	100	2.9	90	6.6	90
Ommatidia	0.5	60	0.5	100	0.4	30	1.2	80	0.7	10
Limacina shells	0.0	0	0.0	10	0.0	0	17.9	80	0.0	0
Setae	**30.7**	100	**34.6**	100	**25.2**	100	**18.7**	100	**17.7**	50
Foraminiferans	**0.1**	20	**0.1**	30	**0.0**	10	**0.2**	60	**0.3**	20
Other	**19.0**		**15.0**		**21.3**		**16.6**		**24.4**	
Unidentifiable	10.3	100	7.7	100	11.2	100	14.0	100	13.1	100
Bolus	8.6	90	7.3	100	10.1	100	2.3	100	11.0	10
Articles counted (total)	**1694**		**4294**		**2709**		**6128**		**1176**	

* diatoms identified to genus level but not listed as such because of low occurrence

Chapter 4
Stable nitrogen isotopes

Introduction

A major obstacle in understanding natural ecosystem processes is the determination of trophic relationships (Paine 1988). This is particularly true of aquatic systems where the accurate determination of predator-prey relationships may be virtually impossible using conventional techniques (Hobson and Welch 1995). Many trophic interactions cannot be observed visually and techniques traditionally employed, including feeding experiments and gut content analysis, have their limitations (see Chapters 3, 5 and 6). An alternative method of measuring the trophic positions of organisms has been developed using stable nitrogen isotopes.

Nitrogen occurs naturally as two isotopes, ^{14}N and ^{15}N. Fractionation between ^{14}N and ^{15}N occurs during food assimilation as the lighter isotope is metabolised more readily and is preferentially excreted as a by-product of protein synthesis, leaving the animal enriched in ^{15}N relative to its diet (Dunton *et al.* 1989; Kling *et al.* 1992; Holmes 1996). As this effect is cumulative, stable nitrogen isotope ratios ($\delta^{15}N$) can be used to identify trophic position (Minagawa & Wada 1984; Dunton *et al.* 1989; Sugisaki *et al.* 1991; Wada *et al.* 1991; Kling *et al.* 1992; Hobson 1993; Thomas and Cahoon 1993; Gu *et al.* 1994; Michener and Schell 1994; Hobson and Welch 1995; Jennings *et al.* 1997). Minagawa and Wada (1984) reported an average stepwise increase of $3.4 \pm 1.1‰$ $\delta^{15}N$ between trophic levels across a wide range of habitats including terrestrial, freshwater and marine.

The strength of stable-isotope analysis is that it is an accurate time-integrated measure of food assimilation, or feeding history (Kling *et al*. 1992; Hobson 1993). The time period reflected by the isotopic analysis is determined by the turnover rate of the proteins in the tissue examined (Tieszen *et al.* 1983).

For this study, the stable nitrogen isotope ratios of *Euphausia vallentini* adults and juveniles, *E. longirostris* and *Nematoscelis megalops* were analysed to assess the relative trophic positions of these three species.

Materials and methods

Stable nitrogen isotope analysis was carried out on the same euphausiid individuals that were used for the gut content analysis (Table 4.1). This included *E. vallentini* juveniles and adults from the *ad hoc* tow, Box 3, and adult specimens of the three species of euphausiid collected at the 24 h station (Table 4.1). In addition to these specimens, the copepod *Calanus simillimus* and the hyperiid amphipod, *Themisto gaudichaudii* were selected from the same samples as the euphausiids. *Ceratoscopelus warmingi*, a small planktivorous fish was sampled aboard the rv *Africana* in March 1994 (Station A15917-119, tr 18). These additional species were chosen to form a framework of species with known dietry habits within which the euphausiids could be placed.

Samples collected on the *SA Agulhas* were preserved in buffered Formalin (4 %) for six months before analysis. The fish *C. warmingi*, were initially preserved in formalin and later transferred to 70 % alcohol. All samples were rinsed and then submerged in distilled water for six hours to leach remaining preservative from the specimens (Mullin *et al.* 1984). Stomachs were removed from all specimens with the exception of *C. simillimus* where the removal of the stomachs was not possible because of their small size.

All samples were dried at 60ºC for 48 hours. Individual animals were weighed using a Sartorius microbalance and then manually pulverised using a mortar and pestle. Twenty *C. simillimus* specimens were pooled for each sample. Muscle tissue was dissected from the fish for analysis.

Subsamples of between 0.6 and 0.7 mg were weighed out for nitrogen isotope analysis. Ten replicate individuals were analysed for each species, with the exception of *E.*

longirostris for which n = 8. Replication was also performed on individual specimens using between 2 and 5 subsamples. Two internal standards (namely Merck gelatine and Valine) were analysed after every tenth sample to allow for accurate calibration of the mass spectrometer.

Measurements of stable nitrogen isotope ratios were made using a dual inlet automated elemental analyzer coupled to an isotope ratio mass spectrometer (Finnigan MAT 252). Stable nitrogen isotope ratios were expressed as parts per thousand (‰) according to the following relationship:

$$\delta^{15}N = [(Rsample/Rstandard) - 1] \times 10^3$$

where R is the corresponding $^{15}N/^{14}N$ ratio. The standard for nitrogen is atmospheric N_2 (air) which is assigned an arbitrary $\delta^{15}N$ value of 0‰ (Holmes 1996).

An ANOVA was used to determine whether there were significant differences between groups for the dry weight and nitrogen isotope ratios data. A Newman-Keuls multiple range test was used to identify group interactions.

Results

Results from the isotope analyses are presented in Table 4.1. Dry weight and nitrogen isotope ratios differed significantly between groups (results of the Newman Keuls multiple range analysis are presented in Table 4.2). As expected, the lowest $\delta^{15}N$ value was found in the herbivorous copepod, *C. simillimus* (Table 4.1). Among the euphausiids, *E. vallentini* juveniles had a low mean $\delta^{15}N$ value, slightly but significantly higher than *C. simillimus* (Table 4.2), which suggests that they exhibit a largely herbivorous diet, supplemented by a minor heterotrophic component.

Euphausia vallentini adults from both stations had significantly higher $\delta^{15}N$ values than the juveniles (Tables 4.1. and 4.2). *Euphausia vallentini* adults from Box 3 had slightly higher isotope ratios than those taken from the downstream station, but the difference between them was not significant (Table 4.2). *Euphausia vallentini* adults from both

stations were closely grouped with the hyperiid, *T. gaudichaudii*; they were slightly, but not significantly depleted in ^{15}N in comparison to it. *Themisto gaudichaudii* had a $\delta^{15}N$ 2.41 ‰ higher than that of *C. simillimus*. *Euphausia longirostris* and *N. megalops* had very similar mean $\delta^{15}N$ values 2.7 ‰ above the hyperiid. Finally, a stepwise increase of 2.7 ‰ was observed between these two euphausiids and the panktivorous fish which was found to have the highest $\delta^{15}N$ value.

Within sample replication had a standard deviation of between 0.12 and 0.41 ‰.

The dry weights of all groups analysed are presented in Table 4.1. Four size groups were identified from the multiple range analysis (ANOVA $p<0.05$; Newman-Keuls $p<0.05$) (Table 4.2). *Calanus simillimus* specimens and *E. vallentini* juveniles were smallest in size and were not significantly different from each other ($p>0.05$). *Euphausia vallentini* adults from Box 3 were slightly, but not significantly larger than those collected from the downstream station (Table 4.2). *Euphausia vallentini* adults from both stations, *T. gaudichaudii* and *N. megalops* all grouped out together with mean values between 9.01 and 14.00 mg, while *Euphausia longirostris* was significantly larger (mean of 24.9 ± 3.36). *Ceratoscopelas warmingi* was the largest group with a mean dry weight of 33.89 (± 13.27).

Discussion

Absolute isotopic values vary among systems depending on the value at the base of the food web (Kling *et al.* 1992). The $\delta^{15}N$ values reported here are relatively depleted when compared with marine zooplankton sampled elsewhere (e.g. Sugisaki *et al.* 1991). This coincides with the relatively low nitrogen isotope ratios reported for source nitrogen by Altabet and Francious (1994) at 46° S in the south-western sector of the Indian Ocean.

The transfer of nitrogen through trophic levels is characterized by a systematic increase in $\delta^{15}N$ with each trophic level, and the results here show that the euphausiids investigated were trophically different. Although *Calanus simillimus* has been observed to feed on microzooplankton (Ward *et al.* 1996), it may be considered predominantly

herbivorous. *Euphausia vallentini* juveniles grouped out with the copepod, but were slightly enriched in comparison to it. This suggests that this group was principally herbivorous but may exhibit a small degree of omnivory. *Euphausia vallentini* adults grouped out with the hyperiid amphipod, *T. gaudichaudii,* which has been described as an opportunistic predator (Pakhomov and Perissinotto 1996b). As discussed earlier, Minagawa and Wada (1984) reported an average stepwise increase of 3.4 ± 1.1 ‰ between trophic levels. Considering this, the isotope value observed for *T. gaudichaudii* was lower than expected (2.41 ‰ above the copepod). To offer a possible explanation for this, pigments from phytoplankton have been observed in the stomachs of *T. gaudichaudii* (Pakhomov and Perissinotto 1996b) and other hyperiid amphipods (*T. japonica*; Sugisaki *et al*. 1991). However, it is not certain whether the phytoplankton was consumed directly by the amphipod or whether the pigments were secondary in origin (i.e. consumed by the prey prior to capture). The isotope signature obtained here suggests that, irrespective of the origin of the material, it is assimilated into the organism and the isotope ratio reflects that of an omnivore.

The results for *E. vallentini* show that there is a dietry shift between the two size classes from a principally herbivorous to an omnivorous diet. Similar dietry shifts have been observed in other euphausiids. For example, Mauchline and Fisher (1969) found that the diets of young *Meganyctiphanes norvegica* and *Thysanoëssa raschi* were different from the diets of older individuals, with older and larger individuals of both species demonstrating a higher degree of carnivory. A study by Wiegmann (1970 in Mauchline 1980) also supports these findings as crustacean remains were more common in the stomachs of larger individuals of *Euphausia diomedeae*, *E. sibogae* and *E. tenera*.

The remaining two euphausiids, *E. longirostris* and *N. megalops* group out above the amphipod at a single trophic position, suggesting that the relative contribution of carnivory to the diets of these two euphausiids was similar.

Many have attempted to use the theory of stepwise increases to delineate trophic position (eg Hobson 1993; Hobson *et al.* 1995) while others have found that the relative enrichment between predatory zooplankton and their prey, will depend on the extent of omnivory by the organism (Dunton *et al.* 1988; Kling *et al.* 1992; Jennings *et al.* 1997;

France *et al.* 1998). France *et al.* (1998) suggested that because of this, trophic continua are more appropriate than discreet trophic levels. Because only 6 species (or 7 groups) have been considered in this study, it is difficult to know if they represent discreet trophic levels, or isolated points of a continuum, resulting from varying degrees of omnivory (Fig 4.1).

Body size has been presented as an important factor in determining trophic position (France *et al.* 1998). In these results (Fig 4.2) it is clear that $\delta^{15}N$ was positively correlated with dry weight (log transformed data; $r^2 = 0.73$). There was however an interesting anomaly. The dry weights of *E. vallentini* adults, *T. gaudichaudii* and *N. megalops* were closely grouped (Table 4.2; $p < 0.05$) yet *N. megalops* had a significantly higher mean $\delta^{15}N$ value (Table 4.2; $p < 0.05$). This trophic position was shared with that of the larger euphausiid, *E. longirostris*. The extended second appendage of *N. megalops* is a morphological adaptation which assists in predation (Mauchline 1967), therefore, while body size is important in determining the trophic position that an organism occupies, species morphological adaptations cannot be overlooked.

Conclusions

The results of the stable nitrogen isotope analysis provided a clear indication of the trophic status of the three euphausiids. A dietry shift from herbivory to a largely omnivorous feeding habit was evident for the two size classes of *E. vallentini. Euphausia longirostris* and *N. megalops* were shown to occupy a similar trophic position, which was distinctly higher than that of *E. vallentini* adults.

Table 4.1 Results of stable nitrogen isotope ratios for each group analysed (A = adult; J = juvenile)

Species	**n**	**Station**	**Mean dry weight ± SD (mg)**	**Mean $\delta^{15}N$ ± SD (‰)**
Calanus simillimus	10	MS3-34	0.09 ± 0.01	1.72 ± 0.15
Euphausia vallentini (J)	10	Box 3	1.39 ± 0.31	2.38 ± 0.19
Euphausia vallentini **1** (A)	10	Box 3	14.00 ± 2.90	3.60 ± 0.51
Euphausia vallentini **2** (A)	10	MS3-36	9.01 ± 2.86	4.02 ± 0.74
Themisto gaudichaudii	10	MS3-33/-34/-36*	9.21 ± 4.63	4.13 ±0.50
Euphausia longirostris	8	MS3-33/-34/-36	24.90 ± 3.36	6.88 ±0.60
Nematoscelis megalops	10	MS3-36	10.85 ± 1.83	6.83 ±0.78
Ceratoscopelas warmingi	10	"Africana"	33.89 ± 13.27	9.55 ± 1.14

*majority of specimens from these stations

Table 4.2 Results of Newman-Keuls multiple range tests for dry weight and stable nitrogen isotope ratios (A = adult; J = juvenile; 1 = Box 3 station; 2 = MS3-36)

Species	**Dry weight Homogenous groups ($p < 0.05$)**	**$\delta^{15}N$ Homogenous groups ($p < 0.05$)**
Calanus simillimus	X	X
Euphausia vallentini (J)	X	X
Euphausia vallentini **1** (A)	X	X
Euphausia vallentini **2** (A)	X	X
Themisto gaudichaudii	X	X
Euphausia longirostris	X	X
Nematoscelis megalops	X	X
Ceratoscopelas warmingi	X	X

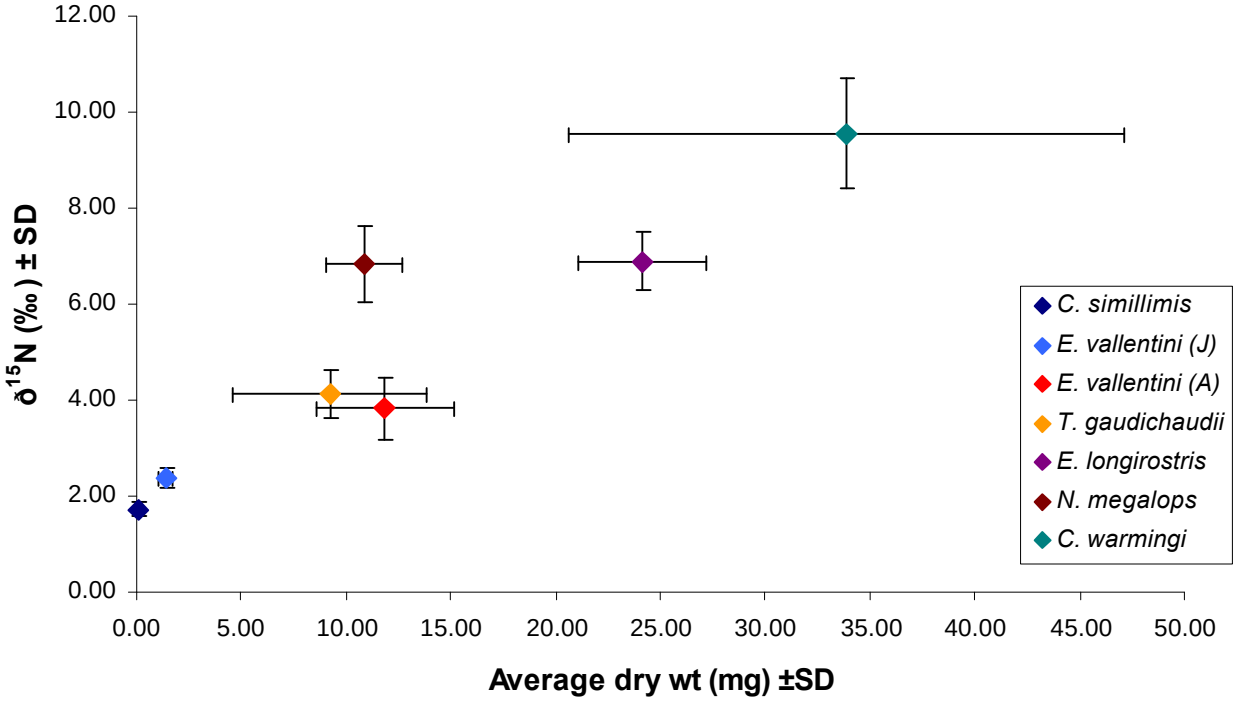


Figure 4.1 Graph of dry weight and stable nitrogen isotope ratios (A = adult; J = juvenile).

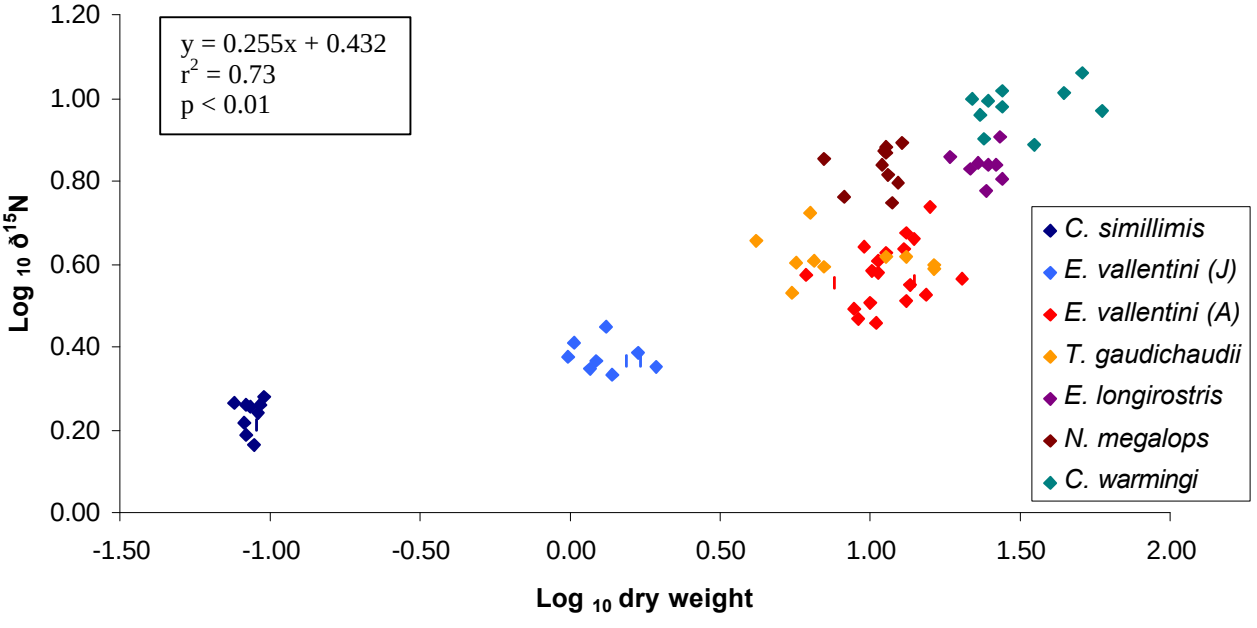


Figure 4.2 Graph of the relationship between stable nitrogen isotope ratios and dry weight (A = adult; J = juvenile).

Chapter 5
Gut fluorescence technique

Introduction

The gut fluorescence technique (Mackas and Bohrer 1976; Conover *et al.* 1986) has been widely used for the measurement of *in situ* feeding rates of zooplankton on phytoplankton (e.g. Kiørboe and Tiselius 1987; Tsuda and Nemoto 1987; Peterson *et al.* 1990; Atkinson *et al.* 1992; Pakhomov *et al.* 1997a and b; Perissinotto *et al.* 1997; Tirelli and Mayzaud 1999). Alternative methods for estimating gut evacuation rates include faecal pellet production (Clarke *et al.* 1988; Irigoien *et al.* 1996; Dilling *et al.* 1998) and radioactive tracers, which give results comparable to the gut fluorescence technique (Ellis and Small 1989). An advantage of faecal pellet production experiments is that in addition to an estimation of egestion, they provide a measure of digestive efficiency and an indication of the quality of the ingested food (Clarke *et al.* 1988). Filtration or clearance rates have been estimated using decline in chlorophyll *a* with time in closed (Meyer and El-Sayed 1983 in Knox 1994) and open systems (Antezana *et al.* 1982). Changes in cell numbers over time in *in vitro* incubations (Stuart 1986) or flow through systems (Morris 1984 in Knox 1994) have also been used to estimate zooplankton ingestion rates of phytoplankton (Boyd *et al*. 1984; Quetin and Ross 1985) and microzooplankton (Froneman *et al.* 1996) as well as other mixed assemblages, which included zooplankton prey items (Atkinson and Snÿder 1997).

The advantage of the gut fluorescence technique is that it is quick and inexpensive, and provides accurate estimates of *in situ* feeding rates. The method requires two measurements; the gut pigment content of recently captured animals, and an estimate of the gut clearance or evacuation rate (Ellis and Small 1989). Both of these parameters are determined fluorometrically. The gut pigment content is measured immediately after capture, while the gut evacuation rate is usually estimated experimentally by monitoring the decline in gut pigments over time in freshly collected animals incubated in filtered seawater (Perissinotto and Pakhomov 1996). Multiplication of these two values gives an

estimate of short-term *in situ* ingestion rate which is presumed to be equivalent to ingestion rates at steady state if degradation of chlorophyll derivatives to non-fluorescing products during gut passage have been accounted for (Baars and Helling 1985; Conover *et al.* 1986; Dagg and Walser 1987; Kiørboe and Tiselius 1987).

In this study, measurements of gut pigment content, gut evacuation rate and pigment degradation were combined in an attempt to provide estimates of *in situ* individual grazing rates for *Euphausia vallentini, E. longirostris* and *Nematoscelis megalops*.

Materials and methods

Specimens collected in the 500 μm mesh net were used for gut pigment content and for feeding experiments. A 2 litre cod-end was attached to the net to minimize damage to zooplankton. Three 24 hr stations were occupied in 1998 (MS3-3 to 8, MS3-29 to 38 and MS3-46 to 53), and one in 1999 (MS4-33 to 42). At these stations six tows were conducted at 4 hour intervals with the aim of studying diel patterns in feeding activity.

Gut pigment content

Euphausiid specimens were sorted immediately after capture. Three to 10 individuals were picked per taxon and placed in polyethylene tubes (one animal per tube) with 8 ml of 90 % acetone and stored in the dark at -20° C for 24 h. The tubes were then centrifuged for 5 minutes at 5000 rpm. The pigment content of the supernatant of the acetone extract was measured with a Turner 111 fluorometer before and after acidification (Mackas and Bohrer 1976). Chlorophyll *a* and phaeopigment contents per individual were calculated using the equations of Strickland and Parsons (1968), modified by Conover *et al.* (1986) and were expressed as total pigments (G, ng pigm.ind^{-1}). Background fluorescence estimates were made in 1998 by incubating euphausiids in filtered seawater with charcoal particles for 24 hours and measuring the fluorescence levels as described above. All gut pigment content measurements were corrected accordingly. Non-parametric Mann-Whitney U tests were performed to investigate

differences of initial gut pigment content between years for each species (or group for *E. vallentini*).

Gut evacuation rates / gut passage time estimates

Gut evacuation experiments were carried out using freshly collected specimens which were placed immediately after capture in 20 litre containers filled with 0.2 μm filtered seawater. Non-fluorescent charcoal particles (of ≤ 100 μm diameter) were added to the containers to simulate continuous feeding conditions (Willason and Cox 1987; Perissinotto and Pakhomov 1996). The particles were added in concentrations equivalent to ambient seston wet weight to reduce the effect of a change in the concentration of food before and after capture (Perissinotto and Pakhomov 1996). The incubations were kept on deck to simulate ambient light and temperature conditions. Incubation time ranged from three to eight hours. Three to five replicate animals were removed at 10-15 minute intervals for the first hour and at 20 minute to 1 hour intervals thereafter. Gut evacuation rates (k, h^{-1}) were derived from the slope of the natural logarithm of decline in gut pigments versus time (Dam and Peterson 1988). The first phase of this pigment decline can be described as a negative exponential function of time (Perissinotto and Pakhomov 1996):

$$G_t = G_0^{-kt}$$

where G_t = the Gut pigment content at time t (ng pig.ind^{-1}); G_0 = the gut pigment content at the start of the experiment (ng pig.ind^{-1}); k = the gut evacuation rate (h^{-1}); t = the time interval (h).

There is, as yet, no consensus as to when gut evacuation rate experiments should be terminated. Much of the debate over this issue has focussed on experiments with copepods. Some argue that an experiment should end after a certain percentage of initial gut fluorescence (Kiørboe and Tiselius 1987; Atkinson 1996), or after a certain amount of time (Ellis and Small 1989; Peterson *et al.* 1990; Atkinson and Snÿder 1997), or by fitting a regression through those points that fall on a straight line when plotted on a semi log scale (Kiørboe and Tiselius 1987; Dam and Peterson 1988). Gut evacuation rate

estimates using each of these proposed methods yield different results. There is agreement that the initial period of the experiment is the most accurate representation of the *in situ* evacuation rate (Perissinotto and Pakhomov 1996), however most of these experiments consider animals which are not under continuous feeding conditions. This issue was addressed by Perissinotto and Pakhomov (1996) who formulated a non-subjective model for a cut off point for these experiments. Their recommendation was that the cut off point should be determined where $\Delta G / \Delta t > 2$. For this study I have used this criterion.

An experiment was designed in 1998 at the Box 3 station to compare the feeding rates of adult and juvenile *E. vallentini*. In addition, in 1999, a gut evacuation experiment was carried out at station MS4-13 to compare the rate for feeding and non-feeding *E. vallentini* adults.

Gut pigment destruction

For gut pigment destruction measurements, freshly caught animals were first allowed to empty their gut of pigments for 24 hours in filtered seawater to which charcoal particles had been added. At the start of the experiment, 1 litre of well mixed ambient seawater containing a natural phytoplankton assemblage was divided into two equal portions. A single animal was incubated in 500 ml of the water, while the remaining 500 ml, the control, was allowed to stand for the same amount of time. The specimens were incubated for one hour under ambient light and temperature conditions. At the end of the experiment specimens were removed and placed in 90 % acetone to extract all pigments. The experimental and control water assemblages were then gently filtered through a 0.2 μm Nucleopore filter to extract all pigments. Pigments from specimens and filters were measured using a Turner 111 fluorometer. Six to 10 replicate animals were used for each experiment. Two experiments were conducted for adults of each species and two experiments were conducted for *E. vallentini* juveniles.

Pigments may be lost from the grazing experiment by digestion to non-fluorescent residues or by disintegration or leakage of faecal pellets (Perissinotto 1992). No faeces

were found at the end of any of the experiments. Calculations of the loss of pigment due to absorption (b') were calculated from:

$$b' (\%) = \{[(Gt - Pb)/P]^{-1}\}100$$

where Gt = gut pigment content ind^{-1}; Pb = background fluorescence ind^{-1}; and P = total amount of pigment ingested ind^{-1} (calculated from the difference between the control and the experimental bottles at the end of the experiment).

Ingestion rates and daily ration estimates

Ingestion rates were calculated from the equation:

$$I = kG (1/(1- b'))$$

where k is the average evacuation rate for a particular year (h^{-1}); G is the gut pigment content integrated over 24 hours (ng $pigm.ind^{-1}d^{-1}$); and b' is the gut pigment destruction (%).

These daily ingestion rates were converted to units of carbon assuming a carbon to chlorophyll ratio of 50:1 (Atkinson 1996). The ingestion rates were then expressed as a percentage of body carbon per day. Carbon content of euphausiids was assumed to correspond to 45 % of total dry weight (Ikeda and Mitchell 1982; Ikeda and Bruce 1986; Atkinson 1996). The mean weight was estimated from the mean length of individuals from each species (or group in the case of *E. vallentini* adults and juveniles) using the length-weight regressions (see chapter 2) from both years combined.

Results

Chlorophyll *a*

Chlorophyll *a* concentrations in 1998 were particularly low in the vicinity of the islands, ranging from 0.05 to 0.40 $\mu g.l^{-1}$ (mean 0.22 $\mu g.l^{-1}$), and were dominated by nano- and picophytoplankon (Fig 5.1a). In 1999 slightly higher chlorophyll *a* concentrations were observed ranging from 0.11 to 0.56 $\mu g.l^{-1}$ (Mean 0.30 $\mu g.l^{-1}$)(Fig 5.1b), excluding stations MS4-9, MS4-10 and MS4-14 where chlorophyll *a* concentrations were particularly high (2.4, 1.32 and 1.34 $\mu g.l^{-1}$ respectively) and were equivalent to bloom conditions for this area (Allanson *et al.* 1985; Perissinotto and Duncombe Rae 1990; Perissinotto 1992). Under these conditions, a high contribution of microphytoplankton to the total chorophyll *a* is usually found, as seen in Figure 5.1b.

Zooplankton grazing

Gut pigment content

Gut pigment levels for *E. vallentini* adults in both 1998 and 1999 showed a diel pattern, with the highest values recorded at night (Fig. 5.2 a, b). *Euphausia vallentini* juveniles did not exhibit a clear diel pattern in 1998 (Fig. 5.2 c). In 1999, a diel pattern was evident with the highest gut pigment levels recorded in the early morning (Fig. 5.2 d). Significantly higher initial gut pigment levels were found in 1999 compared to 1998 for *E. vallentini* adults ($p<0.001$) and juveniles ($p<0.001$)(Table 5.1). Unfortunately no initial gut pigment concentration measurements were made for *E. longirostris* specimens during the day during either cruise due to a lack of specimens, however, high gut pigment levels were measured during dark hours (Fig 5.2 e, f). The highest absolute values for gut pigment content were found in this species, and these were significantly higher in 1999 than in 1998 ($p<0.001$) (Table 5.1). In 1999, the highest gut pigment levels were found in *E. vallentini* and *E. longirostris* specimens which were collected at or in close proximity to the stations where bloom conditions were found (stations MS4-10, MS4-13 and MS4-14). No clear diel pattern in gut pigment content was evident from the data for *N. megalops* for either year (Fig 5.2 g, h). In contrast to the pattern observed for *E. vallentini*

and *E. longirostris*, gut pigment levels were significantly lower in 1999 compared with 1998 ($p<0.01$) (Table 5.1).

Mean background fluorescence measurements for *E. vallentini* adults and juveniles were 3.65 ng pigm.ind^{-1}(SD ± 0.67) and 2.90 ng pigm.ind^{-1} (SD ± 0.25) respectively. *E. longirostris* had the highest background fluorescence, 6.88 ng pigm.ind^{-1} (SD ± 4.11), and *N. megalops* had a value of 3.58 ng pigm.ind^{-1} (SD ± 1.39). All gut pigment levels were corrected by the relevant factor.

Gut evacuation rates

Results of the gut evacuation experiments are illustrated in Figures 5.3 a - l and Table 5.2. The mean rate of evacuation for *E. vallentini* adults in 1998 was 0.54 h^{-1}, which corresponds to a gut passage time of 1.85 h (n = 3). *Euphausia vallentini* juveniles had an mean value of 0.816 h^{-1} (equivalent to a gut passage time of 1.22 h). In 1998, gut evacuation experiments carried out at station Box 3 allowed for a comparison between adult (mean size ≈ 16.6mm) and juvenile (mean size ≈ 11.6) *E. vallentini*. The results (Table 5.2; Fig. 5.3 e and f) show that the juveniles had a slightly faster gut passage time (1.63 h) compared to adults (2.22 h). Results of the experiment conducted to compare the evacuation rate of feeding and non-feeding adults showed that gut passage time was considerably faster for feeding than for non-feeding experimental animals, with gut evacuation rates of 0.992 h^{-1} and 0.360 h^{-1} respectively (Table 5.2; Fig. 5.3 g and h).

Data for gut evacuation rates for *E. longirostris* were only available for 1999 and the mean gut clearance rate was 1.41 h^{-1} (gut passage time of 0.71 h). One gut evacuation experiment was carried out for *N. megalops* in each year. In 1998 the results showed a gut passage time of 2.42 h (0.41 h^{-1}) as compared to 0.93 h (1.08 h^{-1}) in 1999.

Gut pigment destruction

Estimates of gut pigment destruction (b') were made for each of the three euphausiids in 1998. *Euphausia vallentini* adult gut pigment destruction ranged from 43.0 to 94.7 %

(mean 78.0 %). The pigment destruction for juveniles was 94.5 %. Two attempts at measuring the gut pigment destruction for *N. megalops* were made, with only one experiment being successful, and the gut pigment destruction measured was equivalent to 96.8 %. Unfortunately, the two attempts to measure the gut pigment destruction for *E. longirostris* were unsuccessful and therefore a value of 80 % pigment destruction was assumed for this species. A value of 80 % pigment destruction was also assumed for *N. megalops* because of a lack of replication for this species.

Ingestion rates and daily ration estimates

Initial gut pigment measurements taken from specimens captured at different times of the day provide information on diurnal feeding activity, which, when integrated over 24 hours, gives a more accurate estimation of ingestion rates (Perissinotto *et al*. 1997). Therefore, integrated values were used with the gut evacuation rates to estimate ingestion rates of autotrophic carbon for all three species (Table 5.3). Since there were no day time data available for *E. longirostris*, gut pigment levels of 6.62 ng pigm.ind^{-1} in 1998 and of 8.28 ng pigm.$^{-1}$ in 1999 (the first observed evening gut pigment levels for each year) were assumed for the time period extending from the last morning value measured until the first observed value in the evening. This was done to ensure that any associated error would lead to an underestimate of herbivory rather than an overestimate. Pigments lost to absorption during digestion were accounted for using the correction factor (b'), as stated above. As gut evacuation rate measurements were only available for *E. vallentini* juveniles in 1998, and for *E. longirostris* in 1999, these values were used to calculate ingestion rates for both years. Ingestion rates for *E. vallentini* adults calculated for 1998 were 237 ng pigm.ind^{-1}.d^{-1}, and were seven times higher in 1999 (1822 ng pigm.ind^{-1}.d^{-1}). *Euphausia vallentini* juveniles showed a similar pattern with ingestion rates of 980 ng pigm.ind^{-1}.d^{-1} in 1998 and 1924 ng pigm.ind^{-1}.d^{-1} in 1999. Ingestion rates for *E. longirostris* were the highest of all groups considered, with rates of 1862 ng pigm.ind^{-1}.d^{-1} in 1998 and 5495 ng pigm.ind^{-1}.d^{-1} in 1999. *Nematoscelis megalops* showed little variation between years with ingestion rates of 231 and 226 ng pigm.ind^{-1}.d^{-1} in 1998 and 1999 respectively (Table 5.3).

The lowest carbon rations were found for *N. megalops* with values of ≈ 11 µg C. $ind^{-1}.d^{-1}$ for both years (Table 5.3). *Euphausia vallentini* adults consumed on average 11.9 µg C. $ind^{-1}.d^{-1}$ in 1998 and 91.1 µg C. $ind^{-1}.d^{-1}$ in 1999. Daily rations for *Euphausia vallentini* juveniles in 1998 and 1999 were 49 µg C. $ind^{-1}.d^{-1}$ and 96.2 µg C. $ind^{-1}.d^{-1}$ respectively. Highest daily carbon ration estimates were observed for *E. longirostris* with a value of 274.8 µg C. $ind^{-1}.d^{-1}$ in 1999, compared to 93.1 µg C. $ind^{-1}.d^{-1}$ in 1998. Daily ration estimates calculated in terms of body carbon per day are presented in Table 5.4.

Discussion

When using the gut fluorescence technique to estimate grazing there are several assumptions which need to be addressed. Firstly, the results from the experiments are assumed to be a reflection of *in situ* feeding rates. The animals under consideration were collected at depth, and although every effort was made to choose specimens that were healthy, capture, barotrauma and confinement within a 20 litre container may have affected the feeding behaviour of the animals (Wang and Conover 1986; Price *et al*. 1988). Secondly, the method for the gut evacuation experiments relies on the assumption that each individual reflects the feeding of the population as a whole. Increasing the number of replicates reduces the effect of individual feeding patterns, but in most instances five or fewer individuals were used for the estimation of pigment level at each time interval.

In addition, it is impossible to know whether extracted pigments are from direct grazing on phytoplankton or whether they are secondary in origin. Regardless of the origin of the pigment, the rate at which the gut is evacuated will be accurate. However, if the pigments are secondary in origin there would be an overestimation of grazing of zooplankton on phytoplankton. Pilditch and McClatchie (1994) found that the gut pigment content of *Nyctiphanes australis* feeding on the copepod *Acartia* spp. was as much as four times higher than for animals feeding on a strictly herbivorous diet. Estimates of herbivory for omnivorous zooplankton when using the gut fluorescence technique may therefore be over-estimated (Pilditch and McClatchie 1994).

In general, gut pigment levels were higher in 1999 reflecting the higher chlorophyll *a* measured. Highest gut pigment levels were evident for *E. vallentini* and *E. longirostris* at two of the three stations where very high chlorophyll *a* concentrations were observed. This pattern was also observed by Stuart and Pillar (1990) who found that *Euphausia lucens* had maximum gut chlorophyll values at stations where the highest ambient chlorophyll *a* values were observed. Because of the dynamics of the environment, both spatially and temporally, it is difficult to correlate these factors.

Diel variation

Diel vertical migrations have been shown for many euphausiid species (Chapter 2, e.g. Mauchline and Fisher 1969; Perissinotto 1992; Perissinotto and McQuaid 1992; Pakhomov and Froneman 1999a) but in some cases the migration patterns are not reflected in the feeding patterns (e.g. Antezana *et al.* 1982; Perissinotto and Boden 1989). According to Peterson *et al.* (1990), the diel pattern reported in most studies consists of an increase in gut pigment content at sunset and after a 2-3 hour period a value 2-10 times greater than mean daytime values is reached. Following this initial 2-3 hour burst in feeding activity, gut pigment content decreases initally, then levels off and remains constant throughout the night. A diel pattern was observed for *E. vallentini* adults in this study and has been observed in other populations of this species (Antezana *et al.*1996). Because of the lack of *E. longirostris* specimens during day light hours, the diel feeding patterns of this species could not be determined. However, it was evident from the fluorescence data that it feeds extensively on phytoplankton during the dark hours. Data from this study suggested that *N. megalops* does not exhibit any diel pattern in its autotrophic feeding activity.

Gut evacuation rates

Gut evacuation experiments were conducted under ambient temperature and light conditions, and experimental animals were kept under feeding conditions which simulated natural food concentrations as these factors have been found to affect gut

evacuation rates (Durbin and Durbin 1975; Murtaugh 1985; Dagg and Walser 1987; Dam and Peterson 1988; Pond *et al.* 1995; Mayzaud *et al*. 1998; Tirelli and Mayzaud 1999). The effects of food quality (Pond *et al.* 1995; Mayzaud *et al.* 1998), digestive acclimation processes (Mayzaud and Rayzouls 1992) or swarming behaviour (Morris and Ricketts 1984; Priddle *et al.* 1990) could not be addressed during this study.

Perissinotto (1992) reported gut evacuation rates of between 0.59 and 0.81 h^{-1} for *E. vallentini* adults, which is very similar to the evacuation rates reported here (range between 0.45 and 0.99 h^{-1}). Evacuation rates measured for both *E. vallentini* adults and juveniles at 'Box 3' indicated that the gut passage time for *E. vallentini* adults was slightly slower than for *E. vallentini* juveniles (0.450 h^{-1} and 0.612 h^{-1} respectively). A similar pattern was observed for juveniles of *E. superba* (Perissinotto *et al.* 1997) and *E. crystallorophias* (Pakhomov and Perissinotto 1997), which were both found to have faster gut passage times than the adults. Gut evacuation rates of starved animals have been observed to be significantly slower than rates for animals that are maintained under continuous feeding conditions (Kiørboe and Tiselius 1987; Dam and Peterson 1988; Perissinotto and Pakhomov 1996). The results of the experiment at station MS4-13 confirmed these findings. The gut passage time for *E. longirostris* (0.71 h) was faster than for *E. vallentini* but was similar to the gut passage times found for *E. lucens* (range from 0.64 h to 1.5 h) by Stuart and Pillar (1990). As *N. megalops* does not have a feeding basket adapted to filter feeding (Mauchline 1980), maintaining adults in water containing charcoal powder during the incubation may not have provided an adequate substrate for them to feed on, and therefore the results are probably equivalent to non-feeding conditions. The gut passage time for *N. megalops* was notably slower in 1998 (2.42 h) than in 1999 (0.93 h).

Gut pigment degradation

Several studies have suggested that a significant fraction of the chlorophyll *a* in phytoplankton could be degraded into non-fluorescent by-products during transit through the gut, rather than being transformed quantitatively into phaeopigments (Conover *et al.* 1986; Wang and Conover 1986; Lopez *et al.* 1988; Pasternak 1994). Pasternak (1994) found that between 78 and 89 % of ingested chlorophyll was recovered in the guts and

faecal pellets of copepods. On the other hand, some studies have shown that the loss of pigment due to degradation may range from 1- 99 % of total pigment ingested (Kiørboe and Tiselius 1987; Penry and Frost 1991; Head 1992; Mayzaud and Rayzouls 1992; Perissinotto and Pakhomov 1996; Perissinotto *et al.* 1997). Therefore, it is an important parameter to measure when using this technique (Perissinotto *et al.* 1997).

Much of the work on gut pigment degradation has been conducted on copepods. Researchers have linked gut pigment degradation to acclimation to food resources, food concentration (Penry and Frost 1991; Head 1992; Head and Harris 1992; Mayzaud and Rayzouls 1992), ingestion rate (Penry and Frost 1991), feeding history (degree of starvation or level of feeding activity) (Penry and Frost 1991; Head 1992; Head and Harris 1992; Head and Harris 1994), trophic history (Mayzaud and Rayzouls 1992), light intensity (Head 1992), diel feeding behaviour and species composition of the diet (Head 1992). This is important as differences in gut pigment levels under different feeding conditions, which were previously interpreted as differences in ingestion rates, may reflect differences in the degree to which gut pigment destruction occurred (Head 1992). From this evidence it is clear that gut pigment destruction measurements must be taken for each experiment.

The results of this study, where gut pigment destruction levels ranged from 43 to 96.8 % of ingested chlorophyll *a*, support the findings of previous studies which suggested that gut pigment destruction levels for euphausiids are high. Perissinotto (1992) found a gut pigment destruction level of 55 % for *E. vallentini*. The gut pigment destruction for *E. crystallorophias* ranged from 0 – 99 % (Pakhomov and Perissinotto 1997). Pigment destruction levels for *E. spinifera* have also been found to be high, with an average of 97 % destruction (Perissinotto *et al.* in review). Levels for *E. superba* have been found to range between 58.1 and 98.4 % (Perissinotto and Pakhomov 1996; Pakhomov *et al.* 1997a; Perissinotto *et al.* 1997), with average values between 81 and 84%. In instances where gut pigment destruction has not been measured, a value of 84 % (Atkinson and Snÿder 1997 for *E. superba*) was assumed. It therefore seems reasonable to assume 80% destruction in the case of *E. longirostris* and *N. megalops* for which there were no measurements, or too few replicates, from this study.

An important question which arises from assessing this technique is whether the measures will be grossly over-estimated if gut pigment degradation occurs during the time period when gut evacuation rates are measured. This question was addressed by Quetin *et al.* (1987) who found that the loss of fluorescing pigment during ingestion did not affect estimates of clearance time but did lead to an underestimate of total initial pigment and, therefore, if not taken into consideration, field ingestion rates will be underestimated.

Ingestion rates and daily ration estimates

Ingestion rates ranged between 226 and 5495 ng pigm.ind^{-1}.d^{-1} are comparable to observed values for euphausiids in Antarctic waters (Pakhomov *et al.* 1997a; Perissinotto *et al.* 1997). Ingestion rates for *E. frigida* and *E. triacantha* ranged between 543 ng pigm.ind^{-1}.d^{-1} and 639 ng pigm.ind^{-1}.d^{-1} compared with estimates for *Thysanoëssa* spp. juveniles of 153 ng pigm.ind^{-1}.d^{-1} (Pakhomov *et al*. 1997b). Daily ration estimates for *E. crystallorophias* ranged between 0.25 and 6.84 µg pigm.ind^{-1}.d^{-1} (Pakhomov and Perissinotto 1996a).

The highest daily ration estimates were calculated for *E. vallentini* juveniles with 8.2 % body carbon per day in 1998 and 16.2 % in 1999. Similar daily ration estimates were found for *E. vallentini* adults and for *E. longirostris* of between 0.4 and 2.7 % and 1.4 and 4.0 % body carbon per day respectively. A low consumption of approximately 0.3 % body carbon per day was recorded for *N. megalops* in both years.

Conclusions

Euphausia vallentini juveniles consumed the highest daily ration of autotrophic carbon. If it is assumed that euphausiids require between 5 and 6 % of their body carbon to be ingested per day in order to meet metabolic requirements (Clarke and Morris 1983), *Euphausia vallentini* juveniles would be the only group considered in this study able to sustain itself on a completely herbivorous diet.

Table 5.1 Mean and range of gut pigment levels for 1998 and 1999 (n = number of individuals analysed)

	Initial gut pigment content (G_0)			
Species	**1998**		**1999**	
	mean ($ng.ind^{-1}$)	**range** ($ng.ind^{-1}$)	**mean** ($ng.ind^{-1}$)	**range** ($ng.ind^{-1}$)
***E. vallentini* (A)**	4.39 (n = 154)	0 - 26.31	21.79 (n = 108)	0 – 229.41
***E. vallentini* (J)**	3.24 (n = 99)	0 – 25.67	6.51 (n = 36)	0 – 39.53
E. longirostris	15.18 (n = 73)	0 – 98.79	57.05 (n = 42)	0 - 370.64
N. megalops	4.45 (n = 135)	0 – 84.43	1.98 (n = 77)	0 – 29.80

Table 5.2 Evacuation rate experiments for all species in 1998 and 1999. G_0 = initial gut pigment; k = gut evacuation rate; 1/k = gut passage time.

Station	**Species**	**Time of day**	**Chl *a*** (µg chl.l^{-1})	**G_0** (ng.ind^{-1})	**k** (h^{-1})	**1/k** (h)
1998						
Station 1	*E. vallentini* (A) n = 5	20:05	*0.219***	3.49	0.504	1.98 119 min
MS3-5	*E. vallentini* (A) n = 3	23:29	0.213	4.85	0.672	1.49 89 min
Box 3	*E. vallentini* (A) n = 5	22:23	*0.219*	11.98	0.450	2.22 133 min
Box 3	*E. vallentini* (J) n = 5	22:23	*0.219*	3.78	0.612	1.63 101 min
Station 3	*E. vallentini* (J) n = 5	22:34	*0.219*	2.80	0.474	2.11 127 min
MS3-58	*E. vallentini* (J) n = 3	17:38	0.364	2.99	1.362	0.73 44 min
MS3-5	*N. megalops* (A) n = 3	23:29	0.213	5.39	0.414	2.42 146 min
1999						
MS4-13	*E. vallentini* (A) n = 5	01:51	0.443	77.97	0.992	1.01 61 min
MS4-13	*E. vallentini* (A)* n = 5	01:51	0.443	77.97	0.360	2.78 167 min
MS4-10	*E. longirostris* n = 1	22:08	1.328	111.14	1.886	0.53 32 min
MS4-14	*E. longirostris* n = 5	04:26	1.350	229.42	0.936	1.07 64 min
MS4-36	*N. megalops* n = 3	00:06	0.154	1.14	1.08	0.93 56 min

*filtered sea water – no charcoal added to experimental container.
**Chl *a* values in italics are the mean values from that year (no chl *a* data were collected at the *ad hoc* stations in 1998).

Table 5.3 Gut pigment levels integrated over 24 h, ingestion rates and daily ration estimates.

Species	**G** **Integrated initial gut pigments** (ng pigm.ind^{-1}.d^{-1})		**I** **Ingestion rates** (ng pigm.ind^{-1}.d^{-1})		**Daily ration estimate** (µg C.ind $^{-1}$d^{-1})	
	1998	**1999**	**1998**	**1999**	**1998**	**1999**
***E. vallentini* (J)**	66.1	129.7	980	1 913	49.0	95.7
***E. vallentini* (A)**	96.1	404.0	286	1 203	14.3	60.2
E. longirostris	264.0	779.0	1 235	3 645	61.8	182.3
N. megalops	111.7	41.9	231	226	11.6	11.3

Table 5.4 Mean dry weight, body carbon and daily ration estimates.

Species	**Mean dry weight** (mg)	**Body carbon content** (using C:DW 0.45:1) (µg C.ind^{-1})	**Daily Ration estimate** (% body carbon. ind^{-1}.d^{-1})	
			1998	**1999**
***E. vallentini* (A)**	7.31	3.29	8.2	16.2
***E. vallentini* (J)**	1.32	0.59	0.4	2.7
E. longirostris	15.32	6.89	1.4	4.0
N. megalops	10.36	4.66	0.3	0.3

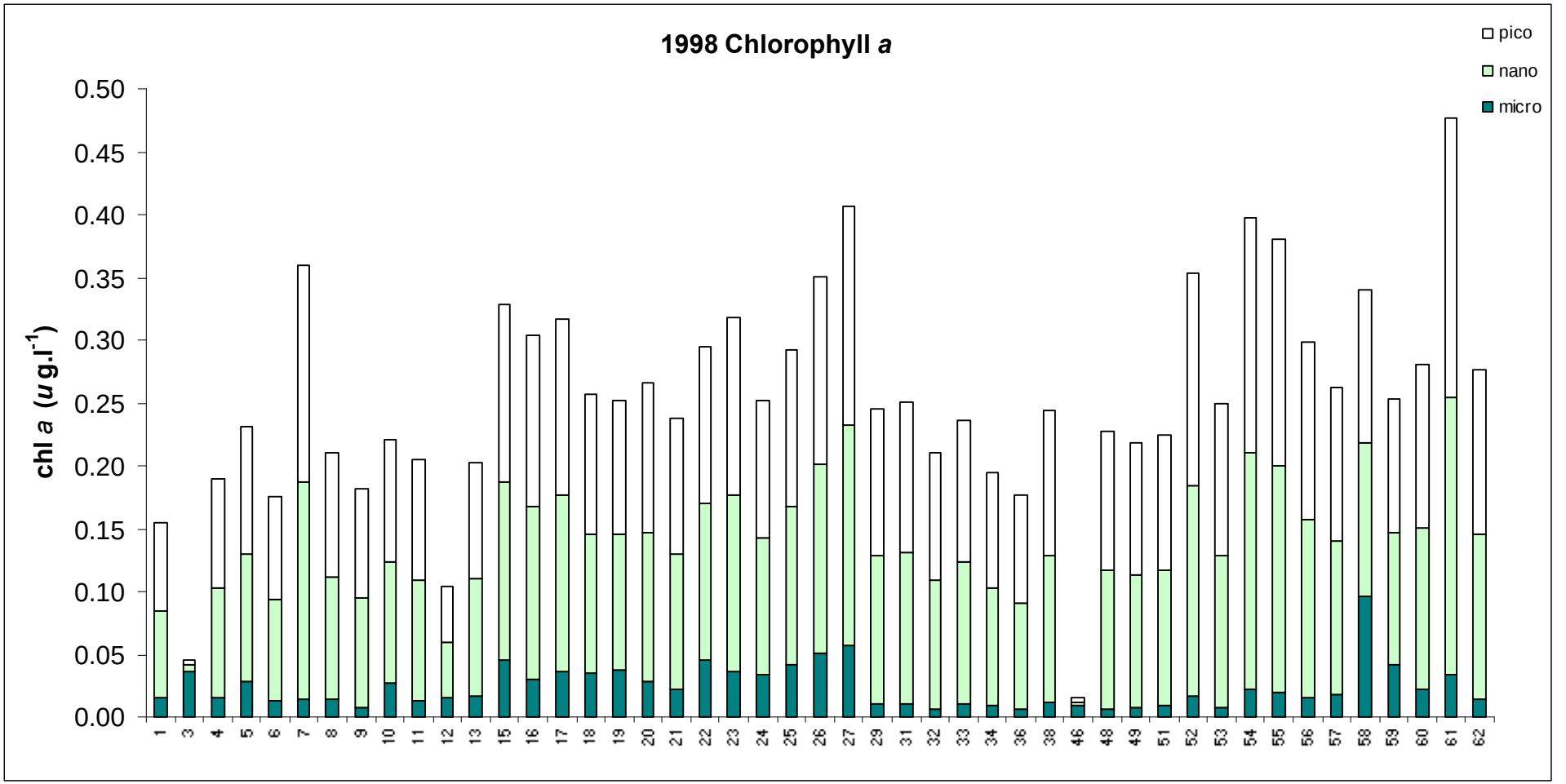


Figure 5.1(a) Size-fractionated chlorophyll *a* at each station in 1998.

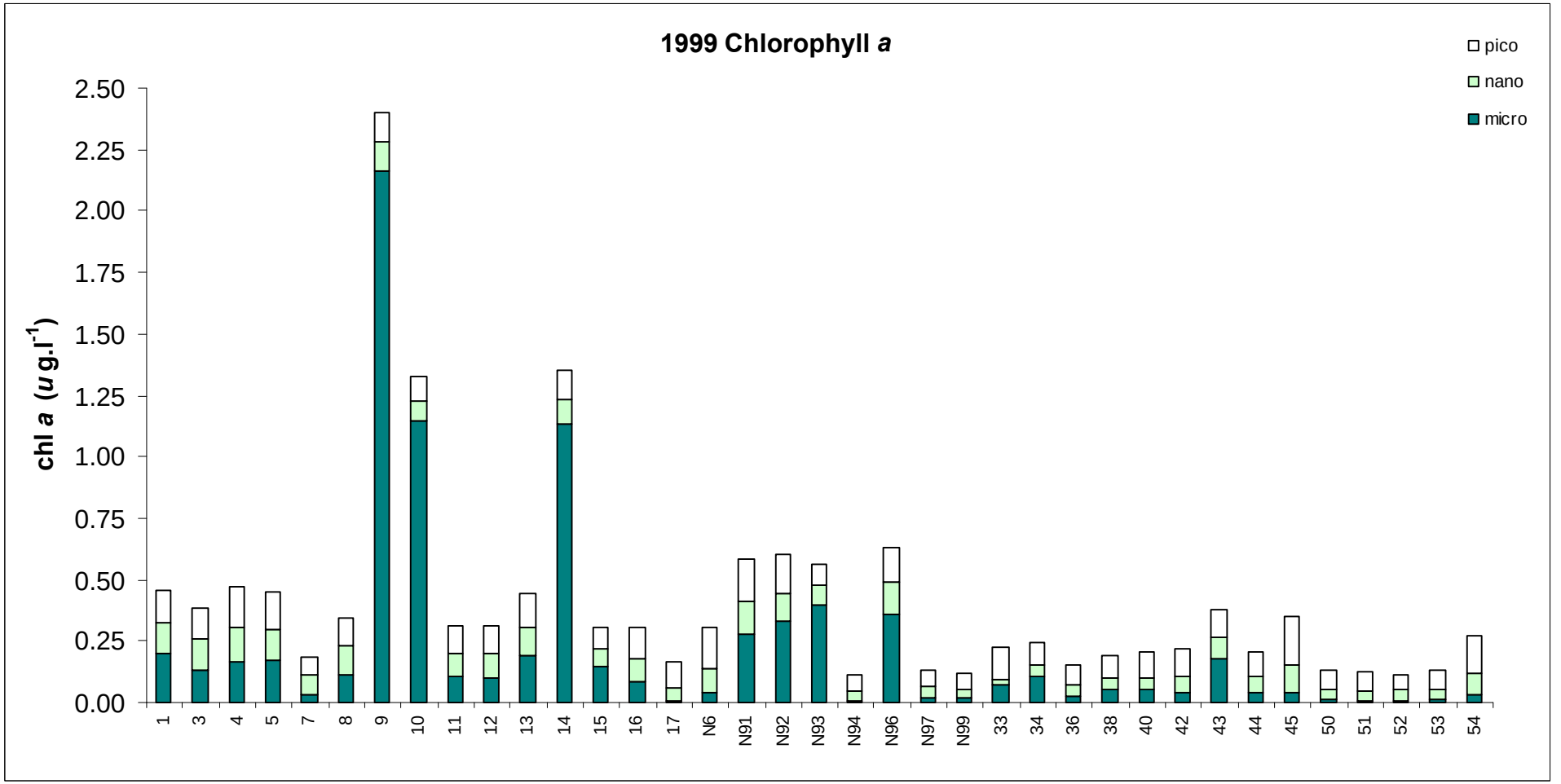


Figure 5.1(b) Size-fractionated chlorophyll *a* at each station in 1999.

Chapter 6
Gut fullness technique

Introduction

Various techniques have been used to estimate daily rations for organisms, but the majority have been conducted in the laboratory with animals being placed in incubations with a selection of food items (Clarke *et al.* 1988; Price *et al*. 1988; Pilditch and McClatchie 1994; Atkinson 1996; Metz 1998). These experimental techniques allow for the estimation of ingestion rate under a variety of conditions. However, the extent to which these results can be applied to the field is difficult to determine (Worobec 1984). The gut fullness technique allows at least part of the data to be obtained from specimens *in situ* and has the advantage of providing insight into temporal feeding patterns which may exist (Pasternak 1995; Pakhomov and Perissinotto 1996b).

The technique of using gut fullness to estimate daily rations was first developed and used for feeding ecology studies of fish (eg. Elliot and Persson 1978; Worobec 1984; Tudela and Palomera 1995; Pakhomov *et al.* 1996). Only a few studies have applied this methodology to marine crustaceans in the field (Pakhomov and Perissinotto 1996b; Maynou and Cartes 1997; Maynou and Cartes 1998) or in the laboratory (Sardà and Valladares 1990). Two measures are required for this method: an integrated stomach fullness index (% of body weight) measured in the field; and an independently measured gut passage time or evacuation rate (k, h^{-1}), which is usually estimated in the laboratory (Worobec 1984). Because the amount of food in the stomach is determined as much by the gut residence time as by the ingestion rate, the rate of movement of material through the gut needs to be measured and not just the ingestion rate (Murtaugh 1985). From these data, daily ration can be estimated as a percentage of body dry weight. When reliable carbon to dry weight conversion relations are available for both the consumer and the diet, these estimates can be converted into percentage of body carbon ingested per day.

In this study, diel feeding patterns and daily carbon rations for adults of *Euphausia vallentini*, *E. longirostris* and *Nematoscelis megalops* were estimated using the gut fullness technique.

Materials and methods

Diel feeding patterns

Individuals were randomly selected from samples taken in 1998 and 1999 at different times of the day for all three species (Table 6.1). Stomachs were carefully dissected out and visual estimates of gut fullness were made. Between three and 20 replicates were used for each time interval depending on the availability of specimens.

Daily ration estimates

Adult specimens of the euphausiids were randomly selected from samples taken in 1999 at different times of the day. The length of the specimen from the tip of the rostrum to the end of the telson was measured and stomachs were carefully dissected out. The stomach contents were separated from the stomach lining and emptied into a small amount of distilled water. A pipette was used to place the stomach contents into a container. The specimen (including stomach lining) and stomach content of each specimen were oven dried at 60°C for 36 h and weighed separately on a Sartorius microbalance. For the analysis of the results at least five replicates were pooled for each time interval.

Gut fullness indices were calculated for each individual as defined by Hureau (1969 in Berg 1979):

$$GFI = [\text{ stomach dry weight / euphausiid body dry weight }] \times 100$$

where GFI is the gut fullness index (%); and the stomach weight and body dry weight are measured in mg.

Ingestion rates were calculated using the average integrated fullness indices for each species and multiplying this value by 12 (the number of hours over which the integrated values were obtained). The gut fullness index (GFI) was then multiplied by the gut evacuation rate (k). Mean k values were obtained for each species from estimates made using the gut fluorescence technique (see Chapter 5). Since the available data only covered a period of 12 hours, these values were assumed to be minimum daily ingestion rates (see Pakhomov *et al.* 1996). Ingestion rates were therefore calculated using the following formula: (adapted from Eggers 1977 in Maynou and Cates 1997)

$$I = k\ (GFI) \times 12$$

where I is the average integrated ingestion rate over 12 hours (%BDW.ind^{-1}.d^{-1}); k is the average evacuation rate for a species (h^{-1}) (calculated from fluorescence data); and GFI is the average integrated gut fullness index (%BDW).

Using a carbon to dry weight ratio of 45 % for the stomach contents (Ikeda and Mitchell 1982, Ikeda and Mitchell 1986), an estimate of daily ration (µg C. ind^{-1}.d^{-1}) was calculated using the following formula:

$$DRE_i = [(I_i \times BDW_i) \times 0.45] \times 1000$$

where DRE is the daily ration estimate (µg C.ind^{-1}.d^{-1}); i is the euphausiid species; I is the ingestion rate (%BDW.ind^{-1}.d^{-1}); BDW is the average body dry weight of the population of species i (mg); and 0.45 is assumed to be the ratio of carbon to dry weight of the stomach contents.

To convert the daily ration from a percentage body dry weight to an estimate of carbon, the average dry weight of the individuals of a species (or a group in the case of *E. vallentini* adults and juveniles) was estimated (see Chapter 5). A carbon to dry weight ratio of 45 % for the stomach contents was assumed (Ikeda and Mitchell 1982; Ikeda and Bruce 1986; Atkinson 1996). Because no gut fullness estimates were made for *E.*

vallentini juveniles, the missing data were assumed to be the same as those calculated for *E. vallentini* adults (i.e. a daily ration of 10.8 % of body dry weight), and daily ration estimates were made accordingly.

Gut evacuation rate / gut passage time

An estimate of gut evacuation rate was made for *E. vallentini* in 1999 using specimens collected at Station MS4-13. This experiment was carried out concurrently with the gut fluorescence technique to allow a comparison of estimates of evacuation rate between the two techniques using specimens from the same net tow under the same conditions. Freshly collected *E. vallentini* adults were placed in 20 litre containers filled with 0.2 μm filtered seawater immediately after capture. The incubations were kept on deck to simulate ambient light and temperature conditions, and lasted three hours. Five replicate animals were removed at 10-15 minute intervals for the first hour and at 20 minute intervals thereafter. The gut evacuation rate (k, h^{-1}) was derived from the slope of the natural logarithm of decline in dry weight of gut content (as an index of body weight) over time. The initial phase of the gut fullness decline was described as a negative exponential function and was used to estimate k, the gut evacuation rate:

$$GFI_t = GFI_0 \, ^{-kt}$$

where GFI_t = the gut fullness index at time t (%BDW.ind^{-1}); G_0 = the gut fullness index at the start of the experiment (%BDW.ind^{-1}); k = the gut evacuation rate (h^{-1}); t = the time interval (h).

Results

Diel feeding patterns

Results from diel feeding investigation are shown in Figure 6.1. A diel pattern was evident for *E. vallentini,* with the highest gut fullness estimates recorded during dark hours (between 20h00 and 4h00). There was a high degree of variability between specimens examined during this period. Low fullness estimates were observed in the

morning until noon, with all examined specimens having gut fullness values < 25 %. Fullness estimates increased after noon and again there was a high variation until 20h00. Visual estimates during this time period ranged between 10 and 65 %. All specimens of *E. longirostris* had consistently high stomach fullness indices. The highest variation occurred between 16h00 and 20h00 where stomach fullness indices ranged between 20 and 100 %. The stomach fullness of *N. megalops* was relatively low throughout the period of observations and showed a high degree of variability between individuals throughout the day. Highest stomach fullness indices were recorded in the late afternoon and early evening (between 16h00 and 20h00).

Daily ration estimates

Integrated fullness indices for the euphausiid species are presented in Table 6.1. The lowest integrated value was recorded for *N. megalops* with a gut fullness index of 0.88 % BDW.h^{-1}. *Euphausia vallentini* and *E. longirostris* had similar values of 1.66 and 1.74 % BDW.h^{-1} respectively. A daily ration of 10.8 % of BDW was calculated for *E. vallentini* adults. The highest daily ration was observed for *E. longirostris,* (19.6 % BDW.d^{-1}) and the lowest for *N. megalops* (7.8 % BDW.d^{-1}). Daily ration estimates converted into units of carbon per day are presented in Table 6.2.

Gut evacuation experiment.

The gut evacuation rate of 0.75 h^{-1} was measured for *E. vallentini* which corresponds to a gut passage time of 1.33 h. This estimate was very similar to the estimates which were calculated using the gut fluorescence technique (1.01 h) on individuals from the same station.

Discussion

Diel feeding patterns

Diel variation in gut fullness has been observed in a number of marine organisms from copepods (Pasternak 1995) to fish (Tudela and Palomera 1995). Problems with interpreting these data include the fact that under non-feeding conditions some euphausiids have been found to retain food in their stomachs. Furthermore, during certain times of the day when the overall feeding activity is greater, individual animals within a group will not feed continuously over these time periods (Mauchline and Fisher 1969).

Clear diel patterns were observed for *E. vallentini* and suggest that this species feeds predominantly at night. Opportunistic feeding may occur during the day from chance encounters with prey. According to Mauchline and Fischer (1969), diurnal changes in the feeding intensity of euphausiids, as observed for *E. vallentini* in this study, are generally associated with the probability that the amount of food available in the surface layers, into which the euphausiids migrate at night, is much greater than in the deeper layers. They state that, in situations where food is available, euphausiids feed by day and night but that an individual animal probably does not feed continuously throughout the day. They also suggest that the amount of time spent resting may be estimated by the percentage of animals with empty or nearly empty stomachs. Applying this theory to the data above, *E. vallentini* spent approximately 53 % of a day feeding while *E. longirostris* fed throughout the night. Using the average percentage of stomach fullness of the population to estimate time spent feeding by one individual suggests that feeding occurred for 86 % of the night.

Average gut fullness for *N. megalops* was highly variable for all time intervals considered. Highest values were observed in the late afternoon but feeding occurred to some extent throughout the day. A similar pattern was observed by Barange *et al*. (1991) with a higher number of copepod fragments found in the stomachs of *N. megalops* in the early evenings. These authors suggested that this species migrates to the thermocline at night and feeds when the bulk of the migratory mesozooplankton community is expected

to cross this layer. The average estimate for the percentage of the day which is spent feeding by this species is comparatively low (31 %).

Daily ration estimates

Although the weight of the stomach contents may provide information on periodicity and quantity of food consumed, it should be remembered that the mass of two different kinds of food may have different calorific values. For more accurate assessments using this technique, a weight to energy regression should be calculated if the value of the food consumed is to be estimated (Berg 1979). The calorific content of food items may not be directly proportional to their dry weight. The calorific content of metazoan food, and even much smaller heterotrophs and protoplankton is much higher than that of diatoms (Ben-Amotz *et al*. 1987; Hitchcock 1982). Therefore, an individual feeding carnivorously may need to consume less material than one feeding herbivorously. Likewise, in situations where the prey is pierced and the body contents sucked out (Mauchline and Fisher 1969), the weight of this material may be low relative to its nutritive value. Also this material may be digested far more readily and estimates of daily carbon ration based on weight will be dramatically underestimated (Sardá and Valladares 1990). This may have some relevance to the results presented here for *N. megalops*. If this species feeds carnivorously, one would expect the quality of the food consumed to be of higher energetic value than that of a herbivore. This would mean that this species is able obtain its full daily carbon ration from a smaller amount of food as estimated using dry weight. *N. megalops* was found to have the lowest daily consumption when compared to the two *Euphausia* species.

A second point to consider is that, although an exponential model of food evacuation is well established in the literature (Maynou and Cartes 1997), the assumption that feeding continues at a steady state throughout the day may not be accurate (ref). Feeding rate has been shown to be dependent on several factors including availability of food, concentration of food items, gut fullness, time of day and type of material which is being digested (Sardà and Valladares 1990). For this study, the mean values of gut evacuation rates measured on at least two separate occasions using the fluorescence technique were used.

The daily ration estimates calculated for the euphausiids during this study (range between 7.8 and 19.6 %) were within range of daily rations estimated for other euphausiid species. Using estimates based on body dry weight, Clarke *et al.* (1988) and Quetin *et. al*. (1993) found relatively high estimates of between 17 and 28 % for *E. superba*. The estimates from this study, however will only be accurate for those species which feed predominantly at night. If feeding continues throughout the day, as may be the case for *N. megalops*, daily ration estimates will be underestimated.

Carbon daily rations ranged between 64 and 1352 µg $C.ind^{-1}.d^{-1}$, with the lowest estimate being found for *E. vallentini* juveniles and the highest for *E. longirostris* (Table 6.2). *E. vallentini* adults and *N. megalops* had similar carbon rations of 355 and 363 µg $C.ind^{-1}.d^{-1}$, respectively. Daily rations of *E. superba* have been measured using a wide range of techniques (Antezana *et al.* 1982; Boyd *et al.* 1984; Schnack 1985; Atkinson and Snÿder 1997; Perissinotto *et al*. 1997), and range between 0.02 and 17.1 % of body carbon per day (summarised in Pakhomov *et al*. 1997a). Similarly, daily ration estimates from *in vitros* for *E. lucens* ranged between 11 and 22 % of body carbon and were dependent on the food source provided (Stuart 1986). The metabolic requirements for *E. superba* have been estimated to be between 5 and 6 % of body carbon per day (Clarke and Morris 1983). The estimated daily ration obtained during this study will therefore be sufficient to meet basic metabolic requirements of the three euphausiid species investigated.

Conclusions

The diel feeding patterns for *Euphausia vallentini* have been confirmed with this species feeding predominantly at night. Although no data were available during the day for *E. longirostris,* this species was found to feed extensively during the dark hours. No clear pattern was observed for *N. megalops* but this species appeared to feed to some extent throughout the day. Carbon rations were sufficient to meet basic metabolic requirements for all three species.

Table 6.1 List of stations used for gut fullness indices, the average length and weight of specimens and the integrated gut fullness.

Species	**Stations**	**n**	**Mean length** (mm)	**Mean dry weight** (mg)	**Integrated gut fullness** (%BDW. $ind^{-1}.h^{-1}$)
E. vallentini	MS3-29; -31; -32; -33; -34 MS4-3; -5; -9; -13; -14; -38 NT 91; -99	64	20.4	9.53	1.66
E. longirostris	MS3-11; -12; -21; -26; -32; -33;-34-52;-53;-58 MS4-3; -4; -5; -9; -36; -38 NT 91; -99	47	24.6	17.19	1.74
N. megalops	MS3-11;-12;-21;-51;-52;-53; -58 MS4-3; -9; 36; 38 NT 91; -99	65	21.9	10.83	0.88

Table 6.2 Mean dry weight of whole population, integrated gut fullness indices, ingestion rates and daily ration estimates (assume no feeding for 12 h).

Species	**Mean dry weight** (mg)	**G Gut fullness** (%BDW. $ind^{-1}.d^{-1}$)	**K* Gut evacuation rate** (h^{-1})	**I = kG Ingestion** (%BDW.$ind^{-1}.d^{-1}$)	**Daily Ration estimate** (using C:DW 0.45:1) (µg $C.ind^{-1}.d^{-1}$)
***E. vallentini* (A)**	7.31	19.9	0.54	10.8	355
***E. vallentini* (J)**	1.32	-	0.816	-	64
E. longirostris	15.32	20.9	0.936	19.6	1 351
N. megalops	10.36	10.5	0.747	7.8	363

* measured using the gut fluorescence technique

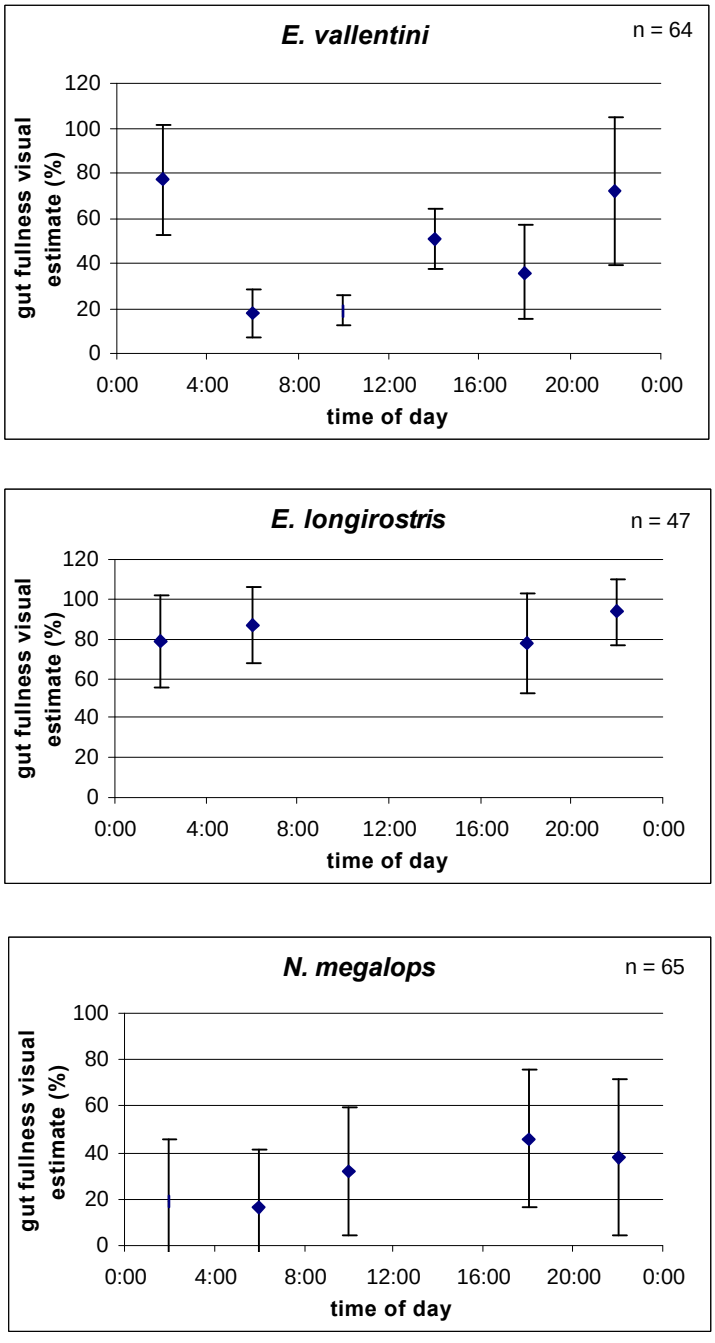


Figure 6.1 Gut fullness estimates made visually from specimens from both 1998 and 1999 (Error bars indicate 1 standard deviation).

Chapter 7
Summary

Important aspects of feeding ecology include gaining a better understanding of diel variation in feeding activity, trophic status and an estimation of daily ration. Few quantitative studies are available comparing herbivorous and carnivorous feeding by euphausiids on natural food items (Ohman 1984; Price *et al.* 1988; Stuart and Pillar 1990; Perissinotto *et al.* 2000). The findings of this study are combined to assess the diel feeding patterns and relative contributions of herbivory and carnivory to the diets of the selected euphausiids in the waters surrounding the Prince Edward Archipelago.

Vertical migrations were observed for *Euphausia vallentini*, both adults and juveniles. Associated with these migrations were clear diel feeding patterns. Due to insufficient data during daylight hours, the diel feeding patterns for *E. longirostris* could not be determined, but high feeding activity did occur during the night. *Nematoscelis megalops* did not show any distinct vertical migration patterns but higher gut fullness indices were observed in the late afternoon suggesting feeding activity may have been highest during this period.

From the evidence presented in this study, *Euphausia vallentini* juveniles were found to be herbivorous. The results of the gut content analysis showed that there was a high contribution of phytoplankton to the diet, and this was supported by the low trophic position indicated by the stable nitrogen isotope results. Combining the results from the gut fluorescence and gut fullness techniques, the estimated contribution of autotrophic carbon to the total daily ration was 76.6 % in 1998 and in excess of 100 % in 1999 (see tables 5.3 and 6.2). *Euphausia vallentini* adults may be considered omnivorous, but the contribution of carnivory to the diet was difficult to determine. The high phytoplankton contribution, and relatively low mesozooplankton components of the gut content analysis, suggested a herbivorous diet. But, the stable nitrogen isotope results showed a high degree of omnivory. When the results of the gut fluorescences and gut fullness techniques were combined, the percentage of the daily ration which was autotrophic in origin, was

estimated to be 3.3 % of body dry weight in 1998 and 25.7 % in 1999. These results support the isotope results and confirm that there was a large heterotrophic contribution to the diet of *E. vallentini* adults. Irrespective of the degree of carnivory, a dietry shift with an increase in size was evident for *E. vallentini*, as has been observed in other euphausiid species (Mauchline and Fisher 1969).

The gut content analysis of *E. longirostris* showed that this species consumed large amounts of both phytoplankton and metazoan prey. The stable nitrogen isotope analysis indicated that this species was carnivorous, and occupied a trophic position above that of *E. vallentini* and similar to *N. megalops*. The combined results from the fluorescence and gut fullness techniques showed that the contribution of autotrophic carbon to the daily ration estimates of *E. longirostris* was 6.9 % of the daily carbon consumption in 1998, and 20.3 % in 1999. Some of the evidence presented here suggests that *E. longirostris* may share a trophic position with *E. vallentini* adults, but the isotope results clearly place this species in a higher trophic position, with a high contribution of heterotrophic carbon in the diet.

The findings for *N. megalops* were more difficult to interpret. Relatively high phytoplankton and metazoan contributions were observed in the stomach contents, suggesting an omnivorous diet for this species. But the trophic position, as identified by isotope ratios, clearly indicated a high heterotrophic carbon component to the diet. This finding was further supported by the calculations combining daily ration estimates using the gut fluorescence and fullness techniques and show that only 3.1 and 3.2 % of the carbon ingested was of autotrophic origin in 1998 and 1999 respectively, which confirms the carnivorous feeding habit of this species.

The findings of this study support findings in the literature and show that *E. vallentini* juveniles are herbivorous and *N. megalops* adults are carnivorous. However, adults of *E. vallentini* and *E. longirostris* appear to have higher contributions of heterotrophic carbon to their diets than had been assumed, and may occupy higher trophic positions than initially predicted. The strength of a study of this nature is the converging techniques which all address the same problem. Each technique has its limitations but the end results allow for a far better assessment of the feeding biology of these organisms.

Ecological implications

Research at the Prince Edward Islands aims ultimately to allow for management of the island system. Modeling is a tool often used for the management of ecosystems, and neither management nor modeling can be successful without assessing the trophic links at species level. Euphausiids have been shown to be an important part of the Prince Edward Island ecosystem, forming a principal component of the diet of many of the predators in the vicinity of the islands (e.g. Brown and Klages 1987; Ridoux 1988; Brown *et al.* 1990; Cooper and Brown 1990; Cooper *et al.* 1992; Bost *et al.* 1994 a, b). Within a food web all elements are linked and no biological interaction occurs in isolation. The effect of predation on the euphausiid community is one factor that determines the abundance of these organisms. This, in turn, has an effect on the grazing and predation pressure that these species may have on the community. Therefore, an understanding of the interactions between species within the food web allows for a better understanding of the food web as a whole. Studies have often suggested that mesozooplankton dominate grazing of phytoplankton, but in fact they rarely consume more than a small fraction of daily primary production (Longhurst 1991). Mesozooplankton grazing in oceanic situations is almost always < 20 % of daily production (Tsuda *et al.* 1989). Oceanic microplankton, on the other hand, frequently consume 70-90 % of daily primary production (Weschmeyer and Lorenzen 1985; Froneman and Perissinotto 1996) and on occasion grazing by this community may exceed 100 % of daily primary production (Goldman *et al.* 1987). When zooplankton consume significant amounts of elements of the microbial food web, as was found in this study, this will have a direct effect on the grazing of phytoplankton by microbes. The predation of euphausiids on copepods will also affect the grazing dynamics of these species within the system. Although there are many parameters which still need to be defined within the Prince Edward Island system, studies such as the present one provide vital information on the trophic interactions within the ecosystem.

Investigating the trophic links within an ecosystem also allows the fate of organically fixed carbon in a food web to be determined. The oceans are the largest global carbon reservoir and play a major role in controlling atmospheric carbon dioxide (CO_2)

(Siegenthaler and Sarmiento 1993). The sequestration of atmospheric CO_2 is controlled by both physical (solubility pump) and biological processes (Longhurst and Harrison 1989; Longhurst 1991; Siegenthaler and Sarmiento 1993). The "biological pump" is a term used to describe the biological activities which are responsible for fixing atmospheric carbon dioxide and transporting it to depth (Longhurst and Harrison 1989, Longhurst 1991), and in so doing depositing it in a relatively long term inactive state. The Southern Ocean has been identified as an important area in terms of the biological pump, as it has high nutrient levels but low production. Proposals of utilizing this potential CO_2 sink are currently being addressed. Because of this, the fate of organic carbon in the Southern Ocean is presently a leading concern in biological oceanographic studies. Sinking phytoplankton cells and grazing by zooplankton are largely responsible for transferring carbon from the surface waters to the interior of the ocean (Froneman *et al*. in press). The relative efficiency of the biological pump is determined by how much of the fixed carbon is sequestered to depth. If primary production in a given area sinks directly out to the ocean floor, then the biological pump may be considered efficient. If, however, the production passes through a number of trophic links, the biological pump will be less efficient, as the majority of the carbon dioxide fixed in the production will be returned to the atmosphere via respiration in the surface waters. The magnitude of carbon flux to deep waters will be determined largely by the partitioning of carbon between the various size classes of herbivores (Fortier *et al*. 1994). Generally large herbivorous zooplankton contribute substantially to the export of carbon from the surface waters at night to depth as they produce large faecal pellets which have a relatively high carbon content and high sinking rates (Schnack 1985, Cadee *et al*. 1992; Fortier *et al*. 1994). The vertical migrations of zooplankton assist in carbon transfer to the deep ocean as food consumed in the surface waters may be transported to depth during the day (Longhust 1991; Fortier *et al.* 1994). Euphausiids, particularly *Euphausia superba,* have been identified as potentially playing an important role in the draw down of carbon to the ocean floor (Tanoue and Hara 1984).

Assessing the efficiency of the biological pump with respect to the euphausiids examined in this study provides conflicting results. The euphausiids did undergo vertical migrations and some of the surface production would have been transported to depth in this manner. *Euphausia longirostris* in particular, was seldom found at depths of < 300 m during the

day and may be an important component in the transfer of carbon from the surface to deep waters. *Euphausia vallentini* may also be an important species to consider within the Polar Frontal Zone as it occurs at high densities and undertakes marked diel vertical migrations (Fig. 2.3 and 2.4). The results from this study provide contrasting implications. On one hand, *E. vallentini* consumes significant amounts of the microbial food web (protozooplankton). This would enhance the drawdown of carbon, as carbon within this microbial system is generally recycled within surface waters (Longhurst 1991; Fortier *et al.* 1994; Froneman and Perissinotto 1996) and represents an inefficient cycle in terms of the biological pump. On the other hand *E. vallentini* has been presumed to be herbivorous when in fact its diet may have a high heterotrophic carbon component. Thus the increased length of the food chain reduces the efficiency of the transfer of carbon through the system. The results for *E. longirostris* are similar in these two respects; it consumes components of the microbial food web, but the heterotrophic component to the diet is higher than was previously assumed. These findings are important in assessing models of carbon flow through the system (e.g. Clarke 1985; Huntley *et al*. 1991; Moloney 1992) where partitioning of carbon between different trophic groups has large scale implications.

Chapter 8

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