UNIVERSITY OF SOUTHAMPTON

NUTRITIONAL REGULATION OF EGG PRODUCTION OF *CALANUS FINMARCHICUS* IN THE NORTH ATLANTIC

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ABSTRACT

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NUTRITIONAL REGULATION OF EGG PRODUCTION OF *CALANUS FINMARCHICUS* IN THE NORTH ATLANTIC

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Ship-board experiments in the North Atlantic were used to study how food quality influences the egg production of *Calanus finmarchicus* feeding on natural planktonic diets. Food quality was expressed in terms of carbon (C), nitrogen (N), and the essential fatty acids eicosapentaenoic acid (EPA; 20:5(n-3)) and docosahexaenoic acid (DHA; 22:6(n-3)). Five consecutive 24 hr bottle incubations were conducted in April and July/August 2002 under *in situ* conditions to determine egg production rates and the ingested quantities of C, N, EPA and DHA. Biomass contributions towards growth were determined and the biochemical composition of the eggs was examined. In order to accurately determine ingestion rates, a method to account for microzooplankton grazing in particle removal experiments was developed.

Balanced physiological budgets were compiled for *C. finmarchicus* in both seasons. The input terms of these budgets consisted of ingestion and the use of biomass, and the outputs were growth, respiration, excretion and egestion. Respiration and excretion were not determined experimentally, and were therefore determined by mass balance and compared to literature-derived values.

In April, close agreement between literature- and mass balance-derived rates of respiration and excretion demonstrated that the experimentally determined components of the budget were accurate. Ingestion rates were low, and > 80 % of the C utilised was derived internally from somatic biomass. The absence of storage fatty acids and the low C:N ratio (~ 4 μg μg^{-1}) of the biomass lost from the females indicated that these animals had been catabolising structural protein and were close to exhaustion. This suggests that when food is scarce, *C. finmarchicus* adopts a semelparous reproductive strategy. In July/August, the observed growth exceeded the estimated ingestion rates. This shortfall was possibly provided by cannibalising eggs.

Assuming that EPA and DHA were used with high efficiency (0.9), the stoichiometric analysis predicted that these compounds were non-limiting in April. Using typical maximum growth efficiencies for C (< 0.6) and N (0.4), the former was predicted to be limiting because the biomass utilised was rich in N, EPA and DHA relative to the demand for C.

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has been produced under the supervision of the following persons:

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DECLARATION OF AUTHORSHIP

I, Daniel Mayor, declare that the thesis entitled 'Nutritional regulation of egg production of *Calanus finmarchicus* in the North Atlantic' and the work presented in it are my own. I confirm that:

- This work was done wholly while in candidature for a research degree at the University of Southampton;

- Part of the work presented in chapter 3 has been submitted for publication in Marine Ecology-Progress Series. This paper is co-authored by Drs T. Anderson, X. Irigoien and D. Pond, although they only contributed by providing comments on the manuscript and scientific advice;

- Where I have consulted the published work of others, this is always clearly attributed;

- Where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work;

- I have acknowledged all main sources of help.

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Chapter 1

Introduction

1.1. Copepods in the North Atlantic. Copepods are thought to be the most numerous multicellular organisms on earth (Mauchline 1998), inhabiting both freshwater and marine environments. Their name originates from the Greek word *kope*, an oar, and *podos*, a foot, perfectly describing their flattened legs which propel them through the water at remarkable speeds. The calanoid copepod *Calanus finmarchicus* dominates much of the northern North Atlantic zooplankton biomass, typically contributing > 50 % of the total (Planque and Batten 2000). North of Iceland, *C. finmarchicus* reaches its northern distribution limits (Planque et al. 1997). Here it co-exists with the Arctic species *Calanus glacialis* and *Calanus hyperboreous*. Towards the southern limits of its distribution in the northeastern North Atlantic, the North Sea and the southern part of the Norwegian Sea (Planque et al. 1997), it is found alongside the more temperate species *Calanus helgolandicus*.

As the predominant copepod, *C. finmarchicus* plays a pivotal role in the planktonic ecosystem of the North Atlantic. It provides a crucial trophic link between the primary producers and planktivorous fish and fish larvae. Well established time-series, such as the Continuous Plankton Recorder record, have recently shown that the survival of larval cod is dependant upon the size and quantity of the available *C. finmarchicus* (e.g. Beaugrand et al. 2003). *Calanus* also plays an important role in the export and remineralisation of carbon and nutrients (Banse 1995). Nevertheless, the abundance of *C. finmarchicus* in the northeast Atlantic and northern North Sea appears to have decreased significantly over the past 50 years (Planque and Fromentin 1996). Because the survival of commercially important fish larvae depends on the availability of *Calanus*, any reduction in its abundance is likely to further reduce the heavily exploited fish stocks (Beaugrand et al. 2003). The reason behind the disappearance of these copepods remains unclear, but it is suggested that climatic warming is forcing a mismatch between the arrival of *C. finmarchicus* in the surface waters and the blooms of algae that they gorge upon during the spring (Edwards and Richardson 2004). If we are to understand and ultimately forecast fluctuations in fish stocks, it is of crucial importance to understand how and why the productivity of these copepods varies.

1.2. Understanding what limits copepod production. In essence, secondary production of copepod communities can be estimated by multiplying the biomass of the population by its growth rate (see Poulet et al. 1995). The growth rate of adult

copepods is quickly and easily determined by measuring the rate at which eggs are produced, assuming that since females have no further moults to undergo, somatic growth ceases and all new biomass produced is therefore in the form of eggs (e.g. Poulet et al. 1995, Runge and Roff 2000). However, this assumption has recently been challenged (Hirst and McKinnon 2001). Although theoretically sound, concurrent data on egg production and changes in body weight are non-existent for high-latitude copepods (Hirst and McKinnon 2001), making this assumption hard to examine in detail. Polar copepods are known to have body weights that increase and decrease over the seasonal cycle, and are capable of producing eggs from internal reserves (Tande 1982, Smith 1990, Hirche and Kattner 1993, Hagen and Schnack-Schiel 1996, Hirche and Niehoff 1996, Niehoff et al. 2002). In cases where eggs are produced in the absence of food, the true net growth rate must be negative as the starved individual will continue to respire. Therefore changes in the animal's biomass over the duration of any egg production experiment must also be considered if an accurate estimate of net growth is to be determined.

The number of eggs copepods produce is influenced by a range of biotic and abiotic factors, including the quantity and quality of the food (Kleppel 1993, Anderson and Pond 2000). Because carbon (C) is required not just for production, but also to meet the energetic demands of respiration, it is intuitive to suspect that C should be limiting, particularly when food is scarce (Sterner 1997). Indeed, egg production of *Calanus* has repeatedly been shown to correlate significantly with food C, as determined by chlorophyll (e.g. Hirche and Bohrer 1987) or the number of available cells (e.g. Marshall and Orr 1955b, Hirche et al. 1997). These correlations between egg production and food C led Hessen (1993) to state that, 'it is fairly evident that food quantity (in terms of carbon or energy) most frequently limits zooplankton production'. However, significant correlations between egg production and phytoplankton biomass are not always found, particularly when the latter is derived from chlorophyll:C conversions (Plourde and Runge 1993, Irigoien et al. 1998, 2000b, Niehoff et al. 1999, Richardson et al. 1999). Considering that non-chlorophyll bearing (i.e. heterotrophic) protists are thought to contribute significantly to the diets of marine zooplankton (e.g. Stoecker and Capuzzo 1990), this is not entirely surprising. In addition, the lack of correlation may also suggest that egg production is not always limited by the quantity of food available, and that food quality is also of importance.

Egg production rates of both *Paracalanus parvus* and *Acartia tonsa* have been shown to correlate positively with food nitrogen (N) (Checkley 1980, Kiorboe 1989). These observations have led to the suggestion that copepod production in the marine realm is limited by this element and that the quality of a particular food can be examined by determining its N content (Roman 1983, Jones et al. 2002). Food quality has also been assessed using other currencies, including protein (Jonasdottir 1994, Jonasdottir et al. 1995, Jonasdottir et al. 2002), amino acids (Cowie and Hedges 1996, Kleppel et al. 1998a, Guisande et al. 2000), cell size (Berggreen et al. 1988, Nejstgaard et al. 1995) and cell toxicity (Ianora et al. 1996, Ban et al. 1997). Specific polyunsaturated fatty acids (PUFAs) of the ingested food have proved particularly useful in explaining variability in both egg production rates (Stottrup and Jensen 1990, Jonasdottir 1994, Jonasdottir et al. 1995, Jonasdottir and Kiorboe 1996, Pond et al. 1996, Jonasdottir et al. 2002, Hazzard and Kleppel 2003, Shin et al. 2003) and also egg viability (Jonasdottir and Kiorboe 1996, Pond et al. 1996, Jonasdottir et al. 2002, Shin et al. 2003) in marine copepods. Together, these data suggest that PUFAs may be good descriptors of food quality. In summary, numerous substrates have been identified as potentially limiting. However, a general consensus on the component that predominantly limits zooplankton production in the marine environment has yet to be reached.

1.2.1. Elemental Stoichiometry. The potential for individual dietary substrates to limit production of marine copepods can be studied on a theoretical basis using elemental stoichiometry (e.g. Anderson and Hessen 1995). The term stoichiometry can be defined as the quantitative relationship between constituents in a chemical substance (Sterner and Elser 2002). In any particular chemical reaction, if the stoichiometric elemental balance of the chemical reagents is known, the composition of the products can be determined given an understanding of the reaction pathways. In the case of copepods, the reagents are the substrates in the food and any body reserves utilised, and the products are growth (including reproduction), CO_2, NH_4 excretion, fecal pellets etc. (Figure 1.1). Stoichiometric models in biological systems typically compare elemental ratios in predator and prey biomass. After taking into account utilisation efficiencies, the compound or element in shortest supply relative to the demand is invoked as limiting. Non-limiting substrates are then in excess by definition and, assuming homeostasis in the consumer, must be returned

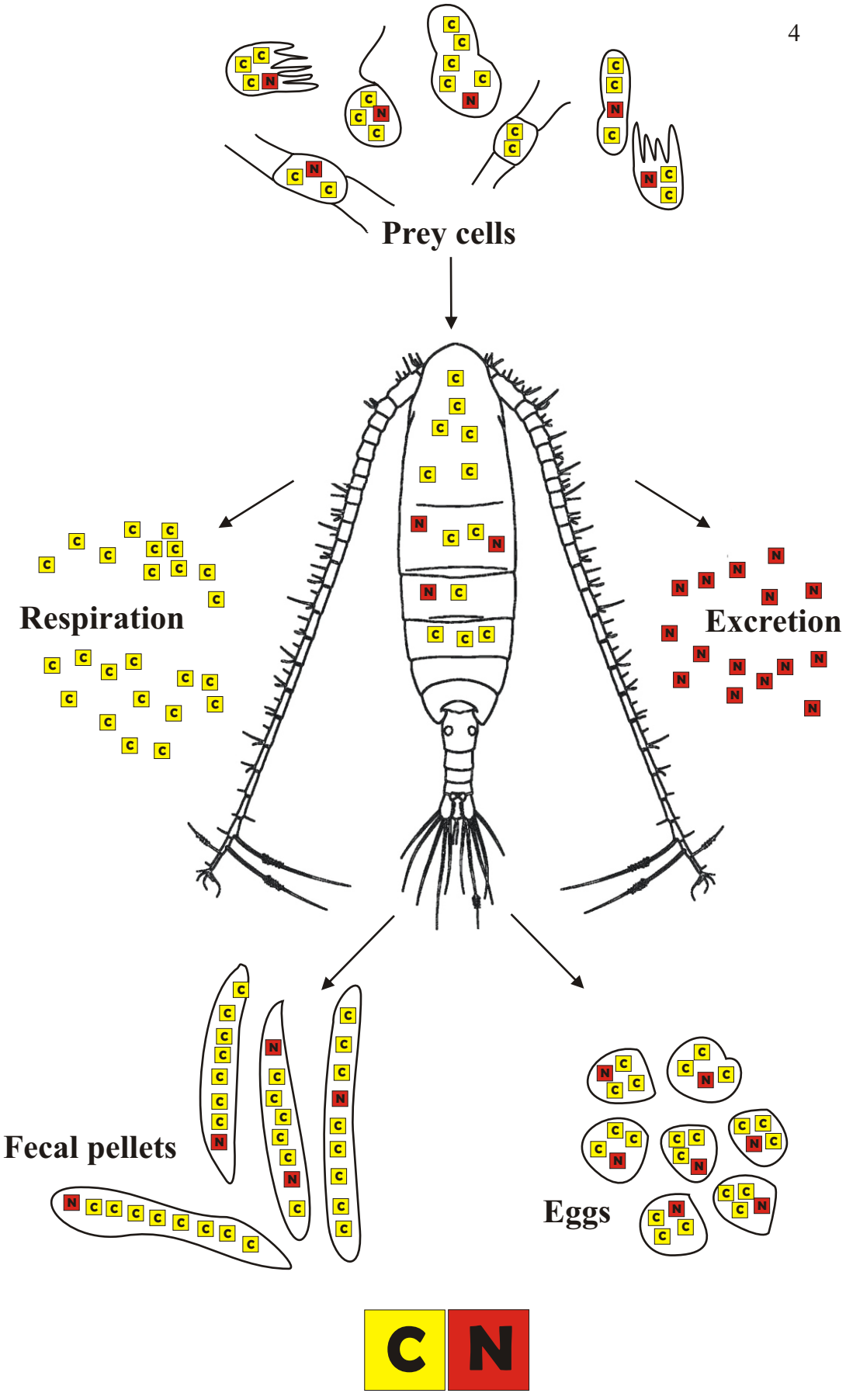

Figure 1.1. Schematic diagram of the carbon (C) and nitrogen (N) budgets in copepods

Note: not to scale

to the environment by pre- or post-absorptive processes. Pre-absorptive processes refer to those which alter the amount of dietary components assimilated. For example, this may be achieved by selective feeding, or by adjusting digestive enzyme activity. Post-absorptive regulation is typically achieved by varying respiration or excretion rates (see Anderson et al. 2005).

The elemental stoichiometric approach (Anderson and Hessen 1995) is based on two key assumptions: (1) substrates are used conservatively for growth and are solely of dietary origin, and (2) grazers are homeostatic, i.e. elements or compounds in the biomass of grazers have fixed ratios which therefore dictate dietary requirements. Carbon and N are immutable, and therefore must ultimately be derived from the diet. In a latitudinal comparison between the elemental ratios of marine copepods, Bamstedt (1986) demonstrated that the C:N ratios of low- and mid-latitude copepods do not differ significantly. These copepods can therefore be considered to be homeostatic with regard to C and N, thereby justifying the second assumption. However, the C:N ratio of high-latitude copepods, such as *C. finmarchicus*, is significantly higher than the low- and mid-latitude copepods (Bamstedt 1986), primarily because they seasonally sequester large quantities of carbon-rich lipid reserves (Sargent and Henderson 1986). Consequently, the total biomass of *Calanus* cannot be assumed to be homeostatic. Nonetheless, although the absolute quantities of C and N allocated to an individual egg may vary (Guisande and Harris 1995), the C:N ratio in the eggs of *Calanus* does appear to remain homeostatic (Pond et al. 1996, Anderson and Pond 2000). The assumption of homeostasis in consumer tissues is therefore justifiable when the eggs produced represent all positive growth. Thus, providing adequate account is taken of different potential sources of substrates (i.e. intake and body reserves), stoichiometry may be an effective tool for understanding the egg production of zooplankton such as *C. finmarchicus*.

1.3. Lipids and fatty acids in *Calanus*. Lipids perform various roles in organisms, notably being a key part of the structure of cell membranes, serving as energy reserves and acting as precursors for various hormones and vitamins. The major building blocks of lipids are fatty acids. These are carboxylic (organic) acids with long aliphatic tails, which may be either unsaturated or saturated i.e. with or without double bonds. PUFAs contain at least two double bonds. All fatty acids are described on the basis of their carbon chain length, the number of double bonds and

the number of carbon atoms between the final CH_3 molecule and the first double bond encountered (n-). For example, the PUFA eicosapentaenoic acid, 20:5(n-3), has 20 C atoms and 5 double bonds, the first of which lies 3 C atoms from the end of the molecule. Lipids consist of fatty acids esterified to a 'backbone' molecule of either glycerol or spinghosine.

The principal components of the energy reserves in *Calanus* are the long-chain monounsaturated fatty acids 20:1(n-9) and 22:1(n-11) (Kattner and Hagen 1995). They are stored in the oil sac, which can occupy most of the central body cavity. These moieties are absent in phytoplankton (e.g. Volkman et al. 1989, Viso and Marty 1993), yet characterise the fatty acid composition of calanoid copepods living in polar regions (Kattner and Krause 1987, Kattner 1989, Kattner and Graeve 1991, Graeve and Kattner 1992, Albers et al. 1996). Both 20:1(n-9) and 22:1(n-11) have a high calorific value (Albers et al. 1996, Scott et al. 2002b), and are thought to be synthesised *de novo* by *Calanus* from carbohydrate and protein precursors in the diet or through the elongation and desaturation of dietary fatty acids (Sargent and Henderson 1986, Sargent and Falk-Petersen 1988, Kattner and Hagen 1995).

Biological membranes consist of phospholipid bilayers and transmembrane proteins, forming a fluid or liquid-crystal mosaic (Singer and Nicolson 1972). The constituent fatty acids in the phospholipid bilayer dictate the temperatures over which the membrane will remain in a liquid-crystalline state, a requirement for the cell to function properly. PUFAs, particularly the n-3 moieties, have lower phase transition temperatures (melting points) than saturated or monounsaturated fatty acids, and the proportion of n-3 PUFAs in the phospholipids increases as environmental temperature decreases, thereby maintaining the membrane in the liquid-crystalline state (Sargent et al. 1989). In the marine environment, the predominant n-3 PUFAs are eicosapentaenoic acid (20:5(n-3) or EPA) and docosahexaenoic acid (22:6(n-3) or DHA) (Klungsoyr et al. 1989, Mayzaud et al. 1989, Parrish et al. 1995, Reuss and Poulsen 2002), which are produced by various phytoplankton groups.

1.3.1. The life-cycle of *C. finmarchicus*. In the North Atlantic, primary production peaks during the spring as the nutrient rich surface waters begin to stratify (Lochte et al. 1993). *C. finmarchicus* nauplii and juvenile copepodites feed on this seasonal delivery of food and sequester large energy reserves in the form of carbon-

rich lipids (Kattner and Hagen 1995). Beginning in early summer, the immature copepodites descend below the convective mixed layer and overwinter in diapause, where they typically remain for > 6 months (Hirche 1996a). During this period metabolism, growth and development are suppressed, and feeding ceases (Hirche 1996a). Upon termination of diapause at the end of winter, the immature copepodites return to the surface waters and undergo their final moult to become adult copepods. Throughout this period, all metabolic demands are met by the stored lipids (Jonasdottir 1999).

1.3.2. The lipid composition of *C. finmarchicus*. The lipids and fatty acids of *Calanus* have been well studied (reviewed by Sargent and Henderson 1986, Sargent and Falk-Petersen 1988). There is strong ontogenic variation in the quantities of lipid present, with the majority of storage lipids being sequestered during the copepodite CIII to CV stages (Kattner and Krause 1987, Tande and Henderson 1988, Hygum et al. 2000). Moulting from CV to adult, and the maturation of the gonad in the females are both energetically costly for *Calanus* (Rey-Rassat et al. 2002a), and CV copepodites allowed to develop into females in the laboratory have been observed to utilise approximately 50 % of their body lipid before the release of eggs (Gatten et al. 1980). Furthermore, wild populations of female *C. finmarchicus* have been observed to lose > 30 % of their biomass between their arrival in surface waters and the onset of the spring bloom (Hopkins et al. 1984). The total quantity of lipid in immature and adult copepodites varies both geographically and seasonally, reflecting the animals feeding and physiological history (Marshall and Orr 1955b, Gatten et al. 1980, Kattner and Krause 1989, Kattner 1989).

Copepods that inhabit the polar regions have been observed to reproduce in advance of the annual phytoplankton bloom, despite low food concentrations (Hirche and Kosobokova 2003). The use of internal reserves to fuel egg production in the sibling species *C. glacialis*, *C. hyperboreous* and *C. helgolandicus* has previously been reported (Smith 1990, Hirche and Kattner 1993, Hirche and Niehoff 1996, Niehoff et al. 2002, Niehoff and Hirche 2005 – see also Hagen and Schnack-Schiel 1996). Females of the more temperate species, *C. helgolandicus,* have also been shown to use carbon and nitrogen from their own biomass to meet metabolic and reproductive costs when food is limiting (Rey-Rassat et al. 2002b).

It is known that the early development of the gonads in *C. finmarchicus* is fuelled by internal reserves (Tande 1982, Sargent and Falk-Peterson 1988, Hirche 1996b, Rey-Rassat et al. 2002a, Pasternak et al. 2004), and a measurable reproductive output has been noted to continue for over 4 weeks when *C. finmarchicus* is starved (Marshall and Orr 1952, Hirche et al. 1997). Prolonged periods of spawning activity have also been observed well in advance of the spring bloom (Niehoff et al. 1999, Richardson et al. 1999, Gaard 2000), and during periods of food scarcity (Plourde and Runge 1993, Ohman and Runge 1994, Hirche 1996b, Jonasdottir et al. 2002), suggesting that the animals can use their biomass to fuel the production of eggs. Egg production with coincidental losses of C and N from the females' biomass before the spring bloom (Tande 1982, Irigoien et al. 1998) supports the idea that *C. finmarchicus* is capable of using somatic reserves to maintain a reproductive output. Indeed, a recent laboratory study (Niehoff 2004) has confirmed that when starved or maintained at low food concentrations, *C. finmarchicus* does utilise internal sources of C and N in addition to the material ingested to maintain egg production. It is hypothesised that reproducing in advance of the spring bloom will enable the lipid accumulation stages of the offspring to coincide with the peak of the bloom, thus maximising their chances of accruing a plentiful energy reserve to survive diapause and mature into an adult the following year (Irigoien 2004). Clearly, if the use of body reserves to fuel egg production are not taken into consideration when examining the limiting potential of specific dietary components, erroneous conclusions are likely to result, since they may be readily supplied from the animal's biomass.

1.3.3. Essential fatty acids in *Calanus*. Essential compounds are those that cannot be synthesised or are synthesised in inadequate quantities to sustain growth and survival and must therefore be obtained at least in part, but not necessarily wholly, from the diet (Spector 1999). Assuming that structural biomass of consumers is homeostatic, it follows that when the demand for an essential compound is not met by the quantities available through ingestion and synthesis, the consumer will face a nutritional imbalance. The compound in most demand relative to supply then limits the production of new biomass (Anderson and Pond 2000).

The PUFAs EPA and DHA are essential for the growth and development of marine animals (Enright et al. 1986). They are principally associated with cell

membranes, but have also been observed to affect specific physiological functions. For example, they serve as precursors to the eicosanoids, a group of chemical 'messengers' that are crucial for a wide range of physiological processes, including the regulation of ionic fluxes, oocyte maturation, spawning and hatching of eggs in invertebrates (Stanley-Samuelson 1987). In the context of this study, EPA and DHA are of particular interest because when maintained on a diet devoid of these compounds, copepods soon cease egg production (Stottrup and Jensen 1990). Furthermore, egg production rates correlate with the quantities of these fatty acids in the diet (e.g. Jonasdottir et al. 2002, Hazzard and Kleppel 2003, Shin et al. 2003).

1.3.4. Extending stoichiometry theory to micronutrients. The fixed proportionality between food N and egg production seen in laboratory experiments does suggest that marine copepods are N limited (Checkley 1980, Kiorboe 1989). However, the C:N ratio of marine seston is characteristically < 10 (Copin-Montegut and Copin-Montegut 1983) and, in combination with the typically low gross growth efficiencies for C in copepods (Straile 1997), this would indicate that N limitation should be uncommon (Anderson and Hessen 1995). Anderson and Hessen (1995) argued that the low growth efficiencies for N (~0.4) are not consistent with limitation by N because limiting elements should be used with high efficiencies. What then causes copepods to only use N with a low efficiency in laboratory experiments, and indeed very possibly in the natural marine environment? A possible solution is that something other than N, but which covaries with it, is limiting. Imbalances in 'micronutrients' have been suggested, and essential amino- or fatty acids have both been identified as dietary substrates with the potential to limit zooplankton production (Anderson and Pond 2000, Anderson et al. 2004). Copepods fed algal monocultures are particularly prone to limitation by essential micronutrients because imbalances between the composition of the prey and the requirements of the consumer cannot be reconciled by selecting a balanced ration based on different food types. The extent to which micronutrient limitation occurs in natural marine systems remains unknown. However, positive correlations between PUFAs and egg production (see above) suggest that it may be prevalent. When examining the limiting potential of essential micronutrients such as EPA and DHA using stoichiometric theory, the key assumptions (Section 1.2.1.) must be re-examined with regard to PUFAs.

1.3.5. The origin of EPA and DHA in copepods. Stoichiometric theory generally assumes that substrates are used conservatively. There is evidence to suggest that EPA and DHA can be synthesized by benthic copepods (Desvilettes et al. 1997, Nanton and Castell 1999), although, it is generally thought that the enzymes involved are slow and inefficient in most crustaceans (see Brett and Muller-Navarra 1997). This is thought to explain why high zooplankton growth rates are typically observed when EPA and DHA are readily available in the diet (Brett and Muller-Navarra 1997, Pond et al. 2005). As a first approximation, it therefore appears reasonable to assume that these PUFAs must be derived from the diet. Nonetheless, the substrates used are not necessarily of dietary origin, nor used conservatively i.e. they may be derived from internal sources or via the alteration of another compound. Consequently, the stoichiometric approach must consider the variable origin of EPA and DHA (diet/biomass/synthesis) in order to provide a realistic understanding of the element or compound that limits production.

1.3.6. Homeostasis of EPA and DHA in *Calanus*. The composition of non-essential fatty acids in *Calanus* is known to change significantly in response to that of the diet (Graeve et al. 1994), but the extent to which EPA and DHA are homeostatic relative to C and N remains poorly understood because such data are typically lacking. At the cellular level, essential PUFAs could well remain homeostatic relative to C and N because small variations in these ratios may disrupt the cells ability to function properly. However, up to 50 % dry weight of *Calanus* can be attributed to lipid reserves (Bamstedt 1986). Homeostasis of EPA and DHA at the animal level may be unlikely because the lipid reserves are sequestered extracellularly and therefore concurrent increases in EPA and DHA are not required in order to maintain cellular homeostasis. If these animals are capable of reproducing from body reserves when food is scarce, internal sources of essential fatty acids will be necessary unless these nutrients are derived from the catabolism of the animals' biomass. However, as previously mentioned, if eggs are produced by *Calanus* without the gain of biomass i.e. eggs represent all positive growth, it is the stoichiometric balance in the eggs rather than the biomass that sets the demands for substrates. It follows that the degree to which EPA and DHA in the eggs are homeostatic must then be considered.

1.3.7. Homeostasis of essential fatty acids in the eggs of *Calanus*. Dietary control over the composition of non-essential compounds in copepod eggs has previously been reported for *Calanus* (Laabir et al. 1999, Lacoste et al. 2001, Helland et al. 2003b), *Acartia* (Ederington et al. 1995) and *Euterpina* (Guisande et al. 1999, 2000). However, C, N, EPA and DHA in the eggs of *C. helgolandicus* do appear to be relatively homeostatic (Pond et al. 1996, Anderson and Pond 2000). Accordingly, each of these constituents has the potential to limit egg production (assuming EPA and DHA cannot be synthesized in appreciable quantities), should demand exceed supply. Homeostasis of other essential compounds in the eggs of *C. finmarchicus* has also been reported. Helland et al. (2003b) found that the composition of essential amino acids in the eggs remained constant, irrespective of maternal diet or season. In contrast, other data suggest that the fatty acid composition of the eggs of *Calanus* (*C. helgolandicus*), including EPA and DHA, varies in relation to the parental diet. However, detectable quantities of EPA and DHA were found in the eggs, even when absent from the diet (Lacoste et al. 2001). This suggests either *de novo* synthesis or maternal control over the levels of these essential fatty acids. That egg production rates rapidly declined to zero for all copepods when either completely starved or fed a diet deficient in EPA and DHA suggests maternal control rather than *do novo* synthesis. It is thought that internal reserves of PUFAs are unlikely to contribute significantly to the provision of these fatty acids for egg production (Anderson and Pond 2000). Therefore, the PUFA composition of the eggs sets the demands for these substrates.

1.4. Food and feeding selectivity. When trying to ascertain the element or compound responsible for limiting copepod production it is essential to accurately determine the quantity and quality of material ingested, rather than the bulk properties of the seston. In turn, because individual microplankton groups differ qualitatively, to understand the quality of the ingested diet requires specific knowledge of the feeding behaviour of *Calanus*.

The process of feeding in *Calanus* was first described by Esterly (1916 c.f. Marshall and Orr 1955a), and has subsequently received considerable attention (e.g. Harvey 1937, Gauld 1966). In brief, the feeding current created by the maxillipedes and maxillulary epipods carries particles forward into the filter-chamber where

particles are intercepted by the maxillary setae. Food items are then combed off the setae by the spines of the maxillulary endites and passed to the mouth. Numerous laboratory studies using cultured phytoplankton have demonstrated that copepods are capable of discriminating between different sized cells as a result of the structure of their filtering apparatus, and thereby preferentially ingest larger prey items (e.g. Marshall and Orr 1955a, Frost 1977, Berggreen et al. 1988). Early investigations into the feeding mechanisms of *Calanus* proposed that the minimum distance between the finest setules on the filtering appendages physically determined the minimum attainable prey size (Ussing 1938 cf. Marshall and Orr 1955b). It was suggested that the smallest ingestible organisms for *Calanus* must be >5.7 μm, and this idea was supported by the finding that cells < 10 μm were cleared by adults at much lower rates than larger cells (Marshall and Orr 1955b). However, using a mechanistic approach to feeding, Boyd (1976) suggested that in order to feed on small cells, copepods might simply increase the beating speed of the feeding appendages. Cowles (1979) subsequently proposed that *Calanus* was capable of increasing the fluid velocity across the particle capture appendages. According to the theory of particle motion in fluid flow (at low Reynolds number), this will increase the capture efficiency of smaller particles (Rubenstein and Koehl 1977). It has also been proposed that copepods can change the intersetule distances, thus altering their spectrum of retainable particles (Wilson 1973). Morphological evidence, based on electron microscope studies of the filtering apparatus of calanoid copepods (Friedman 1977 cf. Cowles 1979), supports this notion. Recently, Irigoien et al. (1998) conceded that at low food concentrations, small cells should be considered as a possible food source for *Calanus*. Indeed, a range of zooplankters have been reported to positively select cells <20μm (Perissinotto 1992). Meyer et al. (2002) highlighted the importance of small cells in the diet of *Calanus* spp., and other recent work has shown *C. finmarchicus* capable of efficiently grazing cells ~5μm (Huntley 1981, Hansen et al. 1994b, Nejstgaard et al. 1997), with such cells maintaining optimal reproductive output (Bamstedt et al. 1999) and constituting the majority of the total carbon ingested at times (Levinsen et al., 2000b).

When copepods are presented a natural microplankton assemblage, trends in feeding selection are not always apparent and sometimes contradictory. For example, the diet of *Calanus* spp. in both the Labrador Sea and in the English Channel was reported to closely reflect that of the available microplankton community (Huntley

1981, Irigoien et al. 2000a), whereas diatoms were strongly selected in the Norwegian Sea (Meyer-Harms et al. 1999). Despite such inconsistencies, a common finding from studies offering natural microplankton assemblages is that in general, epipelagic copepods clear microzooplankton at higher rates than autotrophic cells (Stoecker and Egloff 1987, Gifford and Dagg 1991, Atkinson 1994, 1995, 1996, Verity and Paffenhofer 1996, Irigoien et al. 1998, Zeldis et al. 2002, Bollens and Penry 2003) and strong positive selection is typically shown towards motile prey (e.g. Nejstgaard et al. 2001b, Bollens and Penry 2003).

According to recent prey switching theory (Saiz and Kiorboe 1995, Kiorboe et al. 1996; see also Greene 1988, Jonsson and Tiselius 1990, Tiselius and Jonsson 1990), when the environment is dominated by non-motile prey, copepods adopt a suspension feeding mode in which food items are entrained into the feeding current created by rhythmical beating of the maxillipedes (see Marshall and Orr 1955b). However, the 'jump' escape response typical of ciliates under attack by copepods has been shown to be effective in reducing their mortality (Broglio et al. 2001, Jakobsen 2001). Thus, when *Calanus* adopts a suspension feeding mode, ciliates may be expected to be under-represented in the diet relative to the food environment unless; a) the escape response is ineffective against *Calanus*' feeding current or; b) upon detection (mechanoreception) of ciliates (see Visser 2001), *Calanus* briefly switches to a raptorial mode of feeding. Jakobsen (2001) showed that the level of water disturbance required to elicit an escape response in ciliates was lower than the disturbance created by the feeding current of small copepods. Therefore it is unlikely that they would be ingested if *Calanus* was simply suspension feeding. Upon detection of motile prey, *Calanus* has previously been observed to switch from the characteristic suspension-feeding mode to one of active predation (Conover 1966). Subsequent quantitative experimentation has supported these early observations, illustrating differential feeding behaviours for non- and motile prey in *Calanus* (Landry 1980, Landry 1981).

Studies that only consider the ingestion of autotrophic material (e.g. using the gut fluorescence technique) have frequently shown that the amount of ingested carbon fails to fulfil the metabolic demand, and it is often suggested that heterotrophic microzooplankton are consumed to fulfil this shortfall (Dagg and Walser 1987, Gifford and Dagg 1991, White and Roman 1992, Atkinson 1996, Razouls et al. 1998, Mayzaud et al. 2002a, b). Indeed, copepods derive substantial

proportions of their daily rations from ciliates and other heterotrophic protists (Gifford and Dagg 1991, Kleppel et al. 1996, Rollwagen Bollens and Penry 2003). In addition to their quantitative importance, there is an increasing amount of information illustrating the qualitative importance of microzooplankton in the diet of copepods (Stoecker and Capuzzo 1990, Kleppel 1993). Corner et al. (1976) demonstrated that the copepod *C. helgolandicus* had a significantly higher assimilation efficiency for nitrogen when feeding carnivorously. The faster and more efficient utilisation of the digested components was attributed to the strong similarities between the biochemical compositions of *C. helgolandicus* and their metazoan prey (barnacle nauplii). Both ciliates and dinoflagellates are relatively rich in nitrogen when compared to diatoms (Stoecker and Capuzzo 1990) and it has been suggested that this renders microzooplankton of higher nutritional quality (Gifford and Dagg 1991). Whilst it is acknowledged that the biochemical composition of cultured algae varies depending on the conditions under which it was grown (Ackman et al. 1968, Chuecas and Riley 1969, Dunstan et al. 1993), for a given cell volume, cultured dinoflagellates are estimated to provide 2-6 times more protein, 2.5-3.5 times more carbohydrate, and 1.1-3 times more lipid that diatoms (Hitchcock 1982). Ciliates contain 1.8 times more carbon times that of a dinoflagellate of equivalent volume (Ohman and Runge 1994). Indeed, there appears to be a causal relationship between *in situ* copepod egg production and the abundance of microzooplankton (Runge 1985, White and Roman 1992, Ohman and Runge 1994, Jonasdottir et al. 1995, Pond et al. 1996). Additions of ciliates or rotifers to mono-specific algal diets of copepods causes a reduction in development time, increases the longevity of females, and also increases egg production (Stoecker and Egloff, 1987, Bonnet and Carlotti 2001). This is possibly because protozoa are an important source of essential nutrients, particularly specific PUFAs (Stoecker and Capuzzo 1990). Therefore, in addition to providing information about the physical and behavioural aspects of copepod feeding, determining patterns of food selection can also be used to provide information about the potential quality of the diet of *Calanus*.

1.4.1. *Calanus* and detritus. Detritus features as a dietary component for some copepods (Heinle et al. 1977, Kosobokova et al. 2002, Schnetzer and Steinberg 2002 Kattner et al. 2003), and *Calanus* has been observed to ingest dead phytoplankton cells and copepod fecal pellets (Paffenhofer and Strickland 1970,

Paffenhofer and Knowles 1979). However, the extent to which *Calanus* ingests "marine snow", fragile organic aggregates that are formed by the coagulation of smaller particles such as phytoplankton and fecal pellets (Alldredge and Silver 1988), remains largely unknown. This is primarily because examining this question remains methodologically complex (Dilling and Brzezinski 2004). Early experimental work demonstrated that *Calanus* was unable or unwilling to ingest marine snow (Paffenhofer and Strickland 1970), but more recently, Dilling et al. (1998) suggested that marine snow was ingested in the absence of other food sources. However, in these experiments *Calanus* adopted a 'benthic feeding mode', only ingesting material that had collected on the base of the experimental containers (Dilling et al. 1998). Since this situation does not occur in the open ocean, marine snow is not considered to play an important role in the ingestion of *Calanus* (Irigoien et al. 1998, Meyer-Harms et al. 1999). It is likely that the mechanical process of *Calanus* swimming and feeding in open water causes the fragile aggregates to fragment, deeming them too small for efficient ingestion (see Dilling and Alldredge 2000). *Calanus* is considered to feed primarily on viable microplankton cells (Kleppel 1993, Harris 1996), and therefore determining total POC levels will provide little information about the quantities of available food unless the majority of this POC is comprised of cells that are readily ingestible.

1.5. The use of characteristic 'biomarker' fatty acids. It is well established that algal classes have characteristic fatty acid profiles (Table 1.1) which can be used to study trophic interactions between marine consumers and their food supply (Sargent et al. 1987). The C18 fatty acids, particularly 18:4(n-3) and 18:1(n-9), are primary components of cultured flagellates (Table 1.1), and an increase in their relative abundance is often observed in the field when the communities switch from diatom to flagellate dominance (Kattner et al. 1983, Claustre et al. 1989, Mayzaud et al. 1989, Reuss and Poulsen 2002). Several individual fatty acids have been proposed as indicators of individual flagellate groups (Table 1.1). For example, the fatty acid 18:1(n-9) is abundant in the Prymnesiophycean *Phaeocystis pouchetti* (Nichols et al. 1991) and has been suggested as a possible marker for this genus on the basis of laboratory studies and the prominence of 18:1(n-9) in *Phaeocystis* sp. blooms (Al-Hasan et al. 1990, Claustre et al. 1990, Skerratt et al. 1995, Tang et al. 2001, Reuss and Poulsen 2002). However, it is also present in appreciable quantities in other

Table 1.1. Fatty acid biomarkers and their sources.

	Source	Fatty Acid	Lab. studies	Field studies
	Detritus	18:0		21, 22, 25, 26
		18:1(n-9)		24, 25
Diatoms	Bacillariophyceae	16:1(n-7)	1, 2, 5, 6, 10	12, 13, 14, 19, 20, 21, 24
		16:4(n-1)	1	21
		20:5(n-3)	1, 2, 6, 10	12, 14, 20, 24
		High 16:1(n-7): 16:0 ratio	1, 2, 6, 10	13, 16, 19, 20, 21
Dino-flagellates	Dino-phyceae	18:4(n-3)	1, 2, 3	21
		20:5(n-3)	1, 2, 3, 4, 10,	12, 13, 21
		22:6(n-3)	1, 4, 10,	12, 21
Flagellates	Chloro-phyceae	16:4(n-1)	2,	
		18:3(n-3)	1, 2, 8, 10	
	Crypto-phyceae	18:4(n-3)	1, 2, 6, 10	
		20:5(n-3)	1, 2, 6, 10	
		22:6(n-3)	6	19
	Haptophyceae	18:1(n-9)	2, 6, 7, 10, 11	17, 18, 20, 24
		18:4(n-3)	2, 6, 10, 11	
		18:2(n-6)	2, 10, 11	15, 23
		22:6(n-3)	6	
	General flagellates	18:4(n-3)	1, 2, 6, 8, 9, 10, 11	
		C18	1, 2, 6, 8, 9, 10, 11	12, 14, 24
		22:6(n-3)	1, 3, 4, 6, 8, 9, 10	19
		Low 16:1(n-7): 16:0 ratio	1, 4, 6, 8, 10, 11	13, 16, 18, 20, 24

Lab. studies: [1]Ackman et al. 1968, [2]Chuecas and Riley 1969, [3]Harrington et al. 1970, [4]Nichols et al. 1984, [5]Nichols et al. 1986, [6]Volkman et al. 1989, [7]Nichols et al. 1991, [8]Dunstan et al. 1992, [9]Dunstan et al. 1993, [10]Viso and Marty 1993, [11]Tang et al. 2001, Field studies: [12]Kattner et al. 1983, [13]Claustre et al. 1989, [14]Fraser et al. 1989b, [15]Klungsøyr et al. 1989, [16]Mayzaud et al. 1989, [17]Al-Hasan et al. 1990, [18]Claustre et al. 1990, [19]Parrish et al. 1995, [20]Skerratt et al. 1995, [21]Leveille et al. 1997, [22]Pond et al. 1998a, [23]Hamm et al. 2001, [24]Reuss and Poulsen 2002, [25]Scott et al. 2002a, [26]Hama 1999

Haptophyceans, and also Cryptophyceans and Dinophyceans (Ackman et al. 1968, Cheucas and Riley 1969, Harrington et al. 1970, Volkman et al. 1981, Volkman et al. 1989, Viso and Marty 1993), thus limiting its potential as a genus-specific indicator (see Reuss and Poulsen 2002). In contrast, diatoms are rich in 16:1(n-7) and 20:5(n-3) (Table 1.1). Field studies have shown that these fatty acids correlate well with the biomass of diatoms and also each other (Kattner et al. 1983, Claustre et al. 1989, Skerratt et al. 1995, Reuss and Poulsen 2002).

The 16:1(n-7)/16:0 ratio can be used to understand the relative contributions of diatoms and flagellates to the microplankton community (Claustre et al. 1990, Parrish et al. 1995, Skerratt et al. 1995, Reuss and Poulsen 2002). In culture studies, this ratio is typically > 1 in diatoms (Ackman et al. 1968, Volkman et al. 1989, Viso and Marty 1993), and in flagellates it is generally below 0.5 (Volkman et al. 1989, Al-Hasan et al. 1990, Nichols et al. 1991, Dunstan et al. 1992, Viso and Marty 1993, Tang et al. 2001). These approximate values are also confirmed by field observations (Claustre et al. 1989, Mayzaud et al. 1989, Claustre et al. 1990, Parrish et al. 1995, Skerratt et al. 1995, Leveille et al. 1997). However, it is clear that absolute values of this ratio vary between studies, most likely reflecting the dominant species analysed, and also the environmental conditions under which they grew. For example, 16:1(n-7)/16:0 ratios of *Thalassiosira pseudonana* cultures grown under different light regimes vary between 1.06 and 2.30 (Thompson and Harrison 1992). Unfortunately, because of this variability, it is difficult to ascribe a fixed value above which diatoms dominate flagellates or vice versa (Reuss and Poulsen 2002). Therefore although the 16:1(n-7)/16:0 ratio can provide qualitative information about the relative importance of diatoms and flagellates, it requires confirmation using a complementary technique (e.g. high performance liquid chromatography combined with CHEMTAX analysis (Mackey et al. 1996), inverted microscopy).

In addition to providing information about living cells, fatty acid compositions can be used to examine the presence of detritus. By combining ^{13}C tracer and gas chromatography/mass spectrometry (GC/MS) methods to determine the fatty acid composition of photosynthetic products, fatty acids of phytoplankton and detrital origin have been distinguished (Hama 1991, 1999). Using these techniques, Hama (1999) demonstrated that PUFAs were most prominent in the lipids of phytoplankton origin, whilst 18:0 existed primarily as a component of non-living particles. In almost all cultured phytoplankton, the fatty acid 18:0 usually

accounts for < 2 % of the total fatty acids (e.g. Viso and Marty 1993), yet it is often reported to be a major component (> 10 %) of particulate lipids (Moriss 1984, Hama 1999, Reuss and Poulsen 2002). This demonstrates that detritus contributes significantly to the particulate material in many areas (Hama 1999). The POC:PUFA ratio can therefore be used to draw inferences about the relative contribution that the microplankton biomass makes to total POC measurements. Conversely, the importance of 18:0 in the overall fatty acid composition provides information about the relative importance of detritus in the seston.

Fatty acid biomarkers have been successfully used to provide qualitative and sometimes quantitative information about phytoplankton community species compositions (Kattner et al. 1983, Morris 1984, Claustre et al. 1989, Claustre et al. 1990, Skerratt et al. 1995, Leveille et al. 1997, Reuss and Poulsen 2002). Furthermore, various authors have demonstrated the transfer of non-essential fatty acids from phytoplankton to copepods (Lee et al. 1971, Graeve et al. 1994b, Ederington et al. 1995). For example, Graeve et al. (1994) took *C. finmarchicus* with a fatty acid profile dominated by fatty acids representative of dinoflagellates (e.g. 18:4(n-3); Table 1.1) and fed them with diatoms (rich in 16:1(n-7); Table 1.1). The dinoflagellate lipid pattern was entirely replaced by the characteristic diatom fatty acids within 6 weeks. The fatty acid compositions of consumers have therefore been used to provide information about their diets (Sargent et al. 1985, Fraser et al. 1989a, Klungsoyr et al. 1989, Norrbin et al. 1990, Kattner and Hagen 1995, Falk-Petersen et al. 2002, Scott et al. 2002a, Stevens et al. 2004).

1.6. Objectives of the thesis. The overlying aim of this study was to investigate the significance of food quality, expressed in terms of C, N, EPA and DHA, in influencing the egg production of *C. finmarchicus* when feeding on natural planktonic diets. To achieve this, the following objectives were defined:

1. To undertake experiments at sea with adult *C. finmarchicus* to determine the quantity and quality of food consumed when presented with a natural diet, and the efficiencies with which C, N, EPA and DHA are used for egg production.
2. To investigate the relationship between the biochemical composition of copepod eggs and that of ingested food – does the C, N and fatty acid

composition of the eggs change in response to changes in availability in food?

3. To quantitatively assess the roles of C, N, EPA and DHA in limiting egg production using the stoichiometric theory of Anderson and Pond (2000).

1.7. Summary of the thesis. Ship-board incubation experiments in the northern North Atlantic with female *C. finmarchicus* were undertaken in April and July/August 2002. Egg production and ingestion rates were determined whilst maintaining the females on a diet of natural microplankton under *in situ* conditions. The quantity and quality of the ingested food was determined by its C, N, EPA and DHA content. Similarly, the biochemical content of the females and their eggs was determined, allowing the efficiencies with which each dietary substrate was used for egg production to be assessed. The limiting potential of C, N, EPA and DHA was examined using the stoichiometric theory of Anderson and Pond (2000). The following list provides a brief synopsis of the information presented within each chapter:

- *Chapter 2* details the methods used during this study.
- *Chapter 3* examines in detail the problems associated with particle removal experiments in which copepods graze on natural plankton assemblages. A refined method for the estimation of copepod grazing rates that takes into consideration microzooplankton grazing artefacts is presented.
- *Chapter 4* describes the quantity and quality of the microplankton offered to the animals during the incubations. Seasonal comparisons between the components of the seston, including detritus, are also made. The usefulness of fatty acid biomarkers is assessed by correlating the biomass of individual cell groups with their respective biomarkers.
- *Chapter 5* compares and contrasts the dynamics of C, N, EPA and DHA during the incubations and details the ingested quantities of these dietary substrates.
- *Chapter 6* reports egg production rates and details the changes that occur in the animals' biomass over the duration of the incubations. Homeostasis of the animals and their eggs is examined with regard to N, EPA and DHA. The

extent to which the fatty acid composition of the eggs is determined by that of the diet is also examined.

- *Chapter 7* develops the stoichiometric equations of Anderson and Pond (2000) to incorporate the use of substrates derived from parental biomass. These equations are used to quantify the potential for C, N, EPA and DHA to limit egg production using the data presented in the previous chapters. The efficiencies with which each dietary substrate are used for egg production are also examined.
- *Chapter 8* discusses the experimental limitations of this study and summarises the key findings.

Chapter 2

Experimental and analytical methods

2.1. Collection of samples. All data were collected in 2002 on board RRS *Discovery* under pre- and post-bloom conditions in the northern North Atlantic, on cruises D262 (18/04/02 – 27/05/02) and D264 (25/07/02 – 28/08/02) respectively (Figure 2.1) as part of the Natural Environment Research Council's thematic program 'Marine Productivity' (http://www.nerc.ac.uk/funding/thematics/marprod/). The work presented here focuses on copepod feeding, egg production and fatty acid consumption at stations on the Reykjanes Ridge in April (26 - 30/04/02) and July/August (30/07 – 03/08/02; Figure 2.2). Participation on D260 (06/03/02 – 23/03/02, also North Atlantic) was solely for the purpose of verifying the experimental protocol and to produce samples for trial analyses.

2.2. General procedure. In brief, 5 groups of 10 female *C. finmarchicus* were incubated for 5 consecutive 24 hour periods to determine daily ingestion, egg production and changes in female biochemical composition. Females were collected and live-sorted into replicate groups of 5 to determine their carbon (C) and nitrogen (N) and fatty acid content at the start of the incubation. Groups of 10 females from the same sample were placed into 6 bottles (2.2 l) of pre-screened (90 μm) seawater from the chlorophyll maximum. In addition, 4 bottles (2.2 l) of pre-screened seawater were maintained as controls. All bottles were incubated on a water cooled plankton wheel under ambient conditions for 24 hrs. The females were then carefully removed and placed into bottles of fresh pre-screened seawater and incubated with fresh controls for a further 24hrs. This process was repeated for 5 days (to enable ingested food to be translated into eggs). The experimental and control bottles were sampled at the start and end of each day, for microplankton, C/N, and fatty acid analyses, enabling the quantity (biomass) and quality (biochemistry) of the food consumed to be determined. Eggs were removed at the end of daily incubations for biochemical analysis, permitting the relationship between food quantity/quality and egg production to be determined. At the very end of the experimental period, the females were divided into replicate groups to quantify their final C, N and fatty acid content.

2.3. Experimental protocol

2.3.1. Animals. Copepods were collected using a 250 μm, 1 m diameter plankton net with a non-filtering cod-end, hauled vertically from 100 m. The contents of the cod-end were gently poured into a 20 l bucket of fresh surface sea

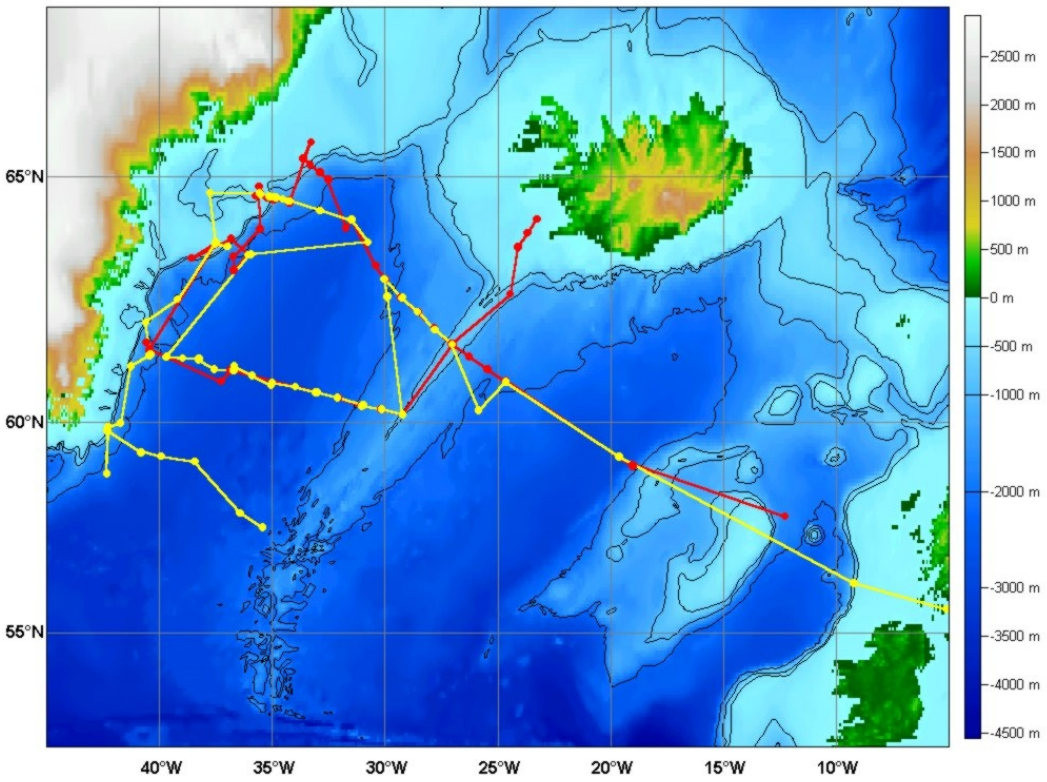

Figure 2.1 Map of the study area (Irminger Basin) with cruise tracks from D262 shown in red and D264 shown in yellow. Scale on right hand side indicates elevation in metres (M).

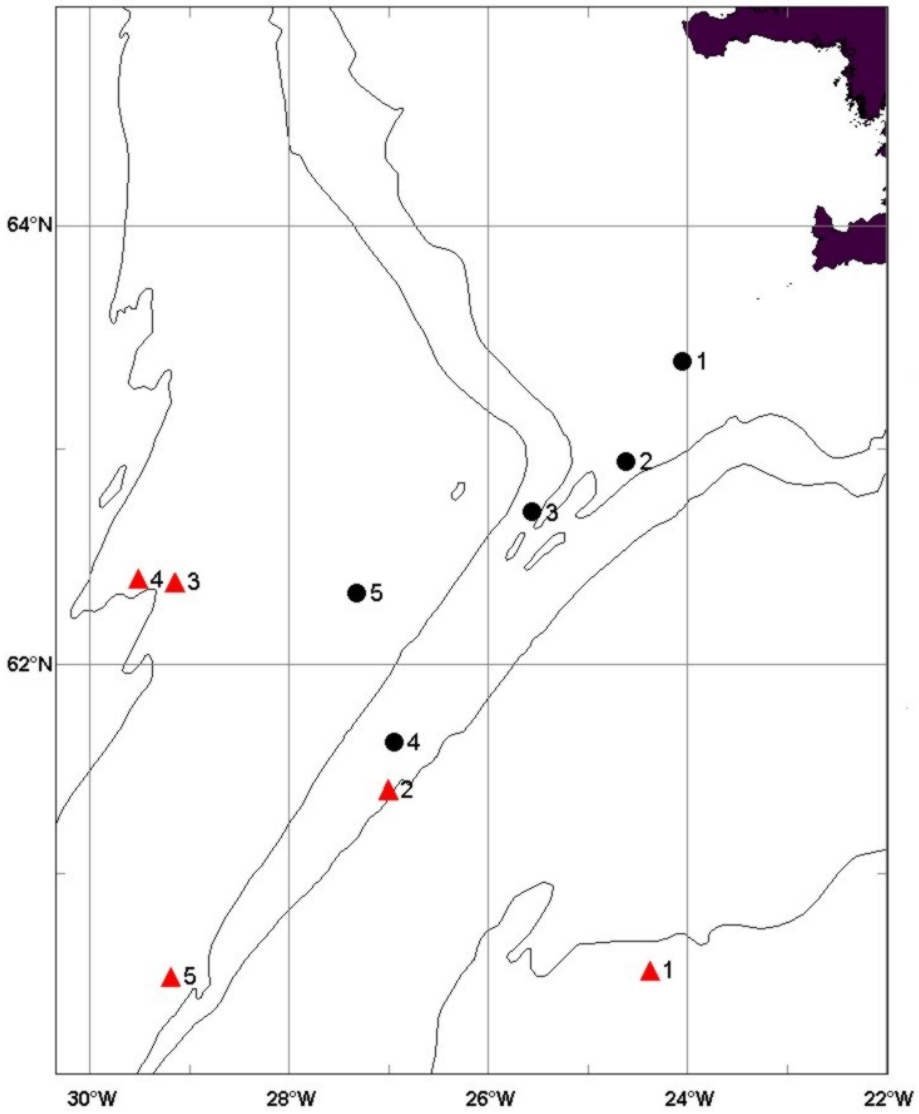

Figure 2.2. Locations of water sampling stations along the Reykjanes Ridge in April (black circles) and July/August (red triangles). Number denotes incubation day. See Figure 2.1 for overview of the study area.

water (from non-toxic supply). Female *C. finmarchicus* for the feeding incubations were sorted into groups of ten under the dissection microscope using a wide-bore pipette. All animals were individually inspected to ensure that the antennules and sensilla were intact and that they were free from parasites.

The biochemistry of the animals at the start of the experiments (initial animals) was determined by sorting 5 replicate groups of 5 females from the same sample for C/N and fatty acid analysis. Upon termination of the 5 day incubation period, the animals from each experimental bottle were split into two groups: 5 for elemental and 5 for fatty acid analysis (final animals). Animals for C/N analysis were stored in tin cups, whilst those for fatty acid analysis were placed into 1.1 ml screw-capped, Teflon septum vials and completely filled with solvent (Chloroform:Methanol 2:1 v/v) before storage. All animals for biochemical investigation were stored at –80º until analysis.

2.3.2. Collection of water. Water containing the natural microplankton assemblage from the chlorophyll *a* maximum (located by examining the downwards fluorescence profile of the CTD cast) was collected in 10 l Niskin bottles with Teflon fittings. Seawater from the non-toxic supply (pumped by means of an impellor from ~5 m below the surface) was used only when the weather was too bad to deploy the CTD rosette. The water was gently screened with a submerged 90 μm mesh to remove other copepods, then carefully transferred via silicone tubing into the 2200 ml clear glass incubation bottles. Each bottle was filled a little at a time to ensure maximum homogeneity between bottles. All incubation bottles (experimental and control) were topped up with the screened seawater and sealed with clingfilm to remove air bubbles.

2.3.3. Incubation protocol. Bottles containing the screened microplankton assemblage and 10 female *C. finmarchicus* copepods are referred to as experimental bottles. Those containing only the microplankton assemblage without copepods are referred to as control bottles. Feeding rates were quantified by incubating 6 experimental bottles alongside 4 controls for 24 hrs on a water-cooled plankton wheel illuminated by natural light at the *in situ* photoperiod. The water was sampled at the start and end of each incubation for 'initial' and 'final' particulates (Section 2.3.5.). After each 24 h incubation period, the copepods were carefully transferred via a dip-tube to bottles of fresh, screened seawater from the chlorophyll *a* maximum and incubated for a further 24 h. The females were incubated for a total of 5

consecutive days, and appeared intact and healthy upon termination of the experiments.

2.3.4. Eggs. Eggs and faecal pellets were removed from the experimental bottles at the end of each incubation by gentle filtration (63 µm). Control bottles were treated correspondingly. The water was then sampled for 'final' particulates (Section 2.3.5.). The eggs from each experimental bottle were counted under a dissection microscope. Half of the total eggs produced each day were stored on a pre-combusted GF/F filter (12 h in muffle furnace at 500 ºC to remove any organic contamination: Feely and members of the working group, 1991) for elemental analysis. The remainder were stored in a 1.1 ml screw-capped, Teflon septum vial filled with solvent (Chloroform:Methanol 2:1 v/v) for fatty acid analysis. All egg samples were stored at –80º.

2.3.5. Particulate sampling. To determine how the composition, abundance and biochemistry of the microplankton community changed during the 24 hr incubations and thus the quantity and quality of food consumed by the copepods, a suite of samples were taken from the water at the beginning and end of the incubations. 'Initial' samples refer to those taken from the screened in the incubation bottles at the start of the incubation period. 'Final' samples refer to those taken from the experimental and control bottles following the removal of eggs and faecal pellets at the end of the daily incubation period. The initial microplankton sample was a single 200 ml aliquot. Final microplankton samples were 100 ml aliquots taken from each of the experimental and control bottles, following the removal of eggs. All microplankton samples were preserved with 10 % acid Lugol's solution (see Appendix 1) and stored in amber medicine bottles in the dark until analysis.

A particulate sample for biochemical analysis (C/N or lipid) consisted of the particulate matter from 1000 ml of water, collected on a pre-combusted GF/F filter under gentle vacuum. Triplicate initial samples for C/N and fatty acid analyses were taken at the start of each day. Following the removal of the final microplankton sample at the end of each incubation period, each bottle yielded a single, final particulate sample for C/N and fatty acid analyses.

Because of the sensitive nature of the elemental and fatty acid analyses, filters were only handled using clean stainless steel forceps (Ehrhardt and Koeve 1999). Those for fatty acid analysis were folded in half, then in half again, slotted into 2 ml screw-capped, Teflon septum vials and completely filled with solvent

(Chloroform:Methanol 2:1 v/v). Filters for C/N analysis were folded in half (sample side inwards). All particulate samples were stored in individual labelled polythene zip-lock bags and maintained at –80 ºC.

2.4. Microplankton analysis

2.4.1. Sample preparation. Two aliquots from Lugol's fixed initial samples and three random samples from experimental and control bottles were analysed from each 24 h incubation period. Each sample bottle was carefully rotated through 360 degrees at least 50 times to ensure that all matter was re-suspended and fully mixed. The samples were initially concentrated in parafilm-sealed, 100 ml measuring cylinders. Entire samples (100 ml) were settled from the spring cruise (D262), whilst only half the sample volume (50 ml) of the Summer Cruise (D264) samples was settled. After the time required to ensure complete sedimentation (24 and 48 h for 50 and 100 ml respectively: Lund et al. 1958, Gifford 1993, Gifford and Caron 2000), the supernatant water was slowly and carefully removed to a clean storage bottle until approximately 20 ml of sample remained. Cells were then re-suspended by rotating the cylinder between the palms of the hands for 30 seconds (see Lund et al. 1958) and transferred to a 25 ml settling chamber (Duncan and Associates: http://www.duncanandassociates.co.uk/).

Because all phytoplankton samples were preserved with 10 % acid Lugol's solution, they required a degree of bleaching (removal of iodine) before accurate identification of the cells could be made. This was achieved by carefully adding drops of a saturated sodium thiosulphate solution (in milli-Q water) to the sample in the 25 ml settling chamber (Sherr and Sherr 1993). The bleaching technique, initially tested on samples from the 'trial cruise' D260, typically required 4 drops of sodium thiosulphate to turn the sample clear. The remaining chamber volume (~4ml) was topped up with the supernatant water (10 % Lugols vol:vol), returning the sample to a 'weak tea' coloured solution, before applying the glass coverslip. Over-addition of the bleaching solution caused the thiosulphate to crystallise on the baseplate of the chamber, and in severe cases, completely obscured the sample. In cases of over-addition, neat Lugol's was dripped into the chamber until the familiar 'weak tea' colour was achieved. Following a final 12 h period of sedimentation, the cells were then enumerated by means of inverted microscopy, the protocol of which is described by Lund et al. (1958).

2.4.2. Cell counts. Cell counts were undertaken on an Olympus IMT-2 inverted microscope in a darkened room. All cells excluding flagellates and cryptomonads were enumerated at X 200. Flagellates and cryptomonads (all < 10μm) were counted at X 400 on a single 'field of view' transect from top to bottom using phase contrast. The area of the flagellate transect was determined as diameter of baseplate (23 mm) multiplied by the width of the field of view (0.048 mm). By expressing this area (1.104 mm^2) as a fraction of the entire baseplate area (415.48 mm^2), the multiplication factor of 376.341 is calculated (415.48/1.104). By assuming that the distribution of flagellates within this single transect was representative of the distribution of flagellates throughout the baseplate, the number of flagellates per volume of sample settled was calculated by applying the multiplication factor to the number of flagellates counted in one transect. In the majority of cases > > 100 cells were counted, providing a 95 % confidence interval of the estimate within ± 20 % $\bar{x}$ (Lund et al. 1958, Venrick 1978).

2.4.3. Reliability of the cell counts. Typically >> 100 cells for each individual group were counted. As discussed by Venrick (1978 and refs therein), counting 100 cells is sufficient to give a 95 % confidence interval of the estimate within ± 20 % $\bar{x}$. Before undertaking any sample analysis, each cell group in 8 samples from experiment 1, D262, were counted, then re-counted and the results statistically compared. So as not to influence the latter counts by the previous ones, group counts were only summed after both counts had been completed. Randomization was achieved by the physical mixing of the samples before settlement (Venrick 1978). It is therefore valid to compare two single sample counts (Parker 1983):

$$d = \frac{[X_1 - (X_1 + X_2)/2] - 0.5}{\sqrt{[(X_1 + X_2)/4]}} \qquad (1)$$

where X_1 and X_2 are the two counts and d is the 'standardized normal deviate' (∞). In all cases, the counts were not significantly different from each other ($p > 0.1$ in all cases).

2.4.4. Cell volume estimations. For each defined group, the appropriate linear measurements of at least 30 fixed cells were made with a calibrated graticule

in the ocular of the Olympus IMT-2 inverted microscope. Cell volumes were estimated using simple geometric formulae (Table 2.1), as suggested by Menden-Deuer and Lessard (2000).

2.4.5. Volume:Carbon regression equations. Strathmann (1967) made the important distinction between the cell volume to carbon (vol:C) relationships for diatoms and for other protists (because of their large vacuoles, diatoms are less carbon dense), demonstrating the need for separate predictive equations. Despite being adopted as a standard method (e.g. Parsons et al. 1984), little attempt has been made to justify the use of these equations. Considering that the cellular C of cultured organisms (typically used in determining conversion factors) is influenced by the culture conditions (Putt and Stoecker 1989, Thompson et al. 1991, 1992, Davidson et al. 2002), and that the relatively few cultured organisms used are rarely the same as those encountered in field based studies, this is somewhat surprising.

Vol:C conversion factors have subsequently been determined for various components of the microplankton, including phototrophic nanoplankton (Verity et al. 1992), flagellates (Borsheim and Bratbak 1987), dinoflagellates (Menden-Deuer and Lessard 2000), ciliates (Putt and Stoecker 1989), diatoms (Strathmann 1967) and various phytoplankton (Mullin et al. 1966; Montagnes et al. 1994). More recently, Menden-Deuer and Lessard (2000) determined highly significant vol:C relationships for marine protists using both new experimental work and all existing data in the literature. The C biomass of diatoms and protists excluding ciliates (see below) was estimated here using the corresponding equations presented by Menden-Deuer and Lessard (2000).

The C density of aloricate (naked) ciliates is on average 43 % more dense than similar sized dinoflagellates (Menden-Deuer and Lessard 2000). Accordingly, aloricate ciliate C biomass is calculated using Putt and Stoecker's (1989) regression for 2 % acid Lugols preserved aloricate ciliates.

2.4.6. Shrinkage effects of Lugol's. Before C biomass was calculated, the cell volumes of all non-ciliate and non-thecate dinoflagellate taxa were adjusted to account for shrinkage due to preservation with acid Lugol's (Appendix 1). Due to the uncertainties in predicting preservation-induced cell volume changes in thecate dinoflagellates (Menden-Deuer et al. 2001), their volume was not corrected.

Table 2.1. Geometric formulae used to estimate cell volume, where L is length (the longest straight line separation between any two points on the cell boundary regardless of orientation), B is breadth (widest distance measured perpendicular to length), H is height and R is radius (i.e. B/2). [§]H is determined by an aspect ratio of 0.5 * B

Cell group	Shape approximation	Formula
Dinoflagelates (naked and thecate)	Prolate spheroid	(Pi/6)*L*B^2
Nitzschia spp.	Two pyramids	(1/3*(B^2)*(L/2))*2
Pennate diatom (*Triponeis* sp.)	Cylinder	Pi*R^2*L
Centric diatom	Cylinder	Pi*R^2*H[§]
Ciliate	Prolate spheroid	(Pi/6)*L*B^2
Silicoflagelate	Sphere	(Pi/6)*L^3
Flagellate < 3.5 µm	Sphere	(Pi/6)*L^3
Flagellate > 3.5 µm	Sphere	(Pi/6)*L^3
Cryptomonad	Prolate spheroid	(Pi/6)*L*B^2

2.5. Organic carbon and nitrogen analysis

2.5.1. Removal of inorganic carbon. The collection of particulate material on glass fibre (GF/F) filters is indiscriminate, retaining both organic and inorganic carbon wherever present. Despite particulate inorganic carbon (PIC) concentrations being generally very low in relation to particulate organic carbon (POC) (e.g. Gordon, 1969), exceptions are not unknown. In the North Atlantic during late spring and summer, large blooms of the calcite forming coccolithophorids are known to occur (Holligan et al. 1983). During such blooms, molar calcite concentrations can reach 25-186 % of the associated POC concentrations (Fernandez et al. 1993). Although ammonium can adsorb to, or form particles, there are no common nitrogen minerals in the marine environment thus particulate inorganic nitrogen (PIN) is considered insignificant (Karl et al. 1991) and shall be ignored here. Despite the potential biases caused by PIC when analysing for POC, no singular method has been established as a universal laboratory standard (Ehrhardt and Koeve 1999, see also King et al. 1998).

Thermal methods of separation rely on the fact that organic carbon will be converted to carbon dioxide under temperatures (e.g. 500 ºC) at which carbonate (PIC) remains stable. However, Froelich (1980) illustrated that the thermal decomposition ranges for PIC and POC are not mutually exclusive. Certain marine carbonates (e.g. high-Mg calcite) have been observed to decompose at temperatures below 400 ºC (Walsh et al. 1991), whilst Gibbs (1977) found that refractory organic matter may not undergo complete oxidation until 1050 ºC.

Alternatively, carbonate can be removed with non-oxidising acids which do not volatilise the POC fraction (see King et al. 1998). Such acids include hydrochloric (Hedges and Stern 1984), phosphoric (Froelich 1980) and sulphurous (Verardo et al. 1990). Direct acidification of the samples (rinsing with concentrated acid) has been tried, but losses of up to 50 % of particulate organic nitrogen (PON) and POC have been reported (Karl et al. 1991, Lohse et al. 2000). The more subtle technique of fuming with concentrated hydrochloric acid vapour in a glass desiccator (24to 48 hrs) proposed by Hedges and Stern (1984) is recommended by various authors (Bodungen et al. 1991, Knauer 1991) and is the prescribed technique for the Joint Global Ocean Flux Study for calculating the POC (UNESCO 1994).

Initial analysis of plankton samples from cruises D262 and D264 showed very low numbers of coccolithophorids (Russell Davidson, pers. comm.). Thus, the

vapour acidification method (suitable for samples with <50 wt% $CaCO_3$) was chosen as the most suitable method. Prior to combustion analysis, samples were freeze-dried for 24 hrs and then immediately placed into a desiccator containing pre-combusted silica gel (550 ºC over night). Inorganic carbon was subsequently removed by fuming the samples for 24 h in a glass desiccating chamber containing a petri-dish filled with 37 % hydrochloric acid (reagent grade).

2.5.2. Preparation of filters for analysis. Due to the nature of the Carlo Erba EA-1108 C/N analyser, the samples have to be in pellet format (approx. 4x4x3 mm, L, W, H), wrapped in tin foil to ensure complete combustion. To achieve this, a special 'capsule press' was commissioned (after Hilton et al. 1986). The freeze-dried, acidified filter disc (25 mm diameter) is placed onto an ultra-light, 30 mm diameter, tinfoil disk (http://www.microanalysis.co.uk), folded in half and then rolled into a cylindrical 'cigar' shape using clean tweezers (cf. Hilton et al. 1986). This is then placed into the 'capsule press' and crushed to the appropriate size. All prepared material was stored in a desiccator within cell culture trays until analysis.

2.5.3. Calibration and running procedures for Carlo Erba EA-1108 C/N analyser. Before the elemental analyser can be used, it requires a simple calibration. Two bypass runs are initially made to check that the combustion tube is allowing the correct flow of gases and that it is not contaminated by previous analyses (e.g. the combustion of graphite is a function of time as well as temperature, and thus may not undergo complete oxidation during a single analysis). Following this, a tin disc containing a small amount of standard (solf. Acid) is run as a 'bypass'. The C and N in the sample is detected, and the column retention times for each element are calculated. These new values can then be entered if they are different to when they were previously calculated (a slight drift in the retention time is quite common). Two blank samples (pre-combusted 25 mm GF/F filter wrapped in tin discs) are then analysed to determine the quantities of C and N present in the filters and tin discs (theoretically zero). Blank samples (as above) containing known amounts of standard (~0.500 mg solf. Acid) are combusted, permitting the analyser to calculate the K-factor. This is a constant multiplier applied to the area of the unknown sample, calculated according to the formula:

$$K_{factor} = \frac{S_{\%} S_W}{S_C B_C} \qquad (2)$$

where $S_{\%}$ is the % of C in the standard, S_W is the weight of the standard added, and S_C and B_C are the C (or N) areas of the standard and blank samples respectively.

This takes into account the values associated with a blank sample and reduces the areas of the unknown samples accordingly. Upon calculation of the K-factor, the analyser was ready to run. The accuracy of the calibration is assessed by running blank samples containing known amount of standard and allowing the machine to calculate the percentages of C and N present. Analysis only continued when the detection limits were < 0.5 % of the theoretical maximum.

The quantities of C and N in the sample are expressed as the areas of their respective peaks (output from the thermal conductivity detector). These values are initially adjusted by the following formula (C given as an example):

$$A = \frac{S_C F_C}{\overline{X}} \qquad (3)$$

where A is the adjusted sample area, S_C and F_C are the C (or N) areas of the standard closest to the theoretical maximum (when using solf. acid this 41.85 % C and 16.27 % N) and the sample respectively, and $\overline{X}$ is the average C (or N) area of the standards analysed before and after sample F.

Using the adjusted areas (A), absolute values (mg) of C and N can be calculated by the following:

$$M = \frac{WA}{S_C} \qquad (4)$$

where M is mg of C (or N), W is mg of C (or N) in the standard closest to the theoretical maximum (S_W = 0.4185 and S_W = 0.1627 for C and N respectively), A is the adjusted sample area, and S_C is the C (or N) area of the standard closest to the theoretical maximum.

Despite being included in the JGOFS protocol for determination of particulate organic carbon and nitrogen (UNESCO, 1994), the relatively large size (25 mm) of the GF/F filter used to collect the particulate material proved some what problematic. Upon combustion, much of the silica-based filter forms an ash which

settles in the combustion tube, forming a concrete-like crust. Eventually, the build up of ash restricts the draw of combustion gases through the catalytic column, allowing a fraction of the sample to remain in the system which then contaminates the subsequent analyses. To reduce this problem, after every five samples, a 'bypass' was run. This operates the machine as normal, but no sample is added so any trace of C or N in these analyses was taken as a warning sign and that a new combustion column was required. After each bypass, a standard was combusted to keep a constant check on how accurately the analyser was functioning. Sample analysis only continued when the standards were between 99 and 101 % (+/- < 0.5 %) of their theoretical maximum.

2.6. Fatty acid analysis

2.6.1. Extraction. Total fatty acids were extracted using Chloroform:Methanol (C:M; 2:1 v/v) following Folch (1957). Animals were homogenised using a micro-mortar and pestle to ensure complete extraction. The mortar and pestle was rinsed with 1 ml solvent (C:M; 2:1 v/v), which was then added to the sample. All samples were topped up with solvent (C:M; 2:1 v/v) until they were exactly 2 ml and transferred into clean 4 ml vials. An additional 'blank' vial (4 ml) was filled with 2 ml of solvent (C:M; 2:1 v/v) and treated exactly as the other samples. To accurately quantify the amount of fatty acid present, a known amount of 21:0 fatty acid internal standard was added to each sample (2.5 μg to eggs and particulates and 5.0 μg to animals).

The non-fatty acid fraction (sugars, urea, amino acids and salts) was removed using phase separation by adding 500 μl of 0.88% KCl. Following whirlimixing (vigorous shaking) and centrifugation (2 mins at 1500 rpm), the top aqueous layer (containing the non-fatty acid fraction) was removed and discarded. The organic layer (containing sample) was evaporated under a constant flow of oxygen free N gas. Any water remaining was subsequently removed by drying the samples under vacuum in a desiccator containing pre-combusted silica-gel.

2.6.2. Alkaline hydrolysis (saponification). This process is primarily to produce free fatty acids, although it also serves to remove some of the unwanted long chain alcohols and sterols. The free fatty acids are produced by adding 500μl of 1M KOH in 95 % ethanol to the dry vials (containing sample) and maintaining them at 78 ºC for 1 hour. After cooling, 500 μl of water was added and the solution was

acidified with a few drops of 0.6M HCl. The free fatty acids were then extracted by two sequential washes with diethylether; after adding 1000 µl of diethylether (500 µl on the second wash), the sample is whilimixed and centrifuged. The upper layer (containing sample) is transferred to a clean 2 ml via. The sample was then evaporated under N gas and dried by vacuum desiccation.

2.6.3. Preparation of PFB esters (fatty acid derivatization). The samples for fatty acid analysis e.g. seston and eggs, contained only small quantities of fatty acids. Therefore, rather than using a Flame Ionisation Detector (FID), the gas chromatograph (GC) was fitted with a highly sensitive electron capture detector (ECD). By halogenating the free fatty acids with pentafluorogenzyl (PFB) estetrs, nanogram quantities of fatty acids could be detected.

After saponification, the free fatty acids were dissolved in 30 µl of acetonitrile and agitated. Following this, 100 µl of 2,3,4,5,6-pentafluorobenzyl bromide (PFP-Br) solution was added and similarly agitated. Finally, after adding 100 µl triethylamine and mixing, the samples were agitated and left to derivitize for 15 minutes at room temperature. The fatty acids were extracted by two sequential washes with isooctane (500 µl). The upper layers, containing the PFP esters, were transferred to a clean 2 ml vial. The sample was then evaporated and dried before being redissolved in isooctane.

2.6.4. Purification. The fatty acids were separated from any remaining contaminants using high performance thin layer chromatography (HPTLC). The silica HPTLC plates (10 x 10 cm) were pre-run, using an 18 ml hexane, 2 ml diethylether and 200 µl acetic acid solvent system. The PFB esters were dissolved in 100µl isooctane and applied to the HPTLC plates via a syringe, alongside a PFB ester standard. Upon completion of the chromatographic separation, the PFB ester standard was labelled with 2,7-dichlorofluorescein (DCF) dissolved in methanol (Christie 1973) and the band visualised under ultra-violet light. The area of silica corresponding to each sample of purified PFB esters was scraped off the plate using a scalpel blade, and transferred to clean 8ml vials containing 2 ml isooctane. After the addition of 1 ml $NaHCO_3$ (2 % W/V) the sample is whirlimixed, centrifuged and frozen at –20 ºC. The upper, non-frozen, isooctane layer was transferred to a clean 2 ml vial and evaporated under N gas. The samples were dissolved in isooctane and stored in 1.1 ml pear shaped vials at –20 ºC until required for injection. The volume of isooctane used depended on the quantity of fatty acid within the sample. The ideal

loading value for gas chromatography was 0.2 µg of fatty acid per µl injected, but without knowing the absolute values of fatty acid within the sample, a degree of trial and error was required to find the correct volume of isooctane.

2.6.5. Injection and identification of fatty acids. Fatty acid PFB esters were analysed by gas chromatography coupled with an electron capture detector (GC-ECD), using a ZB-Wax 30 m x 0.32 mm internal diameter column and hydrogen as the carrier gas. The oven temperature program was as follows; 80 ºC to 190 ºC at 40 ºC min^{-1}, 190 ºC to 230 at 4 ºC min^{-1}, remaining at 230 ºC for 47 minutes. Because the retention times of the column vary slightly over time, a marine fish oil standard (Marinol) containing a full suite of fatty acids, was injected at the start of every day. Individual fatty acids were identified by comparing retention times of those identified on the Marinol trace using ThermoFinnigan Chrom-Card software to those in the sample.

2.6.6. Quantification of fatty acids. The electron capture detector (ECD) quantifies each individual molecule of a particular fatty acid. The corresponding fatty acid peak on the trace is thus directly related to its quantity (moles). Because a known quantity of standard (21:0) was added, the quantity (moles) of any identified fatty acid can be determined using the following relationship:

$$S_M = \frac{S_A}{21:0_A} 21:0_M \qquad (5)$$

where S is the identified fatty acid, $_M$ is the quantity in moles, and $_A$ is the area of the respective peaks. The absolute quantity (µg of lipid) of a particular fatty acid, S_Q, can then be determined:

$$S_Q S_{AM} \qquad (6)$$

where S is the particular fatty acid and $_{AM}$ is the atomic mass (in µg).

2.7. Statistical analysis. Parametric statistics can only be used to compare samples from populations of normally-distributed variables. They are based on estimates of the means and standard variation parameters of a normally distributed population. Before analysis, data were tested for the assumptions of parametric statistics i.e. a

normal distribution (Kolmogorov-Smirnov test) and equal variance (Levene Median test). All parametric statistical analyses were conducted using SigmaStat for Windows Version 2.03. If these assumptions were not met, statistical comparisons were achieved using the non-parametric statistical package PRIMER (Clarke and Warwick 1994). A specific description of the individual tests employed are given in each chapter.

Chapter 3

Quantifying copepod grazing when using natural plankton assemblages

Paper:

Correcting for microzooplankton grazing in copepod feeding incubations

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3.1. AIMS

The objective of this chapter is to illustrate how the total amount of C ingested daily by copepods (daily ration) is underestimated if microzooplankton grazing artefacts are not considered. Cell count data from the five consecutive daily incubations in both April and July/August are used here to illustrate the differences between the traditional (Frost 1972) and proposed methods.

3.2. INTRODUCTION

The diets of copepods have received interest for well over half a century (e.g. Lebour 1923, Marshall 1924), and numerous methods have been developed to determine their grazing rates (see Bamstedt et al. 2000). Many of these techniques assess only herbivory, and it is often apparent that the ingestion of autotrophic prey is not sufficient to support estimated C demands (e.g. Dagg and Walser 1987, Atkinson 1996, Mayzaud et al. 2002a, b). In such cases, microzooplankton are typically proposed as the 'missing source' of C.

The importance of protists in the diets of copepods has only recently been appreciated (Sherr et al. 1986, Stoecker and Capuzzo 1990, Gifford 1991, Kleppel 1993). Acknowledgment of the importance of non-autotrophic prey has been reflected by a renewed interest in food removal experiments, which remain the only means available to quantitatively determine the total amount of material ingested by copepods (Harris 1996, Bamstedt et al. 2000). In their simplest form, these experiments follow the disappearance of prey during a series of replicated bottle incubations under controlled conditions. Experimental bottles contain a natural microplankton assemblage with added copepods, whilst controls contain only the microplankton. Such studies have revealed that indeed, microzooplankton often constitute a large proportion of the diet (Gifford and Dagg 1991, Gifford 1993, Fessenden and Cowles 1994, Atkinson 1996, Kleppel et al. 1996, Levinsen et al. 2000b, Roman et al. 2000, Zeldis et al. 2002).

The seminal paper by Frost (1972) outlined the mathematical procedures for calculating copepod grazing rates from particle removal experiments. These equations, or derivations thereof, have subsequently been recognised as a standard procedure for the analysis of copepod feeding experiments (Bamstedt et al. 2000). They were initially developed to quantify the ingestion of diatom mono-cultures by groups of copepods, using the control bottles to calculate net growth rates of the prey

during the incubation. In this type of experiment, copepods are the only grazers and it is reasonable to assume that, excluding copepod feeding, net growth in the experimental bottles equals that in the control bottles (Frost 1972).

However, when using natural microplankton assemblages, copepods are not the only grazers. Microzooplankton are prolific grazers (see Appendix 2), with specific ingestion rates higher than those of copepods (Hansen et al. 1997) and the ability to ingest cells at least as large as themselves (Hansen et al. 1994a). Size-fractionated (<160 μm) bottle incubations have been shown to yield underestimations of ciliate growth rates due to the 'internal grazing' pressure of similar sized heterotrophic dinoflagellates (Levinsen et al. 1999; discussed by Hansen et al. 1999). It follows that control bottles for copepod grazing experiments should not be depicted as true controls. It is a gross oversimplification to assume that the community dynamics within them will be representative of those in the experimental bottles. As such, they will now be referred to as 'pseudo-controls'. Using optimal predator to prey size ratios (Hansen et al. 1994a), and for simplicity, assuming that ciliates with an equivalent spherical diameter (ESD) of 40 μm represent the microzooplankton community, the problem of 'pseudo-controls' can be conceptualised. A copepod, with an ESD of 800μm, will optimally prey on cells with an ESD of 44μm (predator:prey ratio 18:1) which, alongside other cell groups (e.g. diatoms and dinoflagellates), will include the ciliates. Copepods are known to selectively remove microzooplankton (Turner and Graneli 1992, Ohman and Runge 1994, Atkinson 1995, 1996, Verity and Paffenhofer 1996, Nejstgaard et al. 2001a, b, Zeldis et al. 2002, Bollens and Penry 2003). Selective removal of the ciliates will reduce or even remove microzooplankton grazing pressure on their optimally-sized food cells (ESD of 5μm; ratio of 8:1 for ciliates:prey) in the copepod grazing bottles.

When selective removal of the microzooplankton by copepods is apparent, net growth in the experimental bottles (excluding copepod grazing) no longer equals that in the pseudo-controls because of differential microzooplankton grazing pressure. Upon termination of the experiment, cells that are heavily grazed by microzooplankton in the pseudo-controls may have significantly decreased during the incubation relative to the experimental bottles. Conversely, these cells may have grown in the experimental bottles relative to the pseudo-controls due to a release in microzooplankton grazing pressure. When growth in the experimental bottles is greater than that determined from the pseudo-control bottles, copepod grazing rates

are underestimated using the equations of Frost (1972). In extreme cases, negative copepod grazing rates will be determined (Turner and Graneli 1992, Hansen et al. 1994b, Atkinson 1995, 1996, Nejstgaard et al. 1997, 2001a, b, Zeldis et al. 2002, Bollens and Penry 2003). The frequency of their occurrence highlights the fact that our mathematical representation of bottle incubations is insufficient to describe copepod ingestion and feeding preferences accurately. Despite this, negative rates are typically ignored by assuming them to be zero.

Nejstgaard et al. (1997) were the first to acknowledge this problem. By conducting dilution experiments (Landry and Hassett 1982) concurrent to copepod grazing incubations, the resulting microzooplankton grazing coefficients can be used to correct the copepod grazing coefficients. Corrected copepod grazing rates are often substantially greater than the uncorrected rates (Nejstgaard et al. 1997, 2001b). Although dilution experiments are simple in concept and execution, collecting and processing the samples is extremely labour intensive (Landry 1993, Bamstedt et al. 2000), making them an unattractive addition to copepod feeding experiments. Furthermore, the dynamics of dilution experiments are more complex than typically acknowledged. For example, the assumption that microzooplankton grazing is proportional to the dilution effect on grazer abundance (Landry and Hassett 1982) is theoretically and practically problematic (see Landry et al. 1995).

An alternative method for estimating microzooplankton grazing coefficients from copepod bottle incubations without the need for dilution experiments is presented here. Using this method, the net growth of microplankton prey types in control bottles is divided into its component parts, gross growth and losses due to grazing. Gross growth is calculated using a simple mathematical model, enabling the microzooplankton grazing coefficients to be estimated from the control bottles using a standard exponential growth model, analogous to that used by Frost (1972). The method of Frost (1972) is developed to incorporate these processes, thus providing a more realistic means of estimating copepod ingestion rates.

3.3. THEORY

The equations of Frost (1972) are developed here into a new method of calculating copepod grazing rates in which the impact of microzooplankton grazing is estimated through the use of a simple growth model. Symbols and units for all variables and parameters used in the text and equations are presented in Table 3.1.

Table 3.1. Symbols and units for all variables used in the text and equations

Variable		Units
t	Duration of incubation	day^{-1}
$P_{ini(C)}$	Initial biomass of cell group *P* in the control bottle	µg C ml^{-1}
$P_{ini(E)}$	Initial biomass of cell group *P* in the experimental bottle	µg C ml^{-1}
$P_{f(C)}$	Final biomass of cell group *P* in the control bottle	µg C ml^{-1}
$P_{f(E)}$	Final biomass of cell group *P* in the experimental bottle	µg C ml^{-1}
$\overline{P}_{(C)}$	Average biomass of cell group *P* in the control bottle	µg C ml^{-1}
$\overline{P}_{(E)}$	Average biomass of cell group *P* in the experimental bottle	µg C ml^{-1}
$Z_{ini(C)}$	Initial biomass of microzooplankton in the control bottle	µg C ml^{-1}
$Z_{ini(E)}$	Initial biomass of microzooplankton in the experimental bottle	µg C ml^{-1}
$Z_{f(C)}$	Final biomass of microzooplankton in the control bottle	µg C ml^{-1}
$Z_{f(E)}$	Final biomass of microzooplankton in the experimental bottle	µg C ml^{-1}
$\overline{Z}_{(C)}$	Average biomass of microzooplankton in the control bottle	µg C ml^{-1}
$\overline{Z}_{(E)}$	Average biomass of microzooplankton in the experimental bottle	µg C ml^{-1}
$k_{(C)}$	Net growth coefficient of cell group *P* in the control bottle	day^{-1}
$k_{(E)}$	Net growth coefficient of cell group *P* in the experimental bottle	day^{-1}
$r_{(C)}$	Estimated gross growth coefficient of cell group *P* in the control bottle	day^{-1}
$r_{(E)}$	Estimated gross growth coefficient of cell group *P* in the experimental bottle	day^{-1}
V	Volume of the incubation bottle	ml
n	Number of copepods in experimental bottle	N/A
R_F	Clearance rate, as calculated using Frost (1972)	ml cop^{-1} day^{-1}

Variable		Units
R_M	Clearance rate, as calculated using the new method	ml cop^{-1} day^{-1}
$m_{(C)}$	Microzooplankton grazing coefficient for cell group *P* in the control bottle	day^{-1}
$m_{(E)}$	Microzooplankton grazing coefficient for cell group *P* in the experimental bottle	day^{-1}
$S_{(C)}$	Microzooplankton biomass specific clearance rate in the control bottle	ml [µg µzoo C $]^{-1}$ d^{-1}
$M_{(C)}$	Microzooplankton biomass specific ingestion rate in the control bottle	µg C [µg µzoo C $]^{-1}$ d^{-1}
g_F	Copepod grazing coefficient for cell group *P*, according to Frost (1972)	day^{-1}
g_M	Corrected copepod grazing coefficient for cell group *P*, according to the new method	day^{-1}
I_F	Quantity of cell group *P* ingested per unit time, according to Frost (1972)	µg C cop^{-1} day^{-1}
I_M	Quantity of each prey group ingested per unit time, new method	µg C cop^{-1} day^{-1}
G_F	Total amount of C ingested daily as calculated using Frost (1972)	µg C cop^{-1} day^{-1}
G_M	Total amount of C ingested daily as calculated using new method	µg C cop^{-1} day^{-1}
μ_{max}	Maximum growth rate of each prey type	day^{-1}
f	Scaling factor for photoperiod	dimensionless
f_{inc}	Photoperiod during copepod feeding experiments	hrs
f_μ	Photoperiod used in experiments to determine μ_{max}	hrs
Q_N	Scaling factor for nutrients	dimensionless
N	Nitrate concentration	µMol l^{-1}
K_N	Half saturation constant for nitrate uptake	µMol l^{-1}

According to Frost (1972), the net rate of change of any given prey type, P, in a control bottle (i.e. in the absence of grazing), $k_{(C)}$, can be calculated assuming exponential growth:

$$P_{f(C)} = P_{ini(C)} exp(k_{(C)} t) \quad (1)$$

where $P_{f(C)}$ and $P_{ini(C)}$ are final and initial concentrations of P in the control bottle, and t is the duration of the incubation. It is not possible to determine the corresponding rate of change in the experimental bottles, $k_{(E)}$, because the observed changes in P then also depend on the grazing by copepods. In this case it is simplest to assume that $k_{(E)}$ equals $k_{(C)}$. The instantaneous copepod grazing coefficient (the loss rate of P due to copepod grazing) according to Frost, g_F, is then calculated from:

$$P_{f(E)} = P_{ini(E)} exp(k_{(C)} - g_F) t \quad (2)$$

The average concentration of prey type P throughout the incubation in the experimental bottle, $\overline{P}_{(E)}$, can now be calculated using $k_{(C)}$ and g_F:

$$\overline{P}_{(E)} = \frac{P_{ini(E)}[\exp(k_{(C)} - g_F)t - 1]}{(k_{(C)} - g_F)t} \quad (3)$$

Copepod clearance, R_F, and ingestion, I_F, rates are then:

$$R_F = \frac{V g_F}{n} \quad (4)$$

$$I_F = \overline{P}_{(E)} R_F \quad (5)$$

where V is the volume of the bottle and n is the number of copepods in the bottle.

The net growth rate in the control bottles, $k_{(C)}$, is in reality the sum of two terms, gross growth, $r_{(C)}$, and mortality due to microzooplankton grazing, $m_{(C)}$, assuming other loss terms such as senescence are negligible:

$$k_{(C)} = r_{(C)} - m_{(C)} \quad (6)$$

The fundamental problem with applying Frost's equations to natural microplankton assemblages is that k differs between the experimental and control bottles, due to the selective removal of microzooplankton by copepods. Those prey types that are actively grazed by microzooplankton will have higher net growth rates in the experimental bottles relative to controls when microzooplankton grazing pressure outweighs that of copepods. In other words, $k_{(E)} > k_{(C)}$ and g_F will be an underestimation of the true instantaneous copepod grazing coefficient. Theoretically, only the growth rates of those cells that are ingested by both microzooplankton and copepods need to be corrected for microzooplankton grazing. However, this requires *a priori* assumptions about the diets of both grazers. Considering the complex interactions between copepods and their food, any such assumptions are difficult to justify, particularly when natural seawater assemblages are offered as food. It is therefore simplest to correct all copepod grazing coefficients, g_M, when microzooplankton grazing coefficients in the experimental bottles, $m_{(E)}$, are significantly different from zero.

If $r_{(C)}$ can be estimated – we use a simple mathematical model (below) - $m_{(C)}$ in the control bottles can then be determined by recasting equation (1), dividing k into its component terms:

$$P_{f(C)} = P_{ini(C)} exp(r_{(C)} - m_{(C)})t \qquad (7)$$

If we assume that grazing by microzooplankton scales proportionally to their biomass, their instantaneous grazing rate on prey type P in an experimental bottle, $m_{(E)}$, is then:

$$m_{(E)} = m_{(C)} \frac{\overline{Z}_{(E)}}{\overline{Z}_{(C)}} \qquad (8)$$

$$\overline{Z}_{(C)} = \left(Z_{f(C)} - Z_{ini(C)}\right) \ln\left(\frac{Z_{f(C)}}{Z_{ini(C)}}\right)^{-1} \qquad (9)$$

$$\overline{Z}_{(E)} = \left(Z_{f(E)} - Z_{ini(E)}\right) \ln\left(\frac{Z_{f(E)}}{Z_{ini(E)}}\right)^{-1} \qquad (10)$$

where $\overline{Z}$, Z_{ini} and Z_f are the average, initial and final biomasses of microzooplankton in the control and experimental bottles (after Nejstgaard et al. 2001b). An estimate of copepod grazing that takes into account the differential microzooplankton grazing pressure in control and experimental bottles, g_M, can now be derived by recasting Equation (2) as:

$$P_{f(E)} = P_{ini(E)}exp(r_{(E)} - m_{(E)} - g_M)t \quad (11)$$

Gross growth rates of prey items can reasonably be assumed to be the same in the experimental and control bottles (see Section 3.6), i.e. $r_{(E)}$ equals $r_{(C)}$. The equation to calculate the average concentration of P in the experimental bottle during the incubation, $\overline{P}_{(E)}$, now becomes:

$$\overline{P}_{(E)} = \frac{P_{ini(E)}[\exp(r_{(C)} - m_{(E)} - g_M)t - 1]}{(r_{(C)} - m_{(E)} - g_M)t} \quad (12)$$

Estimates of copepod clearance and ingestion rates that consider microzooplankton grazing, R_M and I_M respectively, can now be made by recasting equations 4 and 5:

$$R_M = \frac{Vg_M}{n} \quad (13)$$

$$I_M = \overline{P}_{(E)}R_M \quad (14)$$

Microzooplankton biomass-specific clearance, $S_{(C)}$, and ingestion, $M_{(C)}$, rates in the control bottles can also be determined by reworking previous equations:

$$S_{(C)} = \frac{Vm_{(C)}}{\overline{Z}_{(C)}} \quad (15)$$

$$M_{(C)} = \overline{P}_{(C)}S_{(C)} \quad (16)$$

where the average concentration of P in the control bottle during the incubation, $\overline{P}_{(C)}$, is calculated:

$$\overline{P}_{(C)} = \frac{P_{ini(C)}[\exp(r_{(C)} - m_{(C)})t - 1]}{(r_{(C)} - m_{(C)})t} \quad (17)$$

3.4. METHODS

3.4.1. Estimating specific gross growth rates (r) with a simple mathematical model. The general approach to modelling specific gross growth rates, r, in dynamic models begins with an estimation of the maximal attainable growth rate, μ_{max} (divisions d^{-1}). This rate is then reduced by factors that prevent the subject organism from realizing this hypothetical maximum (Brush et al. 2002). The specific gross growth rate of prey items P in the control bottles, $r_{(C)}$, is estimated here using a simple mathematical model relating growth to ambient temperature, photoperiod and nitrate using the following equations:

$$r_{(C)} = ln(2)(\mu_{max} f Q_N) \quad (18)$$

$$f = f_{inc}/f_{\mu} \quad (19)$$

$$Q_N = \frac{N}{K_N + N} \quad (20)$$

where μ_{max} is the maximum growth rate, f and Q_N are scaling factors for photoperiod and nutrients, f_{inc} and f_{μ} are the photoperiods in the incubation and that used in experiments to determine μ_{max}, N is nitrate and K_N is the half saturation constant for nitrate uptake (see below). A typical photoperiod, f_{μ}, of 14 hours was used. Ambient photoperiod and nitrate (f_{inc} and N), are shown in Table 3.2.

3.4.2. Maximal growth rates (μ_{max}). Large-scale models typically consider phytoplankton as a single entity to avoid taxonomic complexities, and use the exponential function known as the 'Eppley curve' (Eppley 1972) to approximate μ_{max} as a function of temperature. However, the Eppley curve underestimates growth rates when compared to empirical data from culture and field studies (Brush et al. 2002), and is based on an exponential function which may be inappropriate (Montagnes et

Table 3.2. Grazing incubations in April and July/August. Experiment station (day) number (Stn. #), incubation start date (Date), day length (f_{inc}), incubation temperature (Surface temp.) and daily ambient nutrient concentrations. Nutrient concentrations were determined using a Skalar San^{plus} autoanalyser following the methods outlined in Sanders and Jickells (2000).

					Ambient nutrient concentrations		
		Date	f_{inc}	Surface temp.	Nitrate	Silicate	Phosphate
	Stn. #	(dd/mm/yy)	(hrs light d^{-1})	(ºC)	(µmol l^{-1})	(µmol l^{-1})	(µmol l^{-1})
April (D262)	1	04/26/02	16.3	7.3	12.55	7.58	0.85
	2	04/27/02	16.3	7.3	13.85	7.05	0.92
	3	04/28/02	16.4	7.2	14.23	6.93	0.97
	4	04/29/02	16.2	7.1	13.72	7.19	0.87
	5	04/30/02	16.5	6.8	13.99	6.88	0.87
July/August (D264)	1	30/07/02	17.4	11.4	3.97	0.61	0.35
	2	31/07/02	17.6	10.7	5.46	1.47	0.39
	3	01/08/02	17.8	10.6	4.7	1.19	0.335
	4	02/08/02	17.7	10.3	4.76	1.37	0.28
	5	03/08/02	17.1	10.1	6.12	0.79	0.45

al. 2003). Additionally, for the purpose of this exercise, it would be a gross over simplification to represent the numerous algal groups present in these experiments simply as 'phytoplankton', with a single growth parameter. The growth rates of individual algae vary enormously between species (see Furnas 1990), which reflects the range of their physiological adaptations.

The effect of size and taxonomic differences on maximal algal growth rates was investigated by Tang (1995). The resulting taxonomically distinct (diatoms, dinoflagellates, other) allometric relationships (Tang 1995) are used here to estimate maximal growth rates of the algae present in the incubations either as a function of cell volume or C. Multiple regression analyses combining ambient temperature, cell volume and growth rates have provided a similar range of predictive equations for ciliate maximal growth rates (Montagnes et al. 1988, Muller and Geller 1993, Nielsen and Kiorboe 1994). The equation of Muller and Geller (1993) has been shown to be the best predictor of oligotrich ciliate maximal growth rates when compared to empirical values (Montagnes 1996). Because the majority of ciliates observed in these studies belong to the order Oligotrichida (D. Wilson, pers. comm.), the equation of Muller and Geller (1993) is used here. All growth models used to estimate maximal cell growth rates are presented in Table 3.3.

Despite the common assumption that growth rates increase exponentially with temperature (e.g. Eppley 1972), it has recently been shown that protistan growth rates respond linearly to temperature (Montagnes et al. 2003). However, because the equations of Tang (1995) were developed using data normalised to 20ºC using an exponential (Q_{10}) relationship, it would be inappropriate to re-adjust the predicted maximal growth rates to the experimental temperatures according to a linear relationship. Values of algal μ_{max} were thus corrected for temperature using a Q_{10} of 1.58, as suggested by Tang (1995).

3.4.3. Nutrient limitation. Marine algae are liable to growth limitation by the macronutrients; nitrogen, phosphorous and to a lesser extent, silicon (silicon is only required by diatoms). Liebig's Law states that 'growth is limited not by the total resources, but by the scarcest resource'. Thus, at any particular instant, only one nutrient will be limiting. It is widely accepted that nitrogen is the limiting nutrient for productivity in marine environments. This has been demonstrated by numerous nutrient-enrichment experiments which show enhanced productivity when a nitrogenous substance is added, yet no increased growth when phosphorous is added

Table 3.3. Growth models used to determine maximal growth rates. V = volume (μm^{-3}), C = pg C $cell^{-1}$ (as calculated by Strathmann, 1967), and T = temperature (°C)

Group	Growth model	Reference
Diatoms	μ (divisions day^{-1}) = 5.37 * $V^{-0.17}$	Tang 1995
Dinophyceae	μ (divisions day^{-1}) = 2.26 * $C^{-0.18}$	Tang 1995
Other taxa	μ (divisions day^{-1}) = 3.56 * $C^{-0.19}$	Tang 1995
Ciliates	μ_{max} (day^{-1}) = 1.52 ln$T$ – 0.27 * ln$V$ – 1.44	Muller and Geller 1993

(reviewed by Burton 1980, Valiela 1984). The mathematical model of Tyrrell (1999) also indicates that it is nitrate, not phosphate, that is the nutrient most limiting to instantaneous growth in the surface waters of the oceans. Silicon is thought to be able to limit diatom growth at times (Egge and Aksnes 1992), but because silicon is typically in excess of nitrogen and phosphorous in the sea (Eppley et al. 1973, Thomas and Dodson 1975), nitrogen is considered to be the primary limiting nutrient.

Nitrate uptake rates (Equation 20, specific to nitrate) have been described by Michaelis-Menten enzyme kinetics (Dugdale 1967). The half-saturation constant for nitrate uptake, K_N, is the concentration at which half the maximal uptake rate is achieved. Ideally, experimentally determined K_N values corresponding to each individual algal group present in the experiments should be used (Flynn 2003). However, despite extensive searching, representative K_N values for each algal group could not be found in the literature (the empirical data does not exist). A theoretical approach was subsequently adopted.

It is intuitive that small cells have a greater surface area:volume ratio (SA:V) relative to larger cells. Assuming that nitrate-ion uptake sites occupy a finite area of the cell's membrane, and that this area remains constant irrespective of the algal group, it follows that smaller cells also have a greater uptake site:volume (US:V) ratio, and will therefore have a lower K_N. Indeed, both nitrate and ammonia (Eppley et al. 1969) and orthophosphate (Friebele et al. 1978) uptake rates vary in proportion to cell size (discussed by Malone 1980). Therefore, the data presented in Eppley et al. (1969) were used to determine a predictive relationship between cell surface area and half-saturation constants (K_N). The reported cell diameters (Table 3.4) were assumed to be equivalent spherical diameters, therefore surface area was calculated using the formula $4*Pi*radius^2$. Because cell surface area ranges over several orders of magnitude, all data were $\log_{10}$ transformed and tested for normality and homoscedasticity before linear regression analysis (Figure 3.1). The resulting regression was highly significant (ANOVA, $n = 27$, $p < 0.001$). K_N was thus predicted using the relationship:

$$\text{Log}_{10}\, K_N\ (\mu\text{mol l}^{-1}) = 0.4617 * \log_{10} \text{cell surface area}\ (\mu\text{m}^2) - 1.4235$$

Table 3.4. Half-saturation constants for nitrate uptake (K_N) and cell diamters of cultured marine phytoplankton (taken from Eppley et al. 1969). Surface area (SA) was approximated by assuming each cell was spherical, using the equivalent spherical diameter (ESD).

	Species	K_N	Cell Diameter (μm)	Cell shape	Cell Volume (μm^3)	ESD	SA (μm^2)
Oceanic species	*Coccolithus huxleyi*	0.1	5	sphere	65	5	78.5
	Coccolithus huxleyi	0.1	5	sphere	65	5	78.5
	Chaetoceros gracilis	0.3	5	cube	125	6	120.9
	Chaetoceros gracilis	0.1	5	cube	125	6	120.9
	Cyclotella nana	0.3	5	sphere	65	5	78.5
	Cyclotella nana	0.7	5	sphere	65	5	78.5
Neritic diatoms	*Skeletonema costatum*	0.5	8	cylinder	804	12	418.2
	Skeletonema costatum	0.4	8	cylinder	804	12	418.2
	Leptocylindrus danicus	1.3	21	cylinder	14547	30	2881.8
	Leptocylindrus danicus	1.2	21	cylinder	14547	30	2881.8
	Rhizosolenia stolterfothii	1.7	20	cylinder	12566	29	2613.9
	R. robusta	3.5	85	cylinder	964665	123	47213.8
	R. robusta	2.5	85	cylinder	964665	123	47213.8
	Ditylum brightwellii	0.6	30	cylinder	42412	43	5881.3
	Coscinodiscus lineatus	2.4	50	cylinder	196350	72	16336.9
	Coscinodiscus lineatus	2.8	50	cylinder	196350	72	16336.9
	Coscinodiscus wailesii	2.1	210	cylinder	14547145	303	288183.6
	Coscinodiscus wailesii	5.1	210	cylinder	14547145	303	288183.6
	Asterionella japonica	0.7	10	sphere	524	10	314.2
	Asterionella japonica	1.3	10	sphere	524	10	314.2

	Species	K_N	Cell Diameter (μm)	Cell shape	Cell Volume (μm^3)	ESD	SA (μm^2)
Neritic or littoral flagellates	*Gymnodinium splendens*	3.8	47	sphere	54362	47	6939.8
	Monochrysis lutheri	0.6	5	sphere	65	5	78.5
	Isochrysis galbaba	0.1	5	sphere	65	5	78.5
	Isochrysis galbaba	0.1	5	sphere	65	5	78.5
	Dunaliella tertiolecta	1.4	8	Sphere	268	8	201.1

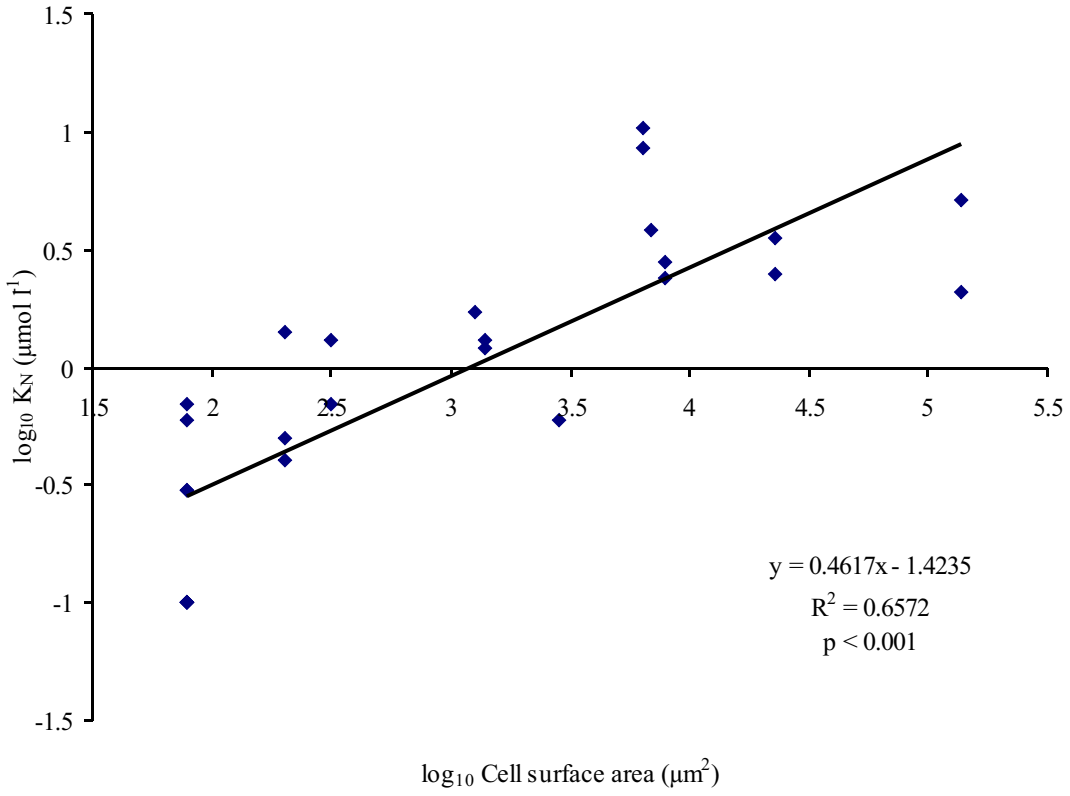


Figure 3.1. Half-saturation constant for nitrate uptake as a function of cell surface area based on data from Eppley et al. (1969). Regression line (Model I) and equation is shown.

3.4.4. Assigning trophic status to protists. Because the proposed method scales microzooplankton grazing directly to biomass (Equation (8)), it is important to correctly establish the heterotrophic component present during these incubations. Microzooplankton include a variety of heterotrophic organisms including ciliates, flagellates and dinoflagellates (Capriulo et al. 1991). These groups were all enumerated using inverted microscopy but, because they were preserved in 10% acid Lugols, epiflourescent microscopy could not be used to distinguish the heterotrophic and autotrophic fractions (Lessard and Swift 1986). Therefore, all ciliates were assumed to be heterotrophic (Burkill et al. 1993), although some may retain chloroplasts (e.g. Stoecker et al. 1989). Flagellates are considered to be autotrophic in the context of this study because, although primarily bactivorous (and thus mixotrophic: reviewed by Capriulo 1990 and Arndt et al. 2000), their optimum prey is expected be smaller than any of the cells counted (Hansen et al. 1994a). It is estimated that approximately 50 % of all species of dinoflagellate are obligate heterotrophs (Gaines and Elbrachter 1987) and therefore half of all dinoflagellates found here are assumed to be heterotrophic.

3.4.5. Calculating copepod grazing and prey preferences. Instantaneous copepod grazing coefficients, g_F and g_M, were calculated (Equations (2) and (11)) for each daily experiment and used to estimate their respective clearance (R_F or R_M: Equations (4) and (13)) and ingestion rates (I_F or I_M: Equations (5) and (14)). The total daily copepod ration, G_F or G_M (G), was calculated by summing I_F or I_M for all prey types. In common with previous studies (Meyer-Harms et al.1999, Irigoien et al. 2000a), negative values of I_F were assumed to be zero (I_M was never negative). Copepod grazing coefficients, g_M, were only corrected for microzooplankton grazing in the experimental bottles when $m_{(E)}$ was significantly different from zero ($p < 0.05$). The total biomass of available prey at the start of the incubations in the control bottles, $A_{ini(C)}$, was calculated by summing $P_{ini(C)}$ for all prey types.

The preference of *Calanus finmarchicus* for different cell groups was estimated using the selection index, E^* (Vanderploeg and Scavia 1979), as presented by Rollwagen Bollens and Penry (2003). Because the relative abundances of prey varies among the samples, E* is thought to be more appropriate than both Chesson's α (Chesson 1983) and Ivlev's E (Ivlev 1961; see Confer and Moore 1987). This index ultimately compares the fraction of each prey type, P, at the start of the incubations (n_i) to the fraction of that prey type in the copepod's diet (r_i). When using

the equations of Frost (1972), the quantity of P ingested is influenced by the estimated net growth coefficient, $k_{(C)}$, which is potentially biased due to microzooplankton grazing artefacts. The proposed method accounts for microzooplankton grazing when estimating the ingested quantity of P, but remains under the influence of the estimated gross growth coefficient, $r_{(C)}$ (equations 2 and 11 respectively). The respective selectivity indices, E^*_F and E^*_M, are therefore influenced by the grazing of *Calanus* and also these parameters. The proportion of prey in the diet, r_i, and in the initial food environment, n_i, were calculated using the following equations:

$$r_i = \frac{I}{G} \tag{21}$$

$$n_i = \frac{P_{ini(C)}}{A_{ini(C)}} \tag{22}$$

where I is the biomass of each prey type p in the diet, determined using either Frost's equations (I_F) or the proposed method (I_M). $P_{ini(C)}$ and $A_{ini(C)}$ are the average biomass of prey type p and the average total available biomass of all prey respectively in the control bottles at the start of the incubation.

E* for each prey type can then be calculated:

$$E^*_i = \frac{W_i - \frac{1}{m}}{W_i + \frac{1}{m}} \tag{23}$$

where m is the number of prey types and W_i is defined by the equation:

$$W_i = \frac{\frac{r_i}{n_i}}{\sum_{j=1}^{m} \frac{r_i}{n_i}} \tag{24}$$

Values > 0 indicate prey selection whilst values < 0 indicate prey avoidance. Neutral preference is indicated by an E* of zero. Separate indices, E^*_F and E^*_M, were calculated using the biomass of each prey type in the diet, I_F and I_M, respectively.

3.5. RESULTS

3.5.1. Microplankton dynamics in the control bottles. Calculated net growth rates, $k_{(C)}$ (Equation 1), modelled gross growth rates of prey, $r_{(C)}$ (Equation 18), the resulting microzooplankton grazing coefficients, $m_{(C)}$ (Equation 7), and microzooplankton biomass-specific ingestion rates, $M_{(C)}$ (Equation 16), in the control bottles for the April (D262) and July/August (D264) experiments are shown in Table 3.5. Net growth rates of microplankton were often negative. Many of these rates were statistically different from zero ($p < 0.05$), indicating net cell loss in these bottles during both the April and July/August incubations. Grazing is generally thought to be the predominant loss process in marine ecosystems (Banse 1994). While it is possible that other forms of mortality, such as cell lysis, may have to some extent been induced due to handling artefacts in the experiments, no evidence of this effect was readily apparent. Modelled gross growth rates of prey, $r_{(C)}$, were greatest for small cells (<10 μm equivalent spherical diameter; ESD) in both seasons, ranging between 0.74 to 1.41 d^{-1} in April and 1.15 to 1.89 d^{-1} in July/August. Conversely, large cells (≥10 μm ESD) were predicted to grow at more modest rates (range 0.32 to 0.49 d^{-1}, and 0.35 to 0.88 d^{-1} in April and July/August respectively). Growth rates in July/August were always higher than their respective values in April. Most notably, ciliates were expected to grow at approximately double their estimated rates in April.

Estimated values of microzooplankton grazing coefficients in the control bottles, $m_{(C)}$, were more variable, but followed a similar pattern to the estimated gross growth rates. Coefficients were higher for small cells in both April and July/August (0.22 to 1.61 d^{-1} and 0.81 to 2.44 d^{-1} respectively) relative to large cells (0.09 to 1.50 d^{-1} in April and 0.01 to 1.12 d^{-1} in July/August).

The $m_{(C)}$:$r_{(C)}$ ratio is thought to be a reasonable proxy for the fraction of primary production consumed by microzooplankton (cf. Calbet and Landry 2004), and enables the degree of coupling between growth and microzooplankton grazing to be assessed. To reduce the biasing of particularly large values, individual ratios were

Table 3.5A. Microplankton dynamics in the control bottles in April. Average instantaneous rates of net growth ($k_{(C)}$), gross growth ($r_{(C)}$), microzooplankton grazing ($m_{(C)}$), the fraction of production consumed by microzooplankton in the control bottles ($m_{(C)}/r_{(C)}$) and microzooplankton biomass specific ingestion ($M_{(C)}$) ± SE. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ for $k_{(C)}$ or $m_{(C)} = 0$, n = 3. Ciliates and 50% of all dinoflagellates are considered as microzooplankton (see Section 3.4.4). Only significant m(C) values ($p < 0.05$) were used to correct copepod grazing rates. Prey types are given with equivalent spherical diameter in parentheses.

Stn. #	Cell group (p)	$k_{(C)}$ (d^{-1})			$r_{(C)}$ (d^{-1})	$m_{(C)}$ (d^{-1})			$m_{(C)}/r_{(C)}$ (%)	$M_{(C)}$ (d^{-1})
1	Centric diatom (32)	-0.33	±0.03	***	0.39	0.73	±0.03	***	183.80	0.10
2	Centric diatom (32)	-0.11	±0.08		0.40	0.51	±0.08	**	127.23	0.04
3	Centric diatom (32)	-0.05	±0.42		0.40	0.45	±0.42		113.10	0.02
4	Centric diatom (32)	-0.39	±0.07	**	0.40	0.78	±0.07	***	196.59	0.06
5	Centric diatom (32)	-0.24	±0.30		0.40	0.64	±0.30		160.75	0.04
1	Ciliates (19)	-0.30	±0.24		0.49	0.79	±0.24	*	160.37	0.62
2	Ciliates (19)	0.41	±0.13	*	0.49	0.09	±0.13		17.69	0.06
3	Ciliates (19)	0.39	±0.24		0.49	0.11	±0.24		21.82	0.02
4	Ciliates (19)	0.22	±0.27		0.49	0.27	±0.27		55.70	0.20
5	Ciliates (19)	-0.06	±0.40		0.49	0.55	±0.40		112.13	0.21
1	Cryptomonad (8)	-0.22	±0.39		0.74	0.96	±0.39		129.87	3.02
2	Cryptomonad (8)	0.52	±0.23		0.74	0.22	±0.23		29.83	0.30

Stn. #	Cell group (p)	$k_{(C)}$ (d^{-1})			$r_{(C)}$ (d^{-1})	$m_{(C)}$ (d^{-1})			$m_{(C)}/r_{(C)}$ (%)	$M_{(C)}$ (d^{-1})
3	Cryptomonad (8)	0.04	±0.17		0.74	0.70	±0.17	*	94.82	0.59
4	Cryptomonad (8)	-0.12	±0.09		0.74	0.86	±0.09	***	116.60	2.00
5	Cryptomonad (8)	0.64	±0.08	**	0.74	0.10	±0.08		12.93	0.11
1	Flagellate (< 3.5)	0.13	±0.25		1.41	1.28	±0.25	**	90.60	2.09
2	Flagellate (< 3.5)	-0.07	±0.12		1.41	1.48	±0.12	***	105.16	1.51
3	Flagellate (< 3.5)	0.34	±0.06	**	1.41	1.08	±0.06	***	76.14	0.74
4	Flagellate (< 3.5)	-0.08	±0.01	***	1.41	1.49	±0.01	***	105.37	1.66
5	Flagellate (< 3.5)	0.37	±0.07	**	1.41	1.04	±0.07	***	73.50	0.66
1	Flagellate (> 3.5)	0.29	±0.07	*	0.88	0.60	±0.07	***	67.73	1.17
2	Flagellate (> 3.5)	-0.21	±0.12		0.89	1.10	±0.12	***	124.15	0.97
3	Flagellate (> 3.5)	0.08	±0.07		0.89	0.81	±0.07	***	90.97	0.63
4	Flagellate (> 3.5)	-0.15	±0.07		0.89	1.04	±0.07	***	117.03	0.51
5	Flagellate (> 3.5)	0.42	±0.33		0.89	0.46	±0.33		52.26	0.10
1	Naked dinoflagellate (19)	-0.13	±0.08		0.31	0.44	±0.08	**	141.70	0.11
2	Naked dinoflagellate (19)	-0.18	±0.13		0.31	0.49	±0.13	*	156.23	0.18
3	Naked dinoflagellate (19)	-0.50	±0.12	*	0.32	0.81	±0.12	**	257.50	0.43
4	Naked dinoflagellate (19)	-0.14	±0.12		0.31	0.46	±0.12	*	144.93	0.26
5	Naked dinoflagellate (19)	-0.14	±0.26		0.31	0.45	±0.26		143.05	0.16

Stn. #	Cell group (p)	$k_{(C)}$ (d^{-1})			$r_{(C)}$ (d^{-1})	$m_{(C)}$ (d^{-1})			$m_{(C)}/r_{(C)}$ (%)	$M_{(C)}$ (d^{-1})
1	Nitzschia (5)	-0.54	±0.03	***	1.07	1.61	±0.03	***	150.09	0.00
2	Nitzschia (5)	-0.39	±0.12	*	1.08	1.46	±0.12	***	135.95	0.02
3	Nitzschia (5)	0.23	±0.11		1.08	0.84	±0.11	**	78.45	0.01
4	Nitzschia (5)	-0.07	±0.10		1.08	1.15	±0.10	***	106.66	0.01
5	Nitzschia (5)	0.51	±0.09	**	1.08	0.57	±0.09	**	52.85	0.00
1	Silicoflagellates (28)	0.01	±0.23		0.37	0.35	±0.23		95.94	0.05
2	Silicoflagellates (28)	-0.39	±0.19		0.37	0.76	±0.19	*	204.76	0.29
3	Silicoflagellates (28)	-1.11	±0.11	***	0.37	1.49	±0.11	***	397.54	0.40
4	Silicoflagellates (28)	-0.62	±0.14	*	0.37	0.99	±0.14	**	265.90	0.20
5	Silicoflagellates (28)	-0.32	±0.12		0.37	0.69	±0.12	**	185.59	0.13
1	Thecate dinoflagellate (17)	0.24	±0.07	*	0.33	0.10	±0.07		29.40	0.01
2	Thecate dinoflagellate (17)	-0.59	±0.05	***	0.34	0.93	±0.05	***	275.80	0.09
3	Thecate dinoflagellate (17)	-0.40	±0.14	*	0.34	0.73	±0.14	**	217.90	0.08
4	Thecate dinoflagellate (17)	-0.15	±0.15		0.34	0.48	±0.15	*	143.24	0.05
5	Thecate dinoflagellate (17)	-0.30	±0.10	*	0.34	0.63	±0.10	**	188.46	0.03

Table 3.5B. Microplankton dynamics in the control bottles in July/August. Average instantaneous rates of net growth ($k_{(C)}$), gross growth ($r_{(C)}$), microzooplankton grazing ($m_{(C)}$), the fraction of production consumed by microzooplankton in the control bottles ($m_{(C)}/r_{(C)}$) and microzooplankton biomass specific ingestion ($M_{(C)}$) ± SE. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ for $k_{(C)}$ or $m_{(C)} = 0$, n = 3. Ciliates and 50% of all dinoflagellates are considered as microzooplankton (see METHODS). Only significant m(C) values ($p < 0.05$) were used to correct copepod grazing rates. Prey types are given with equivalent spherical diameter in parentheses.

Stn. #	Cell group (p)	$k_{(C)}$ (d^{-1})			$r_{(C)}$ (d^{-1})	$m_{(C)}$ (d^{-1})			$m_{(C)}/r_{(C)}$ (%)	$M_{(C)}$ (d^{-1})
1	Centric diatom (35)	-0.01	±0.07		0.41	0.41	±0.07	**	101.35	0.20
2	Centric diatom (35)	0.06	±0.04		0.44	0.38	±0.04	***	86.31	0.16
3	Centric diatom (35)	-0.34	±0.13		0.43	0.77	±0.13	**	180.22	0.31
4	Centric diatom (35)	-0.56	±0.07	**	0.43	0.98	±0.07	***	229.76	0.88
5	Centric diatom (35)	-0.05	±0.04		0.45	0.50	±0.04	***	110.37	0.09
1	Ciliate (22)	0.66	±0.15	*	0.88	0.22	±0.15		24.91	0.06
2	Ciliate (22)	0.15	±0.12		0.88	0.73	±0.12	**	82.67	0.38
3	Ciliate (22)	0.00	±0.13		0.88	0.88	±0.13	**	100.03	0.54
4	Ciliate (22)	-0.39	±0.06	**	0.88	1.27	±0.06	***	144.45	0.80
5	Ciliate (22)	0.87	±0.01	***	0.88	0.01	±0.01		0.60	0.00
1	Cryptomonad (10)	0.06	±0.15		0.83	0.77	±0.15	**	93.05	0.57
2	Cryptomonad (10)	0.51	±0.02	***	0.86	0.35	±0.02	***	40.57	0.61

Stn. #	Cell group (p)	$k_{(C)}$ (d^{-1})			$r_{(C)}$ (d^{-1})	$m_{(C)}$ (d^{-1})			$m_{(C)}/r_{(C)}$ (%)	$M_{(C)}$ (d^{-1})
3	Cryptomonad (10)	0.34	±0.10	*	0.85	0.51	±0.10	**	60.28	2.86
4	Cryptomonad (10)	-0.04	±0.06		0.85	0.89	±0.06	***	104.60	3.14
5	Cryptomonad (10)	0.00	±0.14		0.87	0.86	±0.14	**	99.54	4.04
1	Flagellate (< 3.5)	0.50	±0.12	*	1.88	1.37	±0.12	***	73.32	1.62
2	Flagellate (< 3.5)	-0.02	±0.07		1.89	1.92	±0.07	***	101.32	4.30
3	Flagellate (< 3.5)	-0.56	±0.05	***	1.88	2.44	±0.05	***	129.53	7.56
4	Flagellate (< 3.5)	-0.31	±0.10	*	1.89	2.19	±0.10	***	116.34	6.09
5	Flagellate (< 3.5)	-0.50	±0.17	*	1.90	2.40	±0.17	***	126.48	6.89
1	Flagellate (> 3.5)	-0.38	±0.40		1.14	1.53	±0.40	*	133.56	0.32
2	Flagellate (> 3.5)	0.36	±0.26		1.17	0.81	±0.26	*	69.22	0.36
3	Flagellate (> 3.5)	0.07	±0.15		1.16	1.08	±0.15	**	93.74	1.43
4	Flagellate (> 3.5)	-0.40	±0.01	***	1.16	1.56	±0.01	***	134.36	2.85
5	Flagellate (> 3.5)	0.02	±0.18		1.17	1.16	±0.18	**	98.60	1.94
1	Naked dinoflagellate (17)	0.26	±0.03	**	0.39	0.14	±0.03	*	34.49	0.12
2	Naked dinoflagellate (17)	-0.29	±0.18		0.41	0.70	±0.18	*	169.14	0.54
3	Naked dinoflagellate (17)	-0.32	±0.16		0.40	0.73	±0.16	*	179.82	0.54
4	Naked dinoflagellate (17)	-0.26	±0.12		0.40	0.66	±0.12	**	163.64	0.69
5	Naked dinoflagellate (17)	0.15	±0.04	*	0.42	0.27	±0.04	**	64.45	0.20

Stn. #	Cell group (p)	$k_{(C)}$ (d^{-1})			$r_{(C)}$ (d^{-1})	$m_{(C)}$ (d^{-1})			$m_{(C)}/r_{(C)}$ (%)	$M_{(C)}$ (d^{-1})
1	Nitzschia (6)	-0.41	±0.02	***	1.28	1.70	±0.02	***	132.28	0.08
2	Nitzschia (6)	-0.01	±0.21		1.31	1.33	±0.21	**	101.03	0.06
3	Nitzschia (6)	-0.30	±0.08	*	1.30	1.60	±0.08	***	122.70	0.07
4	Nitzschia (6)	-0.44	±0.17		1.30	1.74	±0.17	***	134.05	0.07
5	Nitzschia (6)	-0.78	±0.49		1.32	2.10	±0.49	*	159.23	0.05
1	Pennate diatom (25)	0.04	±0.08		0.52	0.48	±0.08	**	92.95	0.02
2	Pennate diatom (25)	-0.34	±0.09	*	0.56	0.90	±0.09	***	161.64	0.74
3	Pennate diatom (25)	-0.51	±0.18	*	0.54	1.05	±0.18	**	194.44	0.78
4	Pennate diatom (25)	-0.29	±0.11		0.54	0.83	±0.11	**	152.81	0.10
5	Pennate diatom (25)	-0.52	±0.10	**	0.57	1.09	±0.10	***	192.22	3.99
1	Thecate dinoflagellate (20)	0.09	±0.11		0.35	0.25	±0.11		73.23	0.12
2	Thecate dinoflagellate (20)	-0.07	±0.05		0.37	0.44	±0.05	**	119.06	0.15
3	Thecate dinoflagellate (20)	-0.42	±0.16		0.36	0.78	±0.16	**	216.43	0.29
4	Thecate dinoflagellate (20)	-0.24	±0.17		0.36	0.60	±0.17	*	167.65	0.16
5	Thecate dinoflagellate (20)	-0.23	±0.04	**	0.37	0.60	±0.04	***	160.36	0.37

arctangent transformed before calculating daily averages and the values inverse transformed (tangent(x)) (Calbet and Landry, 2004).

In April, the microzooplankton are responsible for removing, on average, 80% of the daily productivity (84, 66, 70, 114 and 53% at stations 1 to 5 respectively). This increased during the experiments in July/August, with an average of 106% being removed each day (64, 88, 121, 142 and 114% daily).

Microzooplankton biomass specific ingestion rates, $M_{(C)}$, were typically low in both seasons, with values in July/August being greater than those in April. Again, values of $M_{(C)}$ for large cells (April: 0.01 – 0.61µg C [µg microzooplankton C]$^{-1}$ d^{-1}; July/August: 0.00 – 3.80 µg C [µg microzooplankton C]$^{-1}$ d^{-1}) are lower than those of the smaller cells (0.00 – 3.00 µg C [µg microzooplankton C]$^{-1}$ d^{-1} and 0.05 – 7.00 µg C [µg microzooplankton C]$^{-1}$ d^{-1} in April and July/August respectively), particularly flagellates and cryptomonads. Although values of $m_{(C)}$ were high for the small diatom *Nitzschia* in both April and July/August (ESD 5 and 6 µm respectively), corresponding values of $M_{(C)}$ are very low. This reflects the scarcity of this algal group (Table 3.6).

3.5.2. Selective removal of microzooplankton. Calculated copepod grazing coefficients, g_F and g_M, and selectivity indices, E^*_F and E^*_M, April and July/August are presented in Table 3.6. Values of g_F and g_M on ciliates were consistently high in both seasons (up to 0.83 and 1.28 d^{-1} in April and July/August respectively), though selection towards ciliates was much stronger during July/August. Copepod grazing coefficients and selection indices for ciliates were only significantly different from zero ($p < 0.05$) on the second day of experimentation in April. The reduction of ciliates in the grazed bottle relative to the controls was also significant on this day (Table 3.7A). In contrast, both grazing and selection for ciliates were significant on all days except day 4 of the experiment in July/August, and they were significantly reduced in the experimental bottles relative to the controls at all stations except station 3 (Table 3.7B). In both seasons, ciliates were the only prey group reduced on average by greater than 40 % in the experimental bottles relative to controls (Table 3.7). Although high grazing coefficients (g_F and g_M) on dinoflagellates were sometimes observed in both April (up to 0.69 d^{-1}) and July/August (up to 0.88 d^{-1}), no clear trend in selection was apparent.

3.5.3. Microplankton dynamics in the experimental bottles. A number of negative copepod grazing coefficients, g_F (Table 3.6), and thus R_F and I_F (equations 4

Table 3.6A. Copepod grazing dynamics in April. Average prey concentration at the start of the incubations (Initial). Instantaneous copepod grazing coefficients ± SE (g_F and g_M) are calculated according to Frost (1972) and the proposed method respectively. Electivity indices (E^*_F and E^*_M) ± SE are based on ingested biomass, I_F and I_M respectively. $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ for gF, gM, E^*_F and $E^*_M = 0$, n = 3.

Stn. #	Cell group (p)	Initial ($\mu g\ C\ l^{-1}$)	g_F (d^{-1})		E_F^*		g_M (d^{-1})		E_M^*	
1	Centric diatom	0.51	0.06 ±0.30		-0.24 ±0.48		0.16 ±0.26		-0.20 ±0.47	
1	Ciliates	3.18	0.26 ±0.12		-0.03 ±0.19		0.37 ±0.16		0.04 ±0.14	
1	Cryptomonad	12.94	0.13 ±0.41		-0.57 ±0.43		0.13 ±0.41		-0.61 ±0.39	
1	Flagellate (< 3.5)	5.24	0.20 ±0.41		-0.55 ±0.45		0.37 ±0.43		-0.33 ±0.39	
1	Flagellate (> 3.5)	5.68	0.06 ±0.05		-0.54 ±0.29		0.14 ±0.08		-0.42 ±0.32	
1	Naked dinoflagellate	0.87	0.40 ±0.23		-0.13 ±0.44		0.46 ±0.24		-0.18 ±0.41	
1	Nitzschia	0.01	0.20 ±0.09		-0.13 ±0.12		0.42 ±0.13	*	0.14 ±0.06	
1	Silicoflagellates	0.59	0.07 ±0.18		-0.20 ±0.41		0.07 ±0.18		-0.29 ±0.36	
1	Thecate dinoflagellate	0.17	0.27 ±0.12		0.15 ±0.01	***	0.27 ±0.12		0.02 ±0.04	
1	Total C	29.21								
2	Centric diatom	0.25	0.10 ±0.05		-0.41 ±0.31		0.24 ±0.04	**	-0.15 ±0.12	
2	Ciliates	2.38	0.79 ±0.07	***	0.59 ±0.07	***	0.79 ±0.07	***	0.39 ±0.07	**
2	Cryptomonad	4.59	0.36 ±0.13		0.34 ±0.07	**	0.36 ±0.13		0.09 ±0.09	
2	Flagellate (< 3.5)	3.53	-0.19 ±0.08		-1.00 ±0.00		0.22 ±0.10		-0.21 ±0.19	
2	Flagellate (> 3.5)	3.29	0.10 ±0.13		-0.36 ±0.35		0.41 ±0.15	*	0.02 ±0.14	
2	Naked dinoflagellate	1.41	0.18 ±0.07		-0.10 ±0.10		0.32 ±0.07	*	-0.05 ±0.03	

Stn. #	Cell group (p)	Initial (μg C l^{-1})	g_F (d^{-1})		E_F*		g_M (d^{-1})		E_M*	
2	Nitzschia	0.05	-0.16 ±0.07		-1.00 ±0.00		0.24 ±0.05	**	-0.15 ±0.13	
2	Silicoflagellates	1.60	0.04 ±0.08		-0.53 ±0.23		0.25 ±0.07	*	-0.20 ±0.15	
2	Thecate dinoflagellate	0.42	-0.19 ±0.24		-0.70 ±0.30		0.07 ±0.24		-0.65 ±0.32	
2	Total C	17.51								
3	Centric diatom	0.26	0.50 ±0.77		-0.50 ±0.50		0.50 ±0.77		-0.53 ±0.47	
3	Ciliates	1.25	0.83 ±0.33		0.25 ±0.14		0.83 ±0.33		0.08 ±0.11	
3	Cryptomonad	2.86	0.41 ±0.26		-0.21 ±0.28		0.60 ±0.30		-0.07 ±0.12	
3	Flagellate (< 3.5)	1.94	-0.01 ±0.06		-0.78 ±0.12	**	0.29 ±0.05	**	-0.16 ±0.06	
3	Flagellate (> 3.5)	2.51	0.83 ±0.02	***	0.29 ±0.06	**	1.05 ±0.09	***	0.23 ±0.04	**
3	Naked dinoflagellate	2.25	0.45 ±0.22		-0.19 ±0.19		0.68 ±0.30		-0.14 ±0.13	
3	Nitzschia	0.02	0.42 ±0.15	*	0.01 ±0.16		0.66 ±0.20	*	0.07 ±0.11	
3	Silicoflagellates	1.49	0.34 ±0.23		-0.45 ±0.19		0.75 ±0.35		-0.18 ±0.11	
3	Thecate dinoflagellate	0.45	0.49 ±0.12	*	-0.02 ±0.08		0.70 ±0.18	*	-0.04 ±0.05	
3	Total C	13.02								
4	Centric diatom	0.32	0.01 ±0.26		-0.60 ±0.40		0.13 ±0.36		-0.63 ±0.37	
4	Ciliates	2.95	0.38 ±0.41		-0.49 ±0.51		0.38 ±0.41		-0.57 ±0.43	
4	Cryptomonad	8.28	0.15 ±0.14		-0.18 ±0.41		0.28 ±0.23		-0.19 ±0.41	
4	Flagellate (< 3.5)	3.86	-0.05 ±0.05		-1.00 ±0.00		0.16 ±0.13		-0.14 ±0.13	
4	Flagellate (> 3.5)	1.78	0.22 ±0.12		-0.11 ±0.47		0.38 ±0.20		0.07 ±0.32	
4	Naked dinoflagellate	2.13	0.26 ±0.12		0.37 ±0.16		0.33 ±0.18		0.28 ±0.17	

Stn. #	Cell group (p)	Initial (μg C l^{-1})	g_F (d^{-1})		E_F*		g_M (d^{-1})		E_M*	
4	Nitzschia	0.03	-0.25 ±0.12		-1.00 ±0.00		-0.08 ±0.22		-0.71 ±0.29	
4	Silicoflagellates	0.94	-0.07 ±0.21		-0.09 ±0.47		0.07 ±0.09		-0.10 ±0.47	
4	Thecate dinoflagellate	0.35	0.00 ±0.01		-0.90 ±0.06	***	0.07 ±0.06		-0.66 ±0.17	*
4	Total C	20.64								
5	Centric diatom	0.29	0.05 ±0.40		-0.55 ±0.45		0.05 ±0.40		-0.61 ±0.39	
5	Ciliates	1.65	0.56 ±0.51		-0.34 ±0.45		0.56 ±0.51		-0.40 ±0.40	
5	Cryptomonad	3.01	0.26 ±0.08	*	0.19 ±0.02	**	0.26 ±0.08	*	0.08 ±0.06	
5	Flagellate (< 3.5)	1.75	0.08 ±0.12		-0.34 ±0.33		0.30 ±0.08	*	0.14 ±0.07	
5	Flagellate (> 3.5)	0.70	0.16 ±0.05	*	-0.08 ±0.26		0.16 ±0.05	*	-0.17 ±0.27	
5	Naked dinoflagellate	1.45	0.32 ±0.05	**	0.14 ±0.12		0.32 ±0.05	**	0.03 ±0.15	
5	Nitzschia	0.01	0.16 ±0.19		-0.51 ±0.41		0.27 ±0.18		-0.26 ±0.39	
5	Silicoflagellates	0.77	0.08 ±0.16		-0.57 ±0.31		0.22 ±0.22		-0.39 ±0.33	
5	Thecate dinoflagellate	0.20	0.45 ±0.33		-0.09 ±0.45		0.58 ±0.30		0.14 ±0.21	
5	Total C	9.82								

Table 3.6B. Copepod grazing dynamics in July/August. Average prey concentration at the start of the incubations (Initial). Instantaneous copepod grazing coefficients ± SE (g_F and g_M) are calculated according to Frost (1972) and the proposed method respectively. Electivity indices (E^*_F and E^*_M) ± SE are based on ingested biomass, I_F and I_M respectively. $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ for gF, gM, E^*_F and $E^*_M = 0$, n = 3.

Stn. #	Cell group (p)	Initial ($\mu g\ C\ l^{-1}$)	g_F (d^{-1})		E_F^*		g_M (d^{-1})		E_M^*	
1	Centric diatom	3.04	0.82 ±0.24	*	0.14 ±0.07		0.96 ±0.24	*	0.07 ±0.06	
1	Ciliate	1.26	1.21 ±0.37	*	0.36 ±0.03	***	1.21 ±0.37	*	0.22 ±0.03	**
1	Cryptomonad	4.43	-0.10 ±0.11		-0.89 ±0.11	**	0.16 ±0.13		-0.58 ±0.22	
1	Flagellate (< 3.5)	5.54	0.58 ±0.13	*	0.15 ±0.04	*	1.02 ±0.19	**	0.28 ±0.02	***
1	Flagellate (> 3.5)	1.64	-0.46 ±0.08	**	-1.00 ±0.00		0.04 ±0.14		-0.79 ±0.21	*
1	Naked dinoflagellate	4.73	0.55 ±0.08	**	0.09 ±0.06		0.59 ±0.08	**	-0.03 ±0.06	
1	Nitzschia	0.33	0.28 ±0.01	***	-0.31 ±0.07	*	0.84 ±0.06	***	0.05 ±0.04	
1	Pennate diatom	0.26	0.44 ±0.17		-0.16 ±0.17		0.59 ±0.18	*	-0.11 ±0.09	
1	Thecate dinoflagellate	2.95	0.88 ±0.08	***	0.21 ±0.06	*	0.88 ±0.08	***	0.06 ±0.07	
1	Total C	24.18								
2	Centric diatom	2.46	0.82 ±0.13	**	0.27 ±0.06	*	0.90 ±0.12	**	0.17 ±0.06	
2	Ciliate	3.02	0.63 ±0.03	***	0.21 ±0.04	**	0.77 ±0.01	***	0.17 ±0.04	*
2	Cryptomonad	8.10	-0.02 ±0.15		-0.66 ±0.34		0.05 ±0.14		-0.67 ±0.33	
2	Flagellate (< 3.5)	13.83	0.03 ±0.16		-0.59 ±0.24		0.42 ±0.22		-0.26 ±0.34	
2	Flagellate (> 3.5)	2.45	0.08 ±0.24		-0.46 ±0.33		0.24 ±0.23		-0.36 ±0.33	
2	Naked dinoflagellate	5.64	0.26 ±0.24		-0.45 ±0.33		0.41 ±0.26		-0.37 ±0.27	

Stn. #	Cell group (p)	Initial ($\mu g\ C\ l^{-1}$)	g_F (d^{-1})		E_F^*		g_M (d^{-1})		E_M^*	
2	Nitzschia	0.30	0.51 ±0.09	**	0.08 ±0.05		0.78 ±0.13	**	0.15 ±0.03	*
2	Pennate diatom	5.93	0.85 ±0.04	***	0.21 ±0.03	**	1.03 ±0.06	***	0.16 ±0.04	*
2	Thecate dinoflagellate	2.16	0.53 ±0.11	**	0.08 ±0.06		0.62 ±0.12	**	0.01 ±0.03	
2	Total C	43.89								
3	Centric diatom	2.94	0.22 ±0.14		-0.35 ±0.35		0.38 ±0.17		-0.27 ±0.26	
3	Ciliate	3.79	1.08 ±0.60		0.39 ±0.00	***	1.26 ±0.67		0.29 ±0.04	**
3	Cryptomonad	29.11	0.32 ±0.25		-0.17 ±0.42		0.42 ±0.29		-0.24 ±0.38	
3	Flagellate (< 3.5)	24.55	0.05 ±0.12		-0.60 ±0.35		0.55 ±0.22		0.00 ±0.17	
3	Flagellate (> 3.5)	7.86	0.47 ±0.15	*	0.22 ±0.11		0.69 ±0.24	*	0.20 ±0.09	
3	Naked dinoflagellate	5.40	0.08 ±0.15		-0.73 ±0.27		0.22 ±0.21		-0.63 ±0.24	
3	Nitzschia	0.32	0.09 ±0.29		-0.33 ±0.35		0.41 ±0.38		-0.25 ±0.38	
3	Pennate diatom	5.92	0.36 ±0.35		-0.21 ±0.40		0.57 ±0.42		-0.26 ±0.38	
3	Thecate dinoflagellate	2.81	0.23 ±0.02	***	-0.13 ±0.15		0.39 ±0.07	**	-0.09 ±0.11	
3	Total C	82.70								
4	Centric diatom	7.13	0.10 ±0.11		-0.56 ±0.26		0.27 ±0.11		-0.33 ±0.13	
4	Ciliate	4.66	0.41 ±0.07	**	0.13 ±0.07		0.61 ±0.08	**	0.10 ±0.05	
4	Cryptomonad	22.02	0.04 ±0.13		-0.67 ±0.33		0.19 ±0.13		-0.47 ±0.25	
4	Flagellate (< 3.5)	19.65	0.33 ±0.08	*	0.04 ±0.13		0.68 ±0.10	**	0.19 ±0.07	
4	Flagellate (> 3.5)	13.51	0.05 ±0.05		-0.63 ±0.20	*	0.31 ±0.04	**	-0.17 ±0.06	
4	Naked dinoflagellate	7.27	0.34 ±0.06	**	0.08 ±0.10		0.45 ±0.06	**	-0.01 ±0.07	

Stn. #	Cell group (p)	Initial ($\mu g\ C\ l^{-1}$)	g_F (d^{-1})		E_F^*		g_M (d^{-1})		E_M^*	
4	Nitzschia	0.31	0.54 ±0.06	***	0.24 ±0.08	*	0.83 ±0.07	***	0.21 ±0.06	*
4	Pennate diatom	0.86	0.28 ±0.22		-0.19 ±0.40		0.41 ±0.21		-0.26 ±0.36	
4	Thecate dinoflagellate	1.89	0.48 ±0.16	*	0.17 ±0.15		0.58 ±0.16	*	0.05 ±0.12	
4	Total C	77.31								
5	Centric diatom	1.14	0.18 ±0.09		-0.19 ±0.15		0.27 ±0.10	*	-0.15 ±0.11	
5	Ciliate	1.21	0.67 ±0.10	**	0.57 ±0.01	***	0.67 ±0.10	**	0.42 ±0.01	***
5	Cryptomonad	28.86	0.35 ±0.10	*	0.16 ±0.06		0.50 ±0.12	*	0.15 ±0.06	
5	Flagellate (< 3.5)	22.27	-0.25 ±0.11		-1.00 ±0.00		0.15 ±0.05	*	-0.39 ±0.14	
5	Flagellate (> 3.5)	10.28	0.16 ±0.06	*	-0.18 ±0.12		0.36 ±0.08	*	0.04 ±0.07	
5	Naked dinoflagellate	4.21	0.29 ±0.08	*	0.11 ±0.08		0.34 ±0.09	*	0.00 ±0.09	
5	Nitzschia	0.23	-0.19 ±0.27		-0.62 ±0.38		0.16 ±0.22		-0.50 ±0.35	
5	Pennate diatom	28.77	0.11 ±0.02	**	-0.42 ±0.07	**	0.30 ±0.04	**	-0.15 ±0.04	*
5	Thecate dinoflagellate	4.14	0.08 ±0.04		-0.55 ±0.19	*	0.18 ±0.05	*	-0.33 ±0.10	*
5	Total C	101.12								

Table 3.7. Percentage loss of prey C in the experimental bottles relative to control bottles ± SE in April (A) and July/August (B). Ciliates and 50% of all dinoflagellates are considered as microzooplankton. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ for H_0 = biomass in control and experimental bottles are not significantly different, n = 3.

A	Total	Autotrophic	µzoo.	Ciliate	Total Dino.	Thecate Dino.	Naked Dino.
Stn. #	C	C	C	C	C	C	C
1	11.04 ±18.10	9.60 ±19.47	26.63 ±9.39	26.35 ±9.00	28.07 ±14.74	22.86 ±9.18	29.57 ±16.57
2	21.81 ±5.98	14.91 ±7.15	48.03 ±2.25 *	55.44 ±3.01 *	10.60 ±9.32	-27.27 ±28.40	17.91 ±5.65 *
3	33.17 ±5.62 **	29.38 ±4.91 *	48.17 ±13.99	53.98 ±14.71	34.94 ±12.60	39.31 ±7.30 **	33.98 ±13.81
4	12.63 ±8.85	9.13 ±6.01	25.14 ±19.48	26.65 ±22.74	19.71 ±7.80	2.01 ±1.34	22.68 ±9.01
5	21.66 ±4.61	19.06 ±4.53	36.37 ±14.88	38.38 ±22.02	31.58 ±4.86	30.56 ±19.44	31.70 ±3.37 ***
Avg.	20.06	16.41	36.87	40.16	24.98	13.49	27.17

B	Total	Autotrophic	µzoo.	Ciliate	Total Dino.	Thecate Dino.	Naked Dino.
Stn. #	C	C	C	C	C	C	C
1	36.11 ±5.69 **	31.89 ±5.39 *	54.33 ±5.79 **	66.57 ±12.15 *	47.85 ±3.87 **	58.72 ±3.39 ***	42.05 ±4.22 ***
2	15.39 ±2.24 **	12.19 ±2.38 *	37.90 ±5.13 *	47.17 ±1.61 *	27.55 ±12.64	40.78 ±6.55 **	21.45 ±16.74
3	22.09 ±12.55	20.33 ±12.63	37.19 ±13.33	56.18 ±17.05	12.39 ±8.87	22.53 ±1.72 ***	7.59 ±12.83
4	15.14 ±4.30	13.80 ±4.52	32.31 ±1.46 *	33.33 ±4.36 **	31.41 ±1.14	38.42 ±10.18 *	29.54 ±4.16 **
5	14.47 ±1.12	13.38 ±1.04	30.48 ±4.49 **	48.26 ±5.54 **	17.88 ±3.78 *	7.26 ±3.77	25.07 ±6.40 *
Avg.	20.64	18.32	38.44	50.30	27.42	33.54	25.14

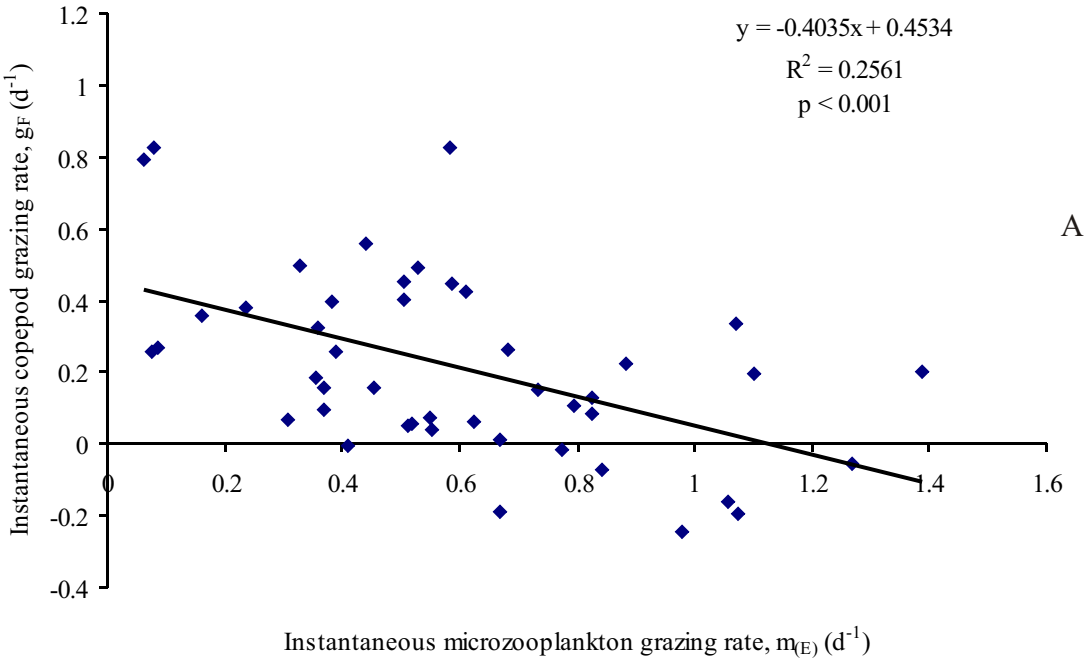
and 5) were calculated using Frost's equations, particularly for cells <10µm. In July/August, all negative incidences of g_F were at stations where ciliates were reduced significantly ($p < 0.05$) by copepod grazing (Table 3.6B). Although the removal of ciliates was only significant at station 2 during the incubations in April (Table 3.6A), negative grazing coefficients were calculated at stations 2, 3 and 4.

The negative correlation between instantaneous copepod grazing coefficients, g_F, and instantaneous microzooplankton grazing coefficients in the experimental bottles, $m_{(E)}$, was highly significant in April (ANOVA, $p < 0.001$, $n = 45$; Figure 3.2A) and July/August (ANOVA, $p < 0.001$, $n = 45$; Figure 3.3A). By contrast, g_M and $m_{(E)}$ were not correlated in April (ANOVA, $p = 0.217$, $N = 45$; Figure 3.2B) or July/August (ANOVA, $p = 0.239$, $N = 45$; Figure 3.3B). Daily copepod C rations, G_F, were seriously underestimated in both April (average > 40 %: Table 3.8A) and July/August (average > 70 %: Table 3.8B) when using the equations of Frost (1972).

3.5.4. Sensitivity analysis. To assess how sensitive the total copepod daily ration, G_M, was to changes in the modelled growth rates, the nitrate half-saturation constant, K_N, and maximal growth rate, μ_{max}, were individually changed by 50%. It is assumed that methodological errors are constant across all cell groups, thus although their absolute values will change, the relative differences between groups will remain constant. Varying K_N by 50% caused only marginal changes in the daily ration (Table 3.9), suggesting that this parameter is of minimal importance to the overall outcome of the method. Changing μ_{max} by 50% had a larger effect, with the resulting corrected daily ration, G_M, differing by an average of 24 and 25% respectively (Table 3.9).

3.6. DISCUSSION

3.6.1. Microplankton dynamics in the control bottles. The frequency of significant ($p < 0.05$) negative net growth rates indicates net cell loss during many of the incubations. Because these cells have been removed, it is reasonable to assume that these losses are attributable to microzooplankton grazing. All values of modelled growth and estimated microzooplankton grazing coefficients were of the same order as experimentally determined values from dilution experiments conducted at similar latitudes and season, where similar community compositions were encountered (Nejstgaard et al. 1997, 2001a, b). Unlike dilution experiments that derive group-



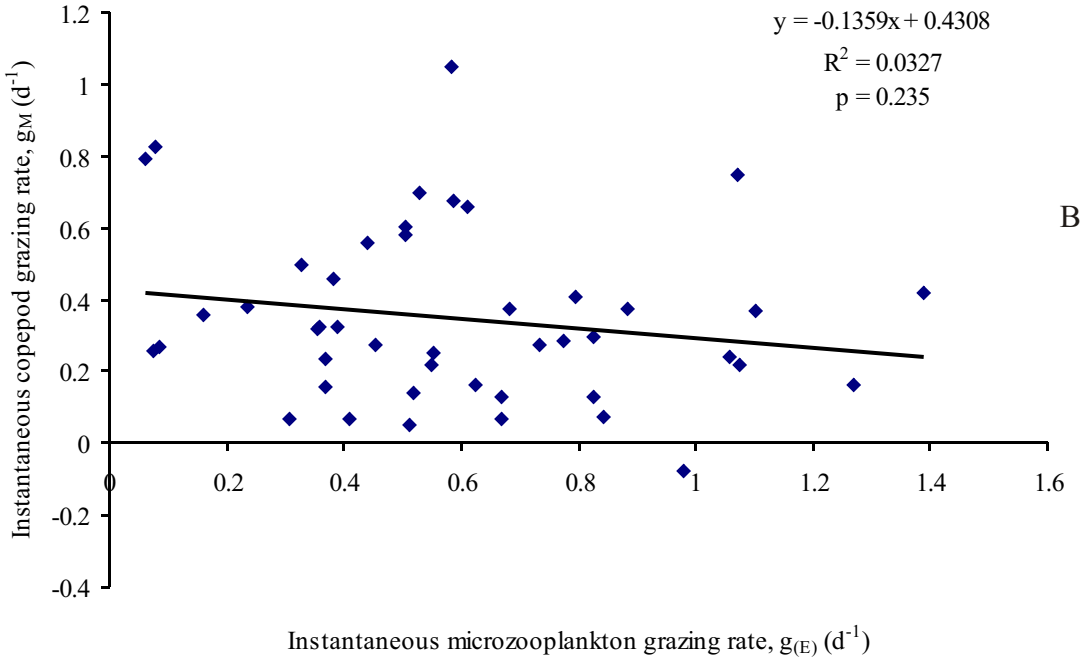


Figure 3.2. Correlation between instantaneous microzooplankton grazing coefficients, $m_{(E)}$, and *Calanus finmarchicus* grazing coefficients, g_F, (A) and g_M (B) in April. All data are included. Regression lines (solid lines) and their equations are shown.

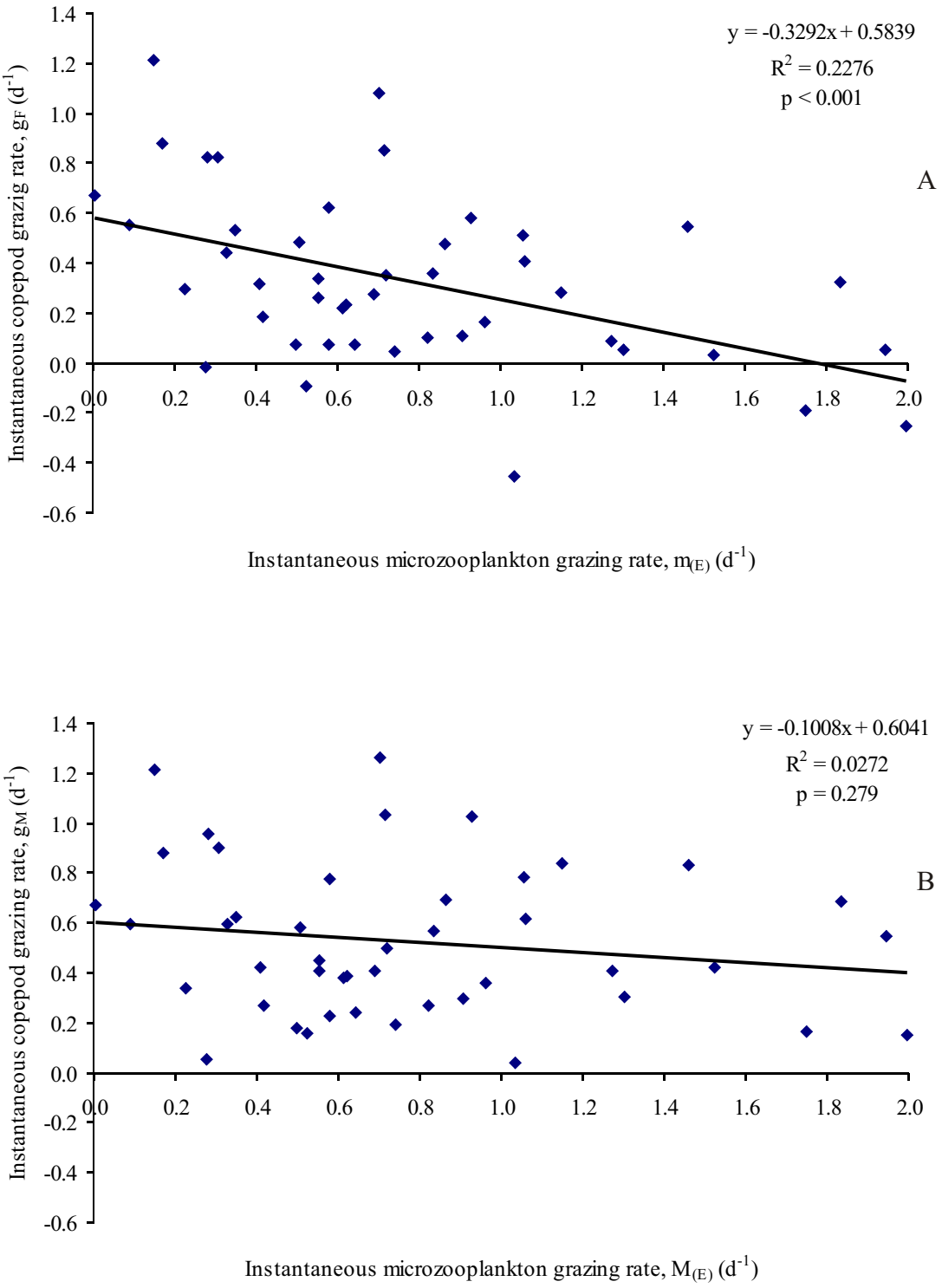


Figure 3.3. Correlation between instantaneous microzooplankton grazing coefficients, $m_{(E)}$, and *Calanus finmarchicus* grazing coefficients, g_F, (A) and g_M (B) in July/August. All data are included. Regression lines (solid lines) and their equations are shown.

Table 3.8. Daily rations in April (A) and July/August (B). Total daily amount of C ingested (µg C [copepod]$^{-1}$ d^{-1}) as calculated by Frost (1972; G_F) and the proposed method (G_M). Differences are expressed as a percentage (%).

A	G_F		G_M		
Stn. #	(µg C cop^{-1} d^{-1})		(µg C cop^{-1} d^{-1})		%
1	1.12	±0.81	1.39	±0.89	37.8
2	0.89	±0.16	1.31	±0.24	48.3
3	0.92	±0.21	1.40	±0.31	51.5
4	0.62	±0.38	1.01	±0.71	50.0
5	0.50	±0.13	0.60	±0.16	19.6
Avg.	0.81		1.14		41.4

B	G_F		G_M		
Stn. #	(µg C cop^{-1} d^{-1})		(µg C cop^{-1} d^{-1})		%
1	2.21	±0.21	3.06	±0.36	37.8
2	2.35	±0.22	3.65	±0.60	54.1
3	4.05	±1.63	7.07	±3.09	74.2
4	2.62	±0.42	5.23	±0.58	102.2
5	3.24	±0.58	6.06	±0.72	91.9
Avg.	2.90		5.02		72.1

Table 3.9. Sensitivity of the daily ration to 50 % changes in nitrate half-saturation constant (k_N) and maximal growth rate (μ_{max}) in April (A) and July/August (B), expressed as absolute values (µg C [copepod]$^{-1}$ d^{-1}) and percentage difference (%) relative to values of G_M without changes to growth parameters (see Table 3.8).

A	k_N < 50 %		μ_{max} > 50 %	
Stn. #	G_M (µg C cop^{-1} d^{-1})	%	G_M (µg C cop^{-1} d^{-1})	%
1	1.43	3.8	1.85	40.7
2	1.39	6.6	1.59	23.0
3	1.46	4.6	1.63	16.1
4	1.07	4.6	1.21	16.5
5	0.62	3.2	0.76	24.2
Average	1.20	4.6	1.41	24.1

B	k_N < 50 %		μ_{max} > 50 %	
Stn. #	G_M (µg C cop^{-1} d^{-1})	%	G_M (µg C cop^{-1} d^{-1})	%
1	3.15	3.0	3.77	23.1
2	3.70	1.2	4.53	25.4
3	7.16	1.3	8.65	26.0
4	5.32	1.6	6.43	23.4
5	6.17	1.8	7.68	26.3
Average	5.10	1.8	6.21	24.8

specific coefficients by using HPLC analysis (e.g. Burkill et al. 1987), those of Nejstgaard et al. (1997, 2001a, b) originate from inverted microscopy cell-counts. The estimated average percentage of primary production removed daily by the microzooplankton (80 and 106% in April and July/August respectively) illustrates the tight coupling between growth and grazing, as previously found in the North Atlantic (e.g. Burkill et al. 1993, Verity et al. 1993b, Gifford et al. 1995).

Microzooplankton biomass specific ingestion rates (0 to 3 d^{-1} and 0 to 7 d^{-1} in April and July/August respectively) were only occasionally high, and always fell within the confidence limits of maximal microzooplankton body volume specific ingestion (BVSI) rates estimated using the equations of Hansen et al. (1997). These values also agree well with the microzooplankton BVSI rates reported by Nejstgaard et al. (2001b). High rates were only reported for small cells (< 10 μm), which may be expected considering the size relationships between microzooplankton and their optimal sized prey (Hansen et al. 1994a).

The method to determine microzooplankton grazing coefficients ($m_{(C)}$) presented here represents an interesting alternative to dilution experiments (Landry and Hassett 1982). Although the application of the dilution technique has dramatically increased over recent years (see figure 1 in Dolan et al. 2000), leading to its adoption as the standard for determining microzooplankton herbivory (e.g. Bamstedt et al. 2000), it is not without criticism. The extent to which the underpinning assumptions are actually met remains theoretically and practically ambiguous (Gallegos 1989, Evans and Paranjape 1992, Landry et al. 1993, 1995). Dilution experiments have a complex effect on the community structure, with the combined effects of selective mortality of grazers in dilute treatments (death of those not resistant to periods of sub-threshold food concentrations) and differential growth of grazers in undiluted treatments complicating their interpretation (Dolan et al. 2000). The method developed here is not subject to the assumptions of the dilution technique, although it does require that specific algal gross growth rates ($r_{(C)}$) are representative values. It is envisaged that as our understanding of algal growth rates in relation to the biological, physical and chemical characteristics of the water increases, more realistic models to describe this growth will be possible. Using a series of concurrent dilution and seawater assemblage incubations, the suitability and accuracy of these two techniques could be compared and assessed.

3.6.2. Selective removal of microzooplankton. The equations of Frost (1972) assume that the net growth in the experimental bottles, $k_{(E)}$, is equal to that in the controls, $k_{(C)}$ (cf. Equation 2). This assumption is violated when microzooplankton are selectively removed by copepods, and in such cases, g_F provides an underestimation of copepod grazing. Selective removal of ciliates was evident in both seasons, particularly during experiments in July/August. That ciliates and other microzooplankton are selectively removed by copepods over a wide range of conditions is becoming increasingly apparent in the literature (Turner and Graneli 1992, Ohman and Runge 1994, Atkinson 1995, 1996, Verity and Paffenhofer 1996, Nejstgaard et al. 2001a, b, Zeldis et al. 2002, Bollens and Penry 2003), consolidating the notion that the equations used to estimate copepod grazing coefficients when incubating natural microplankton assemblages should also acknowledge the high grazing potential of the microzooplankton (Hansen et al. 1997) and the implications of differential microzooplankton grazing rates in the control and experimental bottles.

3.6.3. Microplankton dynamics in the experimental bottles. Negative clearance (R_F) and ingestion (I_F) rates are often reported from copepod bottle incubations (Turner and Graneli 1992, Hansen et al. 1994b, Atkinson 1995, 1996, Nejstgaard et al. 1997, 2001a, b, Zeldis et al. 2002, Bollens and Penry 2003). Because negative clearance (R_F) and ingestion (I_F) rates are impossible, their occurrence strongly suggests a methodological problem. Negative rates result when $k_{(E)}$ is enhanced relative to $k_{(C)}$, either due to a reduction in microzooplankton grazing pressure (Nejstgaard et al. 2001b) in experimental bottles (caused by selective grazing by copepods) or to nutrient excretion by copepods (Roman and Rublee 1980). These processes are not mutually exclusive.

In a recent study of copepod grazing (Levinsen et al. 2000b), nanoflagellates (<20µm) significantly increased in the experimental bottles containing *Calanus hyperboreus*, relative to the controls during a post-bloom period. It is hypothesised that the dramatic increase in nanoflagellates was most likely due to a combination of NH_4^+ excretion and a reduction in ciliate grazing pressure by the copepods, thus promoting algal growth in the nutrient poor, post bloom water. Unfortunately, because nutrients were not reported (Levinsen et al. 2000b), it is not possibly to test this hypothesis. Similarly, the influence of nutrient excretion is difficult to assess in these experiments, because nutrient dynamics in the bottles were not studied.

Nutrient limitation of algal growth was however unlikely in the experiments, considering that macronutrients were high at the start of the incubations (Table 3.2), and algal biomass was typically low (Table 3.6). Additionally, the close coupling between microzooplankton and their prey in the control bottles (Table 3.5) indicates that nutrients are rapidly recycled during the incubations (see Cushing and Horwood 1998). Considering that both nutrients and light were similar in control and experimental bottles, although $K_{(C)}$ and $K_{(E)}$ differ, the assumption that gross growth rates, $r_{(C)}$ and $r_{(E)}$, are equal appears reasonable. However, where excretion and remineralisation effects cause the nutrient dynamics to differ significantly between experimental and control bottles (in low nutrient environments), specific gross growth rates would have to be calculated separately (equation 18) for the different treatments, i.e. giving separate values for $r_{(C)}$ and $r_{(E)}$. Nutrient additions may be considered in order to alleviate these problems (Landry and Hassett 1982, Landry 1993).

A key finding of this work is that instantaneous copepod grazing coefficients, g_F, and instantaneous microzooplankton grazing coefficients in the experimental bottles, $m_{(E)}$, are significantly correlated in both April and July/August (Figures 3.2A and 3.3A). This artefact of microzooplankton grazing was also reported by Nejstgaard et al. (2001b). The negative slope of these relationships illustrate that g_F is only realistic when microzooplankton are not present. Furthermore, values of g_F are increasingly underestimated as the microzooplankton community grazing pressure increases until eventually they become negative. If $m_{(E)}$ remains sufficiently low as to prevent negative results, these effects are likely to pass unnoticed leading to an underestimation of copepod daily rations.

In contrast, instantaneous copepod grazing coefficients, g_M, and instantaneous microzooplankton grazing coefficients in the experimental bottles, $m_{(E)}$, were not correlated (Figures 3.2B and 3.3B), demonstrating that g_M provides a realistic estimate of actual copepod grazing coefficients regardless of microzooplankton community grazing pressure. All but one value of g_M are positive, and this single outlier may reflect an error introduced by the enumeration technique (inverted microscopy), rather than a error in the proposed mathematical approach.

In all cases where the microzooplankton grazing coefficient, $m_{(E)}$, was significantly ($p < 0.05$) different from zero the corresponding value of $g_{(M)}$ is larger than $g_{(F)}$. Statistical differences between g_F and g_M are found where the

microzooplankton biomass is significantly ($p < 0.05$) reduced in the experimental bottles relative to the controls (Table 3.7). This is because $m_{(C)}$ scales directly to biomass (equation 8). Alternatively, if microzooplankton are not significantly reduced in the experimental bottles relative to the controls, although g_F and g_M differ (Table 3.6), such differences will not be statistically distinguishable. This is not to say that the different results do not have significant ecological implications. Lack of significance may again be attributable to inaccuracies associated with the enumeration technique (Venrick 1978).

This study clearly illustrates that daily copepod C rations are seriously underestimated (> 40 % in April and > 70 % in July/August; Table 3.8) when microzooplankton grazing artefacts are not considered. Equally large discrepancies have been reported elsewhere (Nejstgaard et al. 1997, 2001b). The extent to which the daily ration from the proposed method, G_M, differs from G_F, will depend on the microzooplankton grazing coefficients, $m_{(C)}$. These are ultimately dependent on the cell growth rates (equation 7). The sensitivity analysis demonstrates that the overall outcome of the method is relatively insensitive to changes in the half-saturation constant for nitrate uptake, K_N, (tables 12 and 13). Changing the maximal growth rate, μ_{max}, by 50% resulted in the corrected daily ration, G_M, differing by an average of 24 and 25% in April and July/August respectively (Table 3.9). Nevertheless, although there are uncertainties associated with the model, the potential error introduced when microzooplankton grazing artefacts are not corrected for (i.e. when using Frost's equations) appears to be considerably greater than that associated with the new approach, at least in this instance. Considering that the daily rations calculated with the original (Frost 1972) and the new methods differ by approximately three times the difference caused by adjusting the maximal growth rate by 50 % (Table 3.9), the proposed method appears to provide a reasonably robust approach to determining copepod grazing rates.

It is noteworthy that the nutrient limitation factor, Q_N, of the growth model presented here does not contain a silicate component. When silicate concentrations fall below a threshold of approximately 2 μmol l^{-1}, as found in July/August (Table 3.2), diatoms lose the ability to dominate the phytoplankton (Egge and Asknes 1992), suggesting nutrient limitation. However, considering that diatoms represented only a small percentage of the available microplankton biomass in July/August (Table 3.6), a silicate component was considered unnecessary. Estimations of diatom ingestion in

the July/August incubations are thus maximal values. For situations where diatoms represent a more significant fraction the diet, equation 18 can be easily expanded to accommodate a silicate component, similar to that for nitrate (equation 20).

3.7. CONCLUSIONS

The common usage of Frost's (1972) equations in the literature to estimate copepod daily rations when offered autotrophy prey cells in the absence of microzooplankton demonstrates their suitability. However, comparison of the new method with that of Frost (1972) illustrates that when natural plankton assemblages are used, the copepod daily ration may be seriously underestimated if microzooplankton grazing interactions are not accounted for, as originally demonstrated by Nejstgaard et al. (1997). This suggests that our current understanding of the importance of copepods and their effect on primary production (e.g. Calbet 2001) might also be an underestimation. The method developed here provides a simple and realistic alternative to running concurrent dilution experiments. Because the growth model only considers changes over individual 24 h periods, estimates of growth have little time to deviate from their actual values. The sensitivity analysis suggests that if cell growth rates are estimated to within 50 % of their true values, corrected ingestion rates will be more realistic than those estimated using the traditional equations. The proposed method is adopted for all calculations relating to copepod feeding, unless otherwise stated.

Chapter 4

Nutritional quantity and quality of the particulate environment in April and July/August

4.1. AIMS

The work presented in this chapter examines in detail the microplankton species composition and the biochemical characteristics of the seston offered to *C. finmarchicus* in April and July/August. By determining the quantities of chlorophyll *a*, POC, PON, cell biomass and the fatty acid composition of the seston it is possible to establish the fraction of the particulate material that is likely to be available to a non-detrital feeder such as *Calanus* (Section 1.4.1). In addition, the relative contribution of detritus to the particulate environment can also be assessed. These data provide information about how useful individual particulate descriptors (e.g. chlorophyll *a*, POC) are for describing the food environment in terms of quantity and quality. The latter is addressed relative to the quantities of EPA and DHA.

Data on the fatty acid composition of natural oceanic seston are only rarely collected in parallel with microplankton species composition data. Because these data were collected, a correlative study was undertaken to assess whether or not specific fatty acid biomarkers can be used to reliably identify the presence of individual microplankton groups.

4.2. METHODS

The underlying methods used for the collection of samples are outlined in the methods chapter (Chapter 2). Microplankton samples refer to 200 ml of seawater from each station preserved in 10% acid Lugol's. Cells from 50 to 100 ml subsamples were settled onto glass baseplates and enumerated using inverted microscopy (n = 2 at each station). Cell volume was estimated from average linear measurements of each cell group (Section 2.4.4) and adjusted for shrinkage due to preservation in Lugol's iodine (Appendix 1). Microplankton biomass was subsequently derived by using representative cell volume:carbon equations (Section 2.4.5). Particulate samples refer to replicate 1 litre samples of seawater that were filtered onto GF/F filters (0.7 μm). Three replicates (n = 3) for both CN and fatty acid analyses were taken at each station.

4.2.1. Stastical methods. Microplankton species abundance (μg l^{-1}) and particulate fatty acid composition data (μg l^{-1}) were not normally distributed (Kolmogorov-Smirnov test, $p < 0.05$), and thus parametric tests were not appropriate. Therefore, to examine the intra- and extra-seasonal differences between samples, the

non-parametric ANOSIM (ANalysis Of SIMilarity) test was used (Clarke and Warwick 1994). This test is based on Bray-Curtis similarity coefficients (calculated from un-transformed data), and compares the similarities between replicates within each season to similarities between each season. The ANOSIM test is roughly analogous to the parametric ANOVA test. Since absolute quantities of both individual cell groups and individual fatty acid moieties were used, two samples were considered perfectly similar only if they contained the same cell groups or fatty acid moieties in *exactly* the same quantities (Clarke and Warwick 1994). All species (in this case, fatty acid moieties) contribute to the definition of similarity with the Bray-Curtis similarity coefficient, and more common species are given greater weight than the rare ones (Krebs 1998). In ANOSIM pairwise comparisons, the 'r' values gives an absolute measure of how different the groups are, on a scale of 0 (indistinguishable) to 1 (*all* similarities within groups are less than *any* similarity between groups). The non-parametric SIMilarity of PERcentage analysis (SIMPER; Clarke and Warwick 1994), which compares the percentage composition of samples within and between stations, was employed to qualify intra and extra seasonal similarities (and dissimilarities) as a percentage, and also to identify which fatty acid moieties primarily accounted for the observed differences between samples.

The relationships between the particulate descriptors (POC, PON, cell biomass, total fatty acids) were examined using correlation analysis. Total values of chlorophyll a, fatty acids and PUFAs were not normally distributed (Kolmogorov-Smirnov test, $p < 0.05$). The non-parametric Spearman rank order correlation test was therefore used to determine the degree of association between un-transformed particulate variables. Similarly, because the quantitative fatty acid and cell biomass data ($\mu g\ l^{-1}$) were not normally distributed (Kolmogorov-Smirnov test, $p < 0.05$), the Spearman rank order correlation test was used to determine the relationships between specific fatty acids and individual cell groups.

4.3. RESULTS

4.3.1 The particulate environment. Total concentrations of chlorophyll *a*, organic carbon (POC and cell biomass), nitrogen and fatty acids from the particulate samples in April and July/August are presented in Table 4.1.

Table 4.1. Seasonal water conditions above the Reykjanes Ridge in 2002. Station number (Stn.), chlorophyll *a* (Chl. *a*), particulate organic carbon (POC), particulate organic nitrogen (PON), cellular carbon determined via inverted microscopy (Cell C), total fatty acid (Total FA), saturated, monounsaturated and polyunsaturated fatty acids (SFA, MSFA and PUFA respectively) ± SE. ND = not determined, * = single value

	Stn.	Chl. *a* ($\mu g\ l^{-1}$)	POC ($\mu g\ l^{-1}$)	PON ($\mu g\ l^{-1}$)	Cell C ($\mu g\ l^{-1}$)	Total FA ($\mu g\ l^{-1}$)	SFA ($\mu g\ l^{-1}$)	MSFA ($\mu g\ l^{-1}$)	PUFA ($\mu g\ l^{-1}$)
APRIL	1	0.68	197.8±6.7	24.4±0.1	29.3±6.9	13.7±2.2	6.97±0.69	4.84±0.85	1.92±0.75
	2	0.77	136.0±9.8	15.0±1.3	17.7±2.1	18.5±0.7	8.19±0.26	6.39±0.21	3.88±0.39
	3	0.86	167.2±4.1	15.0±0.8	13.2±1.5	7.9 *	5.27 *	1.67 *	0.97 *
	4	0.75	125.4±5.8	17.1±0.7	20.8±3.8	23.7±2.6	10.53±1.45	7.21±0.95	5.99±0.26
	5	0.74	119.8±11.6	13.2±2.0	9.9±0.3	11.1±1.0	5.68±0.38	3.83±0.23	1.56±0.44
		Average:	**149.2±14.7**	**16.9±2.0**	**18.2±3.4**	**15.0±2.8**	**7.3±1.0**	**4.8±1.0**	**2.9±0.9**
JULY/AUGUST	1	1.13	296.0±11.8	49.9±2.4	25.1±6.3	66.4±0.8	22.33±0.91	18.09±0.85	25.99±0.89
	2	0.99	237.0±4.9	36.9±0.5	44.6±7.4	61.2±7.5	19.46±2.42	15.62±1.76	26.12±3.30
	3	0.72	226.3±5.3	30.7±0.1	83.6±6.8	57.8±2.0	18.77±0.98	14.22±0.93	24.78±0.22
	4	0.74	250.1±15.7	38.8±0.9	77.9±5.8	59.5±0.7	20.62±1.03	14.91±0.85	23.96±0.03
	5	ND	273.3±5.6	46.0±1.4	102.4±19.4	62.5±4.3	20.83±2.55	15.62±0.94	26.03±1.02
		Average:	**256.5±12.6**	**40.5±3.4**	**66.7±14.0**	**61.5±1.5**	**20.4±0.6**	**15.7±0.7**	**25.4±0.4**

4.3.1.1. April. Chlorophyll *a* concentrations were low, ranging between 0.68 and 0.86 μg l^{-1}. The majority of chlorophyll-containing particles were <5 μm (R.D. Davidson, unpublished MarProd data). Nitrate, silicate and phosphate concentrations were all high (Table 3.2), suggesting that the annual diatom bloom had yet to occur. Total particulate organic carbon (POC) ranged between 119.8 and 197.8 μg l^{-1}, with the POC:PON mass ratios ranging from 7.3:1 to 11.2:1 (average 9.0:1).

4.3.1.2. July/August. Chlorophyll *a* concentrations were more variable (0.72 to 1.13 μg l^{-1}) than in April. Size-fractionated analysis of chlorophyll *a* showed that, again, most of the pigment was associated with particles <5μm (D. Wilson, unpublished MarProd data). Nutrient concentrations had decreased to approximately one third of April values (Table 3.2), revealing a substantial draw down during the interim period. This suggests that the experiments in July/August were conducted during post-bloom conditions. Concentrations of total POC were 114 to 247 % of values found in April, ranging from 226.3 to 296.0 μg l^{-1}. These levels were significantly higher than observed in April (ANOVA, $n = 10$, $p < 0.001$). The POC:PON ratios were also lower than in April (range: 5.9:1 to 7.4:1, average 6.4:1).

4.3.2 Microplankton community composition and biomass. The characteristics of each cell-group enumerated in April and July/August are presented in Table 4.2. Substantial quantities of marine snow were commonly observed amongst the settled microplankton, particularly in the samples collected in April, where amorphous particles were often more abundant than viable cells. However, because of the nature of this material, no quantitative measurements were possible. Daily microplankton community compositions and the biomass of individual cell groups enumerated in April are shown in Figures 4.1 and 4.2 respectively. The corresponding data for July/August are presented in Figures 4.3 and 4.4.

4.3.2.1. April. The microplankton community was dominated by flagellates < 10 μm equivalent spherical diameter (ESD) on all days. The total C biomass of these flagellates varied between 5.5 and 24 μg C l^{-1}, representing from 54 to 81 % of the total biomass present. Cryptomonads (Cryptophyceae) accounted for between 39 and 58 % of the flagellate <10 ESD biomass (2.9 to 12.9 μg C l^{-1}), and up to 44 % of the total community biomass. Ciliates (1.3 to 3.2 μg C l^{-1}) and dinoflagellates (1.1 to 2.9

Table 4.2. Cell characteristics for the groups enumerated from samples collected above the Reykjanes Ridge in April and July/August 2002. Equivalent spherical diameter (ESD), not present (NP). *Rhizosolena* sp., tintinnids and radiolarians were also infrequently observed.

	APRIL			JULY/AUGUST		
Cell-Group	Cell Volume (μm^3)	ESD (μm)	Cell carbon (pg C $cell^{-1}$)	Cell Volume (μm^3)	ESD (μm)	Cell carbon (pg C $cell^{-1}$)
Thecate dinoflagellate	3252	17	316.6	5782	22	562.7
Naked dinoflagellate	3419	19	449.1	2471	17	331.1
Nitzschia	86	5	10.7	125	6	14.4
Pennate diatom	NP	NP	NP	8342	25	435.7
Silicoflagellates	11290	28	1378.7	NP	NP	NP
Centric diatom	17758	32	804.0	21753	35	947.8
Ciliates	3799	19	722.0	5705	22	1084.0
Flagellate (< 3.5 μm)	6	2	1.1	6	2	1.1
Flagellate (>3.5 μm)	90	6	14.8	87	5	14.3
Cryptomonad	260	8	40.0	453	10	67.3

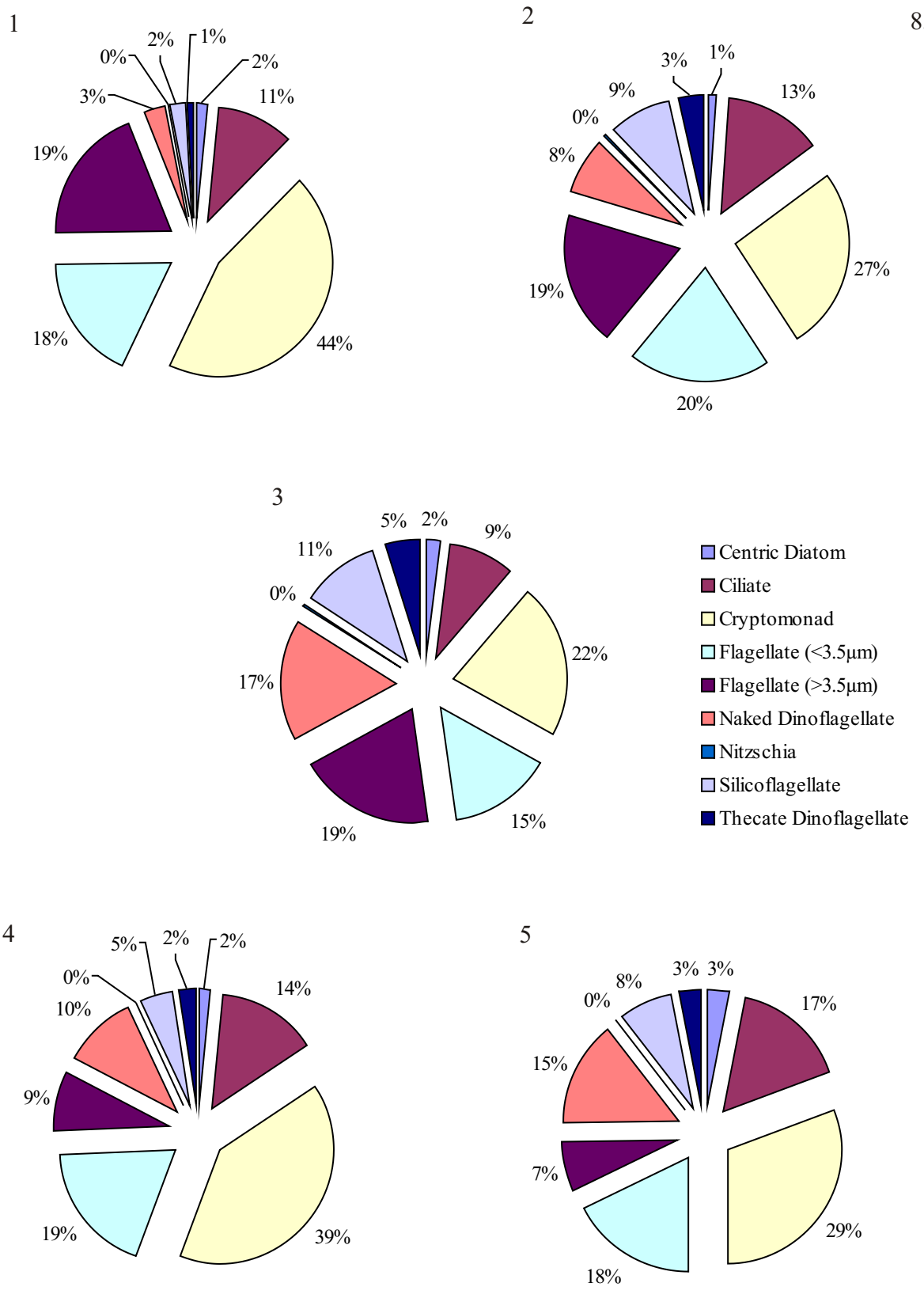


Figure 4.1 . Average contribution (%) of individual cell groups to the total cellular carbon present at the water stations sampled in April. Station number shown at top left hand side of each individual pie

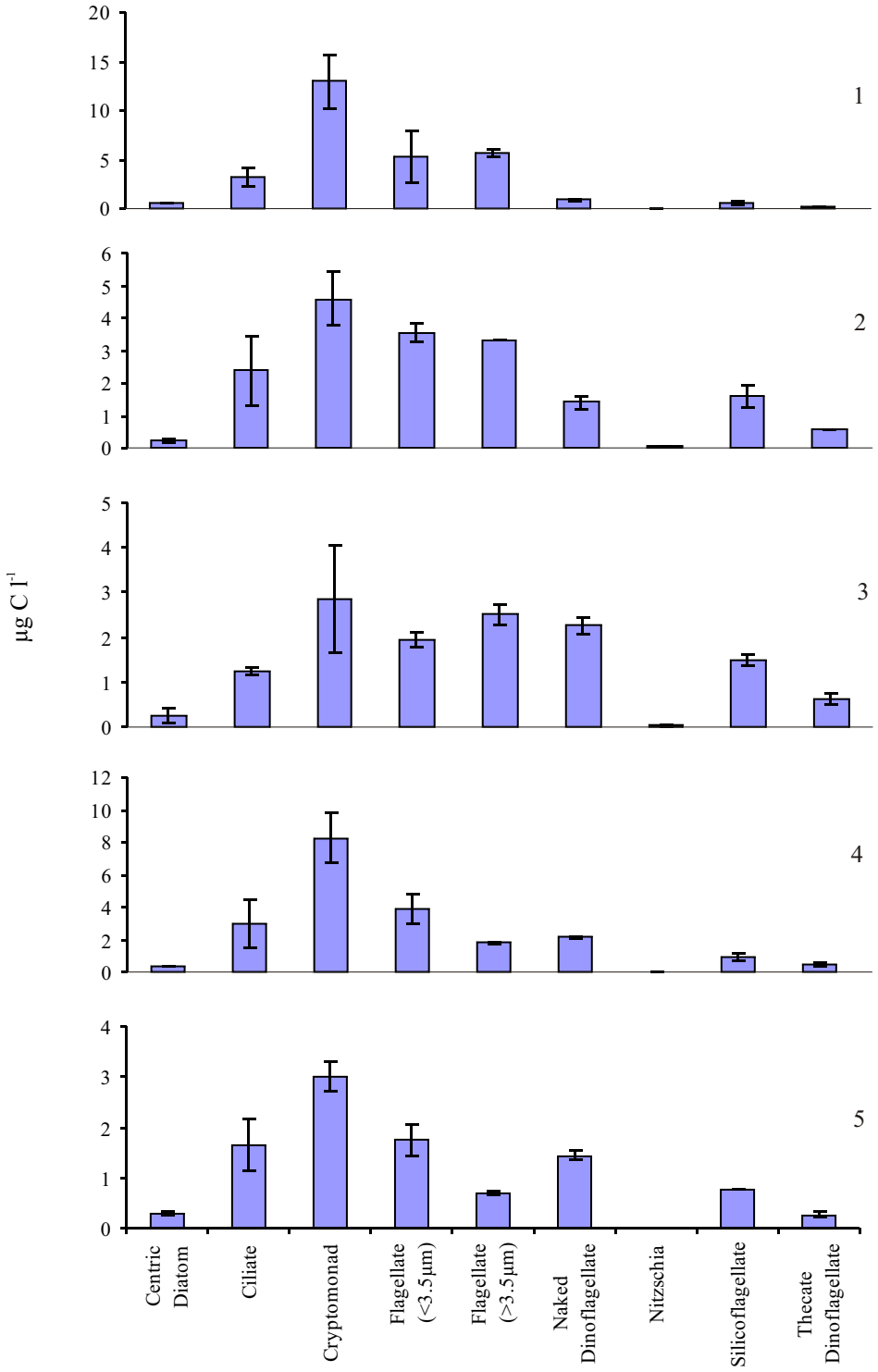


Figure 4.2 . Biomass of individual microplankton groups present at the water stations sampled in April ±SE. Note variable scale on Y-axis. Station number is shown to right hand side of each individual chart.

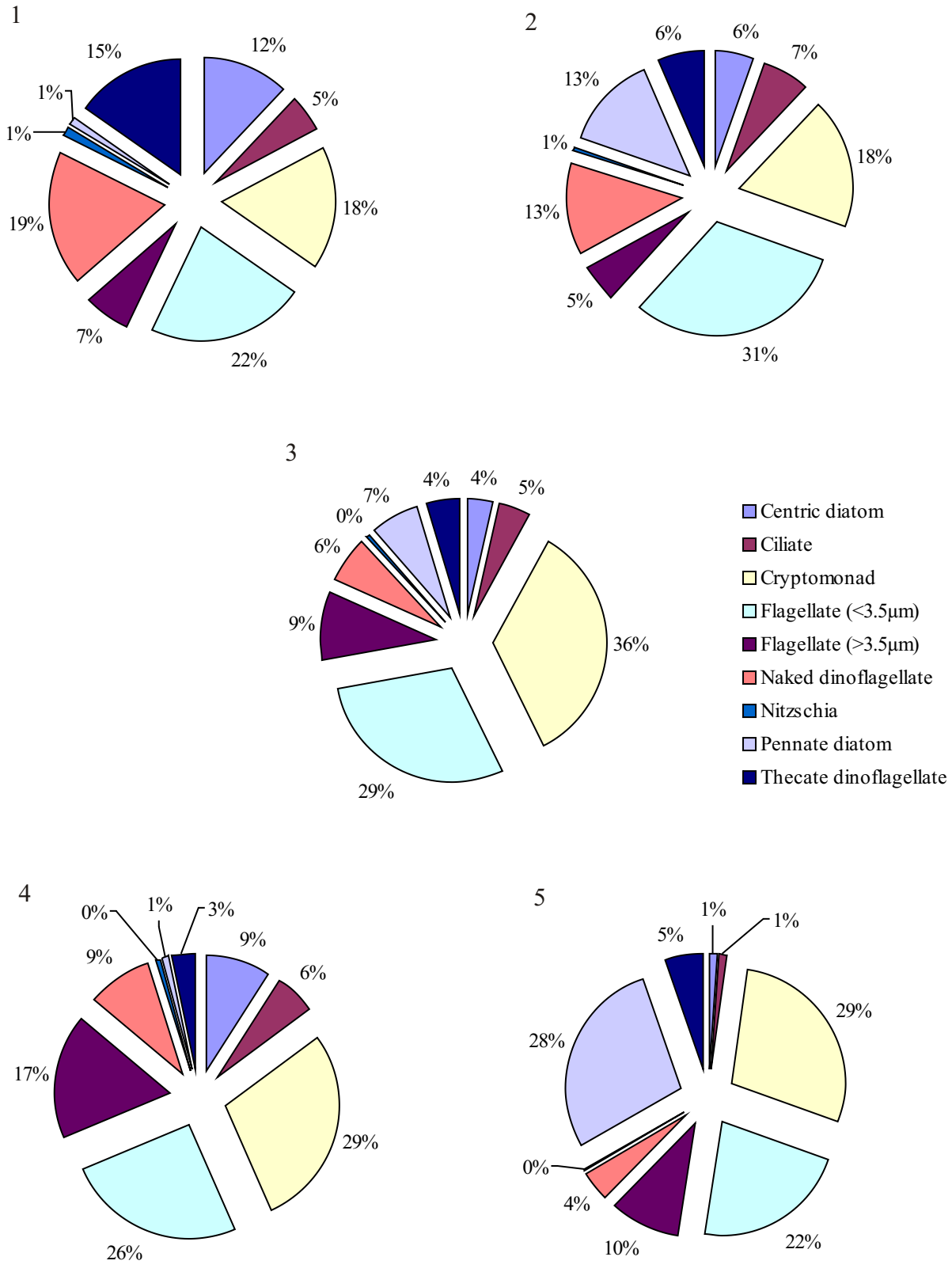


Figure 4.3. Average contribution (%) of individual cell groups to the total cellular carbon present at water stations sampled in July/August. Station number shown at top left hand side of each individual pie.

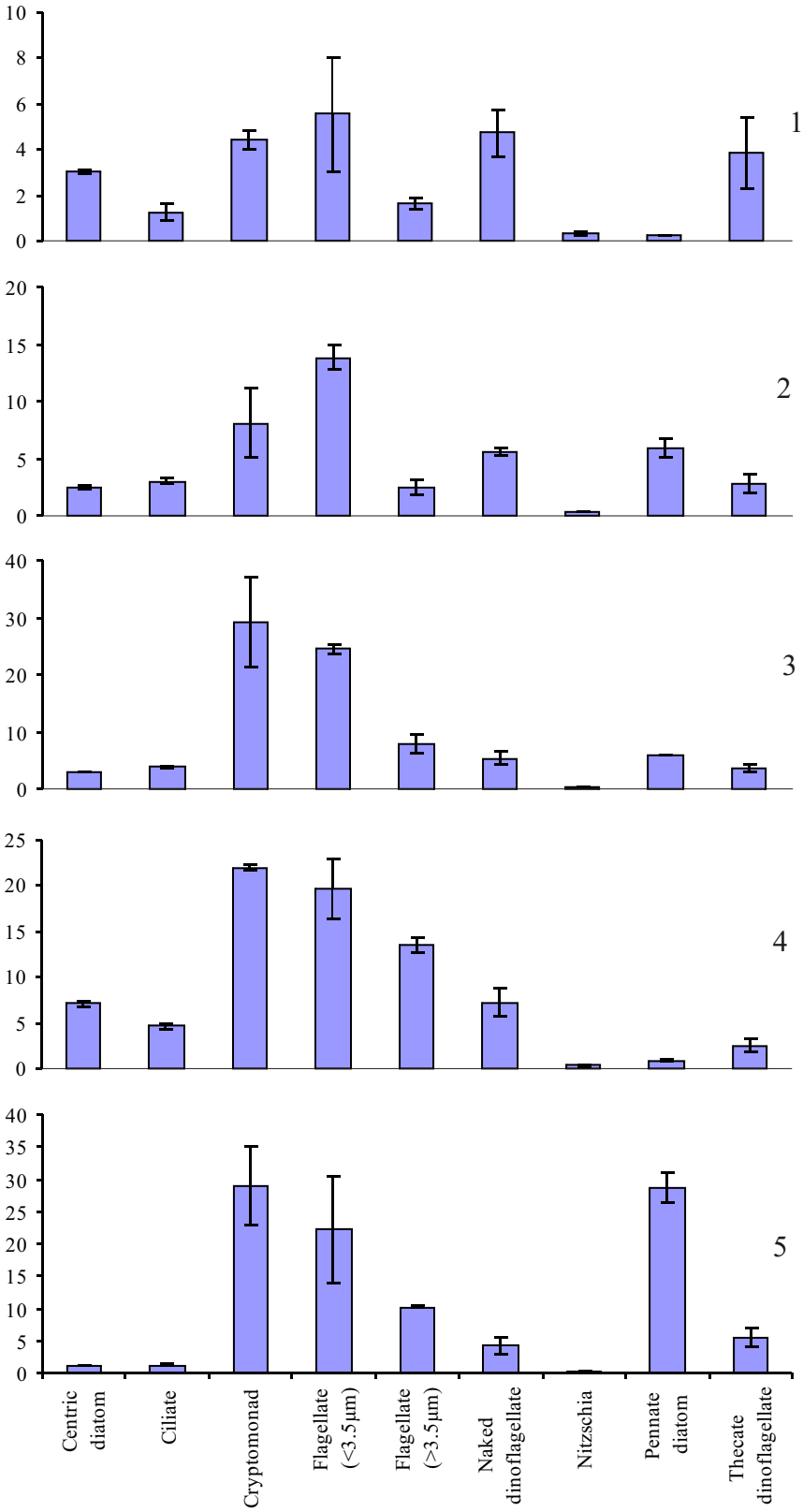


Figure 4.4. Biomass of individual microplankton groups present at the water stations sampled in July/August ±SE. Note variable scale on Y-axis. Station number is shown to right hand side of each individual chart.

µg C l^{-1}), thus the microzooplankton (see Appendix 2), were also an important component of the microplankton, typically comprising > 20 % of the total daily community biomass. In contrast, diatoms were only present in low numbers (0.3 to 0.5 µg C l^{-1}), contributing < 3 % daily to the total community biomass. Estimations of total organic C derived from inverted microscopy cell counts were low at each station, ranging from 9.9 to 29.3 µg C l^{-1} (average = 18.2 µg C l^{-1}, Table 4.1). This accounted for between 8 to 17 % (average 12 %) of the total particulate organic carbon (POC), as determined by elemental analysis (Table 4.1). Because biomass estimations were only based on two replicate cell-counts, inter-comparison between stations was not possible.

4.3.2.2. July/August. The microplankton community in July/August was also dominated by flagellates < 10 µm ESD. The C biomass of small (< 3.5 µm ESD) flagellates, 5.5 to 24.6 µg C l^{-1}, was similar to that of the cryptomonads (4.4 to 29.1 µg C l^{-1}), comprising 22 to 31 % and 18 to 36 % of the total community biomass respectively. Ciliates and dinoflagellates were an important component of the microplankton, together representing between 10 and 39 % (9.9 to 14.5µg C l^{-1}) of the total biomass present. Diatoms were more abundant than in April, representing between 10 and 30 % of the community C biomass (3.6 to 30.1 µg C l^{-1}). A pennate diatom, tentatively identified as *Tropodineis* sp., was particularly abundant on days 2 and 5, reaching up to 28.8 µg C l^{-1}. Community biomass estimates based on cell counts varied dramatically between stations, ranging from 25.1 to 102 µg C l^{-1}. This accounted for between 8 to 37 % (average 26 %) of the total particulate organic carbon (POC), as determined by elemental analysis (Table 4.1). Despite the variability in total cellular biomass between the stations, the average biomass of the microplankton community in July/August was significantly greater than in April (ANOVA, n = 10, p = 0.01).

4.3.3. Quantitative comparison of the microplankton assemblages observed in April and July/August: Multivariate approach. On average, the daily community species-assemblages were 68 % similar in April and 62 % similar in July/August (SIMPER analysis). Significant inter-seasonal differences were found (ANOSIM, r = 0.769, p < 0.001), differing by an average of 61 %. Differences in the biomass of small flagellates (< 3.5 µm), cryptomonads and pennate diatoms in April

and July/August were the major factors forcing the seasonal differences. The multi-dimension scaling (MDS) ordination of the Bray-Curtis similarity coefficients clearly illustrates these differences, showing distinct clusters for the April and July/August microplankton communities (Figure 4.5).

4.3.4. Particulate fatty acid composition and quantity. The total fatty acid composition of the April particulate samples, expressed as mol %, is shown in Figure 4.6. Because an internal standard (21:0) was added to the samples prior to processing, data for each moiety can also be expressed as absolute mass (μg l^{-1}; Figure 4.7). The corresponding data for July/August are presented in Figures 4.8 and 4.9 respectively (See Appendix 3).

4.3.4.1. April. The total quantities of fatty acids in the samples collected in April were low, ranging between 7.9 and 23.7 μg l^{-1} (Table 4.1). POC:cell biomass ratios (μg $μg^{-1}$) were high, on average 9:1 (Table 4.3). This suggests that detritus was a significant component of the seston in April. Saturated fatty acids, in particular 14:0, 16:0 and 18:0 dominated each day, together accounting for between 38 and 60 % of the total fatty acid composition (mol %). Another dominant moiety was the flagellate biomarker, 18:1(n-9) (Table 1.1), which contributed a similar percentage (10 to 16 %) to the overall composition as the dominant saturated fatty acids. The presence of large quantities of flagellates was confirmed by the low 16:1(n-7)/16:0 ratio (range 0.07 to 0.3) and the relative dominance of C18 fatty acids (42 % on average). The large quantities of 18:0 is consistent with the observation of substantial amounts of detritus. In contrast, polyunsaturated fatty acids (PUFA) were relatively scarce, together constituting between 12 and 25 % of the total fatty acid content (1.6 to 6 μg l^{-1}). This was also reflected by the high POC:PUFA ratios (average 82:1, Table 4.3). EPA and DHA were only present in small quantities (Figure 4.7), together contributing < 8 % to the total composition (average 4.8 %). The diatom biomarker, 16:1(n-7), was also only present in small amounts, and did not exceed > 5 % of the total fatty acid composition (Figure 4.6). This indicates that diatoms were only present in small numbers in April, as suggested by the microplankton compositional data and the low 16:1(n-7)/16:0 ratio.

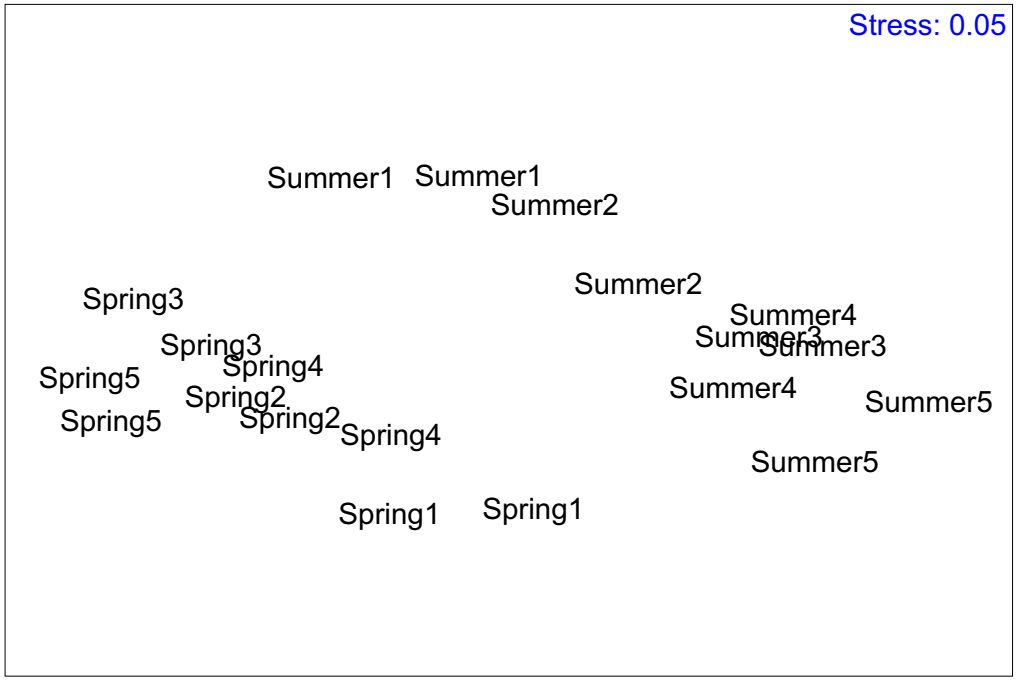


Figure 4.5. 2-dimensional non-metric multi-dimensional scaling (MDS) ordination of un-transformed microplankton community species content (µg C l^{-1}) data from April (spring) and July/August (summer). n = 2 for each station.

1-way ANOSIM, $p < 0.001$.

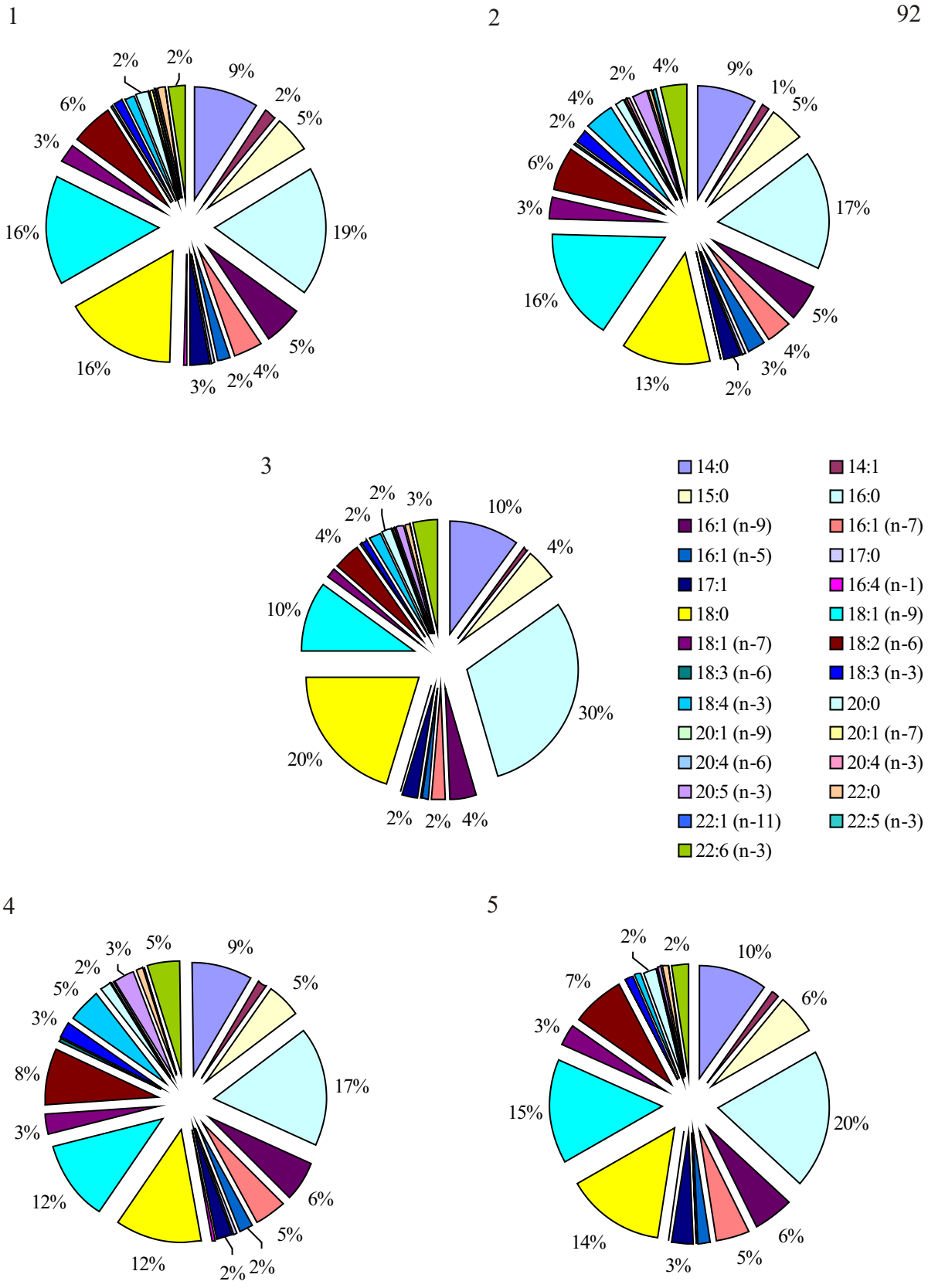


Figure 4.6. Fatty acid composition (mol%) of the particulate environment at the beginning of each experimental day in April. Station number shown at top left hand side of each individual pie.

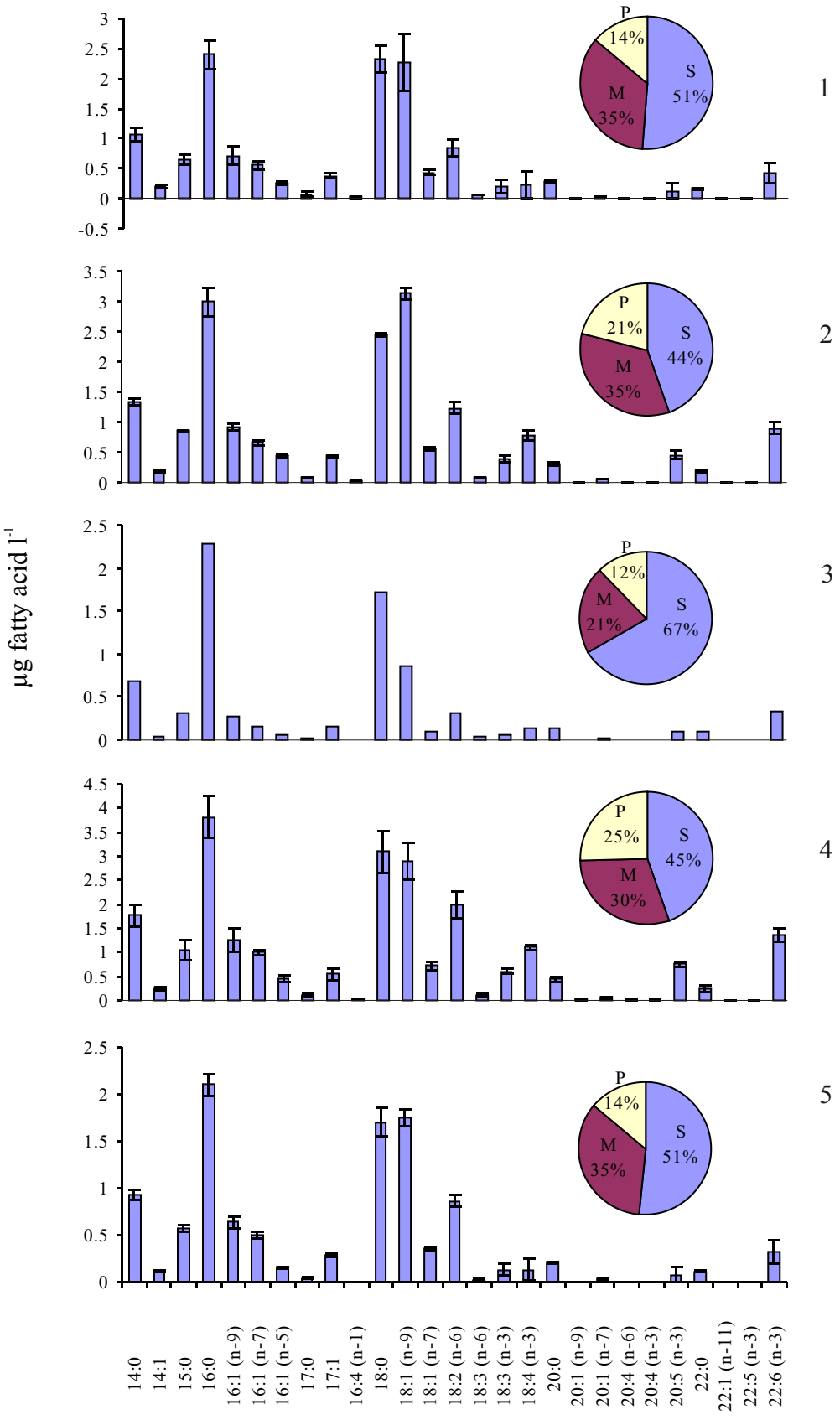


Figure 4.7 . Fatty acid content ±SE of the particulate environment at water stations sampled in April. Note variable scale on Y-axis. Inset pie shows relative contributions (weight %) of saturated (S), monounsaturated (M) and polyunsaturated (P) fatty acids. Station number is shown to right hand side of each individual chart.

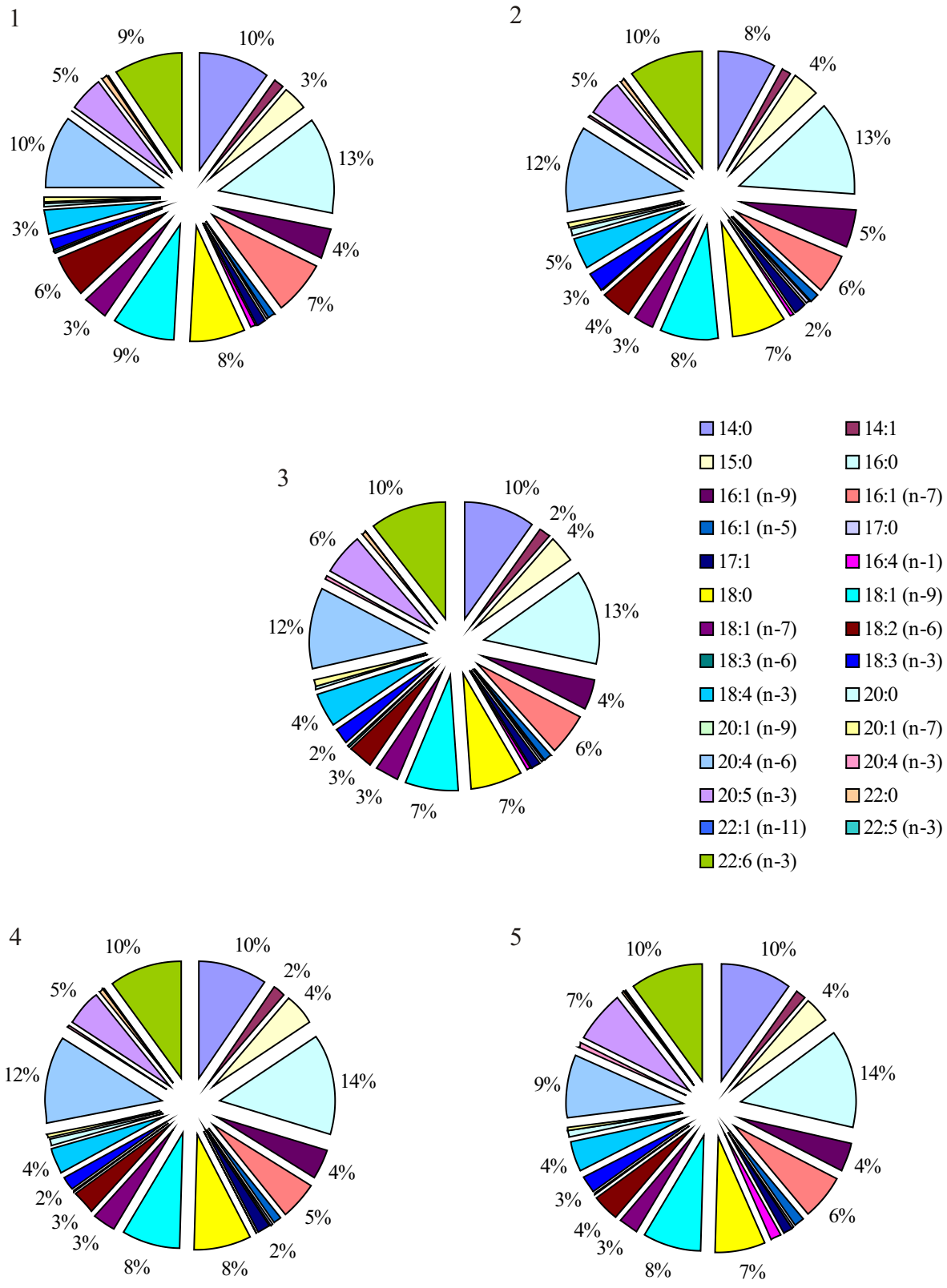


Figure 4.8. Fatty acid composition (mol%) of the particulate environment at each station in July/August. Station number shown at top left hand side of each individual pie.

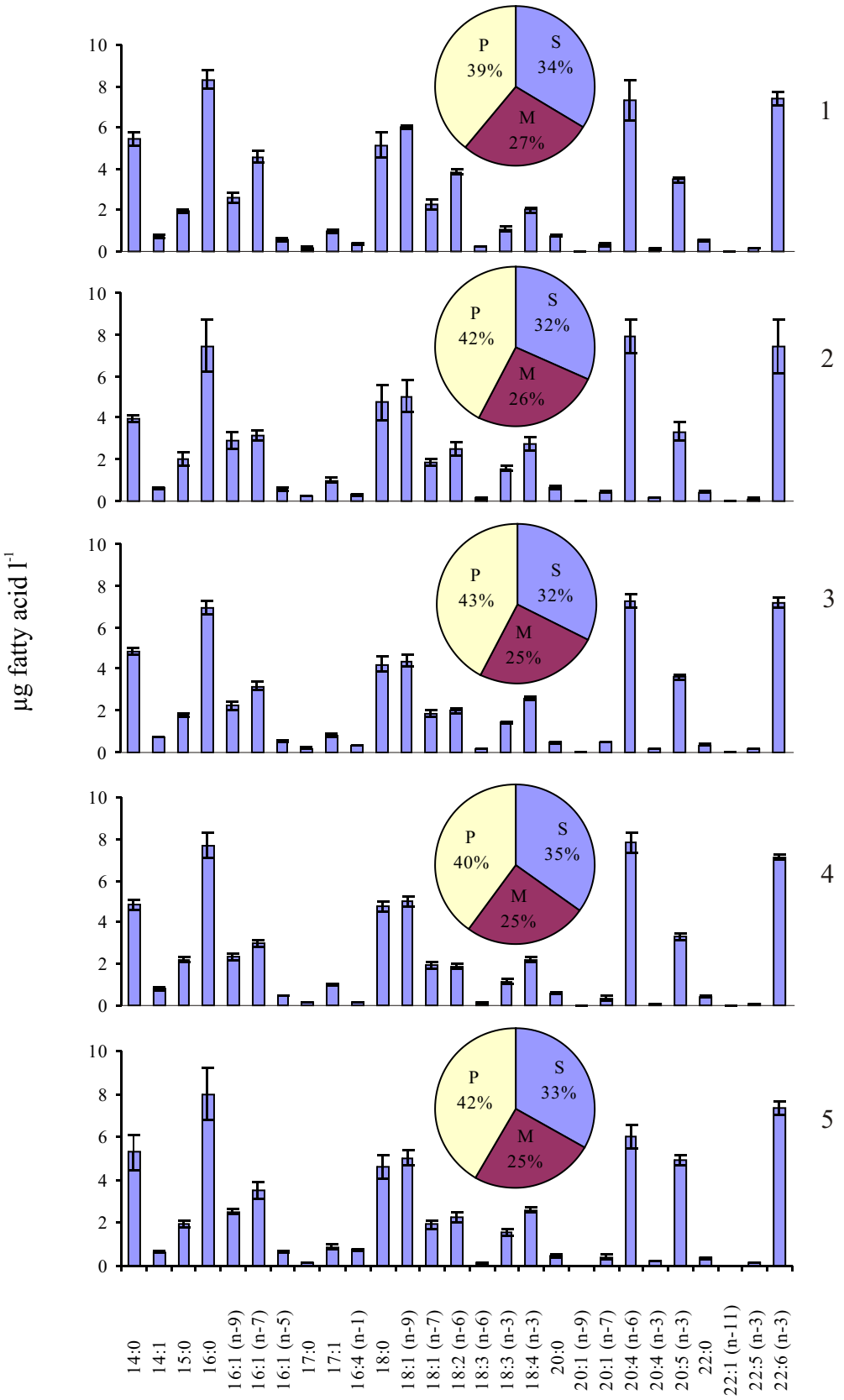


Figure 4.9. Fatty acid content ±SE of the particulate environment at water stations sampled in July/August. Inset pie shows relative contributions (weight %)of saturated (S), monounsaturated (M) and polyunsaturated (P) fatty acids. Station number is shown to right hand side of each individual chart.

Table 4.3. Specific cell biomass:fatty acid ratios (μg l^{-1}) in April and July/August (±SE).

	Stn.	POC:PON	POC:cell biomass	POC:PUFA	Cell biomass:PUFA
APRIL	1	8.1	6.8	103.0	15.3
	2	9.1	7.7	35.1	4.6
	3	11.2	12.7	172.4	13.6
	4	7.3	6.0	20.9	3.5
	5	9.1	12.1	76.7	6.4
	Average:	**9.0** ± 0.7	**9.1** ± 1.4	**81.6** ± 27.0	**8.7** ± 2.4
JULY/AUGUST	1	5.9	11.8	11.4	1.0
	2	6.4	5.3	9.1	1.7
	3	7.4	2.7	9.1	3.4
	4	6.5	3.2	10.4	3.3
	5	5.9	2.7	10.5	3.9
	Average:	**6.4** ± 0.3	**5.1** ± 1.7	**10.1** ± 0.4	**2.7** ± 0.6

4.3.4.2. July/August. The total fatty acid content of the particulate environment had increased significantly by July/August (ANOVA, n = 10, $p < 0.001$), and now ranged between 57.8 and 66.4 μg l^{-1} (Table 4.1). This was reflected in the much lower POC:cell biomass ratios, which had an average of 5:1 (Table 4.3). The overall composition of the fatty acids was far more evenly distributed between the identified fatty acids, with approximately 33, 25 and 42 % of the total mass being attributable to saturated, monounsatured and polyunsaturated fatty acids respectively (Figure 4.9). C18 fatty acids remained important, representing approximately 30 % of the total fatty acids. The detrital biomarker, 18:0, was also present in appreciable quantities (4.2 to 5.2 μg l^{-1}), yet comprised only 7 to 8 % of the total composition, supporting the observation that although absolute quantities were greater in July/August, the relative abundance of detritus was greater in April. The total mass of PUFAs ranged between 24.0 and 26.1 μg l^{-1}, with DHA contributing between 7.1 and 7.4 μg l^{-1} (9 to 10 %). Average quantities of EPA and DHA in the particulate samples from July/August were both significantly greater, relative to April (ANOVA, n = 10, $p < 0.001$ in both cases). This was reflected by a significantly lower POC:PUFA ratio (average 10:1; ANOVA, n = 10, $p < 0.05$). The relative contribution of 16:1(n-7) to the fatty acid composition was also significantly greater in July/August (ANOVA, n = 10, $p < 0.01$), indicating that diatoms were more prominent in the microplankton relative to April. However, the 16:1(n-7)/16:0 ratio remained low (0.4 to 0.6), supporting the observation that flagellates again dominated the microplankton community.

4.3.5. Quantitative comparison of individual fatty acids in the particulates between April and July/August: Multivariate approach. The SIMPER analysis indicates that within each season, the quantities of individual fatty acids (μg l^{-1}) were highly similar (77 and 90 % similar in April and July/August respectively). April and July/August fatty acid compositions were significantly different (ANOSIM, $r = 0.981$, $p < 0.001$), as illustrated by the MDS ordination (based on Bray-Curtis similarity coefficients; Figure 4.10). Seasonal dissimilarities (59 %) were primarily driven by the PUFAs 20:4(n-6) and 22:6(n-3), and to a lesser extent, the saturated fatty acids 16:0 and 14:0.

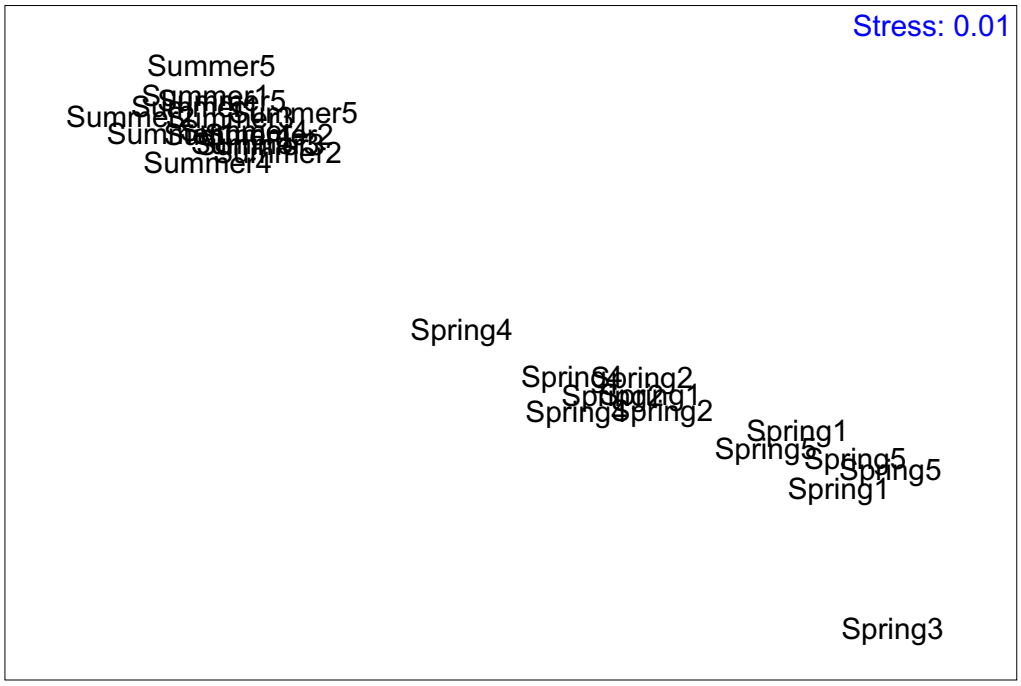


Figure 4.10. 2-dimensional non-metric multi-dimensional scaling (MDS) ordination of un-transformed particulate fatty acid composition (µg l^{-1}) data from spring (April) and summer (July/August). n = 3 for each station.
1-way ANOSIM, $p < 0.001$.

4.3.6. Correlation analyses. Correlation analysis was used to examine the relationships between the biological and biochemical characteristics of the seston.

4.3.6.1. Particulate descriptors. To obtain a sample size large enough to enable a meaningful statistical comparison, the data from April and July/August were pooled (n = 10). Levels of POC showed a significant positive correlation with all variables excluding chlorophyll *a* (Table 4.4). Phytoplankton exude a large fraction of photosynthetically-fixed C, subsequently promoting bacterial growth (Larsson and Hagstrom 1979). These positive correlations may reflect that increases in the microplankton biomass are followed by a concurrent increase in the bacterial or detrital loading of the water column. Total fatty acid concentrations were positively correlated with saturated, monounsaturated and polyunsaturated fatty acids. Cellular C values derived from inverted microscopy were also significantly correlated with all fatty acid groups and POC. Importantly, the relationship between cell C and the PUFA content of the seston was particularly significant ($p < 0.01$), indicating that PUFAs are indeed closely associated with viable cells (Hama 1991, 1999). Chlorophyll *a* did not correlate with any of the variables investigated.

4.3.6.2. Microplankton species assemblage and fatty acid biomarkers. Concurrent data on the fatty acid composition of the seston and microplankton biomass estimations are scarce. Both of these data sets were collected in this study, and it was therefore possible to evaluate the use of individual fatty acids as indicators of specific algal classes. The relationships between the quantities of individual fatty acids (μg l^{-1}) and individual cell-groups (μg l^{-1}) were assessed using linear correlation analysis by combining the seasonal data sets. The resulting correlation coefficients are shown in Table 4.5.

Correlation between the biomass of diatoms and the 16:1(n-7)/16:0 ratio was positive and highly significant ($p < 0.001$). Similarly, the quantities of 16:1(n-7) was also positively correlated with diatom biomass ($p < 0.01$). These two significant relationships demonstrate that fatty acid biomarkers can provide qualitative information about the overall contribution of diatoms to the microplankton biomass. Similarly, the biomasses of dinoflagellates and small flagellates (< 3.5 μm ESD) were both significantly correlated with the quantity of C18 biomarker fatty acids,

Table 4.4. Correlation analysis of untransformed particulate variables. Spearman rank order correlation coefficients are shown. Data were pooled from April and July/August (n = 10, except for Chl. *a* where n = 9). $^{*}p < 0.05$. $^{**}p < 0.01$, $^{***}p < 0.001$. See Table 4.1 for explanation of variable abbreviations.

	POC	PON	Cell C	Total FA	SFA	MSFA	PUFA
Chl. *a*	0.276	0.218	-0.276	0.377	0.301	0.377	0.335
POC		**0.960*****	**0.733***	**0.842*****	**0.855*****	**0.833****	**0.745***
PON			**0.778****	**0.924*****	**0.936*****	**0.915*****	**0.872*****
Cell C				**0.679***	**0.709***	**0.681***	**0.758****
Total FA					**0.988*****	**0.997*****	**0. 939*****
SFA						**0.979*****	**0.981*****
MSFA							**0.948*****

Table 4.5. Linear correlation (Spearman rank) analysis of absolute quantities of individual fatty acids and phytoplankton cells (µg l^{-1}), as determined from inverted microscopy cell counts. Data were pooled from April and July/August (n = 10). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

	16:1 (n-7)/ 16:0	16:1 (n-7)	18:1(n-7)/ 18:1(n-9)	16:4(n-1)	C18	18:2(n-6)	18:3(n-3)	18:4(n-3)	18:1(n-9)	20:5(n-3)	22:6(n-3)
Diatoms	**0.83*****	**0.806****	**0.879*****	**0.842*****	**0.758****	**0.661***	**0.927*****	**0.879*****	**0.733***	**0.879*****	**0.782****
Dinoflagellates	**0.673***	**0.685***	**0.806****	**0.77****	**0.673***	0.539	**0.745***	**0.745***	**0.661***	**0.782****	**0.673***
Total flagellates	**0.624***	**0.624***	**0.806****	**0.733***	0.576	0.442	**0.842*****	**0.83*****	0.552	**0.806****	**0.648***
Flagellates (< 3.5 µm)	**0.77****	**0.77****	**0.879*****	**0.855*****	**0.721***	0.576	**0.891*****	**0.879*****	**0.709***	**0.903*****	**0.77****
Flagellates (> 3.5 µm)	0.0788	0.164	0.418	0.345	0.127	-0.115	0.442	0.43	0.164	0.418	0.176
Cryptomonad	0.491	0.467	**0.733***	0.588	0.394	0.285	**0.709***	**0.648***	0.37	**0.673***	0.43
Flagellates exc. cryptomonads	0.576	**0.648***	**0.77****	**0.721***	**0.648***	0.418	**0.818****	**0.806****	**0.661***	**0.758****	**0.661***
Ciliates	0.0909	-0.00606	0.37	-0.0303	0.0788	-0.0667	0.176	0.224	0.0667	0.0424	0.0909
16:1 (n-7)										**0.927*****	
18:4(n-3)											**0.927*****

particularly 18:3(n-3) and 18:4(n-4) ($p < 0.05$ in both cases for dinoflagellates and $p < 0.001$ in both cases for small flagellates). These data confirm the usefulness of C18 fatty acids as flagellate biomarkers. Highly significant correlations were also found between the biomass of various flagellate groups (including dinoflagellates) and EPA. Ciliate biomass was not significantly correlated with any of the variables investigated, reflecting their highly variable fatty acid composition (Harvey et al. 1997).

4.4. DISCUSSION

4.4.1. Environmental data. Nutrient, chlorophyll and microplankton species composition data provide complementary evidence to support the notion that the spring bloom had not occurred by the time of the experiments in April (i.e. 'pre-bloom' conditions). SeaWiFS satellite observations (Peter Miller, PML, RSG) confirm that chlorophyll levels in the region were low in April (Figure 4.11). The corresponding environmental data from the July/August suggest that the spring bloom occurred during the intervening period, thus 'post-bloom' conditions refer to those under which the July/August experiments were conducted. Indeed, the satellite data illustrates that chlorophyll levels were at their highest in June (Figure 4.11).

4.4.2. Particulate descriptors. Absolute quantities of POC in April (range 120 to 198 μg l^{-1}) and July/August (range 226 to 296 μg l^{-1} respectively) closely reflect values previously reported in North Atlantic waters under similar conditions (120 to 230 μg l^{-1}; Weeks et al. 1993, Irigoien et al. 2000b). Similarly, the mass-specific C:N ratios presented here (9.0 and 6.4; Table 4.3) also agree well with pre- and post-bloom values reported in the Norwegian sea (~9 and 5 respectively; Irigoien et al. 1998). The quantities of total fatty acids (TFA) in the particulates reported here (average 15 and 62 μg l^{-1} in April and July/August respectively) show good agreement with previously determined values in North Atlantic waters outside bloom conditions (18 to 65 μg l^{-1}; Klungsoyr et al. 1989), and were generally lower than values reported during a spring bloom off West Greenland (30 to 132 μg l^{-1}; Reuss and Poulsen 2002). The difference in the quantities of TFA in April and July/August can be explained in part by the different densities of microplankton encountered in the two seasons. This is reflected by a significant positive correlation ($p < 0.05$) between the total cell biomass and quantities of TFA (Table 4.4). Significant correlations between these variables have previously been reported during spring bloom, diatom domination and post bloom conditions in the waters west of Greenland, (Reuss and Poulsen 2002). The seasonal differences may also reflect environmental factors, since light, temperature and nutrient availability, and the physiological stage of the algae are all known to cause the fatty acid composition of phytoplankton to vary (Ackman et al. 1968, Chuecas and Riley 1969, Dunstan et al. 1993).

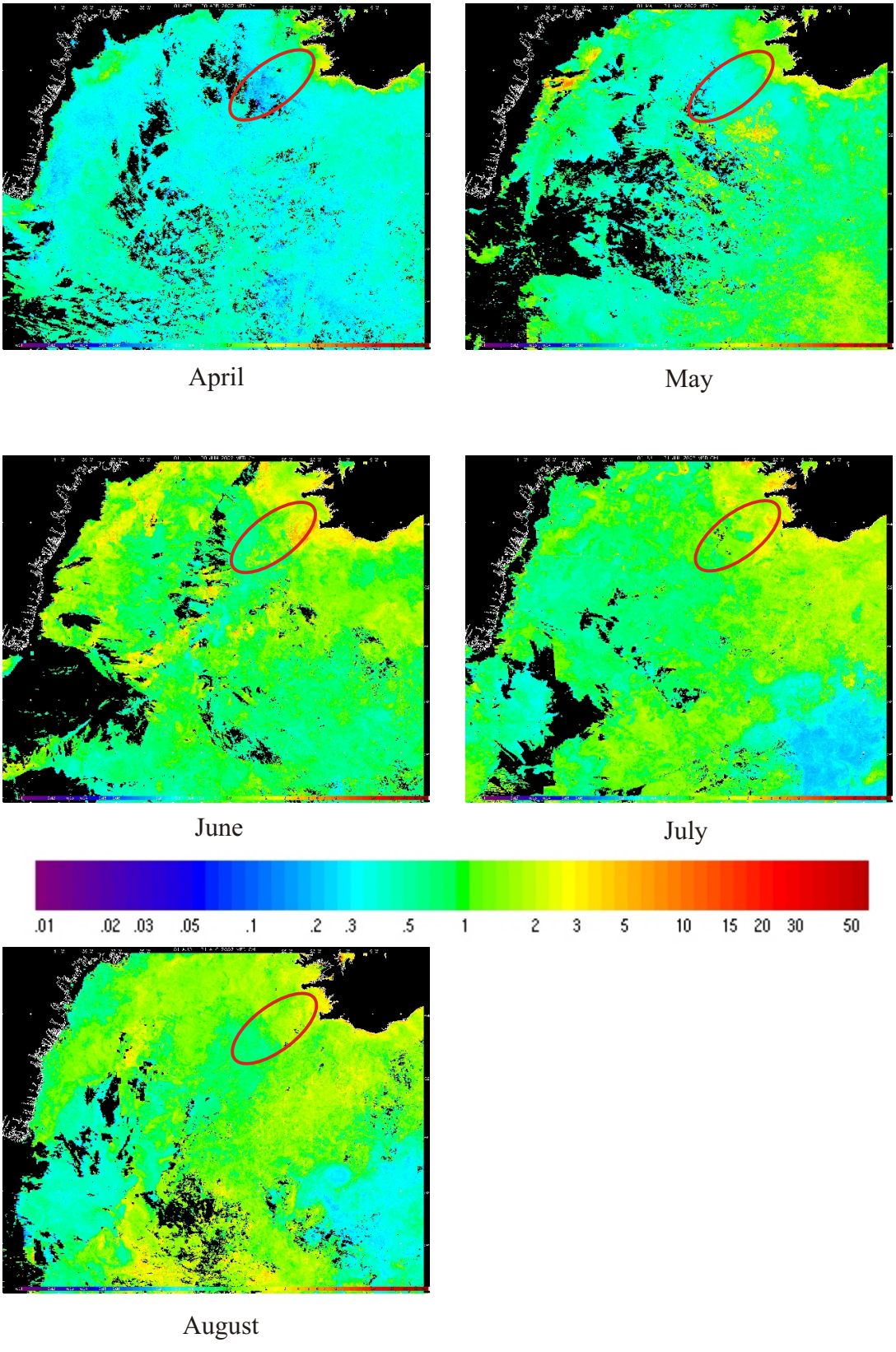


Figure 4.11. Monthly seaWIFS images of the study area (red elipse). Satellite images were received by the NERC Dundee Satellite Receiving Station and processed by Peter Miller at the Plymouth Marine Laboratory Remote Sensing Group (www.npm.ac.uk/rsdas/). SeaWiFS data courtesy of the NASA SeaWiFS project and Orbital Sciences Corporation. Scale ranges from 0.01 to 50 mg C m^2

4.4.3. Microplankton community composition. The quantities of microplankton and the community assemblages described here for April and July/August relate well to those previously observed in North Atlantic waters, where the majority of the community biomass outside of bloom conditions is characteristically represented by cells <10µm ESD (Huntley 1981, Murphy and Haugen 1985, Li and Wood 1988, Sieracki et al. 1993, Verity et al. 1993a, b, Stoecker et al. 1994, Gifford et al. 1995, Irigoien et al, 1998, Hansen et al. 1999, Meyer-Harms et al. 1999, Levinsen et al. 2000b, Irigoien et al. 2003). Huntley (1981) found that cells 5 to 10 µm typically dominated community biomass in the Labrador Sea in April and May. Elsewhere in the North Atlantic, Verity et al. (1993a) reported that in 1989 the spring phytoplankton community was dominated by prymnesiophytes <10µm ESD, with similar sized or smaller athecate heterotrophic dinoflagellates being abundant the following year. The observation that both pre- and post-bloom microplankton assemblages were dominated by flagellates was supported by the fatty acid compositions of the particulate samples. Both seasons were characterised by a low 16:1(n-7)/16:0 ratio, indicative of flagellate dominated systems (Claustre et al. 1990, Nichols et al. 1991, Viso and Marty 1993). Furthermore, flagellate biomarkers, particularly 18:1(n-9), were also important components of the April and July/August fatty acid profiles.

Microzooplankton, in particular heterotrophic dinoflagellates and ciliates, also contributed significantly to the microplankton biomass in both seasons, particularly in July/August. Ciliates and dinoflagellates are known to contribute significantly to spring plankton assemblages found in the North Atlantic (Verity et al. 1993a, b, Stoecker et al. 1994). The numerical abundance of heterotrophic protists in summer is reported to be approximately double that of spring (Gifford et al. 1995), which agrees well with the observation that microzooplankton were more prominent in the samples collected in July/August. Unfortunately, the fatty acid profiles of dinoflagellates are very similar to those of other flagellates (Viso and Marty 1993), making it difficult to substantiate the cell-counts of this group with that from fatty acid analyses. Similarly, because the fatty acid composition of ciliates is known to vary depending on their diet (Harvey et al. 1997) and environmental conditions (Sul and Erwin 1998), it is difficult to assess their abundance based on fatty acid profiles alone.

Despite high nutrient concentrations in April, diatoms constituted only a minor proportion (< 3 %) of the total C biomass. Small pennate diatoms (3 μm) have previously been found to be the most abundant phytoplankton in North Atlantic waters during spring (Verity et al, 1993a). It is possible that such small cells were present in the current samples, and over looked as a result of the insufficient resolution of inverted microscopy. However, the paucity of 16:1(n-7), the diatom fatty acid biomarker (see Table 1.1), confirms that diatoms were only a very minor component of the microplankton community. Additionally, the ratio of 16:1(n-7)/16:0 ratio in diatoms is typically > 1 (Nichols et al. 1986, Volkman et al. 1989, Viso and Marty 1993), yet values in April remained around 0.2. By July/August, on average diatoms only represented 17 % of the microplankton biomass and their biomarkers accounted for only 12 % of the total fatty acids. Additionally, the 16:1(n-7)/16:0 ratio was again, well below 1. Nitrate levels (Table 3.2) remained above, but were always close to, typical half-saturation constants (Eppley et al. 1969, Lomas and Gilbert 2000).

The microplankton communities in April and July/August were typically comprised of the same groups of phyto- and microzooplankton. However, seasonal variation in microplankton abundance, particularly of the flagellated groups, caused the two communities to differ significantly. Similarly, the fatty acid composition of the seston in April and July/August were also significantly different. DHA, one of the primary fatty acids forcing these differences, is known to predominate in the fatty acid signatures of certain flagellates (Table 1.1). This supports the observation that different flagellate groups dominated in April and July/August.

4.4.4. Availability of microplankton to adult *C. finmarchicus*. All of the cells enumerated in April and July/August were relatively small (< 40 μm ESD; Table 4.2), and the microplankton biomass was dominated (> 50 %) by cells < 10 μm ESD in both seasons. However, numerous studies have reported that *Calanus* spp. are capable of grazing cells < 10 μm at rates comparable to those reported for larger cells (Huntley 1981, Tande and Bamstedt 1987, Nejstgaard et al. 1997, Turner et al. 2002). Furthermore, cells < 10 μm can dominate the diet (Nejstgaard et al. 1997, Levinsen et al. 2000b) and provide sufficient nutrition to enable optimal reproductive output (Bamstedt et al. 1999). It is therefore concluded that all the cells enumerated using inverted microscopy are available to *C. finmarchicus* as potential prey items.

4.4.5. Understanding the contribution of detritus to the particulate samples. In the absence of planktonic food, *Calanus* has been observed to feed on detritus that has settled on to the base of incubation bottles using a 'benthic feeding mode' (Dilling et al. 1998, Section 1.4.1). However, the seston offered to *C. finmarchicus* in the experiments presented here was maintained in suspension by means of constant rotation on a plankton wheel. Furthermore, the mechanical disturbance of 10 copepods swimming in the experimental bottles is likely to have caused any 'marine snow' to disaggregate into particles smaller than those that can be efficiently retained by *Calanus* (see Dilling and Alldredge 2000). It is therefore assumed that detritus was not ingested during these experiments.

Clearly, particulate matter collected on a GF/F filter contains not only microplankton, but also non-living particles. POC and TFA data therefore provide somewhat confusing information about the quantity and quality of food available to copepods such as *Calanus*, which feed primarily on microplankton (Kleppel 1993, Harris 1996). It is of crucial importance to differentiate between the quantity/quality of the various particulate descriptors (e.g. chlorophyll, POC etc.) and of the food available to *C. finmarchicus*. Interestingly, of the fatty acid groups investigated (saturated, monounsaturated and polyunsaturated), POC had the strongest correlation coefficient with the saturated fatty acids (to which 18:0 belongs), and weakest with the PUFAs (Table 4.4). Conversely, cell biomass has the highest correlation coefficient with the PUFAs. The results presented here are thus in good agreement with the findings of Hama (1999), suggesting that PUFAs are associated with living matter, and that 18:0 is a good indicator of detrital material. It is apparent that in July/August a greater proportion of the POC was attributable to microplankton biomass (see Table 4.3). The average POC:Cell biomass ratio ($\mu g\ \mu g^{-1}$) was much lower (5:1) relative to April (9:1). This trend was also mirrored in the average POC:PUFA ratios, which were much higher in April relative to July/August (82 and 10 respectively). Intuitively, this would suggest that the relative contribution of carbon-rich detritus to the POC was greater in April, as indicated from the analysis of settled microplankton samples. Indeed, the C:N ratio of the particulates ranged between 8 and 11 ($\mu g\ \mu g^{-1}$) in April, much higher than that observed in July/August (range 6 to 7). The Redfield ratio, the ratio in which different chemical elements are present in average phytoplankton biomass, dictates that the C:N ratio ($\mu g\ \mu g^{-1}$) in

phytoplankton is 5.7 (Redfield 1958). The relatively high C:N ratio observed in April therefore suggests that detritus contributed more to the POC than in July/August. In contrast, the C:N of the seston sampled in July/August was much closer to Redfield, suggesting that a greater proportion of the POC was associated with living matter. Examination of the relative importance of the detrital biomarker, 18:0, in the particulate samples further supports the observation that detritus was relatively more abundant in April. Although present in lower absolute quantities in April, the relative contribution of 18:0 to the fatty acid pool (15 %) was approximately double that observed in July/August (7 %), indicating that a much larger fraction of the particulate environment was composed of detritus. Accordingly, much of the particulate material sampled in April would not have been available to *Calanus* (Paffenhofer and Strickland 1970, Dilling et al. 1998).

4.4.6. Assessing the quantity of food available for *C. finmarchicus*. Many studies have quantified particulate chlorophyll levels, and by assuming a constant carbon:chlorophyll ratio, have determined the amount of total POC available for ingestion by mesozooplankton (see Banse 1977). In this study, chlorophyll concentrations did not significantly correlate with the concentrations of cell biomass, PUFAs, or indeed any of the particulate descriptors (Table 4.4). This highlights the fact that, although chlorophyll is quick and easy to determine, it does not always serve as a useful proxy for food availability. Large quantities of detritus, which do not necessarily vary in relation to autotrophic organisms, are most likely responsible for the lack of correlation. Furthermore, the presence of non-chlorophyll bearing protists (e.g. ciliates) will also reduce the significance of any relationship between chlorophyll concentration and other particulate descriptors. It is clear that determining C from previously published chlorophyll:carbon ratios is at best difficult, and highly likely to produce erroneous results unless autotrophic cells dominate the seston biomass.

POC correlated significantly with all the particulate descriptors excluding chlorophyll (Table 4.4), yet the microplankton biomass estimations only accounted for < 40% of the POC in July/August, where the contribution of detritus to the particulates is thought to be relatively low. Although cell volumes were adjusted for shrinkage due to preservation in an attempt to avoid underestimation of microplankton biomass, the cell counts may have still been underestimations because

much of the pico-plankton (0.2 to 2 μm; Sieburth et al. 1978) is beyond the resolution of the inverted microscope. However, a similar study in the Norwegian Sea found that C not associated with living cells was reported to be around 200 μg l^{-1} during a spring bloom (Irigoien et al. 1998). Since the phytoplankton biomass (i.e. the fraction most likely to be ingested by mesozooplankton) in the North Atlantic is reported to represent approximately 15 % of POC, even during bloom conditions (Weeks et al. 1993), the microplankton biomass values presented here appear to be realistic. Clearly, much of the POC in marine waters is typically comprised of detritus and its quantification provides little information about the quantity of food available to *Calanus*, assuming that they do not consume detrital material (Section 4.4.5), or derive much nutrition from it.

A more useful measure of food availability is the biomass of viable cells that are within the ingestible size-spectra. In both seasons, food concentrations were low (9.9 to 29.3 and 25.1 to 102.4 μg C l^{-1} in April and July/August respectively), and well below saturating food concentrations for *Calanus* spp. (Frost 1972). However, such microplankton concentrations are characteristic of North Atlantic waters outside of bloom conditions (Smith 1988, Stoecker et al. 1994, Hansen et al. 1999, Harris et al. 2000, Levinsen et al. 2000b). Considering that egg production of *Calanus* has been shown to correlate with food supply (Marshall and Orr 1955b, Hirche and Bohrer 1987, Hirche et al. 1997), egg production would not be expected to be particularly high during either the incubations in April or July/August. Indeed, it has already been demonstrated that egg production of *C. finmarchicus* is food limited under pre-bloom conditions (Niehoff et al. 1999).

4.4.7. The quality of food available to *C. finmarchicus*. Because C, N and fatty acids are also present in detritus (e.g. Wakeham et al. 1984, Hama 1999), it would be incorrect to assume that the total fatty acid composition of the particulate material is available to *Calanus*. However, the following evidence suggests that it is reasonable to assume that at least the PUFA content of the particulates is primarily associated with viable cells, and are therefore readily ingestible:

1. PUFAs are primarily associated with phytoplankton cells (Hama 1991, 1999).
2. PUFAs disappear rapidly from marine particulates as depth increases, and appear to be selectively degraded relative to saturated fatty acids (de Baar et

al. 1983, Prahl et al. 1984, Wakeham et al. 1984, Neal et al. 1986, Reemtsma et al. 1990, Hama 1991, Fileman et al. 1998).

3. The stability of fatty acid molecules decreases with increasing numbers of double bonds (Farrington et al. 1977 cf. de Baar et al. 1983).

4.4.7.1. April. The quantities of EPA and DHA in the food environment have both been found to correlate positively ($p < 0.05$) with egg production rates (Jonasdottir 1994, Jonasdottir et al. 1995, Pond et al. 1996, Jonasdottir et al. 2002, Shin et al. 2003). Their paucity in the particulates (Figures 4.6 and 4.7), and the high cell biomass:PUFA ratios (Table 4.3) both suggest that the food available in April was of very poor quality, at least in term of availability of PUFAs. Furthermore, the average n-3/n-6 ratio was 1.4 (Appendix 3), similar to previously published ratios for North Atlantic waters (Mayzaud et al. 1989, Parrish et al. 1995). This ratio is known to correlate positively with egg production rates (Jonasdottir 1994, Jonasdottir et al. 1995), with highest rates occurring at ratios > 20, and lowest with a ratio of 3 (Jonasdottir 1994). The extremely low values reported here again suggest that the particulates sampled in April were of low nutritional quality, and only low egg production rates would be expected with such a diet (Jonasdottir 1994, Jonasdottir et al. 1995). Of course, it is possible that *Calanus* can either bio-convert fatty acid precursors (e.g. 18:3(n-3)) into EPA and DHA, or has body reserves of these essential fatty acids, presumably sequestered during the previous year when the food environment was more favourable (Niehoff et al. 1999, Richardson et al. 1999). The possibility that calanoid copepods possess the necessary biochemical apparatus to elongate and further desaturate other PUFAs remains unknown. It is currently thought that the majority of the PUFAs in marine food webs are produced by bacteria, protists and microalgae (Gonzalezbaro and Polloero 1988, Klein-Breteler et al. 1999, Zhukova and Kharlamenko 1999, Nichols 2003), although limited information suggests that some calanoid, cyclopoid and harpacticoid copepods are capable of producing limited quantities of EPA and DHA (Desvilettes et al. 1997, Nanton and Castell 1999, Shin et al. 2003). If, as many suspect, calanoid copepods like *C. finmarchicus* produce insignificant quantities of PUFAs (Fraser et al. 1989b, Henderson and Sargent 2000), limitation by EPA and/or DHA is a possibility that should be considered, unless the reserves deposited during the previous year are sufficient to fuel egg production during periods of low PUFA availability.

4.4.7.2. July/August. In July/August, PUFAs constituted almost 40% of the particulate fatty acids, and the quantities of EPA and DHA were both significantly greater relative to April. Cell biomass:PUFA ratios were on average, four times lower than in April (Table 4.3), suggesting that the microplankton was more nutritious in terms of PUFAs. Furthermore, the 20:5(n-3)/22:6(n-3) ratio was also low (Appendix 3). Low values have previously been shown to indicate a food environment conducive to higher rates of egg production in calanoid copepods (Stottrup and Jensen 1990, Jonasdottir 1994, Jonasdottir et al. 1995, Shin et al. 2003), further demonstrating the greater nutritional quality of the particulates in July/August (Figure 4.9, Appendix 3). The low n-3/n-6 ratio (Appendix 3) would suggest that egg production rates should be low with this diet. However, the roles of these fatty acids are not well known, and the usefulness of this ratio may be questionable. For example, Jonasdottir et al. (2002) found no significant correlation between the n-3/n-6 ratio and egg hatching in *C. finmarchicus*, and Pond et al. (1996) found the ratio in the eggs of *C. helgolandicus* to correlate negatively with egg viability.

4.4.8. Evaluation of particular fatty acids as biomarkers for individual microplankton groups. In general, highly significant positive correlations between the quantities of proposed indicator fatty acids (Table 1.1) and the biomasses of individual cell groups were found (Table 4.5), supporting the findings of previous field investigations (Kattner et al. 1983, Clauste et al. 1990, Skerratt et al. 1995, Hamm et al. 2001), and demonstrating that fatty acid analysis of particulate samples can provide qualitative information about the relative contributions of individual cell groups to the microplanktonic community (though see Hamm et al. 2001).

As discussed above, outside diatom bloom situations, the North Atlantic microplankton community is typically composed of small flagellated cells. Counter intuitively, a positive correlation between the 16:1(n-7)/16:0 ratio and the biomass of flagellates was found. However, although the biomasses of diatoms and flagellates were both greater in July/August, the relative increase in diatom biomass was much greater (700 %) than that of the flagellates (311 %), explaining why flagellates and the 16:1(n-7)/16:0 ratio were positively correlated. The biomass of diatoms was significantly correlated with the 16:1(n-7)/16:0 ratio and the quantities of 16:1(n-7) and 20:5(n-3), thus confirming the usefulness of these markers as indicators of

diatom abundance. However, since the ratio increased from 0.21 in April to only 0.45 in July/August, diatoms would not be expected to be a major component of either community. The C18 fatty acids, particularly 18:3(n-3) and 18:4(n-4), also proved to be useful biomarkers, and were significantly related to the abundance of both dinoflagellates and small flagellates. Additionally, 22:6(n-3) is also prominent in flagellated cells (Table 1.1) and despite some previous studies failing to find a correlation between this fatty acid and the biomasses of dinoflagellates and flagellates (Parrish et al. 1995, Reuss and Poulsen 2002), the data presented here showed significant correlations. Taxonomic distinction between flagellate groups using inverted microscopy was not possible. However, the fatty acid profiles can be tentatively used to provide such information. A significant positive correlation between 18:1(n-9) and the biomass of small flagellates (< 3.5 μm ESD) was also apparent, supporting previous field observations that have noted increases in the abundance of 18:1(n-9) during periods of flagellate (*Phaeocystis* sp.) predominance (Al-Hasan et al. 1990, Claustre et al. 1990, Skerratt et al. 1995, Tang et al. 2001, Reuss and Poulsen 2002). This positive correlation suggesting that at least a proportion of the small flagellates were indeed Haptophytes, and perhaps even single cells of *Phaeocystis* sp.. Independent samples taken above the Reykjanes Ridge during the same cruises (D262 and D264) support this idea, with *Phaeocystis* sp. dominating the microplankton biomass in April and contributing to the flagellate biomass in July/August (Irigoien et al. 2003). Chlorophytes may also have contributed to the flagellate community. The fatty acids characteristic of this group (16:4(n-1) and 18:3(n-3)) were both significantly correlated with the biomass of flagellates.

4.5. SUMMARY

Nutrient (N, Si, and P) concentrations in April were high, yet chlorophyll concentrations remained low. This suggested that at the time of sampling, the spring bloom had yet to occur. This was confirmed by examining satellite observations of chlorophyll *a*, which showed that elevated levels of chlorophyll were not present until June. The microplankton community was typical of a pre-bloom community in the North Atlantic, being heavily dominated by small flagellated organisms. This was confirmed by the abundance of the flagellate biomarker, 18:1(n-9) and the low 16:1(n-7)/16:0 ratio. In addition, microzooplankton also contributed significantly to

the microplankton community, typically representing > 20 % of the total microplankton biomass. In contrast, diatoms were scarce, contributing < 3 % to the daily community biomass. This was also reflected in the low quantities of the diatom biomarker, 16:1(n-7), which did not exceed > 5 % of the total fatty acid composition. The total quantities of fatty acids in the samples collected at the stations in April were low, and EPA and DHA together represented < 8 % of the fatty acid composition.

By July/August, the nutrient concentrations were much lower, suggesting a large draw down in the interim period. Although the prominence of diatoms had increased, the microplankton community was again dominated by flagellates. These observations were reflected by the abundance of diatom and flagellate biomarkers in the seston. Microzooplankton were also a prominent feature of the microplankton, contributing between 10 and 39 % to the community biomass. The stations sampled in July/August were representative of a post-bloom microplankton community. Despite being characteristically small, all the cells enumerated in April and July/August were considered to be potential prey items for *C. finmarchicus*.

Significant inter-seasonal differences in the microplankton communities were found. These were driven by the relative abundance of the individual flagellate groups identified, and also the abundance of diatoms. Similarly, inter-seasonal differences in the fatty acid compositions were significant. The PUFAs 20:4(n-6) and 22:6(n-3) were responsible for much of the observed differences, being greater in July/August. Furthermore, quantities of POC, PON, EPA, DHA and microplankton biomass were all significantly greater in the samples collected in July/August. Large quantities of detritus were observed in the microplankton samples from both seasons. This explains why < 20 % and < 40 % of the POC was attributable to microplankton biomass in April and July/August respectively. The detrital biomarker, 18:0, indicated that although absolute quantities were greater in July/August, the relative abundance of detritus was greater in April. This was confirmed by the higher POC:PON, POC:cell biomass and POC:PUFA ratios in April. *Calanus* is assumed not to ingest detritus, therefore POC data provides limited information about the food available to the copepods during the incubations. Cell biomass, determined by inverted microscopy, was considered to be a much more reliable estimate of the available food. Polyunsaturated fatty acids are primarily associated with viable microplankton, therefore cell biomass:EPA and cell biomass:DHA ratios were used

to assess the relative quality of the food. These ratios were much greater in July/August, indicating that the microplankton sampled after the spring bloom had a greater nutritional value relative to that sampled before the bloom.

Individual fatty acids and fatty acid ratios were significantly correlated with particular algal classes. The 16:1(n-7)/16:0 ratio appears useful in determining the relative contributions of diatoms and flagellates to the microplankton community. Additionally, the C18 fatty acids, 18:3(n-3) and 18:4(n-3), were useful indicators for the presence of flagellated cells. However, the understanding of particulate fatty acid data was greatly enhanced with the addition of cell biomass data, as determined by inverted microscopy.

Chapter 5

Ingestion and food selection of *Calanus finmarchicus* on the Reykjanes Ridge in April and July/August: examining the dynamics of incubation experiments

5.1. AIMS

The aim of the work described in this chapter was to establish the quantities of C, N, EPA and DHA ingested by *C. finmarchicus* feeding in April and July/August in the North Atlantic. The results will be used in chapter 7, in which the potential limitation of egg production by each of these individual components is assessed. The quantities of C ingested daily were determined using particle removal experiments conducted under *in situ* conditions. Specifically, ingestion was quantified by counting the disappearance of viable cells (via inverted microscopy using cell volume:carbon conversions), taking into consideration microplankton growth and microzooplankton grazing during the incubations (Chapter 3). POC was not considered a good indicator of ingestion because of the large quantities of detritus noted in both spring and summer (Chapter 4). The simplest approach to estimate the ingestion of the other nutritive elements and compounds is then to assume that the cellular C:N, C:EPA and C:DHA ratios of the food are the same as those in the seston. This approach however assumes that the N, EPA and DHA in the seston are only associated with viable microplankton C, and that *Calanus* does not select against individual cell groups. The quantities of C, N, EPA and DHA in the seston were followed during the course of each daily incubation, allowing preferences for individual microplankton groups to be independently assessed. In this way, patterns of selectivity were established, and total intake of C, N, EPA and DHA quantified for the grazing experiments in April and July/August.

5.2. METHODS

The experimental protocol is explained in Chapter 2. In brief, the microplankton and biochemical (fatty acid and C/N) compositions of the experimental and control bottles were determined at the beginning and end of each 24 hour incubation. Clearance and ingestion rates were calculated using the method developed in Chapter 3 (Mayor et al. submitted).

5.2.1. Stastical methods. Differences in total values of cell biomass (µg C l^{-1}), POC (µg C l^{-1}), and fatty acids (µg fatty acid l^{-1}) between initial, experimental and control bottles were assessed using 1-way ANOVAs. Before analysis, data were tested for the assumptions of parametric statistics i.e. a normal distribution (Kolmogorov-Smirnov test) and equal variance (Levene Median test). Where the

data did not meet these assumptions, the non-parametric Kruskal-Wallis 1-way ANOVA on ranks was used. To establish differences between individual treatments (initial, experimental or control), pairwise comparisons (initial vs. experimental, initial vs. control and experimental vs. control) were performed using the Tukey test (Sokal and Rohlf 1995).

Feeding selectivity and prey preferences of *Calanus* were assessed in 3 ways: The percentage distribution of prey items in the diet was compared to the percentage distribution of prey available during the incubations using the Chi-squared goodness-of-fit test (Parker 1983). In addition, the electivity index, E^* (Vanderploeg and Scavia 1979), as presented by Rollwagen Bollens and Penry (2003) was used to assess preferences for individual taxa (see Chapter 3). Fatty acid data were collected and analysed separately from the cell biomass data, and therefore provide an independent data set with which to examine food preference. It was hypothesised that if selective feeding was apparent, the fatty acid composition data (mol %) of the particulate samples in the experimental and control bottles should be significantly different, since individual algal groups have specific fatty acid patterns (Table 1.1). The fatty acid data were non-normally distributed (Kolmogorov-Smirnov test, $p < 0.001$). Therefore, to examine the differences between the treatments, the non-parametric ANOSIM test was used (Section 4.2.1, Clarke and Warwick 1994). Pairwise comparisons within the ANOSIM test (analogous to the parametric Tukey test) were used to determine which treatments differed significantly. In ANOSIM pairwise comparisons, the 'r' values gives an absolute measure of how different the groups are, on a scale of 0 (indistinguishable) to 1 (*all* similarities within groups are less than *any* similarity between groups). The non-parametric SIMilarity of PERcentage (SIMPER; Clarke and Warwick 1994) analysis was employed to qualify within and between treatment similarity (and dissimilarity) as a percentage, and also to identify which fatty acid moieties primarily accounted for the observed differences.

5.3. RESULTS

5.3.1. Particulate dynamics

5.3.1.1. April. Biomass estimations derived from inverted microscopy cell-counts showed a similar trend each day (Figure 5.1A). The experimental bottles were always reduced relative to the control bottles, although these differences were only

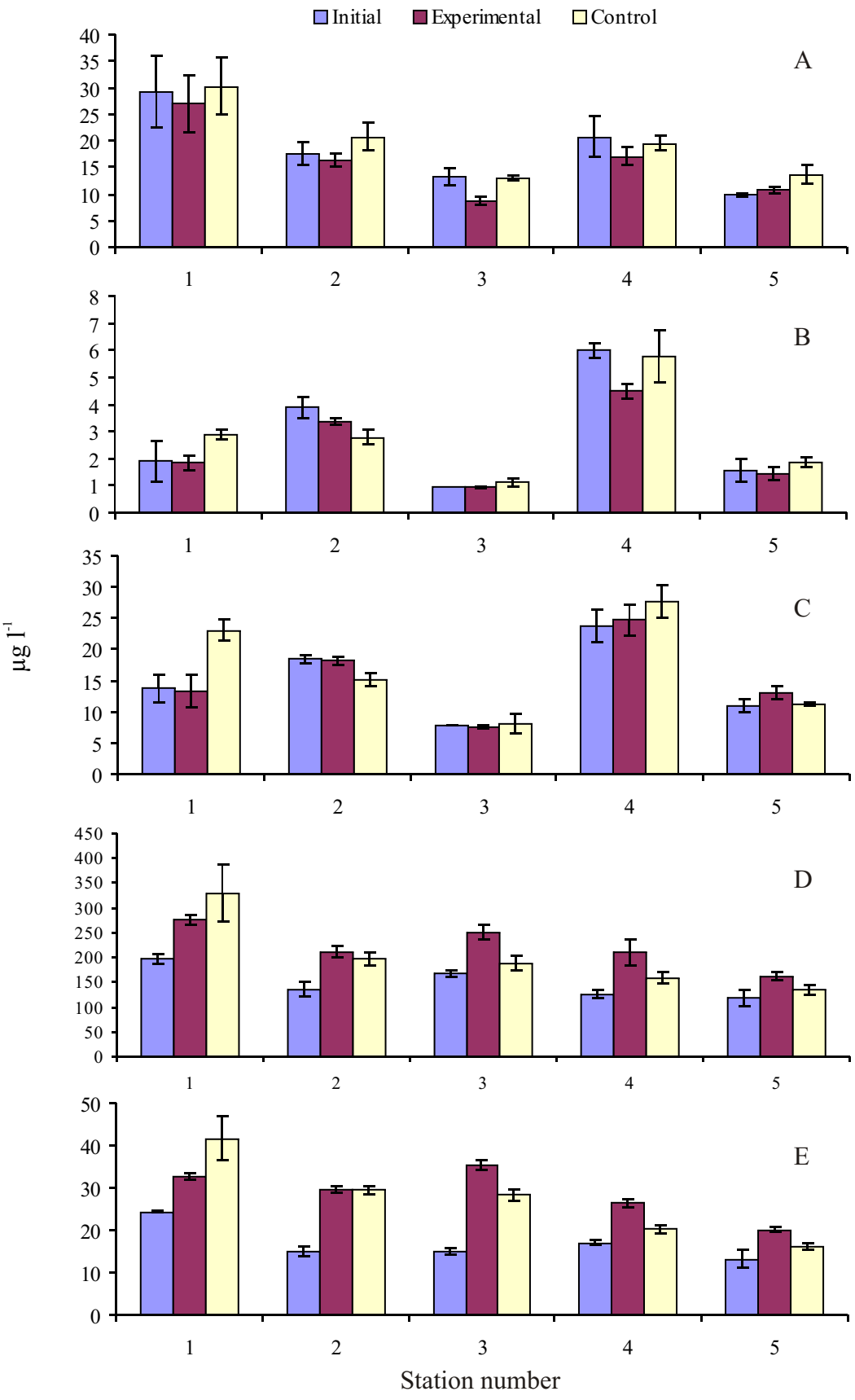


Figure 5.1. Particulate dynamics in the initial, experimental and control bottles during incubations in April (± standard error). A, Cell biomass. B, Polyunsaturated fatty acids. C, Total fatty acids. D, Particulate organic carbon. E, Particulate organic nitrogen. All units in µg l^{-1}. Note variable scales on y-axis.

significant at station 3 (ANOVA, $p = 0.014$). However, the probability of the microplankton biomass in the experimental bottles being reduced relative to the controls on all five days by chance is unlikely (Binomial probability distribution; $p = 0.031$), and therefore the reductions appear to be real. A similar trend was observed in the quantities of PUFAs in the different treatments, with the experimental bottles being reduced relative to the controls at stations 1, 3, 4 and 5 (Figure 5.1B). However, these daily differences were not statistically distinguishable (ANOVA $p > 0.1$, Tukey test $p > 0.1$ in all cases), nor was this general trend (Binomial probability distribution; $p = 0.156$). The total fatty acid data mirrored the PUFA data at stations 1, 2 and 3, but differed at stations 4 and 5 (Figure 5.1D). A reduction in the experimental bottles relative to the controls was observed at stations 1, 3 and 4, although there was only a significant difference between the treatments at station 1 (ANOVA, $p < 0.05$). At this station, the fatty acids in the control bottles had increased relative to the initial and control bottles (Tukey test, $p < 0.05$ in both cases).

The trends observed in the particulate organic carbon and nitrogen data (POC and PON respectively) closely reflected each other, demonstrating a constant C:N ratio (Figures 5.1D and E). At stations 2, 3, 4 and 5, the quantity of C (and hence N) increased in the experimental bottles relative to both the initial and control treatments (Figure 5.1D). This trend apparently contradicts the results of both the cell biomass estimations and the PUFA data (Figures 5.1A and B). However, these differences were only significant at stations 2 and 3 (ANOVA, $p < 0.001$ in both cases). At station 2, the quantities of POC in the experimental and control bottles had both increased significantly relative to the initial bottles (Tukey test, $p < 0.05$). The experimental bottles at station 3 showed a significant increase relative to the initial and control bottles (Tukey test, $p < 0.05$). It is possible that these differences reflect fragments of fecal pellets that were not retained by the sieve when removing the eggs each day (see Chapter 2).

5.3.1.2. July/August. The cell biomass estimations showed the same pattern as found in April, with the experimental bottles being reduced relative to the initial and control treatments (Figures 5.1A and 5.2A). Although these differences were only statistically different at station 4 (ANOVA, $p = 0.019$), the probability of this pattern occurring by chance was again low (Binomial probability distribution; $p = 0.031$). No clear trends were apparent in the PUFA data (Figure 5.2B). The quantities

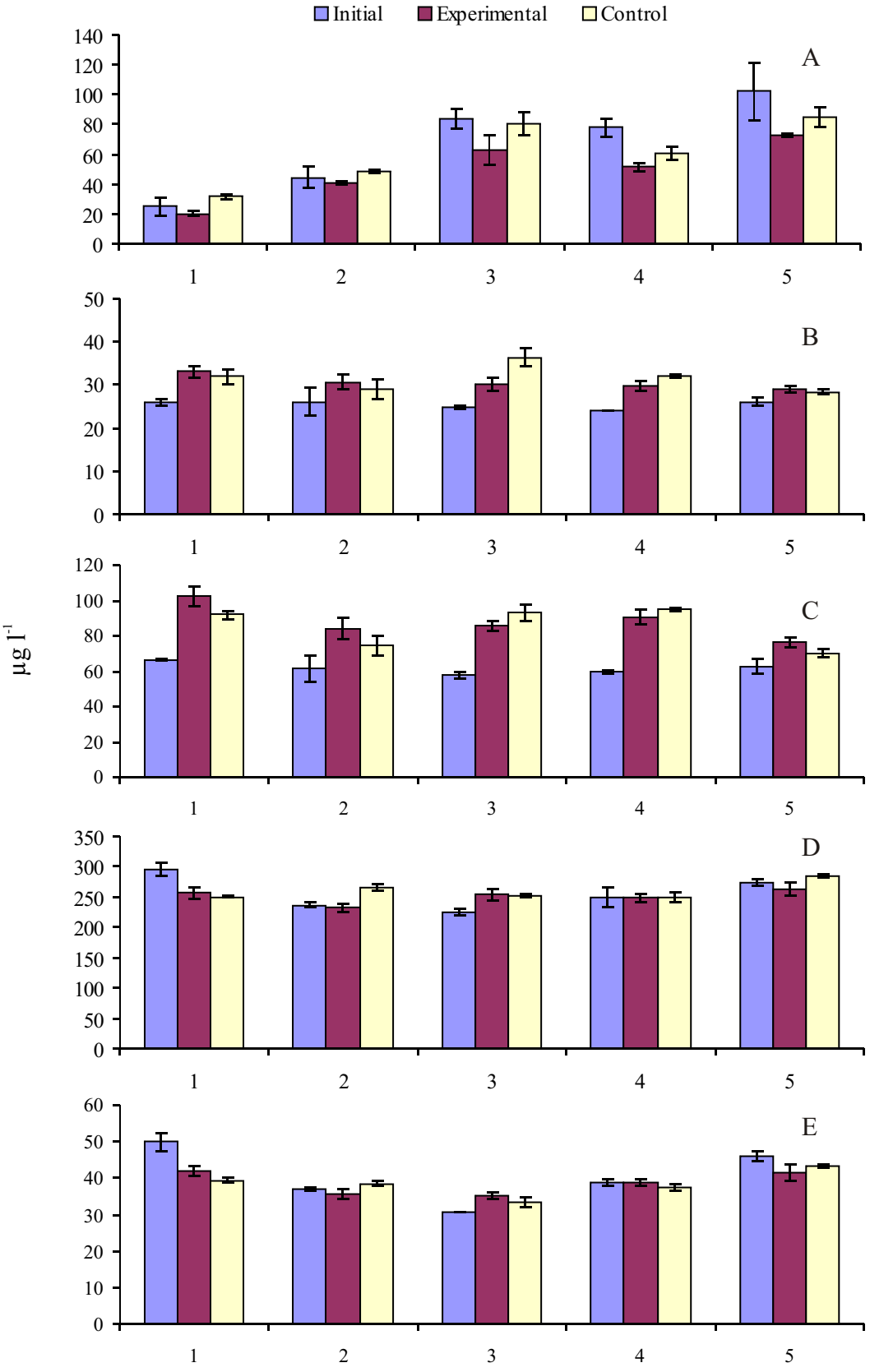


Figure 5.2. Particulate dynamics in the initial, experimental and control bottles during incubations in July/August (± standard error). A, Cell biomass. B, Polyunsaturated fatty acids. C, Total fatty acids. D, Particulate organic carbon. E, Particulate organic nitrogen. All units in µg l^{-1}. Note variable scales on y-axis.

of PUFAs in the experimental bottles were only significantly reduced relative to the controls at station 4 (Tukey test, $p < 0.05$). Similarly, total quantities of fatty acids in the experimental and control bottles never differed significantly (ANOVA, $p > 0.05$, Figure 5.2C). However, the total quantities of fatty acids in the experimental and control treatments were always greater than the quantities in the initial samples. These differences were significant at stations 1, 3 and 4 (Tukey test, $p < 0.05$ in all cases), reflecting microplankton growth over the duration of the incubations. The POC and PON data again closely reflected each other, demonstrating a constant C:N ratio (Figures 5.2D and 5.2E). These data concur with the PUFA and total fatty acid data in that they reveal no apparent trends between the treatments. The quantity of POC in the experimental treatments was only significantly lower than that in the controls at station 2 (Tukey test, $p < 0.05$).

5.3.2. Clearance and ingestion

5.3.2.1. April. Average clearance rates of individual cell groups ranged between 12 and 231 ml copepod^{-1} day^{-1}, with ciliates often being cleared at higher rates than other cells (Figure 5.3). Ingestion rates of individual cell groups ranged between < 0.01 to 0.4 µg C copepod^{-1} day^{-1} (Figure 5.4). However, group-specific clearance rates were highly variable within each daily incubation. Therefore, average clearance and ingestion rates for individual cell groups were not always significantly different from zero (Figures 5.3 and 5.4). The majority of ingested C was of flagellate origin, comprising 47 to 62 % of the total C ingested daily (Figure 5.5). Ciliates and dinoflagellates were also important dietary components, typically contributing > 30 % of the ingested C. In contrast, diatoms only contributed > 2 % to the daily ration at station 1.

On average, a total of 1.14 µg of C was ingested daily by each copepod (range: 0.61 to 1.39 µg C copepod^{-1} day^{-1}, Table 5.1A), corresponding to an average daily ration of 1.5 % body C day^{-1} (Table 5.1A; C, N, EPA and DHA biomass data for the females is presented in the following chapter). Typical daily rations for female *C. finmarchicus* range between 1.1 and 2.3 % (µg C ingested [µg C copepod]$^{-1}$ day^{-1} * 100) under pre-bloom conditions (Table 5.2), demonstrating that the values determined here are realistic. However, rates of respiration and nitrogenous excretion in *Calanus* vary in relation to their life cycle and the availability of food (Conover and Corner 1969, Butler et al. 1969, Butler et al. 1970). The N-biomass specific

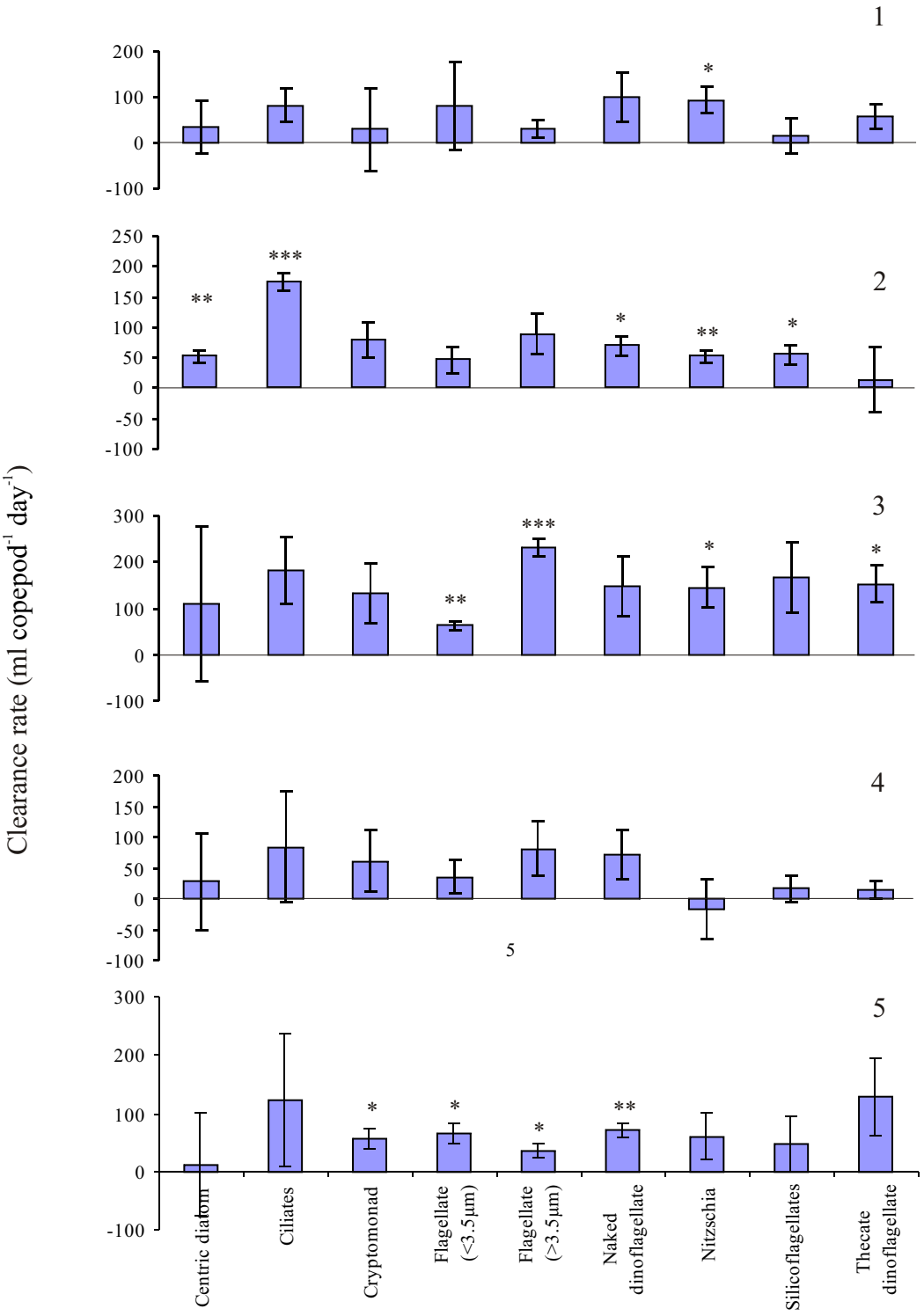


Figure 5.3. Average clearance rates (± standard error) of female *Calanus finmarchichus* in April. Station number are shown on right hand side. Note variable scale on y-axis. T-test H0: Clearance is not significantly different from zero. *P <0.05, **p < 0.01, ***p < 0.001

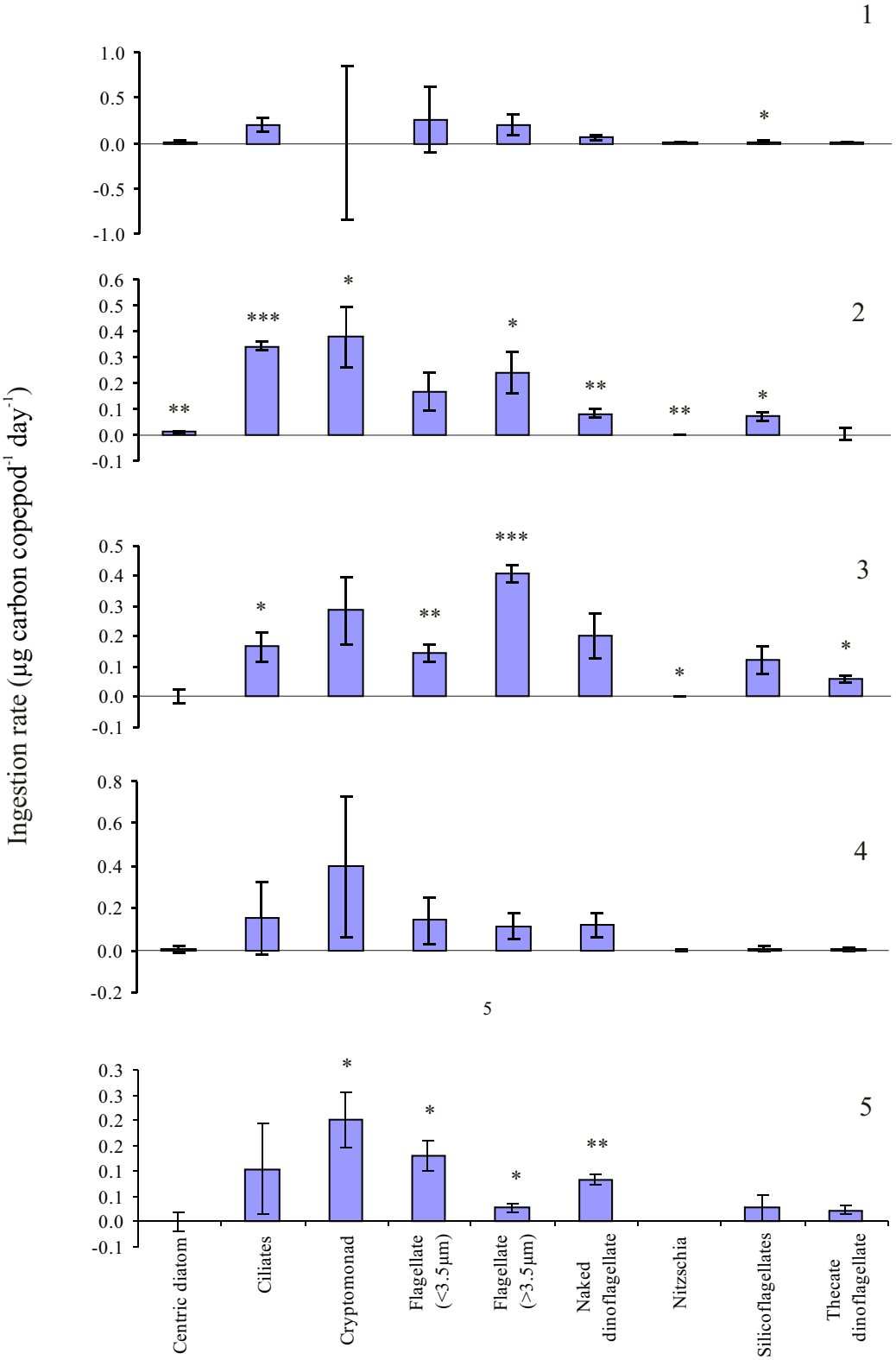


Figure 5.4. Average ingestion rates (± standard error) of female *Calanus finmarchichus* in April. Station numbers are shown on right hand side. Note variable scale on y-axis. T-test H0: Ingestion is not significantly different from zero. *p <0.05, **p < 0.01, ***p < 0.001.

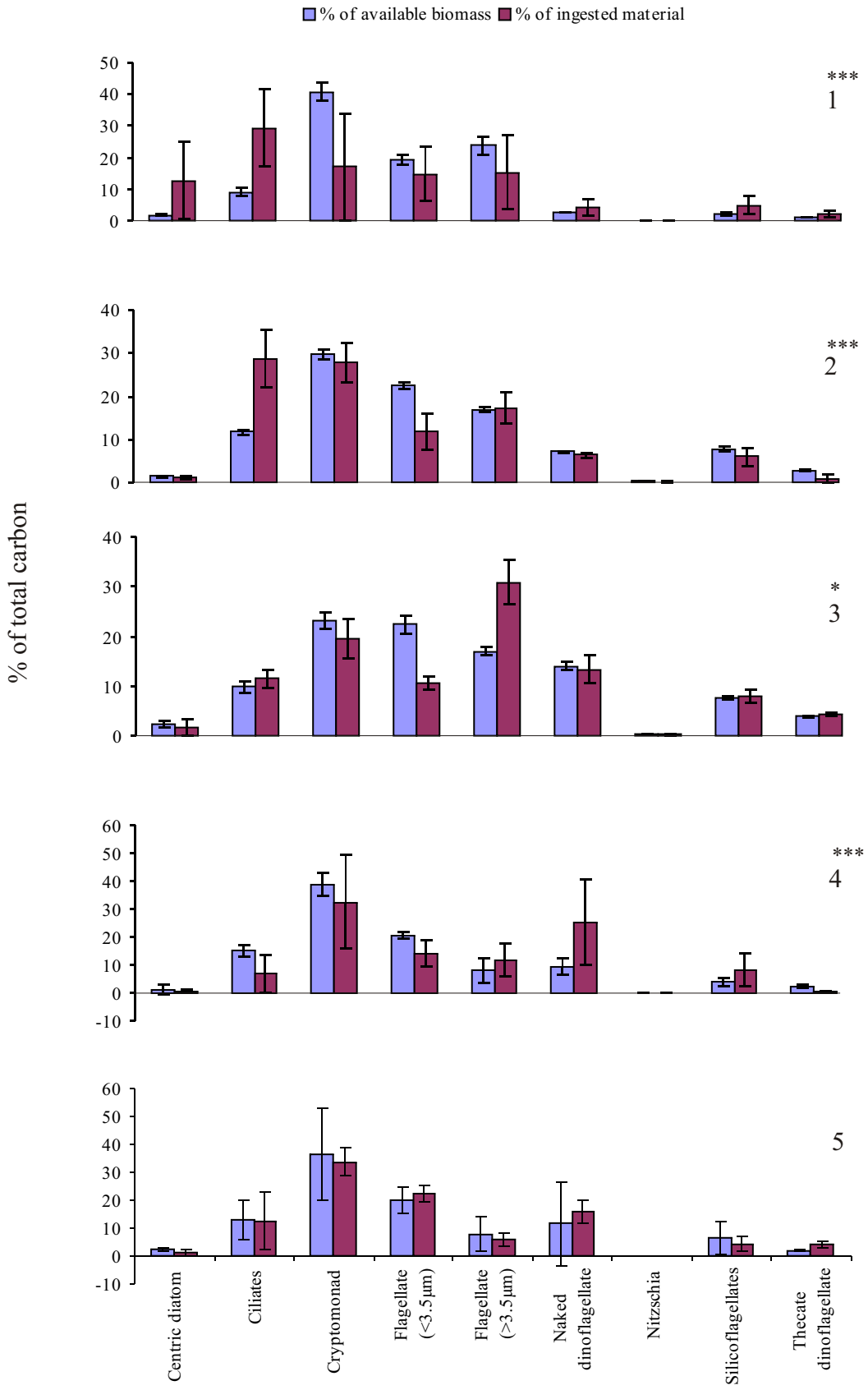


Figure 5.5. Average percentage composition of the food field during the incubations and the material ingested by *Calanus finmarchicus* in April (± standard error). Chi-squared goodness of fit, H0: the percentage distributions of prey items in the diet is not significantly different the distributions of prey available during the incubations. *p <0.05, **p < 0.01, ***p < 0.001.

Table 5.1 Average daily ingestion rates (µg copepod day^{-1}, ± SE) and mass specific daily rations (X_{DR}; µg X ingested [µg X copepod]$^{-1}$ day^{-1} * 100) for *C. finmarchicus* in April (A) and July/August (B), where X is a particular dietary component.

A.

Station #	C	N	EPA	DHA	C_{DR}	N_{DR}	EPA_{DR}	DHA_{DR}
1	1.39± 0.89	0.24	0.01	0.02	2	1	1	2
2	1.31± 0.25	0.23	0.03	0.07	2	1	4	6
3	1.41± 0.31	0.25	0.01	0.03	2	1	1	3
4	1.01± 0.70	0.18	0.04	0.02	1	1	4	6
5	0.61± 0.16	0.11	0.00	0.01	1	1	0	1
Avg.	**1.14 ± 0.15**	**0.20 ± 0.03**	**0.02 ± 0.01**	**0.04 ± 0.01**	**1.5 ± 0.2**	**1.0 ± 0.1**	**2.1 ± 0.9**	**3.7 ± 1.1**

B.

Station #	C	N	EPA	DHA	C_{DR}	N_{DR}	EPA_{DR}	DHA_{DR}
1	3.19± 0.36	0.56	0.44	0.94	3	2	42	67
2	3.74± 0.61	0.66	0.28	0.62	4	3	27	44
3	7.07± 3.05	1.25	0.30	0.61	7	5	29	43
4	5.33± 0.61	0.94	0.23	0.49	5	4	22	35
5	5.90± 0.68	1.04	0.50	0.75	6	4	48	53
Avg.	**5.05 ± 0.71**	**0.89 ± 0.12**	**0.35 ± 0.05**	**0.68 ± 0.08**	**5.1 ± 0.7**	**3.6 ± 0.5**	**33.5 ± 5.0**	**48.5 ± 5.5**

Table 5.2. Literature data on ingestion rate (IR; µg C copepod day^{-1}) and C specific daily rations (DR; µg C ingested [µg C copepod]$^{-1}$ day^{-1} * 100) for *C. finmarchicus* (unless indicated otherwise) at different locations. * = juvenile stages CIV and CV, NS, $^{\&}$ = *Calanus* spp. CV, NS = not stated, NP = natural plankton, BI = bottle incubation, PR = particle removal, GE = gut evacuation, GF = gut fluorescence, IM = inverted microscopy, CC = coulter counter, PF = particulate fluorescence, EP = egg production, average values in parentheses.

Area	Date and Temp (ºC)	IR (d^{-1})	DR (%)	Comments	Reference
Norwegian sea	23 March – 3 May. ~6	1.4	2.3	Pre-bloom. NP, GF, Chl-*a*. analysis. ♀ = 62 µg C.	Irigoien et al. 1998
Norwegian Sea	23 March – 3 May. ~6	0.64	1.1	Pre-bloom. NP, BI, PR, HPLC pigment analysis. ♀ = 60 µg C.	Meyer-Harms et al. 1999
Disko Bay, Greenland	28 April. -1.7	1.9	1.2	Pre-bloom. NP, BI, PR, IM. ♀ = 160 µg C	Levinsen et al. 2000b
Barents Sea	27 May – 13 June. ~-1	2.4 – 26.4	2.8 - 32	Early-bloom and bloom. GE, Chl-*a*. analysis. ♀ = 82 µg C	Tande and Bamstedt 1985
New York Bight, NW Atlantic	March. 12	60	37	Early-bloom. NP, GF, GE. ♀ = 161 µg C	Smith and Lane 1988
New York Bight, NW Atlantic	April. 12	129	80	Mid-bloom. NP, GF, GE. ♀ = 161 µg C	Smith and Lane 1988
Kattegat/Skagerrak, North Sea	4 – 13 April. 1 - 5	9.4	1.5 – 26.1 (10)	Bloom. NP, GF, GE. ♀ = 94 µg C	Kiorboe et al. 1985b
Kattegat/Skagerrak, North Sea	4 – 13 April. 1 – 5	8.9	3.5 – 22.3 (9)	Bloom. NP, BI, PF. ♀ = 80 µg C	Kiorboe et al. 1985b
Kattegat/Skagerrak, North Sea	4 – 13 April. 1 – 5	9.6	7.3 – 15.3 (10)	Bloom. NP, BI, CC. ♀ = 96 µg C	Kiorboe et al. 1985b
Kattegat/Skagerrak, North Sea	4 – 13 April. 1 - 5	7.6	4.2 – 12.0 (8)	Bloom. NP, BI, EP. ♀ = 96 µg C	Kiorboe et al. 1985b

Area	Date and Temp (ºC)	IR (d^{-1})	DR (%)	Comments	Reference
Norwegian sea	20 May – 27 May. ~7.5	14.3	30	Bloom. NP, GF, Chl-*a*. analysis. ♀ = 48 µg C.	Irigoien et al. 1998
Norwegian Sea	20 May – 27 May. ~7.5	9.24	15.4	Bloom. NP, BI, PR, HPLC pigment analysis. ♀ = 60 µg C.	Meyer-Harms et al. 1999
St. Lawrence River estuary, Canada	21 June – 3 July. 7.5	92.7 – 106.3	42 – 48	Upwelling estuary. NP, BI, PR, IM. ♀ = 222 µg C	Ohman and Runge 1994
Polar front, Barents Sea[&]	14 – 28 July. 0	10.6 – 332.6	2.6 – 82.3	Post-bloom. NP, GF, GE. CV = 404 µg C	Hansen et al. 1990a
Gulf of St. Lawrence, Canada	21 June – 3 July. 6.3	2.4 – 9.3	1.1 – 4.2	Post-bloom. NP, BI, PR, IM. ♀ = 222 µg C	Ohman and Runge 1994
Norwegian sea	29 May – 5 June. ~8	7.6	14	Post-bloom. NP, GF, Chl-*a*. analysis. ♀ = 54 µg C.	Irigoien et al. 1998
Norwegian Sea	29 May – 5 June. ~8	2.69	4.5	Post-bloom. NP, BI, PR, HPLC pigment analysis. ♀ = 60 µg C.	Meyer-Harms et al. 1999
Disko Bay, Greenland	20 June. 3.0	4.9	3	Post-bloom. NP, BI, PR, IM. ♀ = 160 µg C	Levinsen et al. 2000b
Fram Straight,	22 – 23 June. 2.7	0.2	1.8	Summer. NP, GF, GE. ♀ = 257 µg C	Smith 1988
Fram Straight,	25 June. 0.6	0.02	0.2	Summer. NP, GF, GE. ♀ = 257 µg C	Smith 1988
Fram Straight,	3 – 4 July. -1.7	0.01	0	Summer. NP, GF, GE. ♀ = 257 µg C	Smith 1988
Norwegian Fjords*	NS 5.0	21	36	Phaeocystis. BI, PR, Chl-*a* ingestion. CIV - CV = 59 µg C	Hansen et al. 1994b

regression of Ikeda et al. (2001) predicts that *Calanus* will respire approximately 3.27 µg C copepod^{-1} day^{-1} at the experimental temperature of 7 ºC (assuming an RQ of 0.8), suggesting that ingested material was not sufficient to support basal metabolic processes in April.

Determining the quantities of N, EPA and DHA ingested was more complicated. Unlike C, quantities of these biochemical constituents in a cell cannot be determined from its volume as such relationships have yet to be determined. The quantities of N ingested during each daily incubation were therefore estimated by assuming that the C:N of the food was at the Redfield ratio (6.625:1; Redfield 1958). This is the ratio in which different chemical elements are present in average phytoplankton biomass. Assuming Redfield, an average of 0.2 µg of N were ingested daily by each female, equivalent to a daily ration 1.0 % body N day^{-1} (µg N ingested [µg N copepod]$^{-1}$ day^{-1} * 100; Table 5.1A). Over the seasonal cycle, female *C. finmarchicus* excrete between 1.9 and 11.4 % of their body N daily, with females under pre-bloom conditions excreting 3.7 to 9.8 % of their body N daily (Butler et al. 1970). The derived quantities of ingested N are therefore not sufficient to meet the expected excretion rates. Furthermore, eggs were produced in April (see Chapter 6), confirming that the overall demand for N could not have been satisfied from the ingested material alone.

Daily ingestion rates of EPA and DHA were estimated in a manner similar to that for N. The C:EPA and C:DHA of the food were determined by expressing the quantities of these PUFAs in the initial seston samples relative to the respective quantities of C associated with viable cells, as determined by inverted microscopy. The average C:EPA and C:DHA ratios in April were 1627:1 and 797:1 (µg µg^{-1}) respectively. Using cell biomass is more realistic than using POC data to determine these ratios because EPA and DHA are primarily associated with viable cells (Hama 1991). Expressing EPA and DHA relative to POC would lead to a gross underestimation of their ingestion rates because of the large quantities of detrital C (see Chapter 4). The derived average daily ingestion rates of EPA and DHA were 0.02 and 0.04 µg copepod^{-1} day^{-1} respectively, corresponding to average daily rations of 2.1 and 3.7 % (Table 5.1).

5.3.2.2 July/August. Average group-specific clearance rates ranged between 9 and 276 ml copepod^{-1} day^{-1} (Figure 5.6). Again, ciliates were consistently cleared

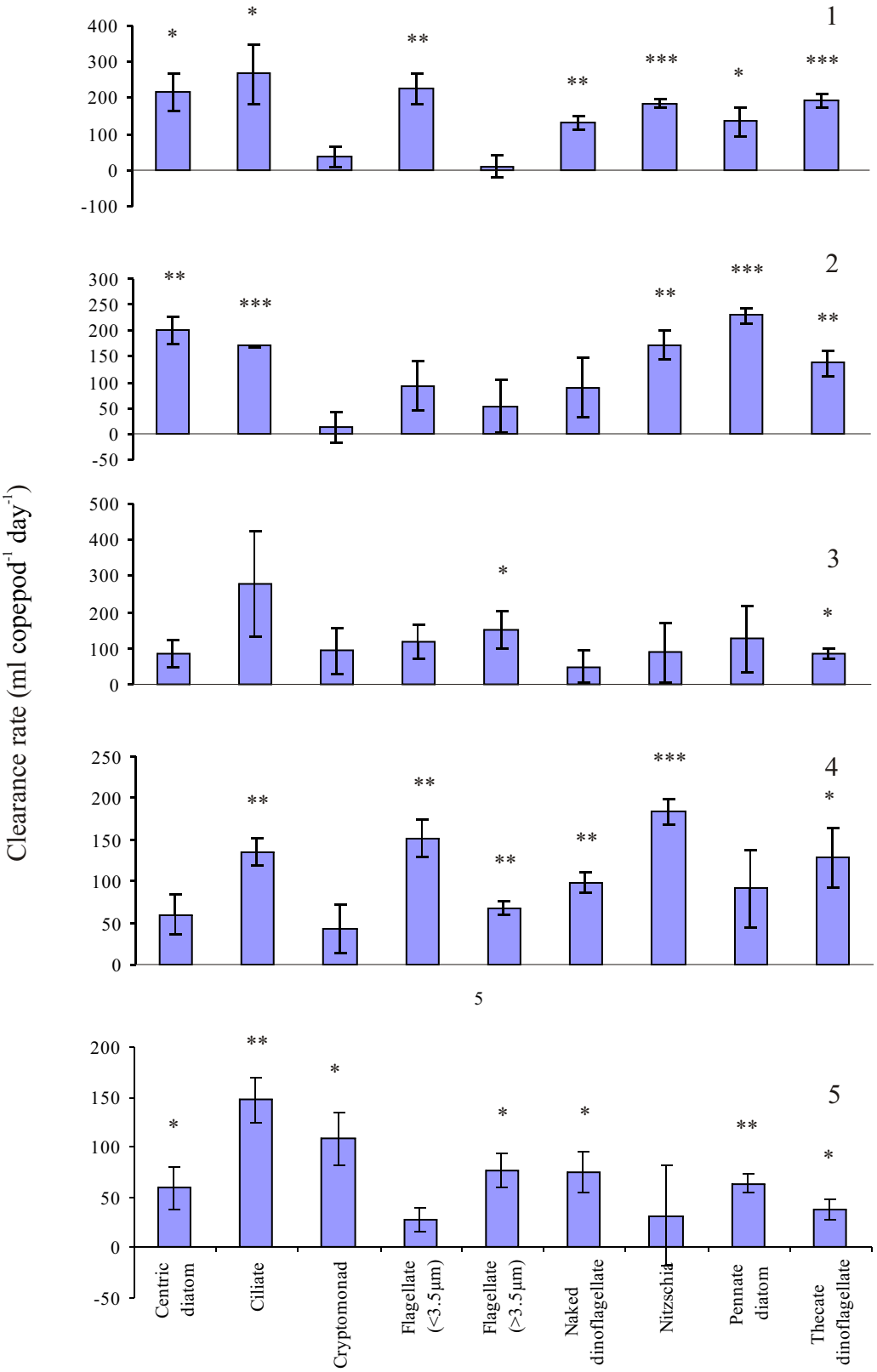


Figure 5.6. Average clearance rates (± standard error) of female *Calanus finmarchichus* in July/August. Station numbers are shown on right hand side. Note variable scale on y-axis.T-test H0: Clearance is not significantly different from zero. *p <0.05, **p < 0.01, ***p < 0.001.

at higher rates. Small flagellates (<3.5 µm ESD) were ingested at high rates, providing between 0.52 and 2.18 µg C copepod^{-1} day^{-1}, Figure 5.7). In general, there was much less variation in the data relative to April, and the majority of clearance and ingestion rates were significantly different from zero (Figures 5.6 and 5.7). Flagellates dominated the biomass of the ingested material (range: 40 to 72 %, Figure 5.8), as was found in April. Ciliates and dinoflagellates were also important components of the diet, together contributing between 11 and 41 % of the total C ingested each day. In contrast to April, diatoms also contributed noticeably to the diet of *C. finmarchicus* in July/August, representing between 7 and 33 % of the ingested C. This reflects their increased contribution to the microplankton biomass in July/August (Figure 4.3).

Average daily ingestion rates in July/August ranged between 3.19 and 7.07 µg C copepod^{-1} day^{-1} (average = 5.1), and 0.56 to 1.12 µg N copepod^{-1} day^{-1} (average = 0.9; Table 5.1B). On average 0.35 and 0.68 µg of EPA and DHA respectively were ingested each day (Table 5.1B). Daily ingestion rates in July/August were significantly higher than the rates determined in April (t-test, $p < 0.001$). This reflects a greater abundance of prey cells in July/August (see below). Average specific daily rations for C, N, EPA and DHA were 5.1, 3.6, 33.5 and 48.5 % day^{-1} respectively (Table 5.1B). These daily rations determined in July/August were noticeably higher than in April, particularly those of EPA and DHA. This reflects an increase in the intracellular concentration of these PUFAs in the microplankton i.e. a decrease in the C:EPA and C:DHA ratios (329:1 and 179:1 respectively, units in µg µg^{-1}). *Calanus* typically ingest between 1 and 14 % of their C biomass daily (Table 5.3), indicating that the biomass-specific ingestion rates determined here are realistic. Furthermore, the specific rations of C and N (Table 5.1B) agree well with literature-derived values of respiration and excretion (Ikeda et al. 2001, see Chapter 6), suggesting that the animals had ingested sufficient quantities to fulfil their basal metabolic demands.

5.3.3. Particulate variables as predictors of copepod ingestion. When data from April and July/August were pooled, ingestion rates were significantly related to POC, cell biomass and total fatty acids (Regression analysis, $R^2 = 0.55$, 0.88 and 0.70 respectively; ANOVA, $p < 0.05$ in all cases; Figure 5.9A to C), but not to Chlorophyll *a* (Regression analysis, $R^2 = 0.004$; ANOVA, $p > 0.1$; Figure 5.9D). The coefficients of determination, R^2, represent the fraction of variability in *y* (ingestion

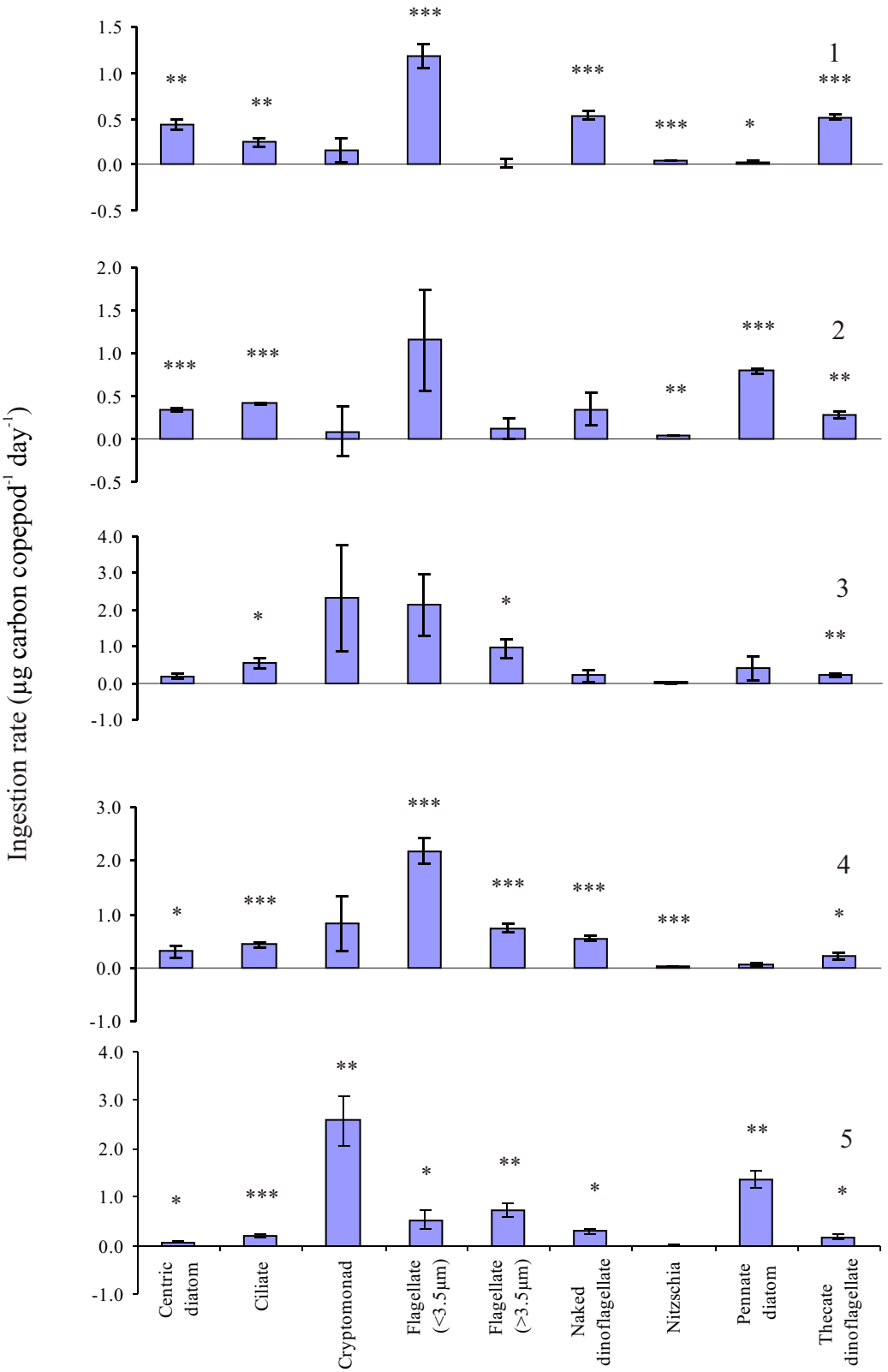


Figure 5.7. Average ingestion rates (± standard error) of female *Calanus finmarchichus* in July/August. Station numbers are shown on right hand side. Note variable scale on y-axis.T-test H0: Ingestion is not significantly different from zero. *p <0.05, **p < 0.01, ***p < 0.001.

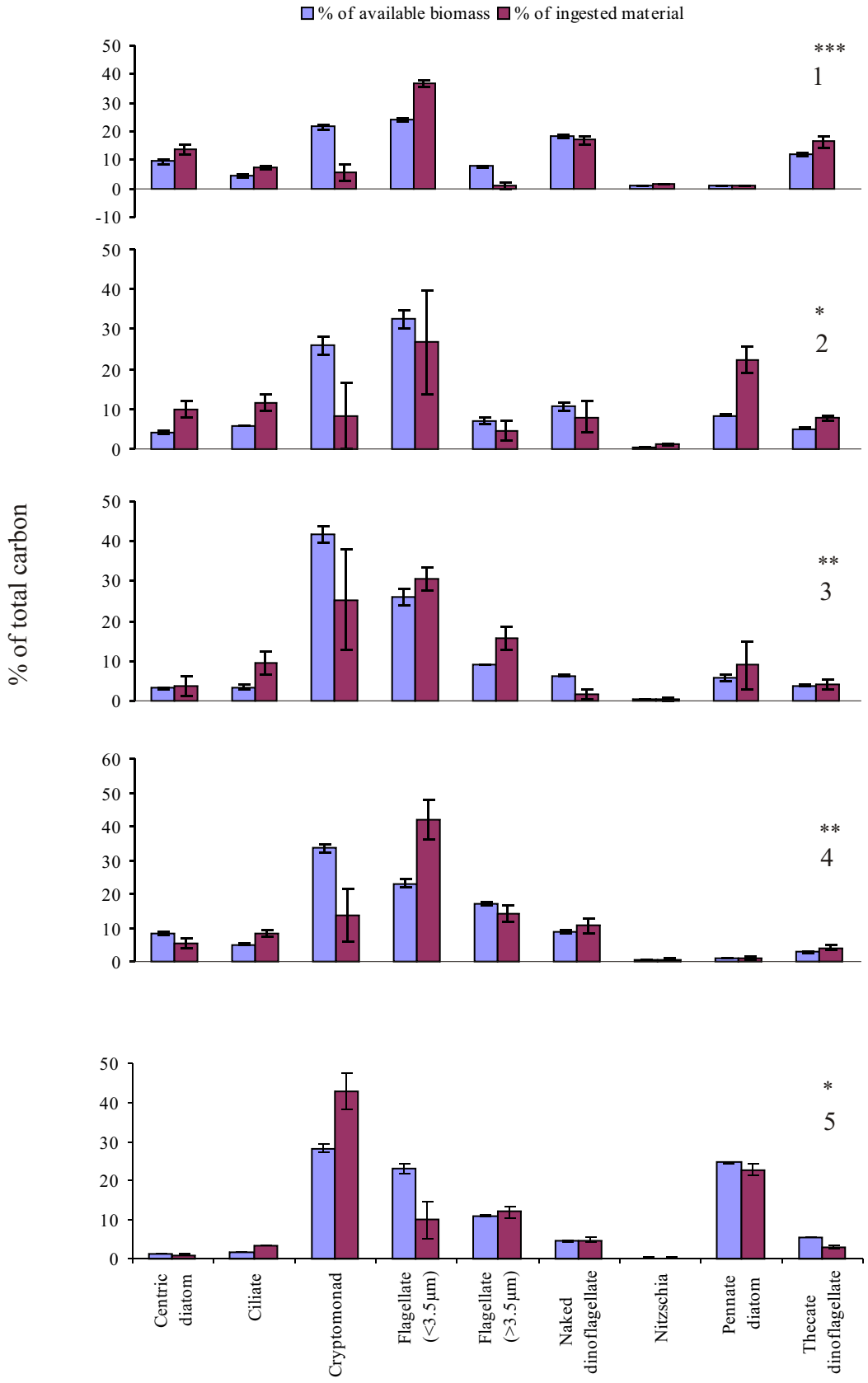


Figure 5.8. Average percentage composition of the food field during the incubations and the material ingested by *Calanus finmarchicus* in July/August (± standard error). Chi-squared goodness of fit, H0: the percentage distributions of prey items in the diet is not significantly different the distributions of prey available during the incubations. *p <0.05, **p < 0.01, ***p < 0.001.

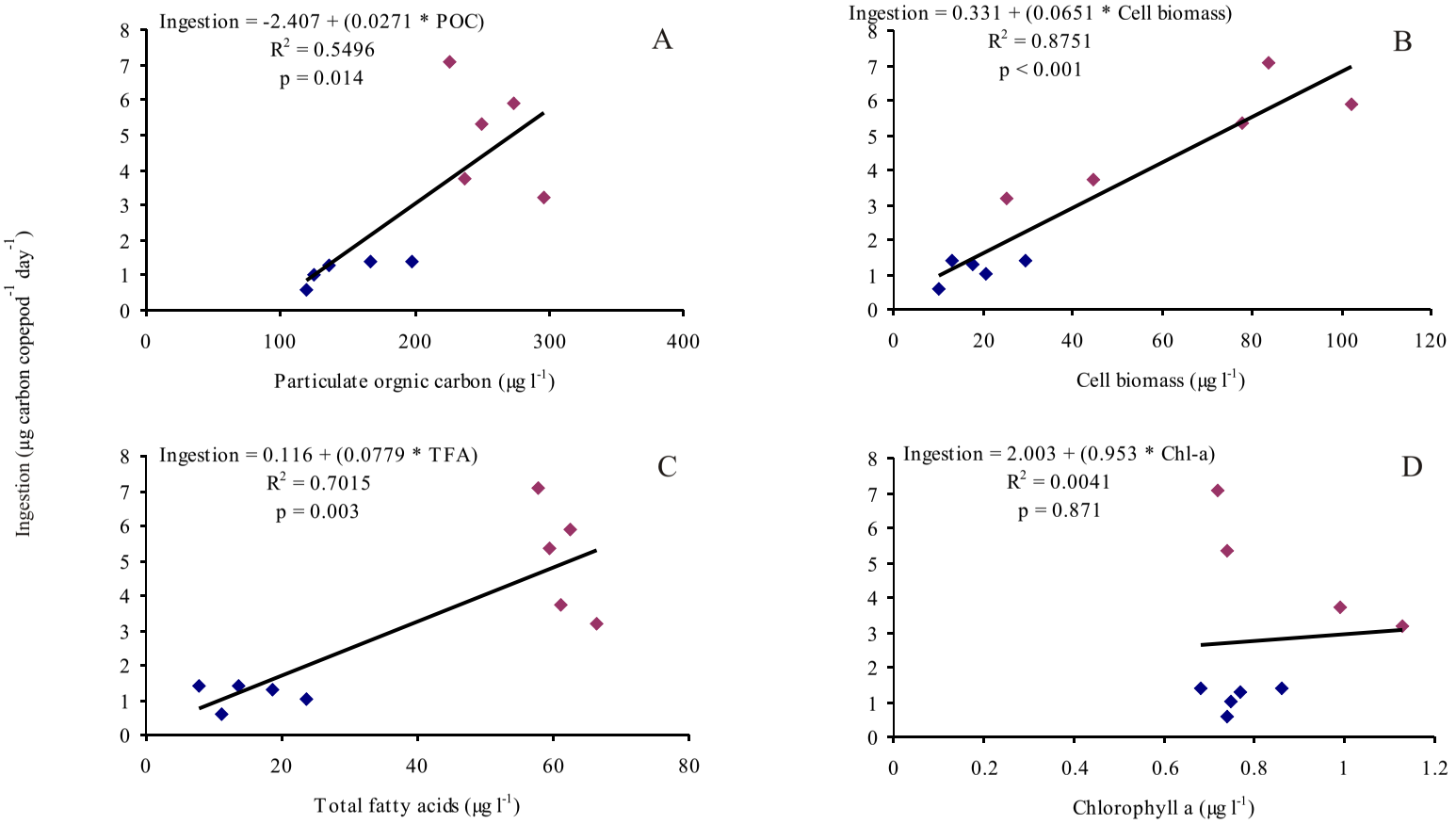


Figure 5.9. Relationships between Particulate organic carbon (A), Cell biomass (B), total fatty acids (C) and Chlorophyll *a* (D), and copepod ingestion rate. Solid lines are lines of best fit. Linear regression equations, their coefficients of determination (R^2) and their significance (ANOVA) are presented. Data from April and July/August incubations are shown in blue and maroon respectively.

rates) that can be explained by the variability in *x* (particulate descriptor). R^2 values for the cell biomass and total fatty acid regressions were both high, indicating that these variables are both useful for predicting ingestion rates. The linear nature of these relationships also indicates that over the concentrations encountered, feeding was not saturated.

5.3.4. Food preference

5.3.4.1 April. During the incubations in April, the Chi-squared goodness-of-fit test found that the percentage distributions of prey items in the diet of *C. finmarchicus* was significantly different to the distributions of prey available during the incubations at all stations excluding station 5 ($p < 0.05$ in all cases; Figure 5.5). However, when the composition of the diet is compared to that of the available food visually (Figure 5.5), it is apparent that in most instances the composition of the diet generally reflects that of the food environment. The electivity index, E^* (Vanderploeg and Scavia 1979), found selection towards ciliates and large flagellates at stations 2 and 3 respectively, and avoidance of small flagellates and thecate dinoflagellates at stations 3 and 4 respectively (Table 5.3). When all the results from the 5 daily incubations are examined together, however, no particular cell group was consistently selected for or against, suggesting that food selection was not present during any of the incubations. This was confirmed by the fatty acid compositional data (Figures 5.10 to 5.14). Although the fatty acid compositions were significantly different between the treatments at all stations (ANOSIM, $r > 0.32$, $p < 0.05$ in all cases), pairwise comparisons revealed that the significant results were driven by differences between the initial and experimental bottles. This reflects the differential growth rates of individual cell groups over the duration of the incubation. The differences between the experimental and control bottles were not significant at any station (ANOSIM, $r < 0.36$, $p > 0.1$ in all cases), differing by only by 3.0 to 9.3 % (SIMPER analysis), suggesting that no detectable patterns in selection were apparent.

5.3.4.2. July/August. The Chi-squared goodness-of-fit test found that the percentage composition of the diet was significantly different to that of the available food at all stations ($p < 0.05$ in all cases; Figure 5.8). However, a visual comparison between the composition of the diet and the available food again reveals that the two are to a large extent similar. The electivity index E^*, demonstrated that ciliates were positively selected for at all stations, with all average values but that from station 4

Table 5.3 Selectivity indices E^* determined in April and July/August. H0 = selection is not significantly different from zero. $^*p = 0.05$, $^{**}p = 0.01$, $^{***}p = 0.001$

		Station number				
	Cell group	1	2	3	4	5
April	Centric diatom	-0.20± 0.47	-0.15± 0.12	-0.53± 0.47	-0.63± 0.37	-0.61± 0.39
	Ciliates	0.04± 0.14	0.40± 0.07**	0.08± 0.11	-0.57± 0.43	-0.40± 0.40
	Cryptomonad	-0.61± 0.39	0.09± 0.09	-0.07± 0.12	-0.19± 0.41	0.08± 0.06
	Flagellate (< 3.5 µm)	-0.33± 0.39	-0.23± 0.20	-0.16± 0.06*	-0.15± 0.13	0.14± 0.07
	Flagellate (>3.5 µm)	-0.42± 0.32	0.01± 0.15	0.23± 0.04**	0.06± 0.32	-0.17± 0.27
	Naked dinoflagellate	-0.18± 0.41	-0.05± 0.03	-0.14± 0.13	0.29± 0.17	0.03± 0.15
	Nitzschia	0.14± 0.06	-0.16± 0.13	0.07± 0.11	-0.71± 0.29	-0.26± 0.39
	Silicoflagellates	-0.29± 0.36	-0.20± 0.15	-0.17± 0.11	-0.09± 0.47	-0.39± 0.33
	Thecate dinoflagellate	0.02± 0.04	-0.67± 0.32	-0.04± 0.05	-0.67± 0.17*	0.13± 0.21
July/August	Centric diatom	0.07± 0.06	0.17± 0.06*	-0.26± 0.25	-0.32± 0.13	-0.14± 0.11
	Ciliate	0.21± 0.03**	0.17± 0.04*	0.29± 0.04**	0.10± 0.05	0.43± 0.01***
	Cryptomonad	-0.57± 0.22	-0.67± 0.33	-0.24± 0.38	-0.46± 0.25	0.15± 0.06
	Flagellate (< 3.5 µm)	0.28± 0.02***	-0.27± 0.34	-0.01± 0.17	0.19± 0.07	-0.47± 0.17
	Flagellate (>3.5 µm)	-0.79± 0.21*	-0.36± 0.33	0.20± 0.09	-0.16± 0.06*	0.03± 0.07
	Naked dinoflagellate	-0.03± 0.06	-0.37± 0.27	-0.63± 0.24	-0.01± 0.07	0.01± 0.09
	Nitzschia	0.05± 0.04	0.15± 0.03*	-0.25± 0.38	0.21± 0.06*	-0.53± 0.36
	Pennate diatom	-0.10± 0.09	0.16± 0.04*	-0.25± 0.38	-0.24± 0.34	-0.15± 0.04*
	Thecate dinoflagellate	0.05± 0.07	0.01± 0.03	-0.10± 0.11	0.04± 0.12	-0.34± 0.10*

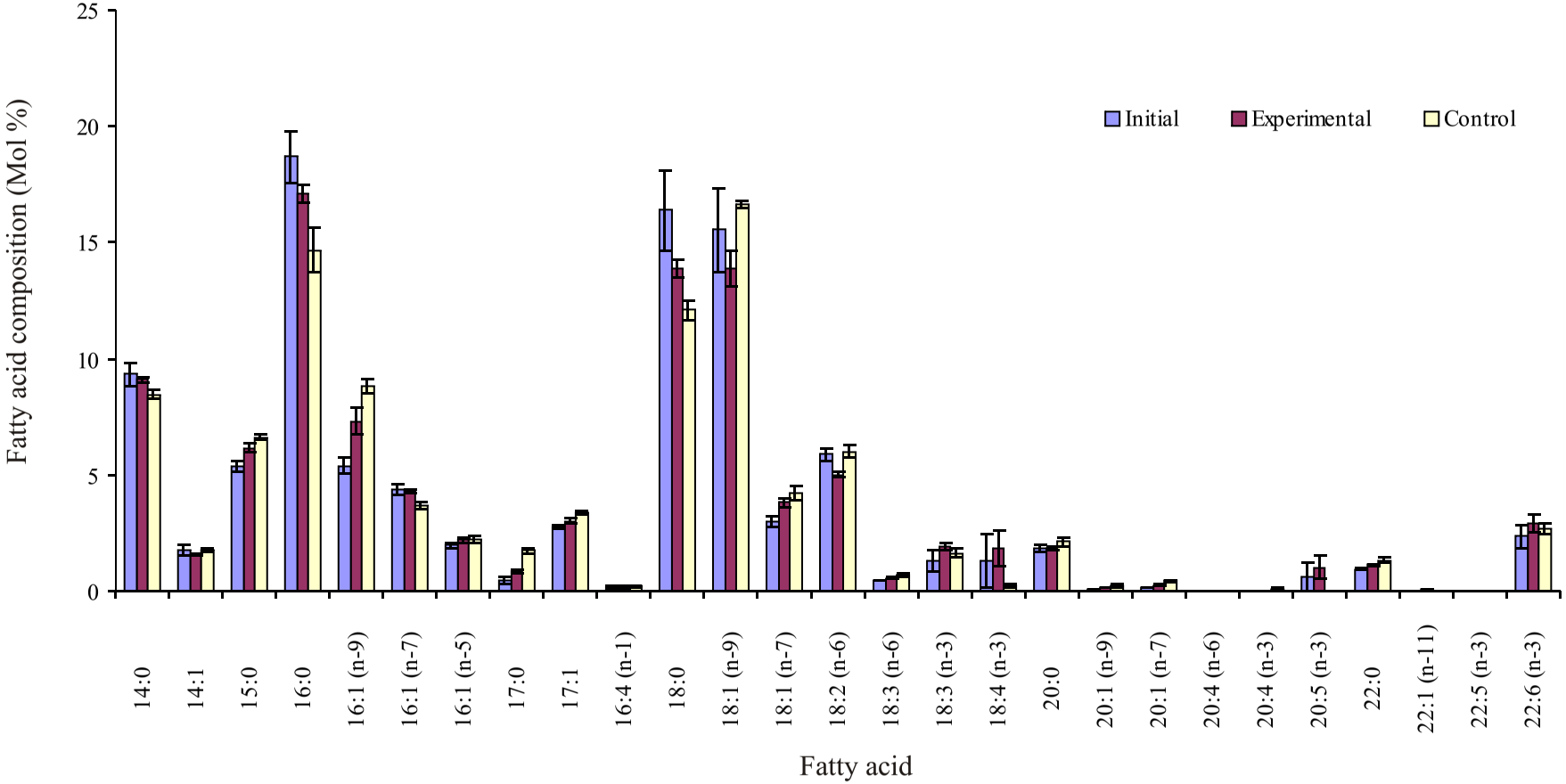


Figure 5.10. Particulate fatty acid composition (mol %) in the initial, experimental and control bottles at station 1 in April (± standard error).

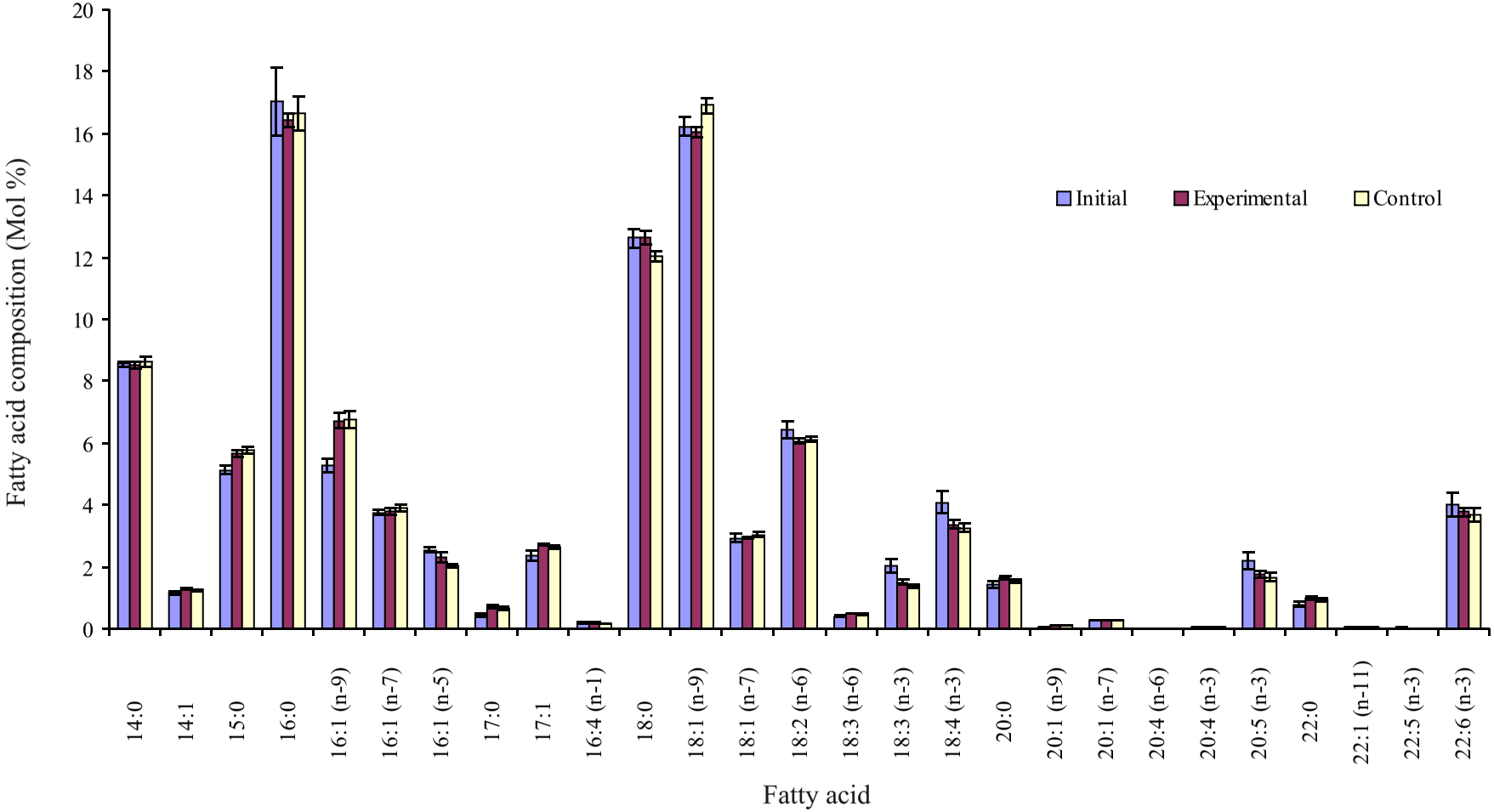


Figure 5.11. Particulate fatty acid composition (mol %) in the initial, experimental and control bottles at station 2 in April (± standard error).

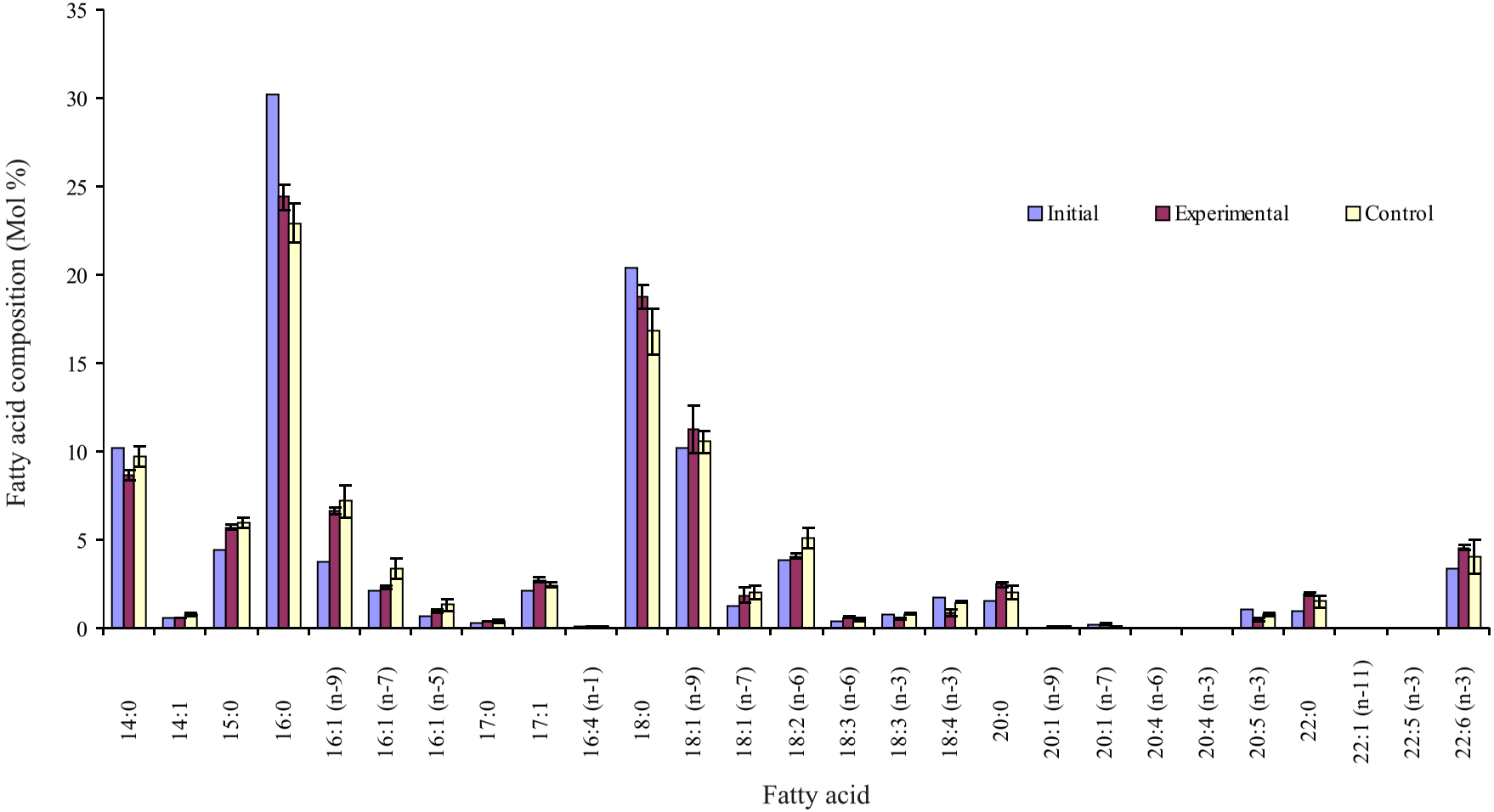


Figure 5.12. Particulate fatty acid composition (mol %) in the initial, experimental and control bottles at station 3 in April (± standard error).

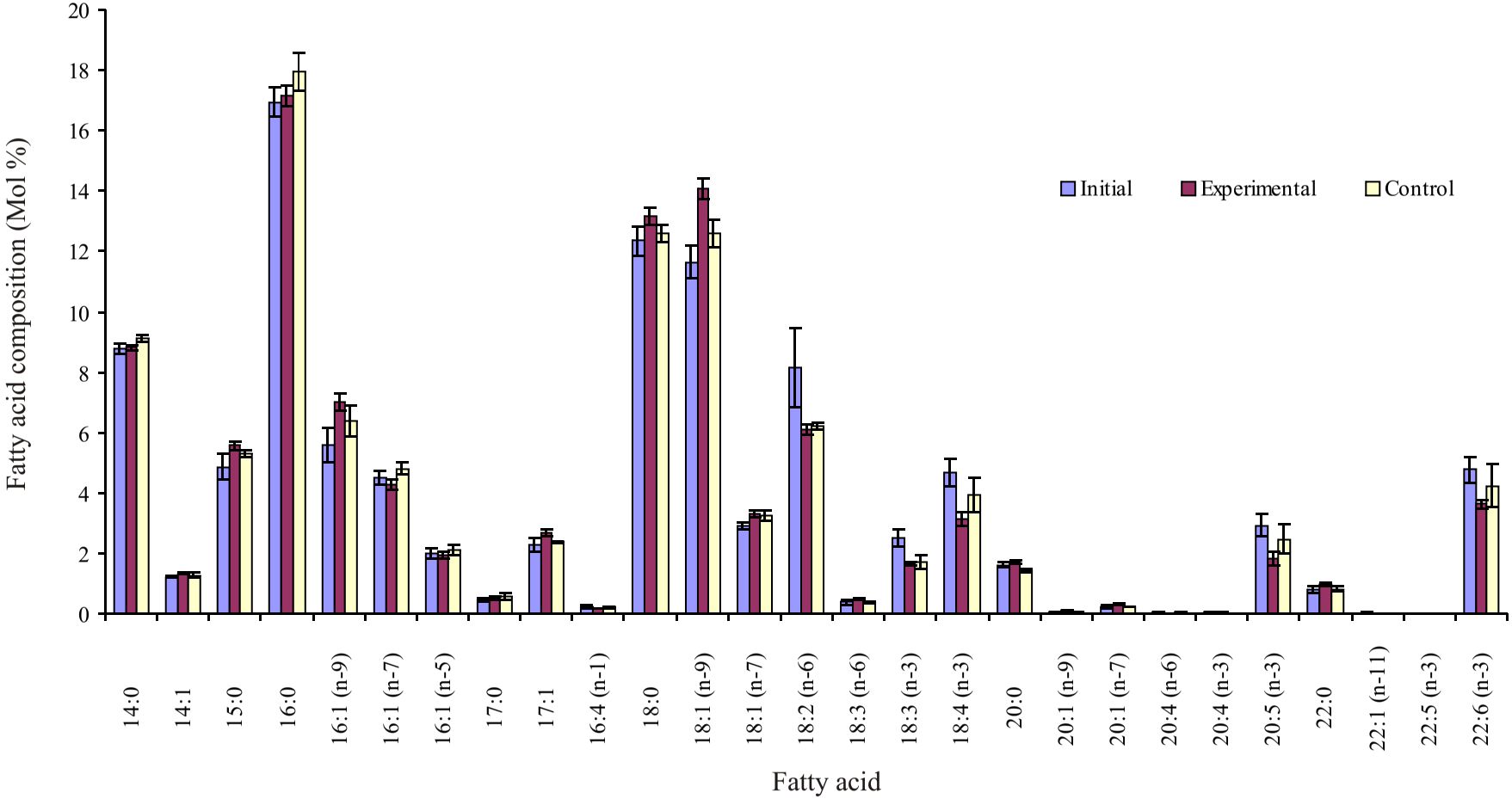


Figure 5.13. Particulate fatty acid composition (mol %) in the initial, experimental and control bottles at station 4 in April (± standard error).

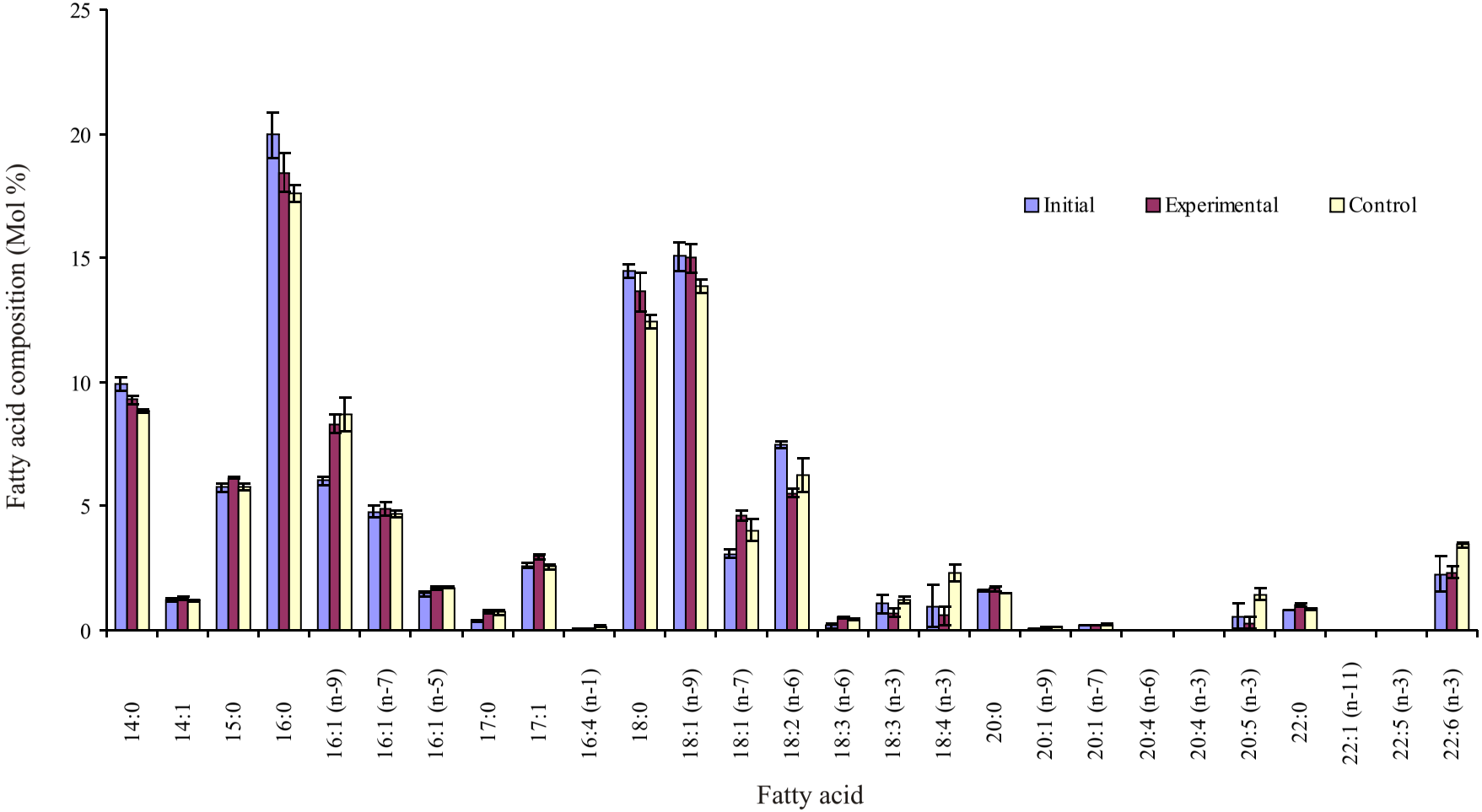


Figure 5.14. Particulate fatty acid composition (mol %) in the initial, experimental and control bottles at station 5 in April (± standard error).

being significantly different from zero (Table 5.2). Unfortunately, because the fatty acid composition of ciliates is variable, reflecting that of their recent prey (Harvey et al. 1997), the fatty acid compositional data in the experimental and control bottles cannot be used as to independently confirm the selection pattern for ciliates. There were no other detectable patterns in selection; both positive and negative values of E^* for each cell group were found. This was again confirmed by the fatty acid data (Figures 5.15 to 5.19). Pairwise comparisons between treatments only found a significant difference between the experimental and control bottles at station 3 (ANOSIM, $r = 0.374$, $p = 0.036$). The difference between these treatments was primarily due to larger quantities of the haptophycean marker, 18:2(n-6) (Table 1.1) in the control bottles (Figure 5.17).

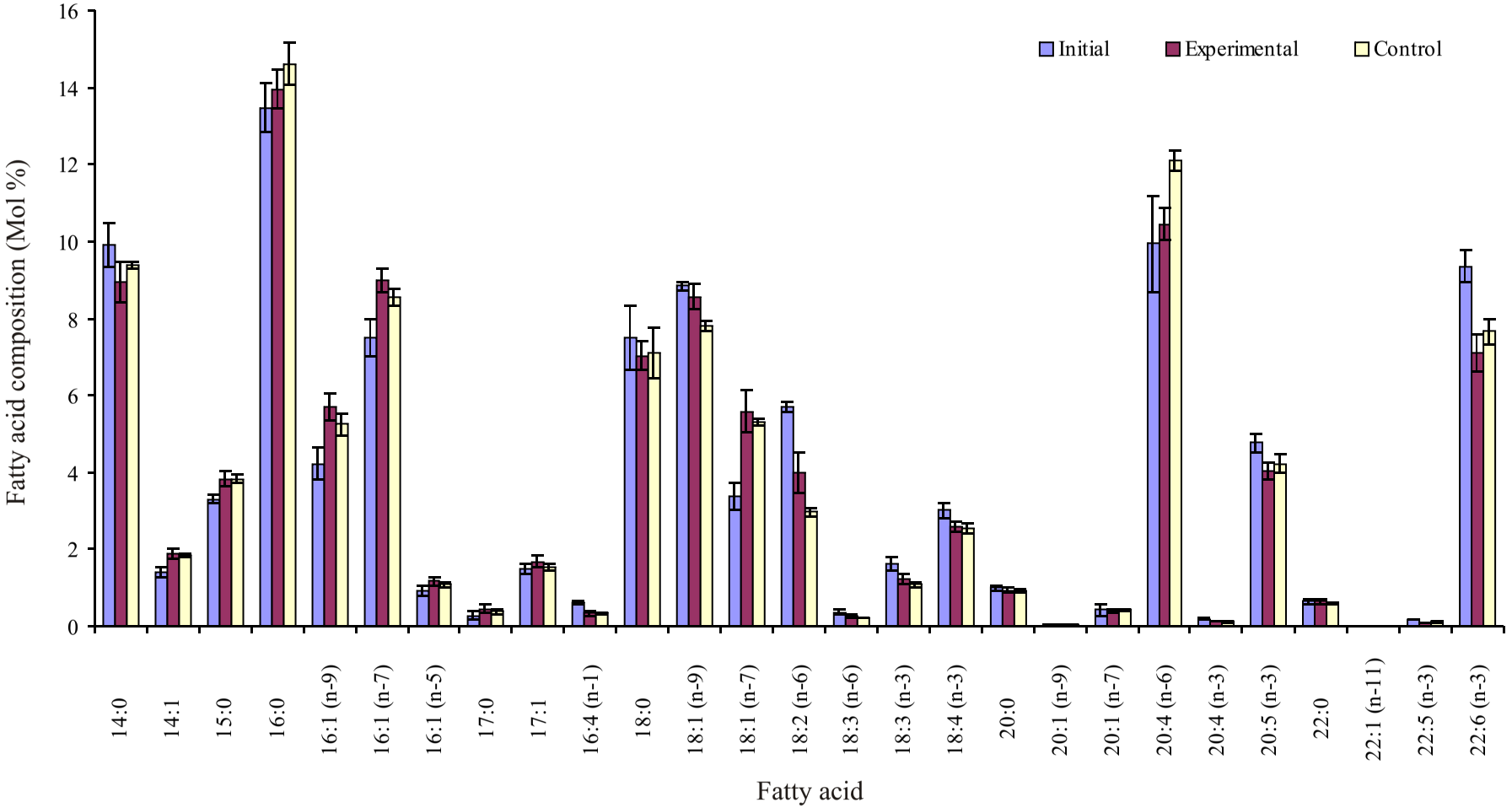


Figure 5.15. Particulate fatty acid composition (mol %) in the initial, experimental and control bottles at station 1 in July/August (± standard error).

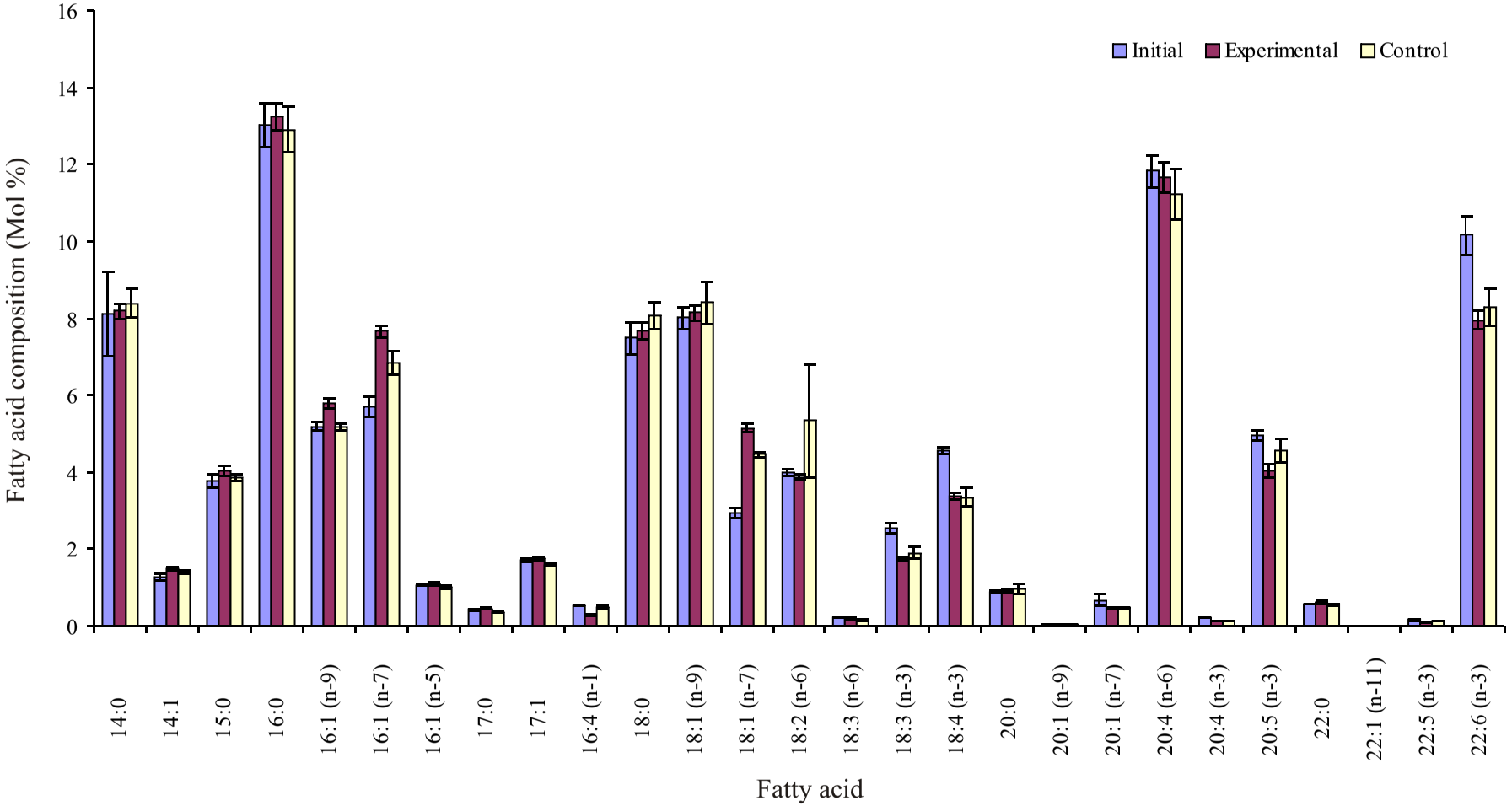


Figure 5.16. Particulate fatty acid composition (mol %) in the initial, experimental and control bottles at station 2 in July/August (± standard error).

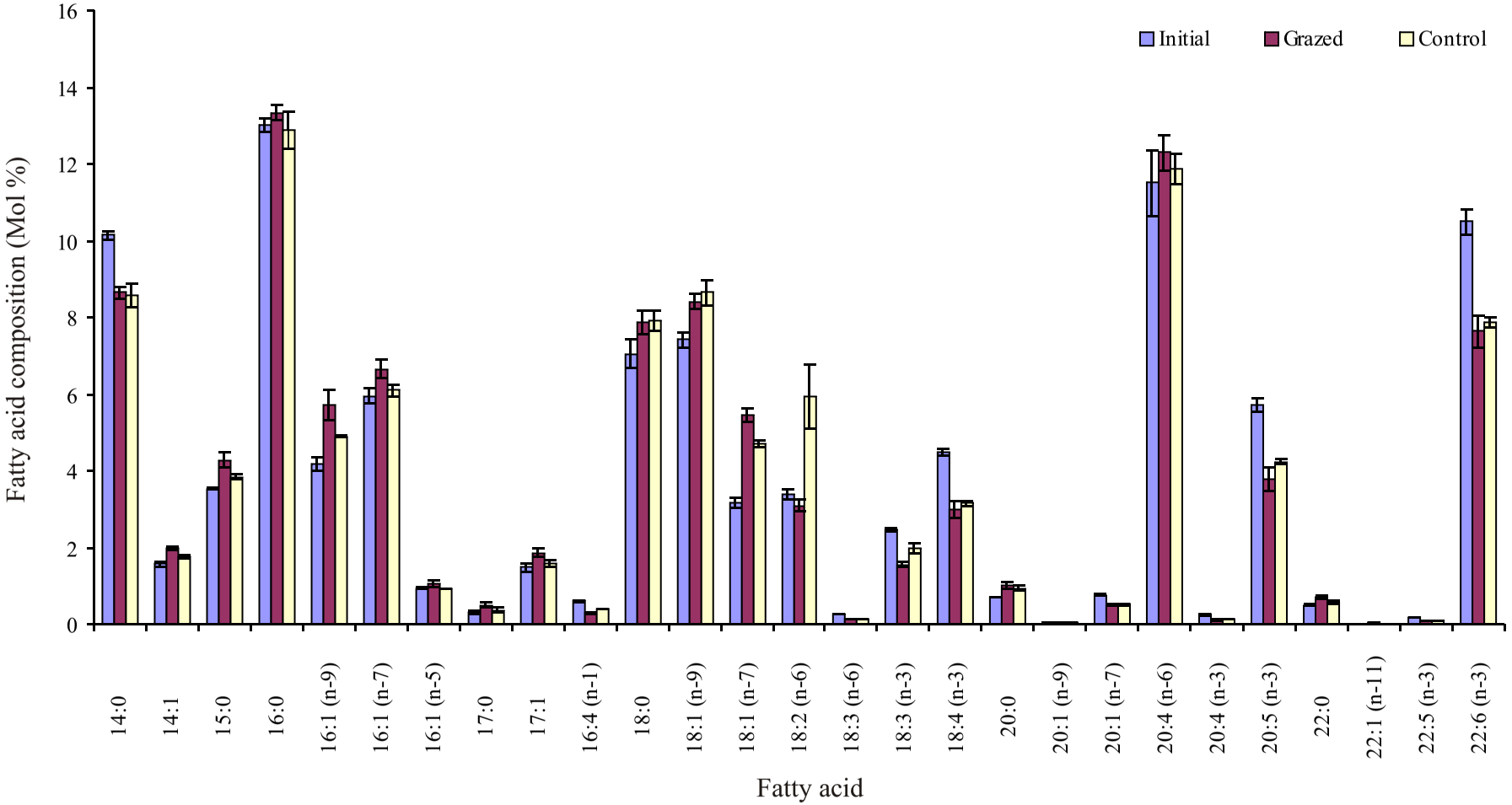


Figure 5.17. Particulate fatty acid composition (mol %) in the initial, experimental and control bottles at station 3 in July/August (± standard error).

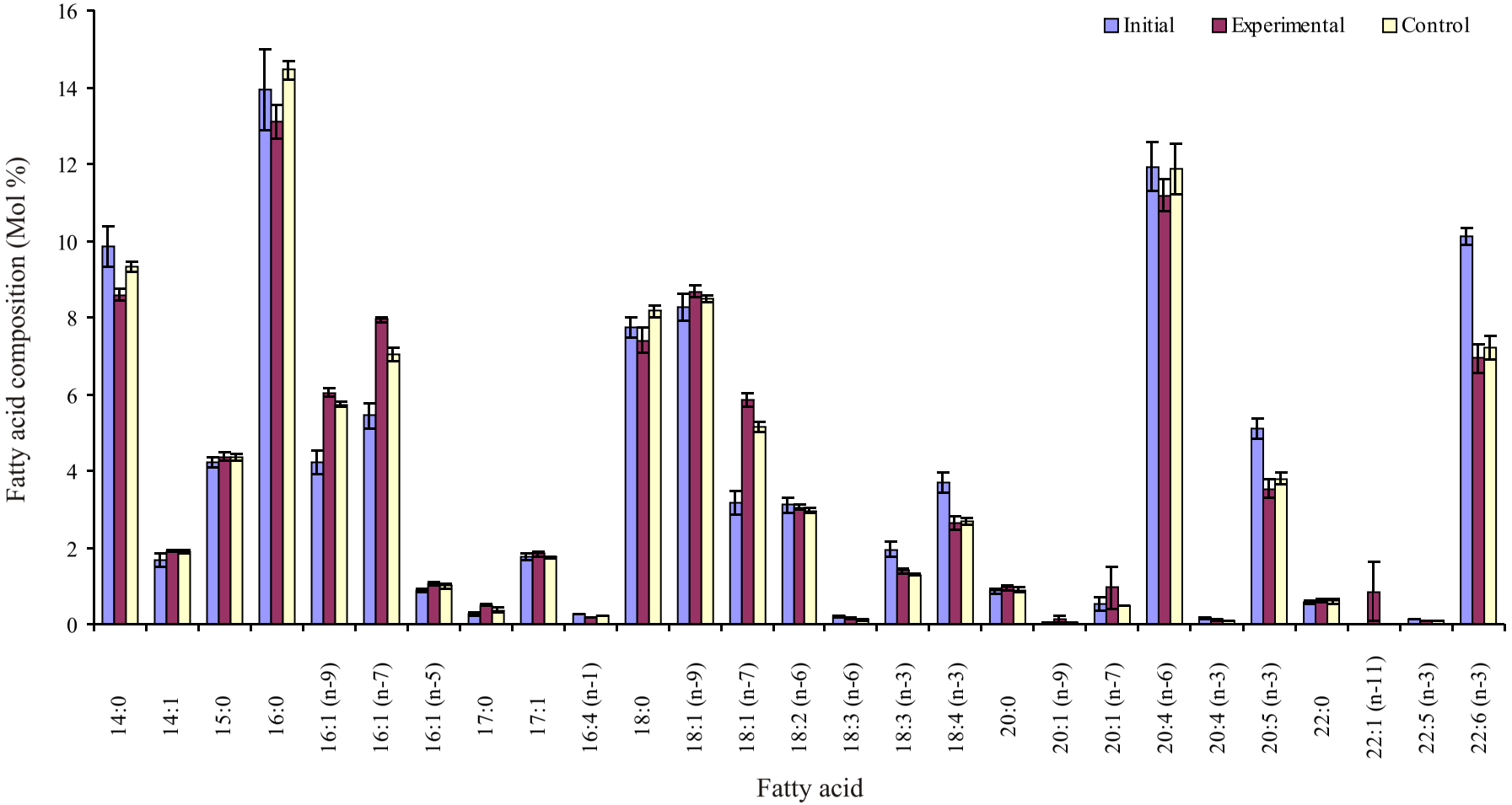


Figure 5.18. Particulate fatty acid composition (mol %) in the initial, experimental and control bottles at station 4 in July/August (± standard error).

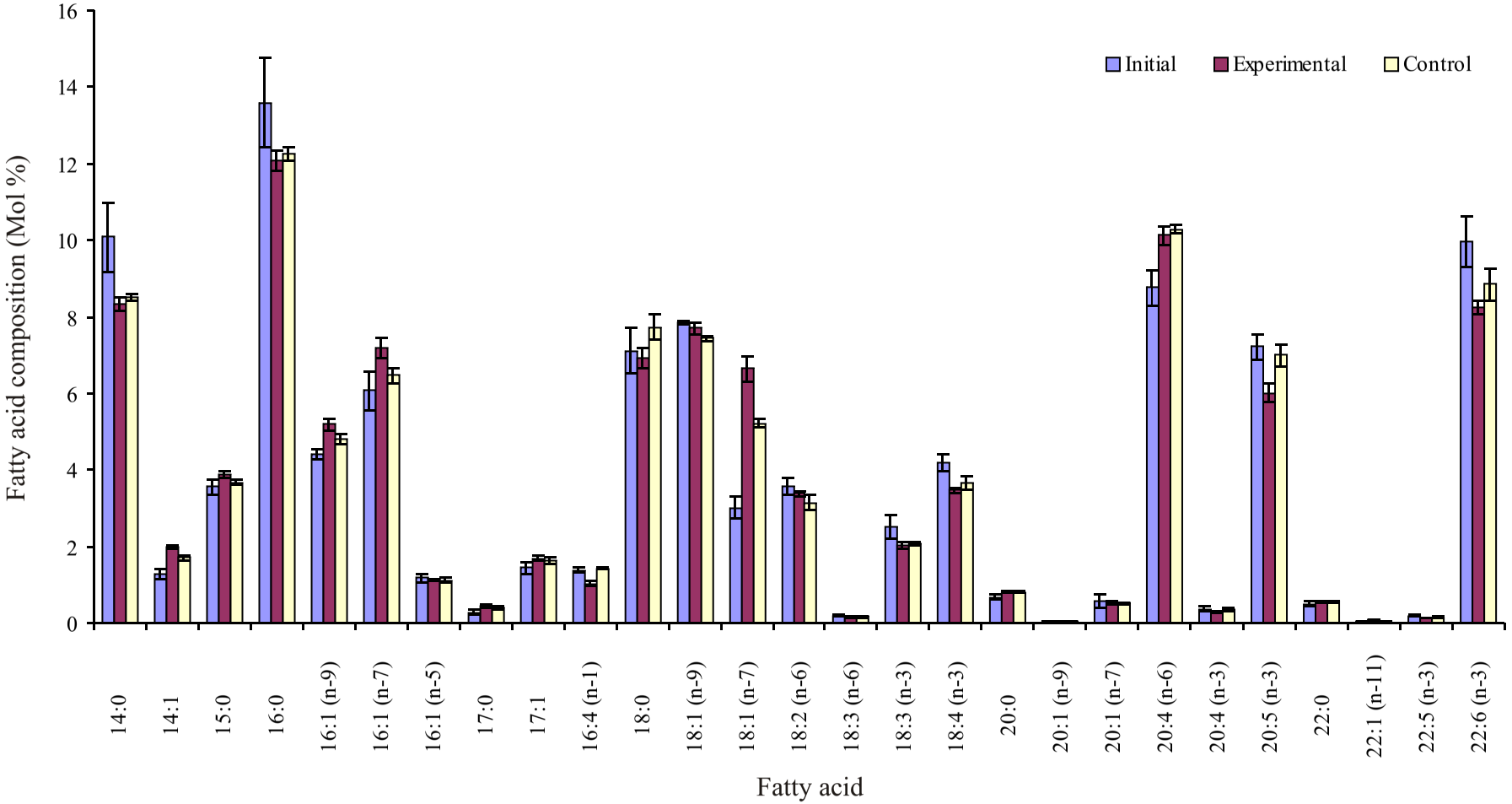


Figure 5.19. Particulate fatty acid composition (mol %) in the initial, experimental and control bottles at station 5 in July/August (± standard error).

5.4. DISCUSSION

5.4.1. Feeding and food preferences. Characterising the dietary intake of copepods feeding on natural plankton assemblages is difficult (Dagg et al. 1982). The work presented here represents the first *in situ* study of the food and feeding preferences of *C. finmarchicus* feeding on natural plankton assemblages under pre- and post-bloom conditions in the Irminger Sea. Clearance rates between 0 and 300 ml copepod^{-1} day^{-1}, as determined here, have previously been reported for *C. finmarchicus* feeding under similar conditions in the North Atlantic (Nejstgaard et al. 1997, Meyer-Harms et al. 1999, Levinsen et al. 2000b, Nejstgaard et al. 2001a, b). In the experiments presented here, *Calanus* was frequently observed to clear ciliates at the highest rates and often showed positive selection towards them, particularly during the July/August incubations. Furthermore, they typically constituted > 10 % of the total daily ration. These data confirm the findings of several other recent studies of *C. finmarchicus* feeding on natural prey assemblages (e.g. Barthel 1988, Ohman and Runge 1994, Nejstgaard et al. 1997, Levinsen et al. 2000b, Nejstgaard et al. 2001a, b). By assuming all flagellates (and cryptomonads) to be autotrophic (see Chapter 3), the heterotrophic component of the microplankton community (here assumed to be only ciliates and 50 % of the dinoflagellates) and estimations of its contribution to the diet are thus conservative. Unfortunately, because of preservation with Lugol's iodine, determination of flagellate trophic status using epifluorescent microscopy was not possible.

Whilst the Chi-squared Goodness of Fit test indicated that selection was generally present, it is suggested that this test is of limited use because it can only be applied to averaged data, and therefore does not take into account the variability within the data set. The electivity index, E^*, failed to reveal any significant trends in selection or avoidance for any particular cell group, other than ciliates. Despite contributing significantly to the diet in both seasons, dinoflagellates were not consistently selected for or against in either season. Moreover, the composition of the diet generally reflected that of the food environment. Excluding ciliates, this suggests that *Calanus* was suspension feeding, a behaviour that can be described as 'fixed' i.e. the composition of the diet is determined simply as a function of predator-prey encounter rate (Greene 1985). This idea is supported by the inconclusive electivity index, E^*, data, and also the lack of difference between fatty acid composition in the experimental and control bottles. The importance of a mixed diet has been

increasingly recognised as a strategy to ensure a complete nutritional ration (Kleppel 1993).

Non-selective feeding behaviour is supported by observations of *C. finmarchicus* feeding during the April bloom in the Labrador Sea, where phytoplankton were removed in direct proportion to their abundance (Huntley 1981). A similar 'fixed' removal of haptophytes and cryptophytes is reported from pre- to post-bloom conditions in the Norwegian Sea (Meyer-Harms et al. 1999). The predominance of flagellates and cryptomonads in the diet of *Calanus* is thus explained by their abundance in the food field, as previously reported under post-bloom conditions elsewhere (Meyer-Harms et al. 1999, Levinsen et al. 2000b). The composition of the diet of *C. helgolandicus* feeding in the English Channel also appears to reflect that of the microplankton community over an annual cycle (Irigoien et al. 2000c).

Typical daily rations of female *C. finmarchicus* range between 1.1 and 2.3 % (µg C ingested [µg C copepod]$^{-1}$ day^{-1} * 100) under pre-bloom conditions, and between 1.1 and 14 % under post-bloom conditions (Table 5.3). The pre- and post-bloom rations determined here generally agree with values previously reported in the literature (Tables 5.1 and 5.2), despite geographical differences in both microplankton assemblages and/or the C content of individual animals. Because no clear trends in selection were apparent other than that for ciliates, assuming fixed ratios for C:N, C:EPA and C:DHA in the ingested material was justifiable. The only existing study that has estimated the quantities of EPA and DHA ingested by a copepod in the field suggested that *Acartia tonsa* had specific ingestion rates of 180 and 236 % day^{-1} respectively (derived from the data in Table 4 of Hazzard and Kleppel 2003). Clearly these rates are much greater than those determined in the present study (Table 5.1). However, specific ingestion rates of *Acartia* are greater than those of *Calanus* (e.g. Kiorboe et al. 1985b). Furthermore, the concentrations of EPA and DHA in the seston reported by Hazzard and Kleppel (2003) were between 1 and 2 orders of magnitude greater than reported here, making comparison between the studies difficult. Regardless of these differences, the data presented here are significant in that they represent the first estimates of EPA and DHA ingested by *C. finmarchicus*.

5.4.2. Particulate dynamics. Considering the complex nature of natural seawater assemblages (see Chapter 3, Appendix 2), it should perhaps not be surprising to find that the different particulate descriptors provided contradictory information about the tropho-dynamics in the experimental bottles. Microzooplankton grazing in the control bottles may explain why cell biomass in the experimental bottles was not always significantly reduced relative to the controls. This can be demonstrated by a simple, coarse examination of the data: Averaged over each seasonal 5 day experiment, the biomass of heterotrophic microzooplankton at the start of the incubations (assumed to be all ciliates and 50 % of dinoflagellates; Chapter 3) was approximately 7.2 and 15.2 µg C in April and July/August respectively (Table 3.6). A conservative estimate of biomass-specific microzooplankton community grazing in the North Atlantic is 2 µg C [µg C µzoo]$^{-1}$ day^{-1} (derived from table 8 in Verity et al. 1993b), suggesting that a total of 14 and 30 µg C day^{-1} would be removed by the microzooplankton community in the control bottles during the April and July/August incubations respectively. By comparison, copepods have much lower biomass specific ingestion rates. *C. finmarchicus* feeding under pre- and post-bloom conditions consume approximately 2 % and 5 % of their body C day^{-1} (i.e. 0.02 and 0.05 µg C [µg C copepod]$^{-1}$ day^{-1} under pre- and post-bloom conditions; Table 5.2). With 10 females in each bottle, each containing, on average 75 µg C in April and 101 µg C in July/August, the copepod community would be expected to remove approximately 15 and 50 µg C day^{-1} in the April and July/August incubations respectively. Evidently, it is quite possible that the copepod and microzooplankton community ingestion rates were similar in the experimental and control bottles during the incubations presented here, possibly explaining why the experimental bottles were not always significantly reduced relative to the controls.

Cell biomass determinations were based solely on intact and healthy looking cells, whereas the samples collected on GFF filters (fatty acid, POC/PON) contained not only the microplankton cells, but also unidentified detrital material. Indeed, a large proportion of the organic C determined in both seasons was attributable to detritus (Chapter 4). Changes in the quantities of POC and PON in the experimental and control bottles therefore do not provide information about grazing on microplankton cells, but rather changes in the detrital loading of the water. POC/PON in the experimental bottles was never significantly reduced relative to the

controls, indicating that detritus did not contribute to the daily ration of *Calanus* in either season. Similarly, because detritus contains primarily saturated and monounsaturated fatty acids (Hama 1991, 1999), these can also be used to provide information about the ingestion of detritus. Total fatty acids were typically not reduced in the experimental bottles relative to the controls, again suggesting that detritus was not ingested by *Calanus*. In contrast, the majority of polyunsaturated fatty acids (PUFAs) in particulate material are associated with living phytoplankton (Hama 1999), and should therefore provide a more realistic understanding of the microplankton dynamics. In April, the patterns in the PUFA dynamics agreed with those observed in the cell biomass data i.e. the experimental bottles were typically reduced relative to the controls. However, in July/August the PUFA dynamics do not mirror those of the microplankton biomass, and their quantities increase in the experimental bottles relative to the controls at three of the five stations (1, 2 and 5; Figure 5.2B). This is quite possibly an indication that at these stations, microzooplankton grazing in the control bottles outweighed that of the copepods. Conversely, these results may be attributable to increased bacterial growth rates in the experimental bottles due to copepod excretion (see Zubkov and Lopez-Urrutia 2003). They may also be due to the microplankton cells producing different quantities of fatty acids in the experimental and control bottles because of some difference in conditions (see Thompson and Harrison 1992). However, there are insufficient data available from these experiments to test these hypotheses.

5.4.3. Predicting ingestion using particulate descriptors. Experimental data suggest that the quantities of autotrophic cells ingested by *C. finmarchicus* can be reliably predicted from ambient chlorophyll levels (Hansen et al. 1990b, Irigoien et al. 2000c). However, considering that recent work has demonstrated that a large proportion of the diets of copepods is in fact derived from non-chlorophyll bearing heterotrophic protists (see Section 1.4), any such estimations are likely to underestimate the actual daily ingestion rate. Since the abundance of heterotrophic microzooplankton is not necessarily proportional to that of the autotrophic phytoplankton (e.g. many heterotrophic flagellates and ciliates are bactiverous), estimations of the daily ingestion rate cannot easily be 'corrected' to incorporate the microzooplankton component of the diet. Therefore, chlorophyll-derived estimates of daily rations are of limited use for bio-energetic studies. The lack of any significant

correlation between chlorophyll *a* concentrations and the daily ingestion rate (ANOVA; $p = 0.871$, $R^2 = 0.0041$, Figure 5.9D) confirms that microzooplankton do not appear in the diet at a fixed ratio with auto trophic prey, and also that ambient chlorophyll concentrations cannot be used to estimate copepod daily rations. Conversely, the significant linear relationship between microplankton biomass and ingestion rates determined here (ANOVA; $p < 0.001$, $R^2 = 0.875$, Figure 5.9B) illustrates that over the range of ambient food concentrations encountered, the feeding rate of *C. finmarchicus* can be predicted from ambient cell biomass concentrations according to the formula:

$$\text{Ingestion } (\mu g\ C\ copepod^{-1}\ day^{-1}) = 0.331 + (0.0651 \times \text{Cell biomass } (\mu g\ C\ l^{-1})$$

The high coefficient of determination (R^2), illustrates that such predictions can be made with a reasonable degree of accuracy. In addition, the strong correlation between these variables indicates that the feeding of *Calanus* did not saturate during the incubations. This agrees well with laboratory and *in situ* data, which both suggest that feeding does not saturate at food concentrations encountered in the natural environment (Frost 1972, Irigoien et al. 1998, 2000c, Bamstedt et al. 1999), even under spring bloom conditions (Huntely 1981).

5.5. SUMMARY

In summary, the particle removal experiments in April and July/August both revealed that cell biomass in the experimental bottles was always reduced relative to the controls. In contrast, the trends observed in the cell biomass, total fatty acid, polyunsaturated fatty acid, POC and PON data generally contradicted each other. Importantly, this illustrates that each of these measurements provides different information about the complex nature of the dynamics that operate within natural seawater assemblages. It was concluded that cell count data provided the most useful information about the grazing rates of *C. finmarchicus* because they were based on viable microplankton cells, rather than bulk properties of the seston.

Clearance and ingestion rates determined in both seasons agree well with values previously determined under similar conditions in the North Atlantic. The female *C. finmarchicus* had specific ingestion rates of 1.5 and 5.1 % day^{-1} in April and July/August respectively. Ciliates were typically cleared at higher rates than

other cell groups, and the electivity index, E^*, indicated that they were positively selected in both seasons, particularly during the post-bloom (July/August) incubations. No other consistent trends in selection were apparent, and the composition of the diet generally reflected that of the microplankton community.

In both seasons, the diet of *Calanus* was dominated by flagellates. Ingestion rates were significantly higher in the July/August incubations. This was a reflection of the increased availability of prey cells, as demonstrated by a highly significant relationship between cell biomass and ingestion rates. The determined ingestion rates of C and N were not sufficient to meet typical respiration and excretion rates in April, whereas during the July/August incubations, ingested material was in excess of basal metabolic demands (discussed later). The daily quantities of EPA and DHA ingested in July/August were approximately 17 times greater than in April. This was because of greater ingestion rates and increased intracellular concentrations of these PUFAs in the microplankton during the summer.

Chapter 6

The biochemical composition of the eggs of *Calanus finmarchicus* and the changes that occurred to the animals during experimentation in April and July/August

6.1 AIMS

This chapter describes egg production rates, and also the biochemical composition of the experimental females and the eggs they produced during the incubations in April and July/August. Homeostasis of essential components is a crucial assumption of stoichiometric theory, and the extent to which this is justified is examined by inter-seasonal comparison of C, N, EPA and DHA in the animals and eggs. The biochemical composition of the eggs will be used in Chapter 7 to set the stoichiometric requirements of the animals. Comparing the biochemical composition of the females before, and after, experimentation in each season offers an insight into the animals physiological condition during the incubations. Furthermore, it provides information on the animals biomass as a possible source or sink for the various biochemical components. These data are subsequently used to construct balanced elemental budgets in Chapter 7.

6.2. METHODS

The methods for collection and incubation of the female *C. finmarchicus* are presented in Chapter 2. Before and after experimentation in April and July/August, replicate groups of 5 females were collected and later analysed for their C/N and fatty acid content. Similarly, at the end of each 24 hr incubation, all the eggs produced were removed for biochemical analysis.

6.2.1. Statistical methods.

All data were tested for the assumptions of parametric statistics (Section 2.7). Differences between absolute quantities of C, N, EPA, DHA and total fatty acids in the pre- and post-experimental animals were examined using t-tests. Similarly, the quantities of these substrates in the eggs collected in April and July/August were compared using t-tests. The non-parametric tests, ANOSIM and SIMPER (Section 4.2.1, Clarke and Warwick 1994), were used to investigate differences in the fatty acid compositions of the pre- and post-experimental females, and also how the fatty acid composition of the eggs differed between the seasons. ANOSIM and SIMPER tests were also used to compare the fatty acid compositions of the eggs to those of the particulates and the parents in both seasons.

6.3. RESULTS

6.3.1. Elemental composition of *C. finmarchicus*. To ensure detectable quantities of material, replicate groups of 5 females were analysed. The C and N content of pre- and post-experimental females from April and July/August are presented in Figures 6.1 and 6.2 respectively.

6.3.1.1. April. Pre-experimental females contained 87.6 (± 2.4) µg of C and 23.9 (± 0.8) µg of N. Upon termination of the 5 day incubation period, the experimental females contained 62.4 (± 1.6) µg of C and 17.8 (± 0.4) µg of N. The loses of C and N were both significant (t-test, $p = 0.003$ and $p = 0.007$ respectively), with daily loss rates averaging 5.8 % and 5.1 % of the C and N biomass respectively. This observation confirms that *Calanus* loses biomass during the months preceding the spring bloom (Tande 1982, Irigoien et al. 1998). The C:N ratio of the biomass lost was 4.1 (µg μg^{-1}), which was very similar to that of protein (Vollenweider 1985). It appears that in April, the animals were using their biomass to meet energetic and reproductive demands not fulfilled by the ingested material (see Section 5.3.2.1.). The average C:N ratio (mass specific) of the females fell from 3.7 at the beginning of the experiment, to 3.5 by the end, although these differences were not significant (t-test, $p = 0.058$).

6.3.1.2. July/August. The elemental composition of the experimental females was more variable in July/August. Prior to incubation, the females contained 88.5 (± 3.8) µg of C and 21.0 (± 1.0) µg of N. After the feeding experiments, the females contained 108.6 (± 6.7) µg of C and 28.5 (± 0.9) µg of N. Over the duration of the experiment, the females had gained significant quantities of C (t-test, $p = 0.036$) and N (t-test, $p < 0.001$). The biomass gained during the incubations had a C:N of around 3 (µg μg^{-1}). In direct contrast to April, this suggests that the females had gained protein. Indeed, at the start of the experimental period, the average C:N ratio of the females was 4.2. As a result of the increase in C and N, the ratio had decreased significantly to 3.8 by the end of the experiment (t-test, $p = 0.037$).

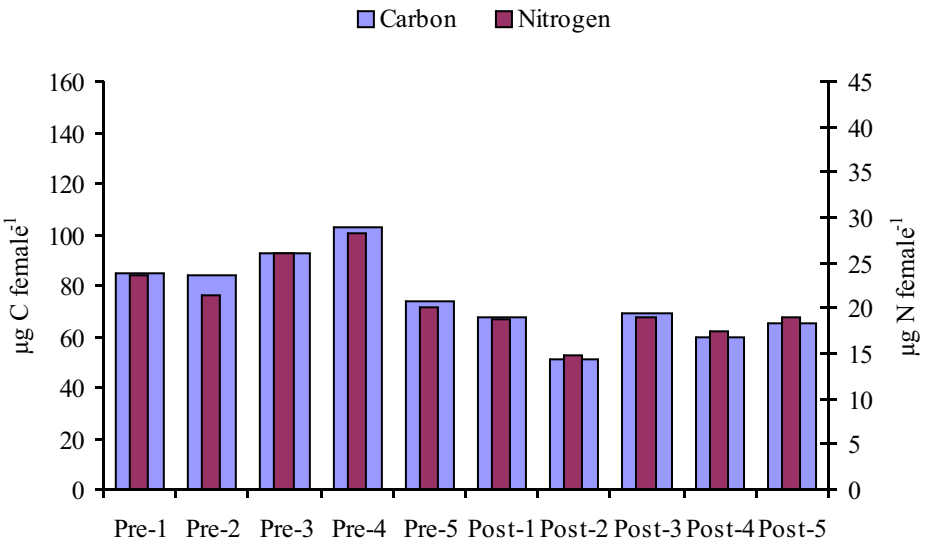


Figure 6.1A. Quantities of carbon and nitrogen in pre- and post-experimental females in April

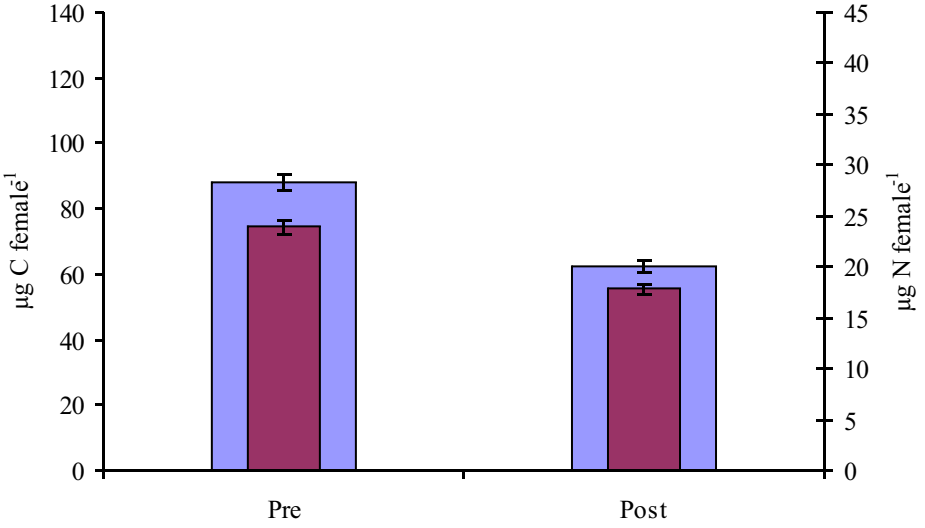


Figure 6.1B. Average quantities of carbon and nitrogen in pre- and post-experimental females in April

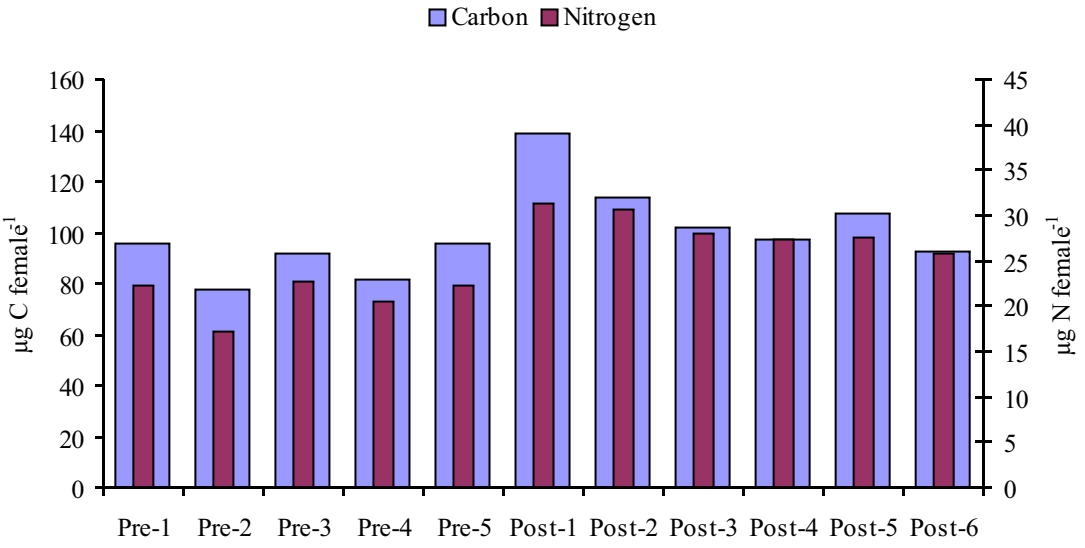


Figure 6.2A. Quantities of carbon and nitrogen in pre- and post-experimental females in July/August.

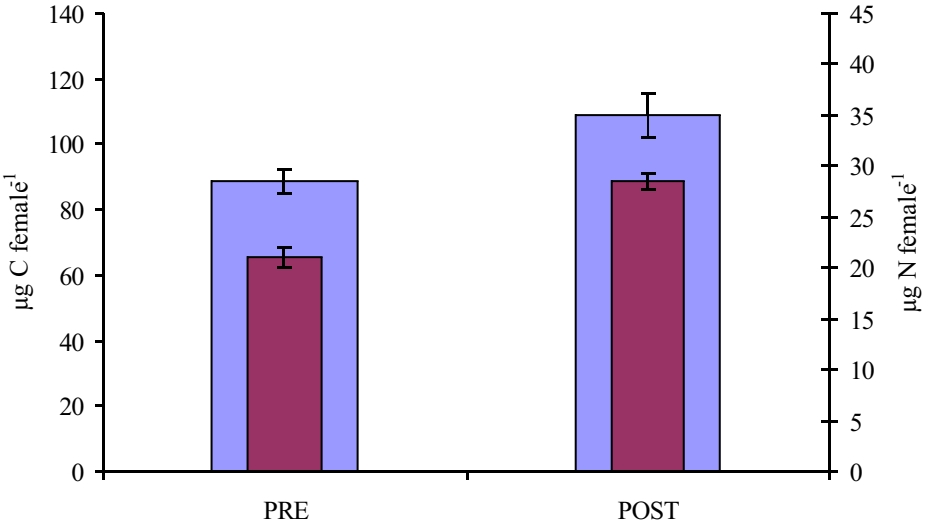


Figure 6.2B. Average quantities of carbon and nitrogen in pre- and post-experimental females in July/August.

6.3.2. Fatty acid composition of *C. finmarchicus*. The quantities of individual fatty acids and the fatty acid composition of pre- and post-experimental females from April and July/August are shown in Figures 6.3 to 6.4, and 6.5 to 6.6 respectively.

6.3.2.1. April. The females collected in April were essentially devoid of the storage fatty acids 20:1(n-9) and 22:1(n-11). Prior to incubation, each female contained 6.55 (± 0.17) µg of fatty acid. The fatty acids 16:0, 20:5(n-3) (EPA) and 22:6(n-3) (DHA) were dominant, each contributing > 15 % to the overall composition. After the 5 day incubation, the total quantity of fatty acid was significantly reduced (t-test, $p < 0.001$), and each female subsequently contained 4.29 (± 0.30) µg of fatty acid. These losses substantiate those observed in the elemental data (above). The fatty acids 16:0, 20:5(n-3) (EPA) and 22:6(n-3) (DHA) remained the dominant moieties in the post-experimental females, despite significant loss of each of these (t-test, $p < 0.05$ in all cases) during the incubation. It is possible that PUFAs are stored, and only catabolised after the major lipid reserves are exhausted, as found here. However, the strong association between PUFAs and cell membranes, and the low C:N of the biomass lost during the incubations strongly suggests that the experimental females were catabolising protein-rich muscle cells in order to sustain metabolic demands and maintain a reproductive output.

6.3.2.2. July/August. Experimental females contained 11.71 (± 1.88) µg of fatty acid before the incubations. As found in April, 16:0, EPA and DHA were the dominant fatty acids. In addition, 14:0, 16:1(n-7), 20:1(n-9) and 22:1(n-11) were also prominent, each contributing > 7 % to the total fatty acid composition (Mol %). By the end of the experiment, each female contained 9.15 (± 1.12) µg of fatty acid, although this apparent loss was not statistically significant (t-test, $p = 0.377$). Similarly, although the average quantities of EPA and DHA were lower in the post-experimental females, these differences were not significant (t-test, $p > 0.3$ in both cases).

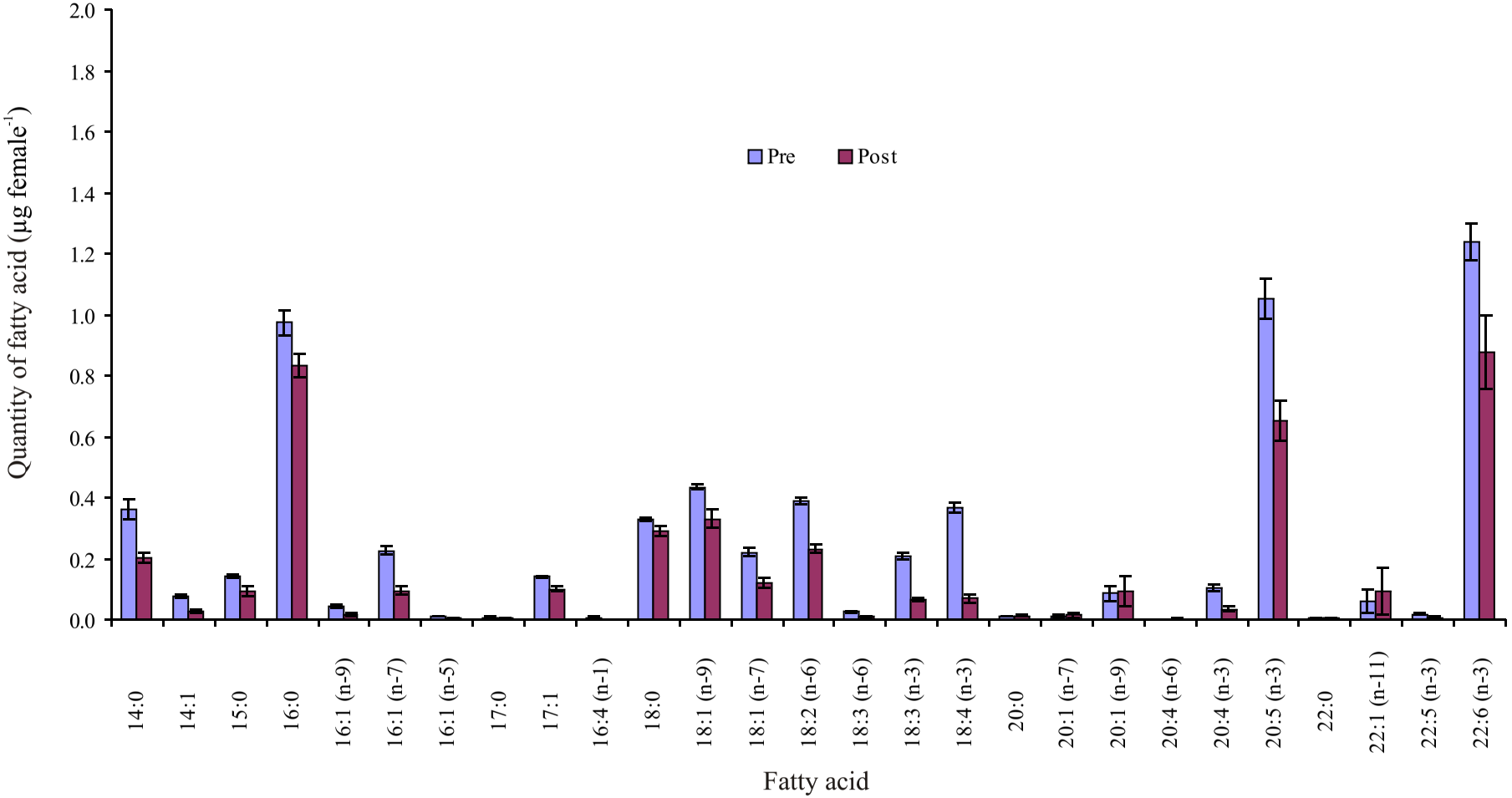


Figure 6.3. Quantities of individual fatty acids (µg female^{-1}) in the pre- and post-experimental females in April (± standard error).

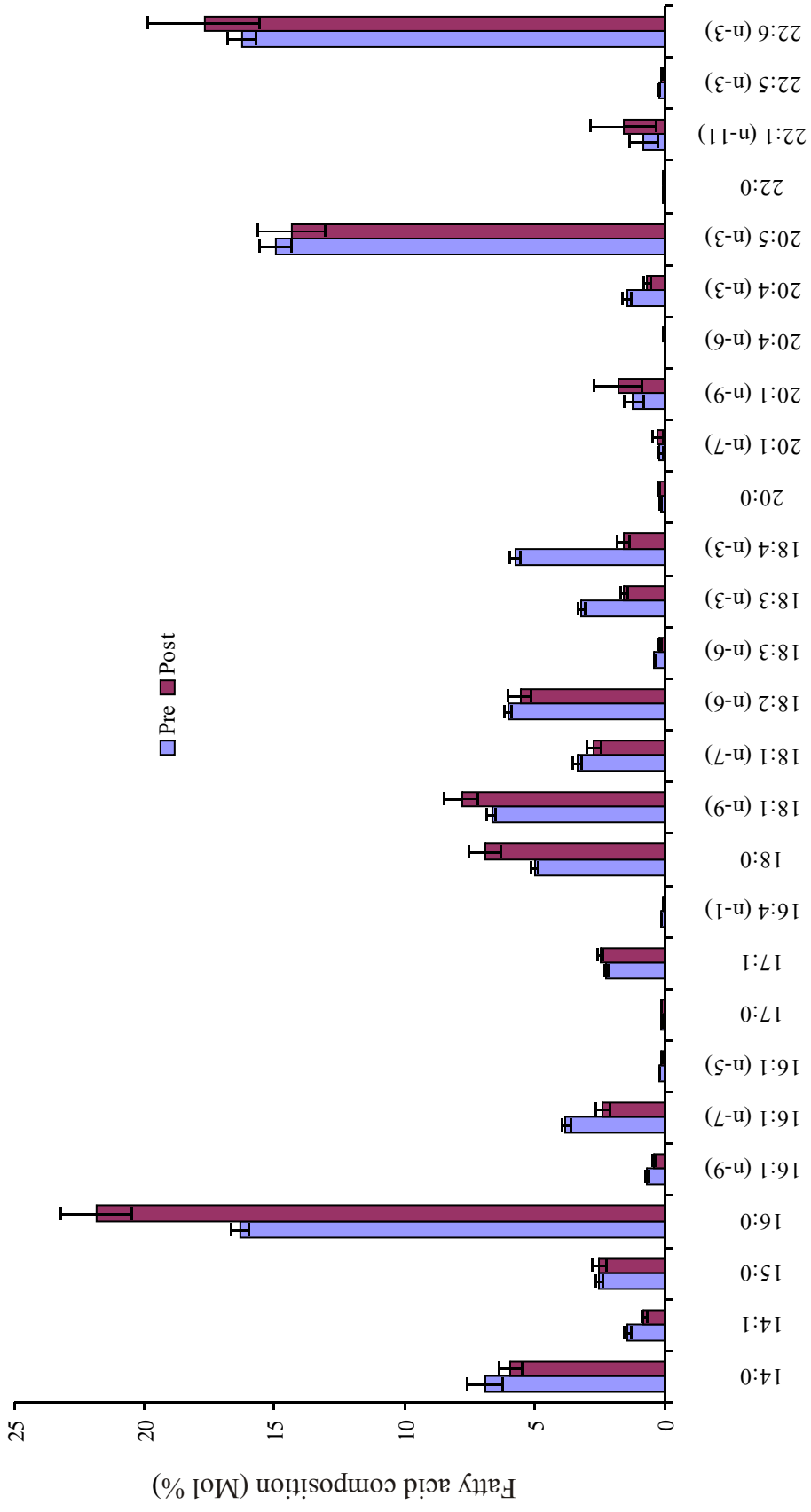


Figure 6.4. Fatty acid composition (Mol %) of the pre- and post-experimental females in April (± standard error).

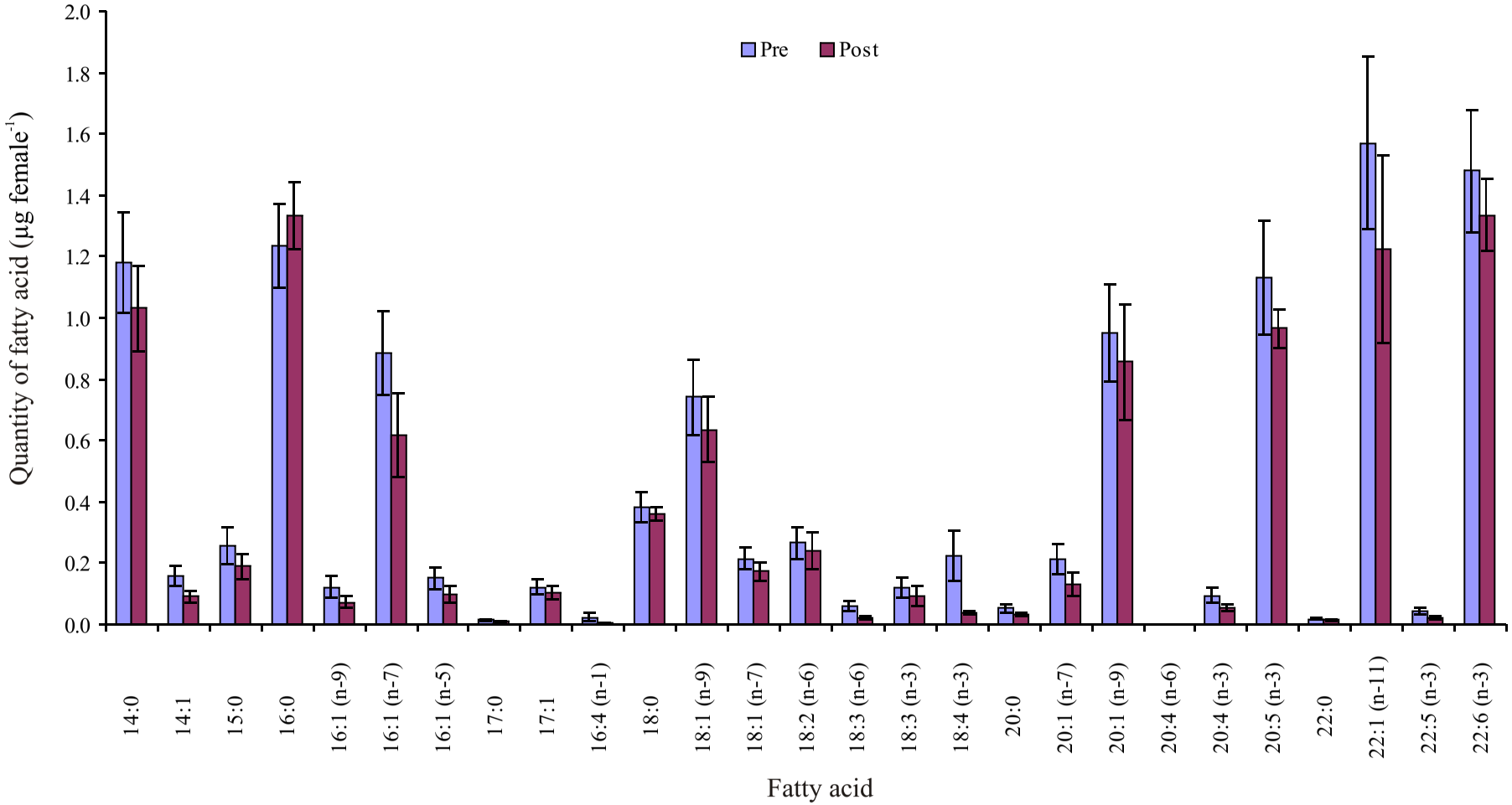


Figure 6.5. Quantities of individual fatty acids (µg female^{-1}) in the pre- and post-experimental females in July/August (± standard error).

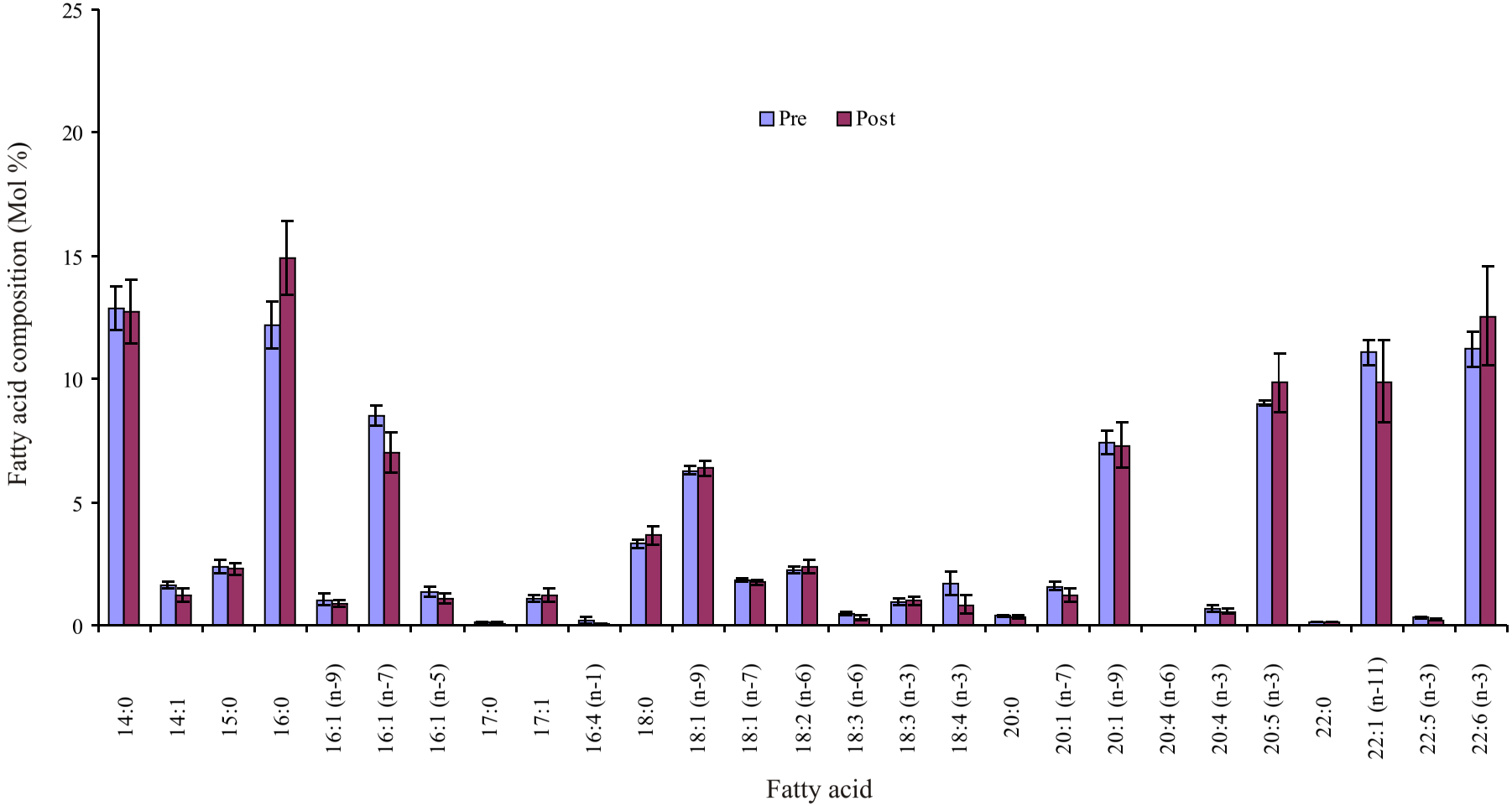


Figure 6.6. Fatty acid composition (Mol %) of the pre- and post-experimental females in July/August (± standard error).

6.3.3. Quantitative comparison of the fatty acid content (µg female^{-1}) and compositions (mol %) between pre- and post-experimental females: Multivariate approach

6.3.3.1. April. The quantities of individual fatty acids (µg female^{-1}) in pre- and post-experimental females (Figure 6.3) were significantly different in April (ANOSIM, r = 0.723, p = 0.004). These differences were primarily attributable to the reduction of DHA, EPA and 18:4(n-3) in the post-experimental females, suggesting that n-3 PUFAs had been preferentially catabolised during the incubations. Interestingly, the quantities of 20:1(n-9) and 22:1(n-11) did not differ between pre- and post-experimental females (t-test, p > 0.05 in both cases), demonstrating that these storage moieties were exhausted prior to experimentation. SIMPER analysis revealed that the fatty acid compositions (Mol %) of pre- and post-experimental females (Figure 6.4) differed from each other by 16.5 %. These differences were also statistically significant (ANOSIM, r = 0.475, p = 0.009), and primarily driven by (in decreasing importance) 16:0, DHA, and 18:4(n-3).

6.3.3.1. July/August. In July/August, the quantities (µg female^{-1}) of individual fatty acids were lower in the post-experimental females (Figure 6.5), although these differences were not significant (ANOSIM, r = 0.008, p = 0.377). This supports the idea that the females had gained protein biomass rather than C storage compounds such as lipid during the incubations. SIMPER analysis revealed that the fatty acid compositions (Mol %) of pre- and post-experimental females (Figure 6.6) only differed by 13.42 %. These differences were also statistically indistinguishable (ANOSIM, r = -0.073, p = 0.771), suggesting that there was little or no net fatty acid storage or catabolism over the duration of the experiments.

6.3.4. Inter-seasonal comparison between pre-experimental females. To avoid any potential bias due to experimental artefacts, a comparison of the females from April and July/August was made using only pre-experimental females.

The quantities of C and N in pre-experimental females from April and July/August did not differ significantly (t-test, p > 0.1 in both cases). Similarly, the quantities of EPA and DHA in pre- and post-bloom females were not significantly different (t-test, p > 0.2 in both cases), suggesting that C, N and these essential fatty

acids occur at fixed ratios in *C. finmarchicus*. Although these data support the idea of homeostasis, they are inconclusive. Homeostasis can only be confirmed by observing the same ratios in animals with significantly different quantities of C (hence N, EPA and DHA).

The total quantity of fatty acid in females collected in July/August was significantly greater (t-test, $p = 0.026$), which appears to contradict the elemental data. Although prosome measurements were not made, these data suggest that the females in July/August were smaller than those from April. The quantities of individual fatty acids were also significantly greater in the females sampled during the post-bloom period (ANOSIM, $r = 0.684$, $p = 0.008$). More than 50 % of the observed differences were attributable to the increased quantities of 22:1(n-11), 20:1(n-9) in the females in July/August (Figure 6.7). These energy-rich storage moieties were more abundant in the females collected in July/August (t-test, $p < 0.001$ and $p = 0.008$ respectively), demonstrating that the quantities of non-essential fatty acids are not homeostatic. The fatty acid compositions (Mol %) of the females were, on average, 93.43 % similar (SIMPER analysis) in April, and 91.02 % similar in July/August. Seasonal differences between the compositions of females were also significant (ANOSIM, $r = 1$, $p = 0.008$), with the animals differing by 31.38 % (SIMPER analysis). Variations in 22:1(n-11), 20:1(n-9) and 14:0 accounted for the majority of the observed inter-seasonal differences (Figure 6.8). In addition, the females in April were relatively rich in 18:4(n-3) and DHA, the trophic markers for flagellated protists (Table 1.1), whereas those in July/August contained a greater proportion of 16:1(n-7), suggesting that diatoms had recently constituted a substantial proportion of their diet. These data confirm that non-essential fatty acids do not occur in fixed ratios in *C. finmarchicus*.

6.3.5. Egg production of *C. finmarchicus*. The total quantity of eggs retrieved from individual experimental bottles at the end of each daily incubation, and daily average egg production rates are shown in Table 6.1.

6.3.5.1. April. The total amount of eggs produced was highly variable, signifying that only a few of the females were producing eggs in any particular bottle. The average daily egg production rate was low, ranging between 3.9 to 7.8

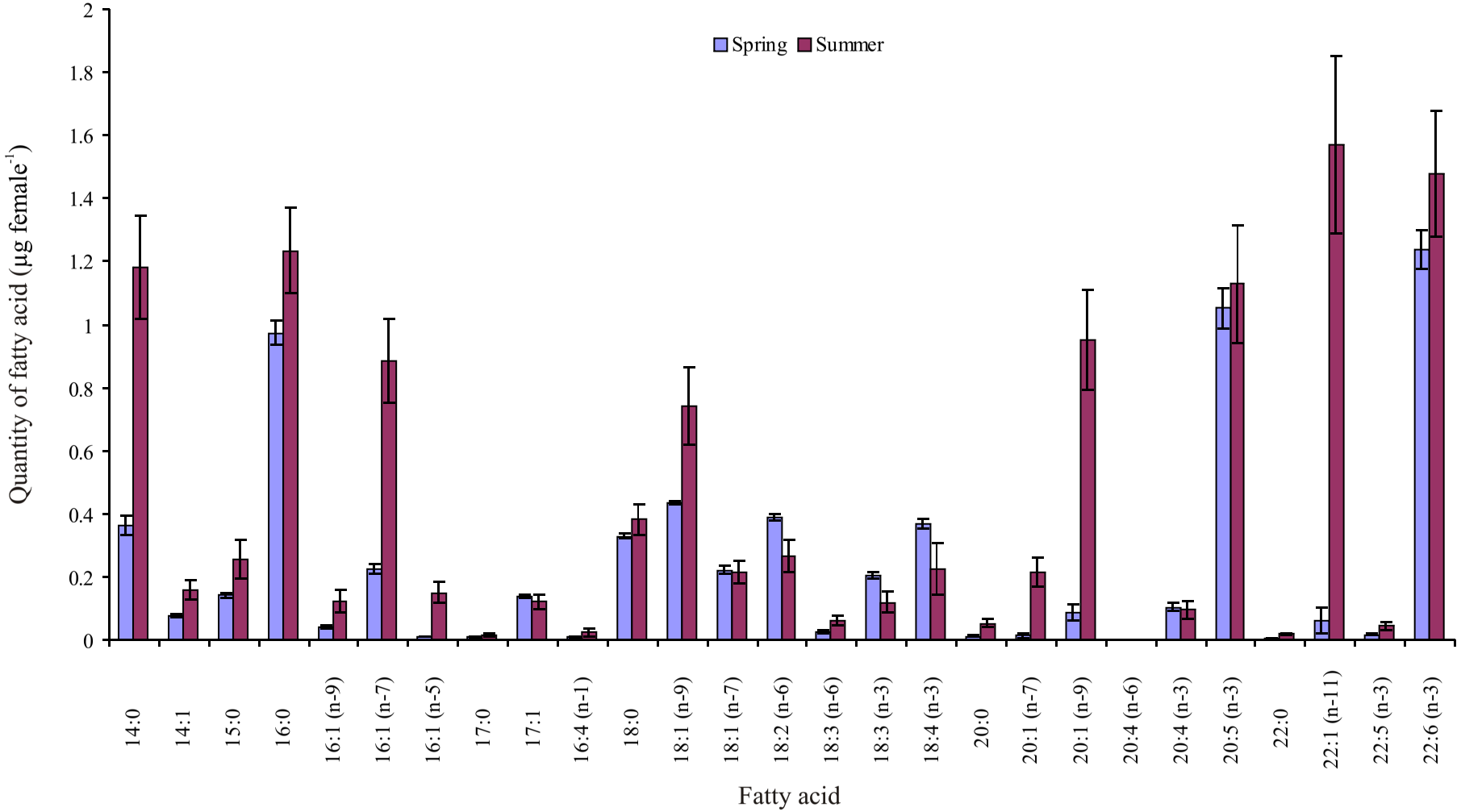


Figure 6.7. Quantities of individual fatty acids (µg female^{-1}) in the pre-experimental females from April and July/August (± standard error).

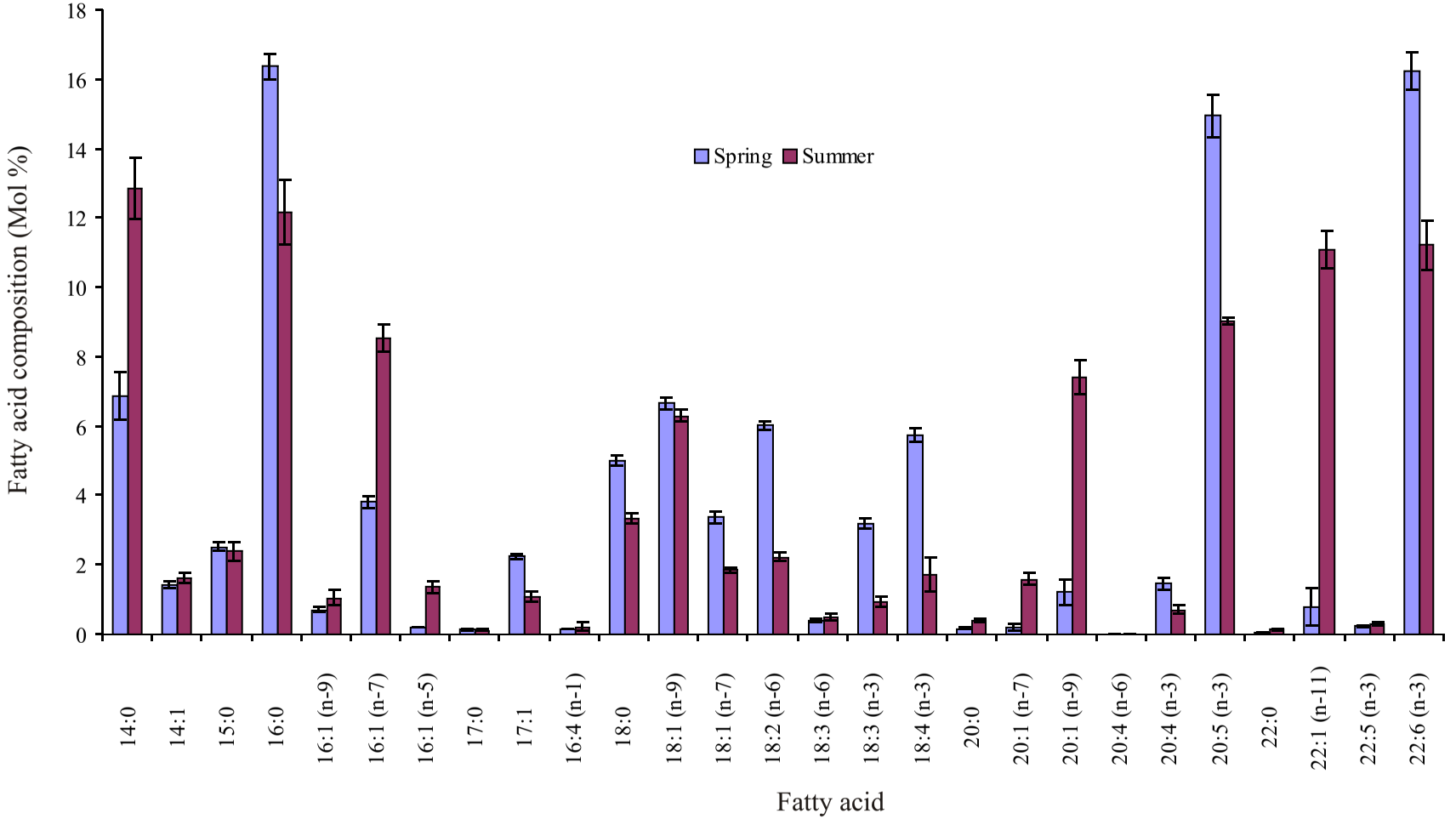


Figure 6.8. Fatty acid composition (Mol %) of the pre-experimental females in April and July/August (± standard error).

Table 6.1 Total eggs produced in each experimental bottle at the end of daily incubations, the average quantity of eggs produced per female per day ($\overline{EFD}$) +/- standard error and carbon-specific egg production rate (C-SEPR) expressed as % of average body C day^{-1}. Bold text signifies column averages.

		Replicate								
	Stn. #	1	2	3	4	5	6	$\overline{EFD}$		C-SEPR (%)
APRIL	1	0	35	0	90	41	101	4.5	± 1.76	2.1
	2	59	72	62	16	2	25	3.9	± 1.17	1.8
	3	100	40	128	77	68	52	7.8	± 1.32	3.6
	4	115	85	25	105	53	73	7.6	± 1.36	3.6
	5	124	13	27	54	29	16	4.4	± 1.71	2.1
							Avg.	**5.6**	± 0.85	**2.6**
JULY/AUGUST	1	42	40	105	66	57	142	7.5	± 1.64	2.3
	2	78	31	60	174	39	157	9.0	± 2.49	2.7
	3	140	85	89	106	166	128	11.9	± 1.29	3.6
	4	49	100	209	133	210	87	11.6	± 2.20	3.5
	5	60	49	108	11	19	21	4.5	± 1.49	1.4
							Avg.	**8.9**	± 1.37	**2.7**

eggs female^{-1} day^{-1}. The overall average egg production rate was 5.6 eggs female^{-1} day^{-1}.

6.3.5.2. July/August. Egg production was also variable in July/August, with daily averages ranging between 4.5 and 11.9 eggs female^{-1} day^{-1}. Overall, each female produced an average of 8.9 eggs day^{-1}. However, this rate was not significantly greater than the overall average egg production rate in April (t-test, $p = 0.078$).

6.3.6. Elemental composition of the eggs. The daily C and N content of the eggs in April and July/August is shown in Figures 6.9 and 6.10 respectively. Since a relatively large number of eggs (> 150) were required to produce detectable quantities of C and N, half of the total number of eggs produced each day were analysed as a single sample, the remainder being analysed as a single sample for their fatty acid content (Section 2.3.4). Elemental data for the eggs produced during the first day of experimentation in both seasons are missing due to analytical problems.

6.3.6.1. April. On average, each egg contained 0.35 µg of C (range: 0.27 to 0.44) and 0.052 µg N (range: 0.050 to 0.055). The C:N of the eggs was typically around 6 (µg µg^{-1}), although at station 3, it was 8.7 (Figure 6.9A). Considering the relative consistency of the C:N ratio at stations 2, 4 and 5 (range: 5.5 to 6.6), it is suggested that the increase noted at station 3 was a result of sample contamination, rather than a real increase in the amount of C allocated to each egg.

6.3.6.2. July/August. The eggs produced in July/August were similar to those produced in April, containing 0.31 µg of C (range: 0.27 to 0.37) and 0.053 µg of N (range: 0.048 to 0.059). The C:N ratio was less variable (average: 5.8), and ranged between 5.3 and 6.3 (Figure 6.10A).

6.3.7. Carbon specific egg production. Carbon-specific egg production rates (expressed as % average C biomass day^{-1}) are presented in Table 6.1.

6.3.8. Fatty acid composition of the eggs. The average quantities of individual fatty acids (µg egg^{-1}), and the average fatty acid compositions (Mol %) of the eggs produced in April and July/August are shown in Figures 6.11 and 6.12 respectively.

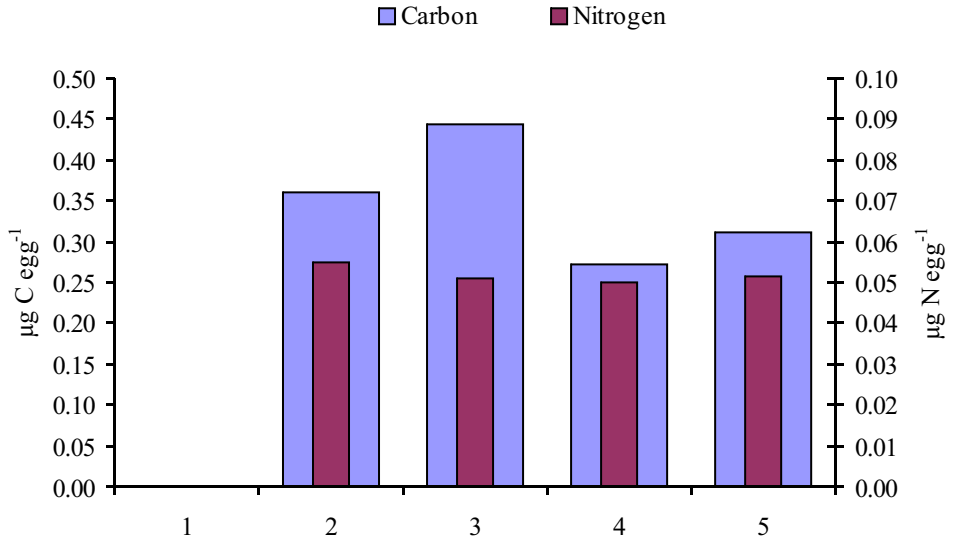


Figure 6.9A. Quantities of carbon and nitrogen in the eggs produced each day in April

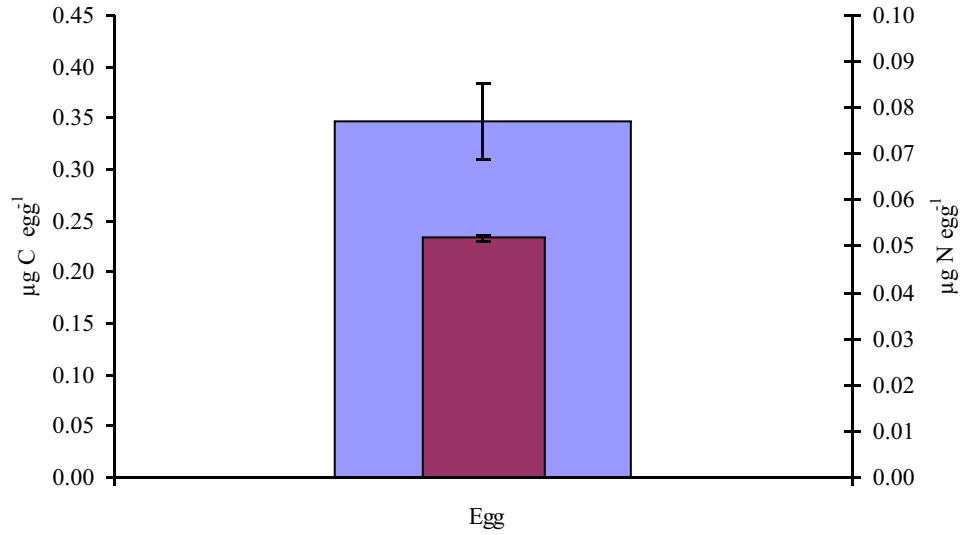


Figure 6.9B. Average quantities of carbon and nitrogen in the eggs in April (± SE).

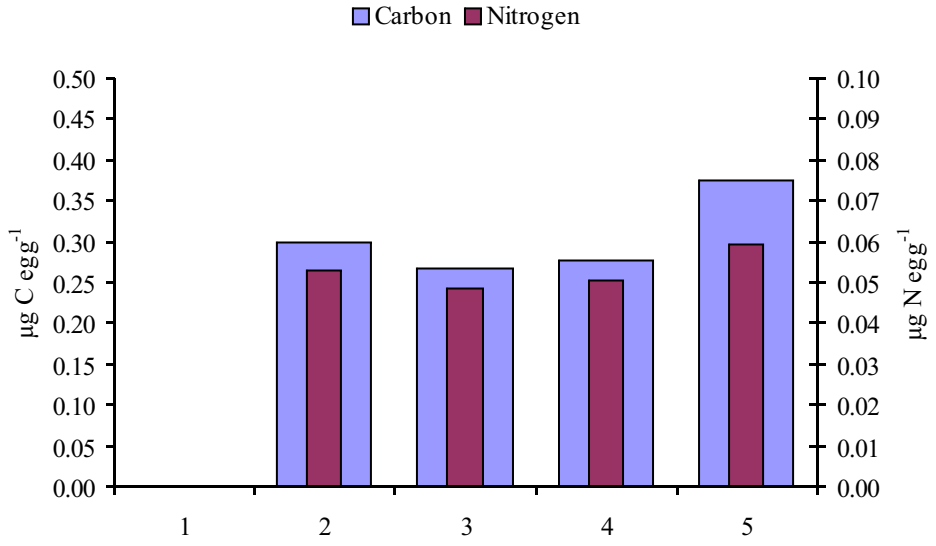


Figure 6.10A. Quantities of carbon and nitrogen in the eggs produced each day in July/August.

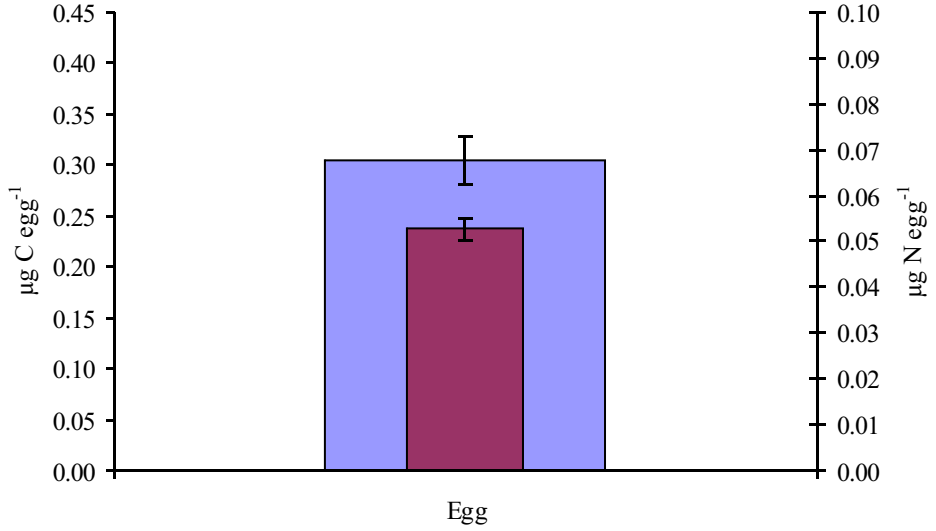


Figure 6.10B. Average quantities of carbon and nitrogen in the eggs in July/August (± SE).

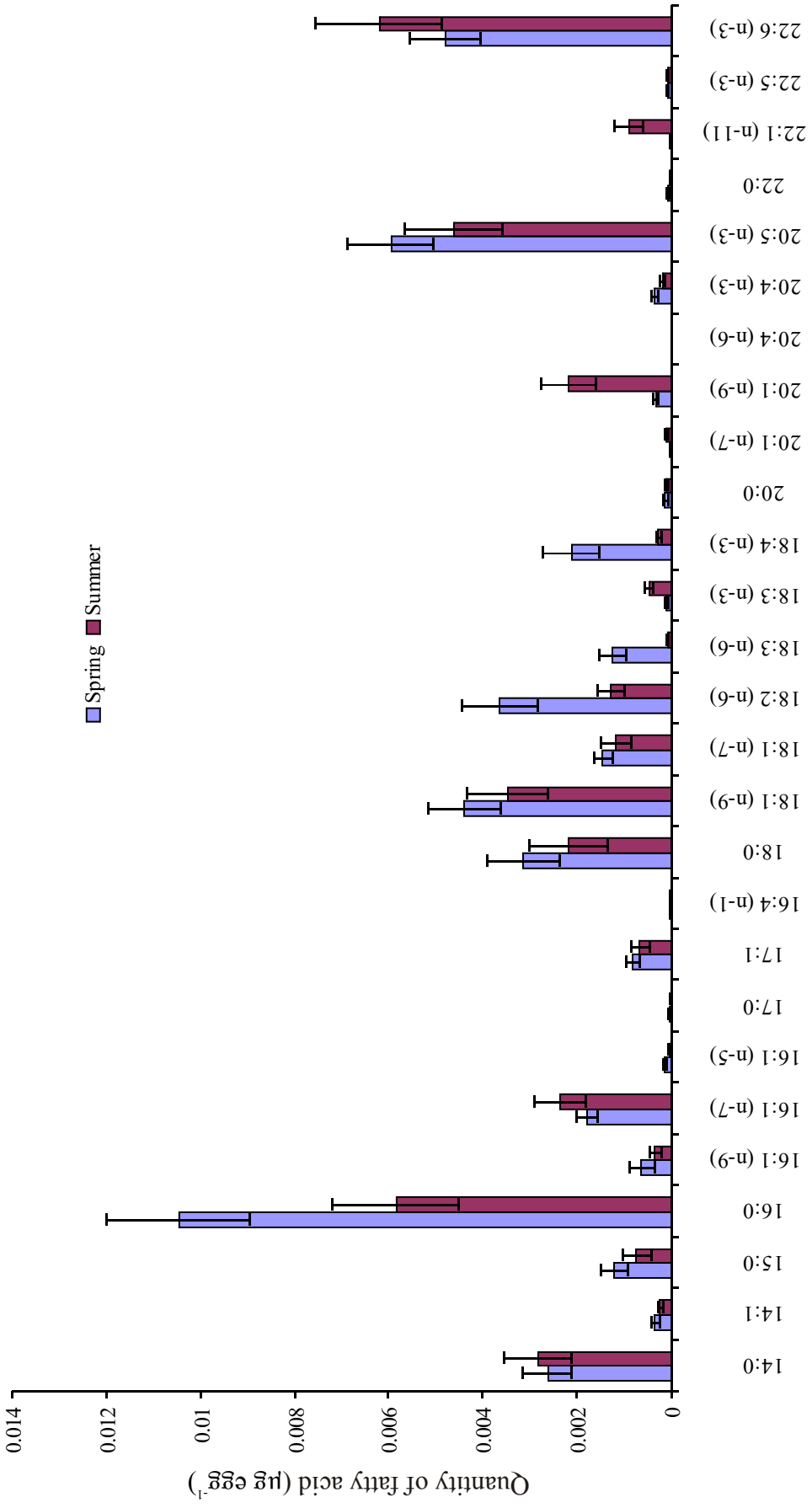


Figure 6.11. Quantities of individual fatty acids (µg egg^{-1}) in the eggs from April and July/August (± standard error).

Figure 6.12. Fatty acid composition (Mol %) of the eggs from April and July/August (± standard error).

6.3.8.1. April. Individual eggs contained 0.046 (± 0.008) µg of fatty acid. The fatty acids 16:0, EPA, DHA and also 18:1(n-9) were dominant, comprising 25, 12, 9 and 9 % of the total fatty acid composition (Mol %) respectively.

6.3.8.2. July/August. The average egg produced in July/August contained 0.037 (± 0.001) µg of fatty acid. The fatty acid composition was again dominated by 16:0, EPA, DHA and 18:1(n-9) (listed in decreasing dominance).

6.3.9. Stoichiometry of the eggs. The quantities of C and N allocated to each egg in April and July/August were not significantly different (t-test, $p > 0.3$). Similarly, quantities of total fatty acids, EPA and DHA in the eggs did not vary significantly between the seasons (t-test, $p > 0.3$ in all cases). This strongly suggests that these elements and compounds occur in the eggs at fixed ratios i.e. homeostatic, as previously reported (Pond et al. 1996, Anderson and Pond 2000). Regression analysis of the quantities of C and N in the eggs pooled from both seasons suggested that there was no relationship between these variables (ANOVA, $n = 8$, $p = 0.245$). However, one of the egg samples collected in April contained a disproportionate amount of C. After removal of this outlier (see Figure 6.13A), the relationship was found to be highly significant (Figure 6.13A, ANOVA, $n = 7$, $p = 0.002$). The relationship between seasonally pooled quantities of EPA and DHA in the eggs normalised to C, and hence N (Figure 6.13B) was also significant (ANOVA, $n = 7$, $p = 0.04$).

SIMPER analysis revealed that on average, the fatty acid composition (Mol %) of the eggs produced each day were 88.20 % and 92.20 % similar in April and July/August respectively. Between the seasons, the fatty acid compositions of the eggs were 77.64 % similar. However, this inter-seasonal difference (22.36 %) was significant (ANOSIM, $r = 0.964$, $p = 0.008$), and primarily caused by variation in the relative importance of 16:0, DHA, 20:1(n-9) and 22:1(n-11) (Figure 6.12), illustrating that the composition of non-essential fatty acids was not homeostatic.

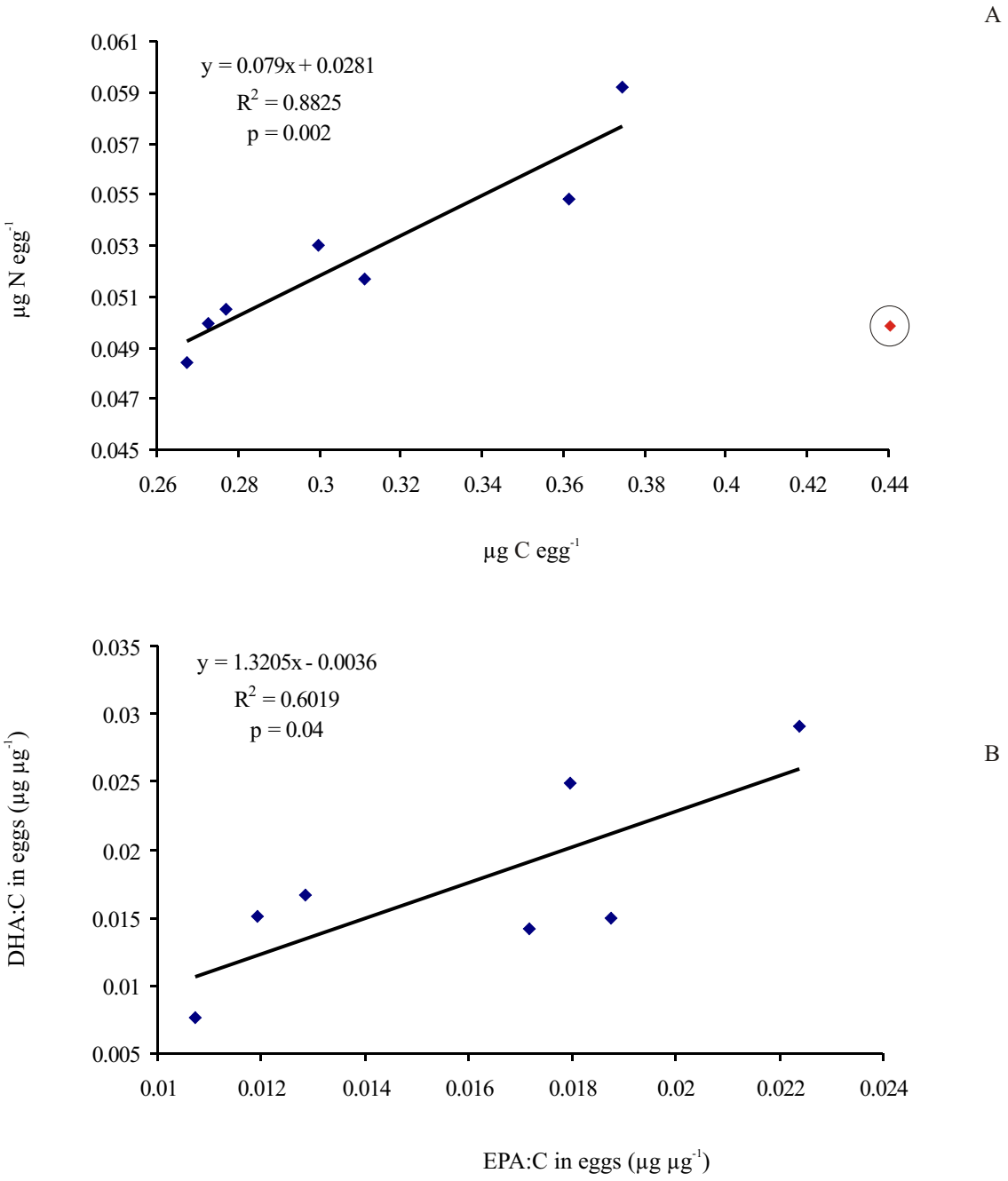


Figure 6.13. The relationship (linear regression) between biochemical constituents in *C. finmarchicus* eggs produced in April and July/August (pooled data). (A) N vs. C. The circled red square is the removed outlier. (B) DHA:C vs. EPA:C. Regression equations, R^2 values and regression statistics (1-way ANOVA) are shown.

6.3.10. The fatty acid composition of the eggs and adults in relation to each other and the particulates.

6.3.10.1. April. SIMPER analysis showed that the fatty acid composition (Mol %) of the eggs was 19.50 % dissimilar (80.50 % similar) to that of the females, and 30.11 % dissimilar to the fatty acid composition of the particulates. These differences were both significant (ANOSIM, r = 1 and r = 0.984 respectively, p = 0.008 in both cases), and in both cases, EPA, DHA and 16:0 were responsible for the observed differences. The fatty acid composition of the females was also significantly different to that of the particulates (ANOSIM, r = 1, p = 0.008), being 36.92 % dissimilar (63.08 % similar). Again, the differences were primarily driven by variation in the importance of EPA and DHA, and also 18:0. Simple linear regression analysis showed that the relationship between the quantities of individual fatty acids in the females and in the eggs was significant (Figure 6.14A).

6.3.10.2. July/August. The differences between the fatty acid composition of the eggs, the females and the particulates in July/August were similar to those in April. The fatty acid composition of the eggs was 22.11 % (77.89 % similar) and 26.72 % dissimilar (SIMPER analysis) to that of the females and particulates respectively. Both these differences were statistically significant (ANOSIM, r = 1, p = 0.008 in both cases). The differences between eggs and females were mainly attributable to the relative importance of 22:1(n-11), 16:0, DHA, and also 14:0 and EPA, whilst the eggs differed to the particulates largely because of the fatty acids 20:4(n-6), EPA, DHA, and 20:1(n-9). The fatty acid compositions of the females and the particulates differed by 31.91 %, and were significantly different (ANOSIM, r = 1, p = 0.008). Differences in the percentage distribution of the fatty acids 20:1(n-11), 20:4(n-6), 20:1(n-9) and 18:0 accounted for the majority of the variance between the animals and the particulates. Simple linear regression analysis again demonstrated that the quantities of individual fatty acids in the eggs was closely related to their quantities in the females (Figure 6.14B).

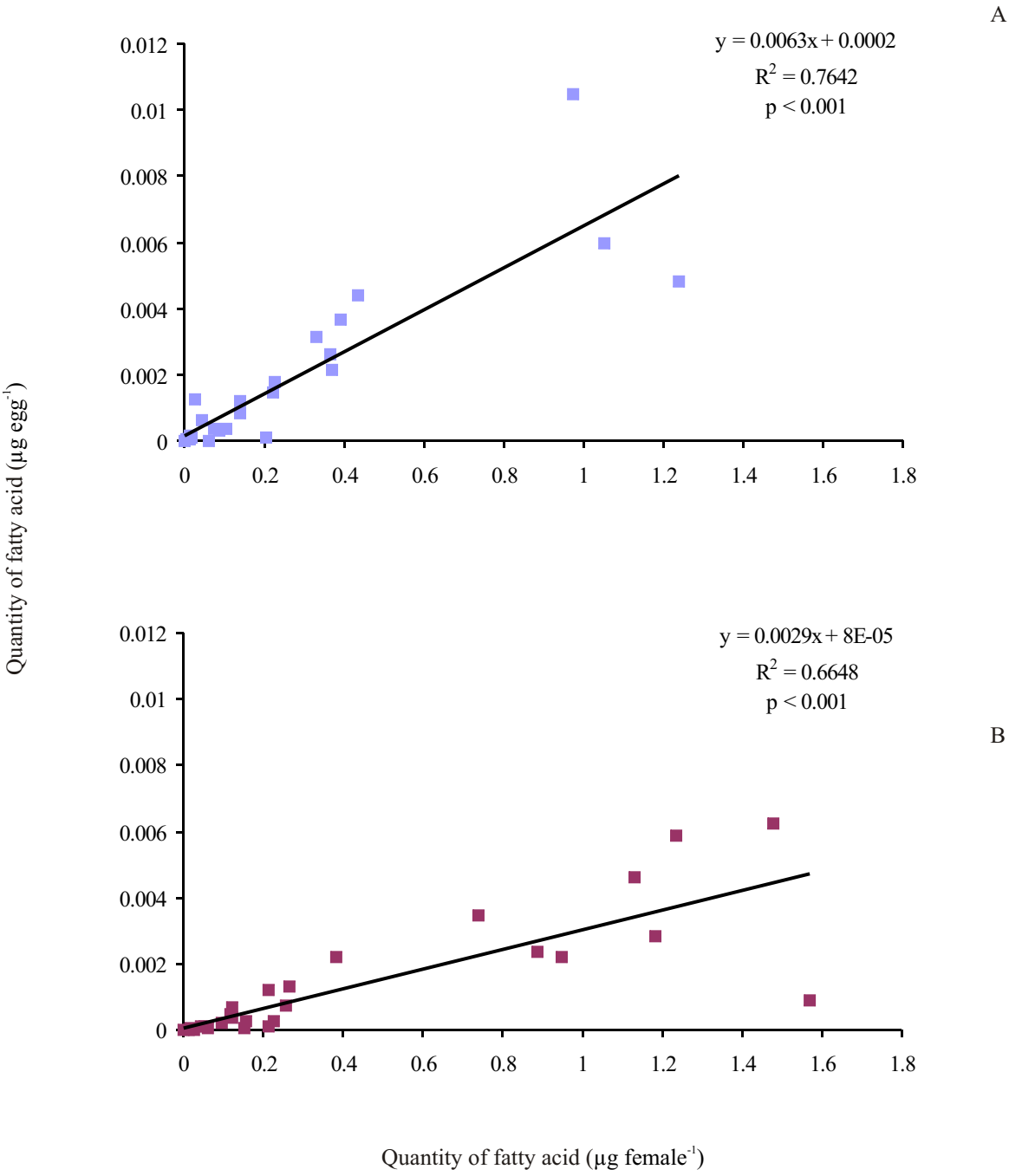


Figure 6.14. The relationship (linear regression) between the quantities of individual fatty acids in the females and their eggs in April (A) and July/August (B). Regression equations, R^2 values and regression statistics (1-way ANOVA) are shown.

6.4. DISCUSSION

6.4.1. Animals. The data presented here on the female elemental and fatty acid compositions before and after a prolonged (> 1 day) feeding incubation are the first of their kind, and provide a crucial insight into the biochemical changes that the females underwent during the experiments. The elemental and fatty acid compositional data determined for the females in the April and July/August agree well with previously published values for *Calanus finmarchicus* at northerly latitudes, particularly those collected during the end of April and July (Tande 1982, Kattner and Krause 1987, Kattner 1989, Kattner and Krause 1989, Graeve and Kattner 1992, Kattner and Hagen 1995, Albers et al. 1996, Irigoien et al. 1998, Scott et al. 2002b).

6.4.1.1. April. The significant losses of C and N during the April incubations suggest that the animals were using their biomass to fuel the costs associated with egg production. It is acknowledged that these losses may also be, at least in part, caused by the potential stresses associated with daily handling. However, the highest average production rates were not recorded until days 3 and 4 of both the April and July/August incubations (Table 6.1), suggesting that the experimental process did not have a negative effect on the females, at least in terms of egg production. In addition, elemental data of female *Calanus* collected at similar latitudes indicate that the loss of biomass observed in the incubations was indeed representative of changes occurring in the wild populations. Irigoien et al. (1998) reported a decrease in both the C content and the C:N ratio in females collected in the Norwegian Sea during pre-bloom conditions. Similarly, in an analysis of the elemental composition of *Calanus* over the seasonal cycle, Tande (1982) demonstrated that the quantities of C and N in females both declined during the months before the spring bloom. It is possible that the females sampled in April had only recently undergone the moult from CV, and therefore that their gonads were still maturing. However, the maturation process requires 40 to 70 µg C (Rey-Rassat et al. 2002a), far more than the observed losses of C during the experiments in April. It is more probable that the fatty acids lost during these incubations were being used to meet the metabolic costs associated with egg production, as observed in other polar copepods (Smith 1990, Hirche and Kattner 1993, Hirche and Niehoff 1996, Niehoff et al. 2002 – see also

Hagen and Schnack-Schiel 1996) and zooplanktiverous fish such as the capelin (Henderson et al. 1984).

Food deprivation is reported to initiate a sequential biochemical breakdown in female *C. finmarchicus* (Mayzaud 1976, Helland et al. 2003a). In the early stages of starvation, lipid depots are preferentially utilised (Mayzaud 1976, Bamstedt and Holt 1978). As lipid reserves are exhausted and the severity of starvation increases, proteins are catabolised at a rate of 4 % copepod^{-1} day^{-1} (Butler et al. 1970, Helland et al. 2003a). The scarcity of 20:1(n-9) and 22:1(n-11) strongly suggests that the animals' storage reserves were spent. Indeed, over the 5-day incubation period in spring, 5.1 % of the females body N was lost each day, indicating that the experimental animals were respiring protein, and thus already under severe starvation. This is confirmed by the C:N ratio of the biomass lost (4.1; µg µg^{-1}), which was very similar to that of protein (Vollenweider 1985). Together, these data strongly suggest that under pre-bloom conditions, the females were not only utilising their structural biomass to maintain a reproductive output (Niehoff 2004), but doing so to their detriment.

6.4.1.2. July/August. By contrast, although the females collected after the spring bloom in July/August contained similar quantities of C, they were significantly richer in the storage fatty acids, 20:1(n-9) and 22:1(n-11). This suggests that the females from July/August were smaller relative to those sampled in April, as found previously in the Norwegian Sea (Arashkevich et al. 2004). Considering that the water was several degrees warmer in the summer months (Table 3.2), this agrees with the well established inverse relationship between copepod body size and temperature (reviewed by Huntley and Lopez 1992). The energy-rich fatty acids 20:1(n-9) and 22:1(n-11) were responsible for much of the observed inter-seasonal differences between the females. Internal reserves are sequestered by *Calanus* primarily between the CIII and CV copepodite stages (Kattner and Krause 1987, Tande and Henderson 1988, Hygum et al. 2000), suggesting that the animals sampled in July/August were healthy and had previously encountered favourable feeding conditions (Sargent and Henderson 1986, Kattner and Hagen 1995). Despite the storage fatty acids differing significantly between the seasons, quantities of EPA, and DHA remained constant. This suggests that unlike 20:1(n-9) and 22:1(n-11), essential fatty acids are not stored in any quantity. Furthermore, the consistent

quantities of C, N, EPA and DHA in the females in both seasons suggest that essential fatty acids are homeostatic relative to C. However, these data cannot be taken as conclusive. Homeostasis can only be confirmed by observing the same ratios in animals with significantly different quantities of C (hence N, EPA and DHA).

The observed C:N of the biomass gained during the incubations in July/August was around 3 ($\mu g\ \mu g^{-1}$), suggesting that the animals had increased their protein content (Vollenweider 1985). Increases in the protein (hence N) content of female *C. finmarchicus* have previously been observed in spring mesocosm and laboratory experiments with newly moulted females (Hygum et al. 2000, Campbell et al. 2001, Helland et al. 2003a). This illustrates that newly moulted females are still capable of structural growth, presumably associated with the maturing of the gonad. It would appear very unlikely that the biomass increase during the experiment in July/August was an indication that the females were sequestering reserves to undergo a further diapause. The overwintering population of *C. finmarchicus* population is dominated by copepodite stages CIV and CV (Pedersen et al. 1995, Irigoien et al. 2000e, Astthorsson and Gislason 2003), with females representing only 1 – 2 % (Hirche 1983, Heath and Jonasdottir 1999, Gislason and Astthorsson 2000). Indeed, if this were the case, it would be reasonable to expect the biomass gained to have a high C:N ratio, as the reserves to survive diapause are stored in the form of C-rich lipids (Kattner and Hagen 1995, Hirche 1996a, Jonasdottir 1999).

6.4.2. Fatty acid biomarkers. When the fatty acid compositions of pre-experimental females from April and July/August were compared, it was apparent that the composition of non-essential fatty acids was not homeostatic. Although the seasonal differences were primarily attributable to increased quantities of 20:1(n-9) and 22:1(n-9) in the females sampled in July/August, these females also contained a greater proportion of 16:1(n-7), suggesting that they had recently ingested large quantities of diatoms (Table 1.1). This supports the results of the feeding experiments, which showed that diatoms constituted up to 33 % of the ingested material (Section 5.3.2.2.). In contrast, the females in April were relatively rich in 18:4(n-3) and DHA, the trophic markers for flagellated protists (Table 1.1). This indicates that these females had previously encountered food environments dominated by flagellates/dinoflagellates, possibly during the preceding summer

months when they are reported to predominate North Atlantic microplankton assemblages (e.g. Hansen et al. 1990a, Gifford et al. 1995).

6.4.3. The eggs of *C. finmarchicus*. The egg production of *C. finmarchicus* has been studied for over half a century (Marshall and Orr 1952), and numerous studies have documented rates under varying physical and chemical conditions. The values determined here under pre- and post-bloom conditions were low, but within values determined under similar conditions (Tables 6.1, 6.2 and 6.3). Determining ingestion rates was an important component of this study, and therefore it was necessary to maintain the prey cells in suspension by means of constant rotation on a plankton wheel. Consequently, it was not possible to separate the females from their eggs. *Calanus* apparently lacks a chemical recognition system to prevent the ingestion of its own eggs (Bonnet et al. 2004), and their egg mortality is dependent upon the density of CV and female copepods (Ohman and Hirche 2001). It is therefore highly probably that a proportion of the eggs produced each day were ingested by the females (Landry 1980), particularly during the July/August incubations, when higher rates of egg production are often recorded (Tables 6.2 and 6.3). Indeed, in the waters Southwest of Iceland during summer, where the experimental data were collected, *Calanus* is reported to produce on average 16 eggs female^{-1} day^{-1}, with a maximum of 46 eggs female^{-1} day^{-1} (Gislason and Astthorsson2000). The average rates determined here (8.9 eggs female^{-1} day^{-1}, Table 6.1) were much lower, suggesting that egg cannibalism was apparent. The egg production rates should therefore be viewed as minimal estimates.

Previous analyses of the eggs of *C. finmarchicus* report that their C and N content ranges from 0.20 to 0.25 µg C and from 0.03 to 0.05 µg N respectively (Ohman and Runge 1994, Runge and Plourde 1996, Cabal et al. 1997). The C content of the eggs produced in April was particularly changeable, although typically greater than the values previously reported. It has been demonstrated that egg size in *Calanus* is related to the amount of available food (Guisande and Harris 1995), and thus the variable allocation of C in the eggs may reflect the variable quantities of food. However, the C content of the eggs did not correlate significantly with the daily amounts of available food (microplankton biomass) or C content of the seston, with or without a 1-day lag ($p > 0.4$ in all cases). Despite discrepancies between the C and N content of the eggs in this and earlier studies (Ohman and Runge 1994,

Table 6.2. Egg production rates (EPR) for *Calanus finmarchicus* determined under pre- early- and post-bloom conditions. *all females had immature gonads. Table adapted from Melle and Skjoldal (1998).

Location	Pre-bloom	Early-bloom	Bloom	Post-bloom	Reference
Nova Scotia, Canada		2	21, 30		Runge (1985)
Greenland Sea	0				Smith (1990)
Norwegian coast		< 10	21 – 33	< 10	Diel and Tande (1992)
Barents Sea	0*				Hirche and Kattner (1993)
St. Lawrence Estuary, Canada	~0	0 – 10	22 – 82	~40	Plourde and Runge (1993)
Gulf of St. Lawrence			21	26	Ohman and Runge (1994)
Barents Sea, Atlantic water	0 – 0.2	2 – 8	24 – 44	0.3 – 4	Melle and Skjoldal 1998
Barents Sea, Polar front water	-	2 – 12	4 – 12	4 – 40	Melle and Skjoldal 1998
Barents Sea, Arctic water	-	9 –18			Melle and Skjoldal 1998
Norwegian Sea, Weathership M	3 – 18		14 – 44	1 – 5	Niehoff et al. (1999)
Faroe-Shetland Channel	~0	~0 – 3	4 – 26		Richardson et al. (1999)
Faroe Shelf	< 3.1		12.2		Gaard (2000)
Reykjanes Ridge, Atlantic water	1 – 5		2 – 46		Gislason and Astthorsson (2000)
Labrador Sea		71.3	47.3	25.9	Head et al. (2000)
West Greenland, Disko Bay	~0	< 5	20 – 25	10 – 22	Niehoff et al. 2002

Table 6.3. Egg production rate (EPR; eggs female^{-1} day^{-1}) and carbon-specific egg production rates (C-SEPR; % body C day^{-1}) determined for *Calanus finmarchicus* from various locations.

Location		EPR	C-SEPR (% d^{-1})	Reference
Norwegian Sea, Weathership M	March – April	3 – 18	2.3	Irigoien et al. (1998)
West Spitsbergen Current, Atlantic water	April	24.4	5.6	Hirche 1990
Georges Bank	April	3 – 73	0.5 – 10.1	Campbell et al. (2001)
Norwegian Sea, Weathership M	May	14 – 44	30	Irigoien et al. (1998)
Labrador Sea	May – June	5 – 37	0.8 – 5.5	Cabal et al. (1997)
Gulf of St. Lawrence	May – September	0 – 82	5.2 – 6.0	Plourde and Runge (1993), Runge and Plourde (1996)
East Greenland Shelf, Polar water	June	19.9	1.3	Hirche 1990
Norwegian Sea, Weathership M	June	1 – 5	14	Irigoien et al. (1998)
Gulf of St. Lawrence	June – July	12 – 45	1 – 5	Ohman and Runge (1994)
Greenland Sea	July	5 – 73	1 – 8.5	Hirche et al. (1997)

Runge and Plourde 1996, Cabal et al. 1997), the average daily carbon-specific egg production rates determined for April and July/August (Table 6.1) agree with those previously determined for *C. finmarchicus* in the North Atlantic (Table 6.3), although higher rates in summer have been reported (Hirche et al. 1997, Irigoien et al. 1998), again suggesting that a degree of egg cannibalism may have occurred.

The total quantities of fatty acids in the eggs of *Calanus* reported here are similar to those reported in the eggs of *C. helgolandicus* (Pond et al. 1996). Furthermore, their fatty acid composition is remarkably similar to those previously presented for *C. finmarchicus* (Sargent and Falk-Petersen 1988, Lacoste et al. 2001), with 16:0, 18:0, 18:1(n-9) and EPA and DHA dominating. It is interesting to note that the total quantities of fatty acids in the eggs of *Calanus* show considerable seasonal variation, ranging from < 30 to > 80 μg of fatty acid egg^{-1} (Figure 8A in Pond et al. 1996). However, the concentration of fatty acids in the eggs is at its lowest under pre- and post-bloom conditions, possibly explaining why no differences were observed in the quantities of fatty acids in the eggs spawned during the April and July/August incubations.

Although the quantities of C, N, total fatty acids, and EPA and DHA in the eggs did not differ significantly between the seasonal incubations, their availability in the food environment was significantly greater during July/August (t-test, $p < 0.001$ in all cases). Considering that total fatty acid concentration in the seston does not correlate with quantities in the eggs of *Calanus* (Guisande and Harris 1995, though see Gatten et al. 1980), this is not surprising. However, both carbohydrate and protein concentrations in the particulates have been shown to correlate positively with their quantities in the eggs (Guisande and Harris 1995), and it may therefore be expected that the eggs produced in the July/August should contain significantly greater quantities of both C and N. Why such a relationship was not found is unclear. One possible explanation is that carbohydrate represents < 13 % of the dry weight of the eggs, whereas C-rich lipid can constitute > 40 % (Guisande and Harris 1995), thereby masking any signal likely to be caused by the changes in carbohydrate levels. However, since N rich protein can represent up to 60 % of the eggs dry mass (Guisande and Harris 1995), this seems an unlikely explanation as to why a significant increase in N in the July/August eggs was not observed.

6.4.3.1. Homeostasis of the eggs. The significant linear relationships between C and N, and EPA:C and DHA:C (Figure 6.13) illustrates that these constituents occur in fixed ratios in the eggs of *C. finmarchicus*. From these data alone it is not possible to conclude that the eggs were homeostatic because absolute quantities of these substrates in the eggs did not differ significantly between April and July/August (as noted above). However, previous studies have demonstrated that the essential components in the eggs of *Calanus* spp. do occur in fixed ratios (Pond et al. 1996, Anderson and Pond 2000, Helland et al. 2003b), thereby supporting the observations presented here.

6.5. SUMMARY

Observing changes in the biochemical composition of the females' biomass during the incubations revealed that in April, significant quantities of C, N, EPA and DHA were utilised by the animals. The storage fatty acids 20:1(n-9) and 22:1(n-11) were essentially absent in these females from the start of the incubations, demonstrating that energetic reserves were exhausted prior to experimentation. Considering the strong association between n-3 PUFAs and cell membranes, significant losses of these compounds suggests that the animals were catabolising somatic biomass during the incubations. This is consistent with the observation that the females did not ingest sufficient material to meet typical respiration and excretion demands (Section 5.3.2.1). The low C:N ratio of the biomass lost during the incubations confirms that the animals had lost protein, rather than C-rich lipids. Together, these data indicate that muscle tissue was being catabolised, which in turn, suggests that the animals were undergoing starvation. It appears that in April, the females were producing eggs to their own detriment.

In contrast, the females in July/August gained significant quantities of C and N during the incubations, whilst maintaining a relatively constant amount of fatty acid. The low C:N of this new biomass indicates that the females were increasing their protein content, rather than storage reserves. It is suggested that this protein gain was associated with the gonad maturation process, and therefore the animals had only recently moulted into adults. The quantities of energy-rich storage fatty acids, 20:1(n-9) and 22:1(n-11), were significantly greater in July/August, illustrating that these animals had previously encountered favourable feeding conditions. Although storage reserves were greater, total quantities of C remained constant between the

seasons, demonstrating that the females in July/August were smaller than those in April. Storage fatty acids were primarily responsible for the inter-seasonal differences between the females, illustrating that the composition of non-essential fatty acids in the females was not homeostatic. However, the quantities of C, N, EPA and DHA remained constant between the seasons, suggesting that these compounds are homeostatic. That EPA and DHA were not significantly greater in the females that contained large quantities of 20:1(n-9) and 22:1(n-11) suggests that PUFAs are not stored in any quantity.

Egg production rates were low in both seasons. The quantities of C, N, EPA and DHA in the eggs were similar to values previously reported, and remained constant between the seasons. This demonstrates that these essential fatty acids occur in fixed ratios in the eggs. Conversely, the composition of non-essential fatty acids showed considerable inter-seasonal variation, as previously reported. There was a highly significant relationship between the quantities of individual fatty acids in the females and in the eggs.

Chapter 7

Physiological budgets of *Calanus finmarchicus* and the stoichiometric analysis of egg production

7.1. AIMS

This chapter aims to collate the data on ingestion, egg production and the changes in the animals' biomass from the previous chapters. Together, these data will be used to construct balanced physiological budgets for *C. finmarchicus* over the 5-day experimental period in April and July/August. Using the stoichiometric theory and equations of Anderson and Pond (2000), these data shall also be used to examine the potential limitation of egg production. An introduction to simple elemental stoichiometry is given first to provide the context for this work. In the previous chapters, all ratios have been expressed as mass ratios, the units typical for zooplankton publications. In contrast, stoichiometric ratios are typically expressed in molar specific terms, and therefore molar units are adopted for this chapter.

7.2. THEORY

A description of all parameters/variables discussed in the text are presented in Table 7.1. Note that rates are normalised to zooplankton biomass in carbon units. First, stoichiometric theory is presented showing how growth, respiration and excretion are calculated considering only two substrates, C and N (Section 7.2.1.). A threshold elemental ratio, the ratio in food that defines the transition between C- and N-limited growth, is also determined (Section 7.2.2.). A set of results is then presented to demonstrate this approach for a set of example parameters (Section 7.2.4.). Finally, the theory is then extended to consider multiple substrates, e.g. C, N, EPA and DHA (Section 7.2.5.).

7.2.1. Stoichiometry. The amount of carbon ingested, I_C, varies (e.g. with food concentration), as does the ratio of C and N in food, θ_f, (e.g. depends on cell-type consumed). For a given I_C, and a given θ_f, the quantity of N ingested, I_N, is then:

$$I_N = \frac{I_C}{\theta_f} \qquad (1)$$

The assimilation efficiency for C, β_C, is fixed, and the amount assimilated, A_C, is then:

$$A_C = \beta_C I_C \qquad (2)$$

Table 7.1 Definition of parameters and variables used in the text, where *i* and *j* can be any of C, N, EPA and DHA.

Paramater/ variable	Description	Units
I_i	Mass specific ingestion rate of *i*	mol *i* (mol C)$^{-1}$ d^{-1}
θ_f	Ratio of components *i* and *j* in food	mol *i* (mol *j*)$^{-1}$
A_i	Mass specific assimilation rate of *i*	mol *i* (mol C)$^{-1}$ d^{-1}
β_i	Assimilation efficiency of *i*	dimensionless (%)
W_i	Mass specific egestion rate of *i*	mol *i* (mol C)$^{-1}$ d^{-1}
K_i	Gross growth efficiency of *i*	dimensionless
G_i	Mass specific growth of *i*	mol *i* (mol C)$^{-1}$ d^{-1}
k_i	Net production efficiency of *i*	dimensionless (%)
B_i	Contribution of biomass to metabolism and growth	mol *i* (mol C)$^{-1}$ d^{-1}
U_i	Gross utilisation efficiency of *i*	dimensionless (%)
u_i	Net utilisation efficiency of *i*	dimensionless (%)
R_i	Mass specific respiration of *i*	mol *i* (mol C)$^{-1}$ d^{-1}
E_i	Mass specific excretion of *i*	mol *i* (mol C)$^{-1}$ d^{-1}
θ_Z	I to j ratio in zooplankton biomass	mol *i* (mol *j*)$^{-1}$
θ^*_f	Threshold elemental ratio (TER), at which both *i* and *j* are limiting. Where $\theta_f < \theta^*_f$, *i* limits and vice verse	mol mol^{-1}
k^*_i	Maximum net production efficiency for *i* (under *i*-limitation)	mol mol^{-1}
ϕ_i	The fraction of the demand for constituent *i* which is met directly by dietary intake	dimensionless (%)

The amount of material egested, W_C, is simply calculated as the fraction of ingested material that is not assimilated:

$$W_C = (1 - \beta_C) I_C \quad (3)$$

Gross growth efficiency (the fraction of ingested material used for growth), K_C, is therefore defined as:

$$K_C = \frac{G_C}{I_C} \quad (4)$$

where G_C is growth (taken to include reproduction). Net production efficiency, k_C, is defined as the fraction of assimilated material that is used for growth, G_C:

$$k_C = \frac{G_C}{A_C} \quad (5)$$

By rearrangement of the equations above, G_C, can be calculated as:

$$G_C = K_C I_C = k_C A_C = k_C \beta_C I_C \quad (6)$$

The equations above assume that food intake is the only source of substrates available for growth. When biomass, B_C, (body reserves when available, but could be structure when reserves are depleted) contributes to growth, gross growth efficiency, K_C, is not particularly meaningful and so the concept of gross utilisation efficiency, U_C, is used instead:

$$U_C = \frac{G_C}{I_C + B_C} \quad (7)$$

Similarly, net production efficiency, k_C, is redefined as the net utilisation efficiency, u_C:

$$u_C = \frac{G_C}{A_C + B_C} \quad (8)$$

Physiological budgets for the experimental copepods can now be constructed:

$$I_C + B_C = G_C + R_C + W_C \quad (9)$$

$$I_N + B_N = G_N + E_N + W_N \quad (10)$$

where R_C is respiration and E_N is excretion. It is assumed that once C has been assimilated across the peritrophic membrane, it is either used for growth, or respiration, and that no C is excreted or stored over the duration of the experiments. We know that $G_C = k_C A_C$ (equation 6), therefore R_C must be:

$$R_C = (1 - k_C) A_C = (1 - k_C) \beta_C I_C \quad (11)$$

The amount of N excreted is calculated in the same manner:

$$E_N = (1 - k_N) A_N = (1 - k_N) \beta_N I_N \qquad (12)$$

7.2.2. The Threshold Elemental Ratio (TER). The following section is based on the equations and theory presented by Anderson (1992) and Anderson and Hessen (1995), who laid the mathematical foundations for the stoichiometric analysis of egg production of copepods in terms of bulk C and N. An extensive freshwater literature also exists on this subject, focussing on P rather than N as the limiting nutrient (e.g. Hessen 1992, Sterner 1993). The limitation of marine copepod production has traditionally been considered in terms of bulk C or N (e.g. Checkley 1980, Kiorboe 1989). Because some of the assimilated C is always required for respiration, k_C will always remain < 1. If proteins are not respired and there is no maintenance requirement for N, then a maximum k_N of 1 is theoretically possible (Anderson 1992, Urabe and Watanabe 1992). Zooplankton are assumed to be homeostatic, i.e. they have a have a fixed C:N ratio, θ_Z. It follows that if G_C is known (the amount of C in the new biomass), G_N (the amount of N in the new biomass) is:

$$G_N = \frac{G_C}{\theta_Z} \qquad (13)$$

The threshold elemental ratio (TER), θ^*_f, is the ratio in food that defines the transition between C- and N-limited growth. If the C:N of the available food, θ_f, is < θ^*_f, there is excess N and C becomes limiting. When substrates are limiting they are used with maximum efficiencies. When C is limiting it is therefore used with its theoretical maximum net production efficiency, k^*_C, i.e. $k_C = k^*_C$, otherwise C is in excess and the realised net production efficiency, k_C, < k^*_C. Conversely, if θ_f is > θ^*_f, N becomes limiting and it will then be used with the maximum net production efficiency, k^*_N. If we consider a hypothetical instance in which all ingested substrates can potentially be allocated to growth, i.e. there are no losses to respiration, excretion, or faecal pellets, then the ideal C:N in food is simply equal to that of the zooplankton biomass, i.e. $\theta^*_f = \theta_Z$. However, if the gross growth efficiency for C, K^*_C, is < 1, i.e. C is required to meet maintenance and respiration costs, then the demand for C increases and the TER becomes:

$$\theta^*_f = \frac{\theta_Z}{K^*_C} = \frac{\theta_Z}{k^*_C \beta_C} \qquad (14)$$

Similarly, if a fraction of the assimilated N is required e.g. for maintenance, maximum gross growth efficiency, K^*_N, decreases and the TER is then:

$$\theta^*_f = \frac{\theta_Z K^*_N}{K^*_C} = \frac{\theta_Z k^*_N \beta_N}{k^*_C \beta_C} \quad (15)$$

Where $\theta_f = \theta^*_f$; both C and N are used with maximum efficiency (K^*_C and K^*_N).

7.2.3. Elemental limitation. When $\theta_f < \theta^*_f$, C is limiting and used with efficiencies K^*_C and k^*_C. Equation 6 now becomes:

$$G_C = K^*_C I_C, \theta_f < \theta^*_f \quad (16)$$

In this situation, N is not limiting, and the realised net production efficiency for N, k_N, is therefore lower than k^*_N. The realised gross growth and net production efficiencies are derived by rearranging equation 15:

$$K_N = \frac{K^*_C \theta_f}{\theta_Z}, \theta_f < \theta^*_f \quad (17)$$

$$k_N = \frac{k^*_C \beta_C \theta_f}{\beta_N \theta_Z}, \theta_f < \theta^*_f \quad (18)$$

Conversely, if $\theta_f > \theta^*_f$, N is limiting. In this instance, N is used with efficiencies K^*_N and k^*_N, and G_N can be determined:

$$G_N = K^*_N I_N, \theta_f > \theta^*_f \quad (19)$$

The realised gross and net production efficiencies for C are then:

$$K_C = \frac{K^*_N \theta_Z}{\theta_f}, \theta_f > \theta^*_f \quad (20)$$

$$k_C = \frac{k^*_N \beta_N \theta_Z}{\beta_C \theta_f}, \theta_f > \theta^*_f \quad (21)$$

These parameters can now be inserted in equations 11 and 12 in order to calculate respiration and excretion.

7.2.4. Examining the fate of C and N under different values of θ_f (Anderson and Hessen 1995). Based on the parameters defined in Table 7.2, the predicted allocations of C and N under varying θ_f can be illustrated (Figure 7.1). Using this parameter set, the TER (θ^*_f) is predicted as 21.74. Interestingly, the molar C:N ratio of marine seston is characteristically < 10 (Copin-Montegut and Copin-Montegut 1983). Assuming that the defined parameters are reasonable, this result

Table 7.2. List of parameters taken from Anderson and Hessen (1995).

Parameter	Description	Value	Units
θ_Z	C:N ratio of consumer tissues	4.7	mol C mol^{-1} N
β_C	Assimilation efficiency of C	0.49	mol C mol^{-1} C
β_N	Assimilation efficiency of N	0.68	mol N mol^{-1} N
k^*_C	Maximum net production efficiency for C (under C-limitation)	0.3	mol C mol^{-1} C
k^*_N	Maximum net production efficiency for N (under N-limitation)	1	mol N mol^{-1} N

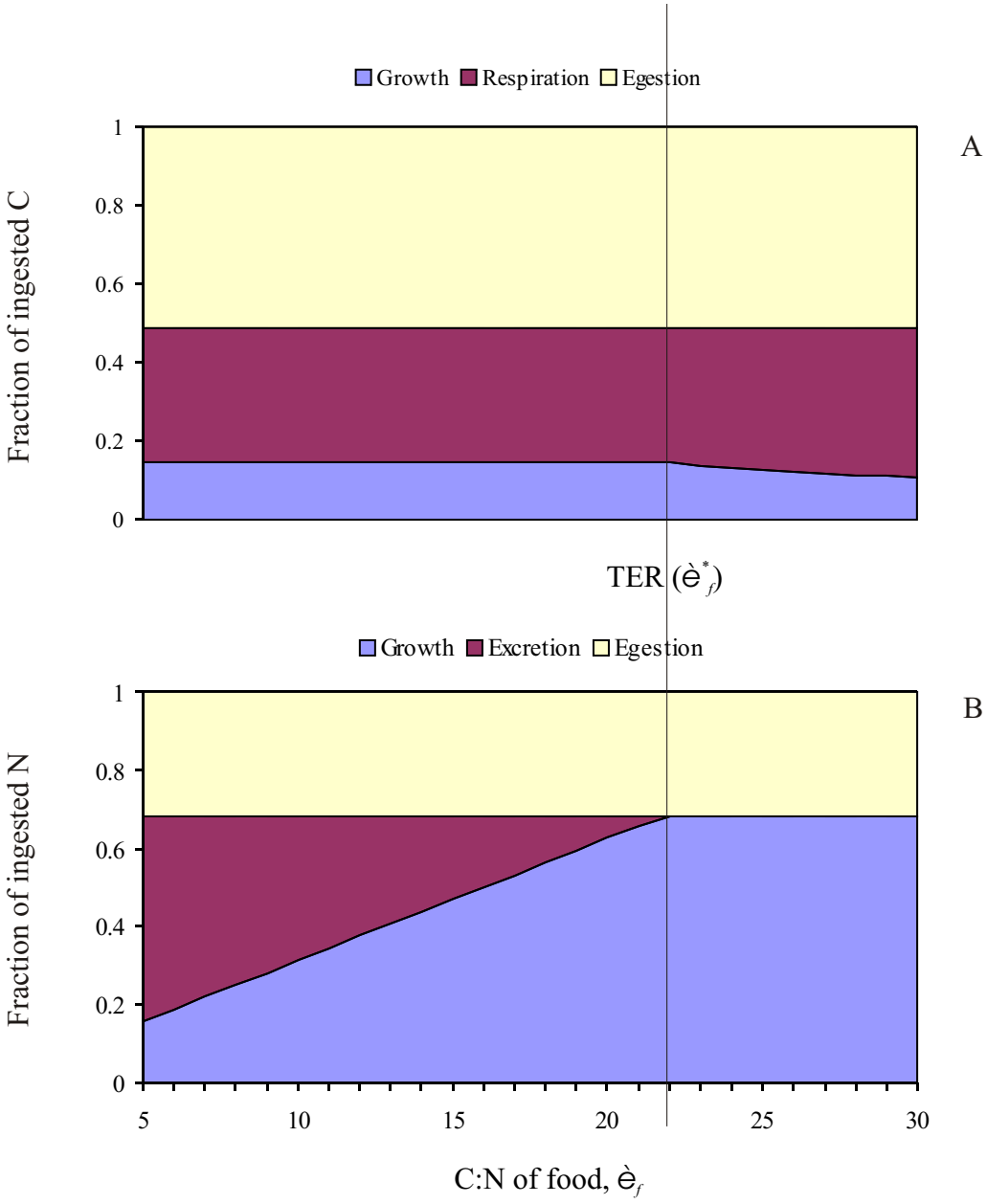


Figure 7.1. Predicted allocations of C (A) and N (B) to growth, respiration, excretion and egestion for $\grave{e}_f$ between 5 and 30. Solid line represents the Threshold Elemental Ratio (TER). Below the TER C is limiting, and above, N is limiting.

strongly suggests that N limitation in marine copepods should be unlikely and that C limitation should be more prominent. It can be seen that when C is limiting ($\theta_f < \theta^*_f$, Figure 7.1A), $K_C = K^*_C$, and a constant fraction of the assimilated C is respired. Above the TER, C is no longer limiting and an increasing fraction must be respired in order to maintain the homeostatic balance. Conversely, under N limitation ($\theta_f > \theta^*_f$, Figure 7.1B), all the assimilated N is available for growth. However, as θ_f decreases away from θ^*_f, a linearly increasing fraction of the assimilated N must be excreted because N is in excess. This is particularly interesting because it contrasts with experimentally determined values of K_N. When fed algal monocultures, experimental copepods display a remarkably constant K_N at around 0.4, over a wide range of θ_f (Checkley 1980, Kiorboe 1989). Stoichiometric theory predicts that when N is limiting ($\theta_f > \theta^*_f$) it is used with a constant and high gross growth efficiency, K^*_N. The constancy of K_N in the experimental copepods does therefore suggest limitation by N, but why should the animals use this element with such a low efficiency if it is limiting in the diet? Under N-limitation K^*_N is equal to β_N, assuming that k^*_N equals 1. Typical values of β_N for copepods range between 0.6 and 0.9 (Corner et al. 1967, Landry et al. 1984, Hassett and Landry 1988), therefore the observed values of K_N are much lower than what may have been expected. What causes the discrepancy between experimentally determined values of K_N and β_N? Was N really limiting production in the experiments?

The low and constant value of K_N observed in the experiments of Checkley (1980) and Kiorboe (1989) may be explained by copepods respiring some proteins (rich in N), even when they are in demand. Protein-sparing is however a well-known phenomenon in organisms (e.g. Arnould et al. 2001, Hervant and Renault 2002), such that excretion of N should decline in the presence of C-rich substrates. Experimental evidence from natural bacterial assemblages has demonstrated that, as stoichiometric theory predicts, when $\theta_f > \theta^*_f$, nitrogenous losses (i.e. excretion) become zero as all assimilated N is used for growth (Lancelot and Billen 1986, Goldman et al. 1987). In the case of bacteria, it therefore appears reasonable to assume that k^*_N equals 1. However, bacteria and copepods do not necessarily share the same physiological requirements for C and N. A recent modelling investigation into the C and N gross growth efficiencies of copepods was able to successfully generate the low K_N seen in laboratory experiments (Kuijper et al. 2004). The two models (Anderson and Hessen 1995, Kuijper et al. 2004) have a crucial difference:

the latter incorporates a maintenance demand for N i.e. a fraction of the assimilated N, A_N, is not available for growth because it is required to meet the turnover of proteins etc. (Kuijper et al. 2004). The close fit between the experimental results (Checkley 1980, Kiorboe 1989) and the output of this model (Kuijper et al. 2004) suggest that copepods do have a maintenance demand for N. Therefore, assuming a k^*_N of 1 appears to be incorrect and the assumption that K^*_N equals β_N when $\theta_f > \theta^*_f$ is not justified. Thus the low K_N in the experiments is consistent with N limitation if copepods have a significant N requirement for maintenance.

Another possible explanation for the disagreement between the theoretically and experimentally derived values of K_N is that something other than bulk C or N is limiting production. In this scenario, N would then be in excess and the net production efficiency, k_N, would therefore be lower than maximum, k^*_N. In light of recent work, it is apparent that amino- and fatty-acids are both capable of influencing copepod reproductive rates (e.g. Kleppel et al. 1998b, Jonasdottir et al. 2002). If, for example, an animal was ordinarily limited by N, then introducing an imbalance of essential amino acids would cause k_N to decrease below k^*_N, the amino acid in least supply relative to demand becoming limiting. Such imbalances may be possible in many terrestrial and aquatic organisms (Anderson et al. 2004). Polyunsaturated fatty acids (PUFAs) in the seston, particularly 20:5(n-3) (eicosapentaenoic acid, EPA) and 22:6(n-3) (docosahexaenoic acid, DHA), correlate well with zooplankton growth and have been implicated as the compounds limiting production (Jonasdottir 1994, Muller-Navarra et al. 2000).

7.2.5. Extending stoichiometric theory to include micronutrients. The elemental stoichiometric approach of Anderson and Hessen (1995) has been developed to include the fatty acids EPA and DHA and their dual origin (diet, synthesis) (Anderson and Pond 2000). This section draws heavily upon the equations and analysis of Anderson and Pond (2000), enabling a simultaneous intercomparison of the limiting potential of both macro (C and N) and micronutrients (EPA and DHA).

If θ_Z and θ_f are redefined as $\theta_{Zi:j}$ and $\theta_{fi:j}$, the ratios of *i:j* in the eggs and in the food respectively, where *i* and *j* can assume any of the dietary components (C, N, EPA or DHA). As above, each substrate can be potentially be used with a maximum gross growth efficiency, K^*_i, although the stoichiometric axiom dictates that only the

limiting substrate will be used thus. We know that gross growth efficiency is the fraction of ingested food that is used for growth (equation 4). Therefore the parameters K^*_{EPA} and K^*_{DHA} refer to the maximum efficiencies with which the ingested quantities of these fatty acids are used for growth, and by definition ignoring internal sources of these compounds (synthesis or body reserves). To account for synthesis of EPA and DHA as a source of nutrition, S_i, a new parameter, ϕ_i, was introduced by Anderson and Pond (2000). This is the fraction of the demand for constituent *i* which is met directly by dietary intake i.e.:

$$\phi_i = \frac{A_i}{(A_i + S_i)} \tag{21}$$

However, numerous *Calanus* spp. are known to be capable of reproducing in the absence of food (e.g. Hirche and Kosobokova 2003). Equation 21 does not acknowledge the possibility that copepods may derive essential substrates from their biomass, B_i. Therefore, the ϕ_i parameter is redefined here as δ_i, representing the fraction of utilised substrates that is derived from ingestion, the remainder being provided by either biomass or synthesis:

$$\delta_i = \frac{A_i}{(A_i + S_i + B_i)} \tag{22}$$

Biomass is typically understood to mean internal reserves. For example, C derived from the animal's biomass suggests that it is from internal lipid reserves. However, it is important to note that material can also be derived from the catabolism of structural components e.g. proteins. C and N are immutable, i.e. cannot be synthesised, and therefore S_C and S_N are by default equal to 0. Similarly, calanoid copepods are thought to be incapable of synthesising EPA and DHA in significant quantities (Nanton and Castell 1999, Dave Pond pers. comm.), and consequently S_{EPA} and S_{DHA} are also set to 0. The equation to determine the TER (Equation 15) can now be recast as:

$$\theta^*_{i:j} = \frac{\delta_i U^*_j \theta_{Zi:j}}{\delta_j U^*_i} \tag{23}$$

At the TER, both *i* and *j* are used with their maximum gross utilisation efficiencies, and it follows that:

$$\delta_j U^*_i \theta^*_{i:j} = \delta_i U^*_j \theta_{Zi:j} \tag{24}$$

When $\theta_{i:j}$ is not equal to the TER, *i* will limit relative to *j* when demand exceeds supply, i.e. $\delta_j U_i^* \theta_{i:j} > \delta_i U_j^* \theta_{Zi:j}$. The strength of limitation of *i* relative to *j*, $S_{i:j}$, is then:

$$S_{i:j} = \frac{\delta_i U_j^* \theta_{Zi:j}}{\delta_j U_i^* \theta_{i:j}} \quad (25)$$

This enables the relative limitation of any pair of substrates to be compared. When *i* is set in turn to represent each of the components for a particular *j*, the most severely limiting component in the diet, *w*, is component *i* corresponding to the greatest value of $S_{i:j}$. That is to say, if all compounds are expressed relative to C, i.e. C:C, N:C, EPA:C and DHA:C, the most limiting compound, *w*, is that which corresponds to the maximum calculated $S_{i:C}$. The realised gross utilisation efficiency of non-limiting substrates can then be derived:

$$U_i = \frac{\delta_i U_w^* \theta_Z}{\delta_w \theta_f} \quad (26)$$

It is easiest to use *w* as the common currency when comparing dietary substrates. The 'limiting potential' of *i*, L_i, ($0 \leq L_i \geq 1$) of each compound is then $S_{i:w}$;

$$L_i = \frac{\delta_i U_w^* \theta_{Zi:w}}{\delta_w U_i^* \theta_{i:w}} \quad (27)$$

The L_i parameter is dimensionless, ranging between 0 and 1. It provides a relative measure of how limiting each dietary substrate is. The limiting potential of *w*, L_w, is always 1, and therefore $U_w = U^*_w$. In the case of other substrates, *i*, $L_i < 1$ and $U_i < U^*_i$ (more than one substrate can simultaneously have $L_i = 1$ if the unlikely scenario that co-limitation occurs).

7.2.6. Examining the limiting potential of macro- and micro-nutrients under varying prey mixtures (Anderson and Pond 2000): Parameterisation. The analysis of Anderson and Pond (2000), incorporating the new parameter δ instead of parameter ϕ, is presented here to illustrate how different components of the diet potentially limit production over a range of hypothetical diatom-dinoflagellate prey mixtures. The biochemical composition of copepod eggs and phytoplankton cultures are presented in Table 7.3 (Jonasdottir 1994, Pond et al. 1996, Anderson and Pond

Table 7.3. Biochemical composition of *C. helgolandicus* eggs and phytoplankton species as used in the stoichiometric analysis. N:C are in mol mol^{-1}, FA:C ratios are in mmol mol^{-1} (after Anderson and Pond 2000).

	N:C	EPA:C	DHA:C
Copepod eggs	0.2	0.400	0.474
DIATOM: *Thalassiosira weisflogii*	0.150	0.722	0.160
DINOFLAGELLATE: *Prorocentrum minimum*	0.145	0.065	0.481

2000). The physiological parameter values and their justification are set out below (after Anderson and Pond 2000):

δ_i To begin with, it is simplest to assume that all material utilised is provided by ingestion. Therefore δ_C, δ_N, δ_{EPA} and δ_{DHA} are all set to 1.0. The effect of substrate supply from the animal's biomass will be examined later.

β_i Assimilation efficiencies for C, N, and PUFAs in marine zooplankton have all been reported to exceed 0.9 (Marshall and Orr 1955a, Corner et al. 1976, Landry et al. 1984, Pond et al. 1995, Xu and Wang 2003), and are set to 0.9.

k^*_i If sufficient C-rich substrates are available for respiration, N, EPA and DHA can all, at least theoretically, be used with a maximum net growth (utilisation) efficiency, k^*_i, of 1.0 (Anderson and Pond 2000). When a fraction of the utilised substrates are derived from biomass, they will be used with a maximum net utilisation efficiency, u^*_i, of 1.0.

K^*_i Maximum gross growth efficiency, K^*_i, is the product of maximum net production efficiency, k^*_i, and assimilation efficiency, β_i. It follows that K^*_N, K^*_{EPA} and K^*_{DHA} are all equal to 0.9. Respiration will always demand a fraction of the assimilated C, and therefore K^*_C cannot equal 1. Maximum net production efficiency is estimated to be around 0.8 for heterotrophs (Calow 1977), so K^*_C equals 0.7 ($k^*_C\beta_C$). When a fraction of the utilised substrates are derived from biomass, they will be used with a maximum gross utilisation efficiency, U^*_C, of 0.7 ($u^*_C\beta_C$).

7.2.7. Model output. The initial parameter and variable set is presented in Table 7.4, and the predicted limiting potentials for each dietary component are shown in Figure 7.2A. Dinoflagellates are relatively deplete in EPA, and when they constitute the majority of the diet, EPA is predicted to strongly limit egg production. Conversely, when diatoms dominate the diet, DHA is limiting because they contain only relatively small amounts of this compound. When a mixture of diatoms and dinoflagellates are ingested, the limiting potentials of C and N show a strong increase and N limitation is predicted to occur with diatom-dinoflagellate ratios between 36:64 and 42:52.

Stoichiometric theory predicts that, when either cell group dominates the diet, much of the available N will be excreted because it is in excess due to limitation by either EPA or DHA. As a result, when only monocultures of these cell types are

Table 7.4. Initial set of parameters and variables used for the initial stoichiometric analysis (after Anderson and Pond 2000).

Parameter/ variable	Description	Value	Units
$\theta_{ZC:C}$	Quantitative relationship between component *i* relative to component *j* in the copepod eggs, where *j* is specified as C	1	mol mol^{-1}
$\theta_{ZN:C}$		0.2	mol mol^{-1}
$\theta_{ZEPA:C}$		0.4	mmol mol^{-1}
$\theta_{ZDHA:C}$		0.474	mmol mol^{-1}
K^*_C	Maximum gross growth efficiency for component *i*	0.7	mol C mol C^{-1}
K^*_N		0.9	mol N mol N^{-1}
K^*_{EPA}		0.9	mol EPA mol EPA^{-1}
K^*_{DHA}		0.9	mol DHA mol DHA^{-1}
ϕC	The fraction of the demand for component *i* that is derived from ingested material.	1	mol C mol C^{-1}
ϕN		1	mol N mol N^{-1}
ϕEPA		1	mol EPA mol EPA^{-1}
ϕDHA		1	mol DHA mol DHA^{-1}
$\theta_{fC:C}$	Quantitative relationship between component *i* relative to component *j* in the diatom *Thalassiosira weisflogii*, where *j* is specified as C	1	mol mol^{-1}
$\theta_{fN:C}$		0.15	mol mol^{-1}
$\theta_{fEPA:C}$		0.722	mmol mol^{-1}
$\theta_{fDHA:C}$		0.16	mmol mol^{-1}
$\theta_{fC:C}$	Quantitative relationship between component *i* relative to component *j* in the dinoflagellate *Prorocentrum minimum*, where *j* is specified as C	1	mol mol^{-1}
$\theta_{fN:C}$		0.145	mol mol^{-1}
$\theta_{fEPA:C}$		0.065	mmol mol^{-1}
$\theta_{fDHA:C}$		0.481	mmol mol^{-1}

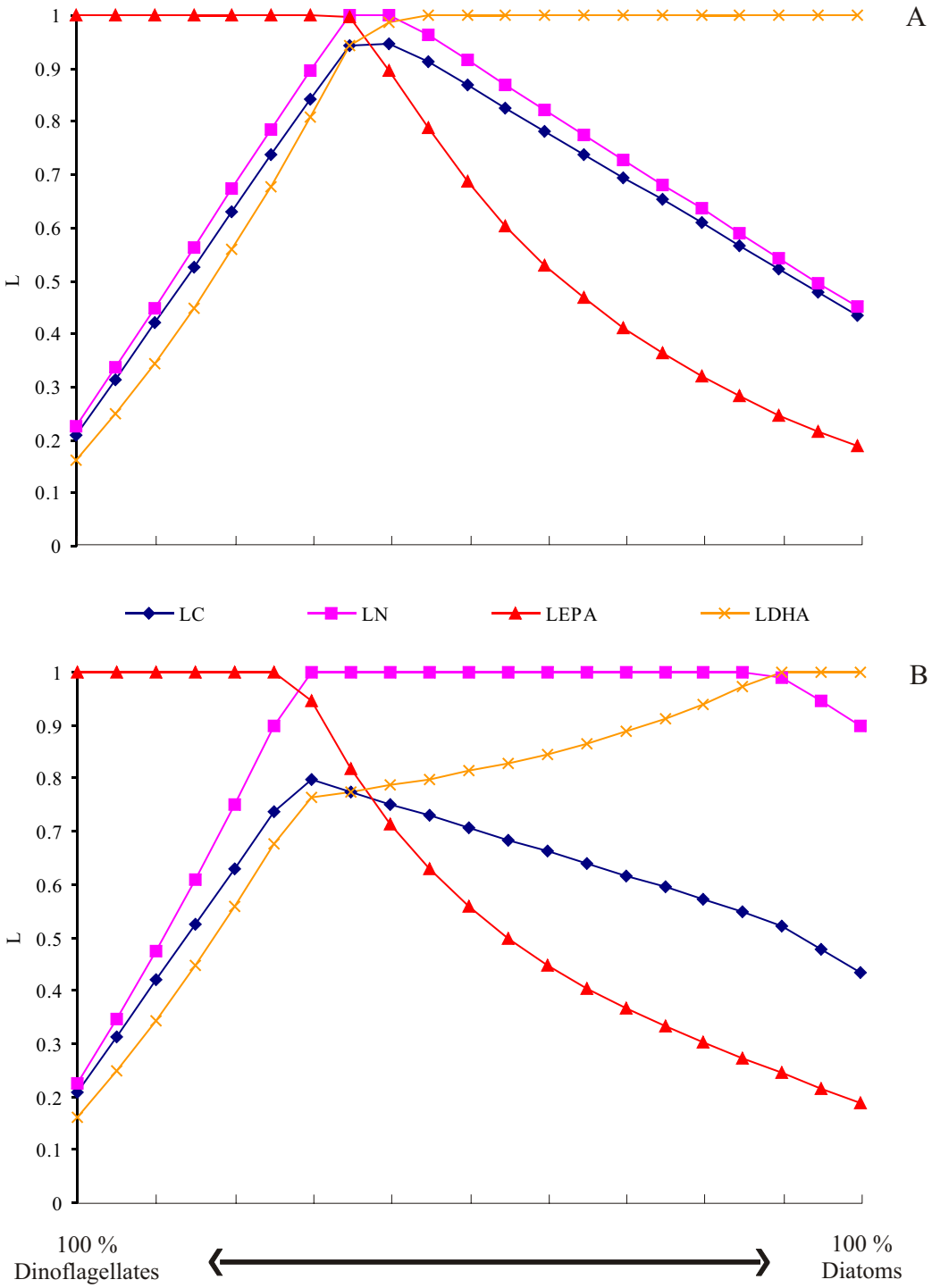


Figure 7.2 A Predicted limiting potentials of each dietary component and how it is influenced by the relative contribution of diatoms and dinoflagellates in the diet. The limiting potential of the component predicted to limit is always 1.0. Parameters as defined by Anderson and Pond (2000): see Table 7.4. Figure 7.2 B Illustrates how halving the N:C ratio of diatoms effects the predicted limiting potentials.

available as prey, the predicted gross growth efficiency for N, K_N ($= L_N K^*_N$), is 0.2 when dinoflagellates are ingested, and 0.4 when only diatoms are ingested. The latter value is very similar to that reported experimentally for copepods when fed diatom monocultures with variable N:C ratios (Kiorboe 1989). It is evident that when copepods are fed solely diatoms, the low and constant observed K_N that apparently suggests N limitation could have been brought about by DHA limitation. However, if the N:C ratio in diatoms is halved ($\theta_{f\,N:C\ diatoms} = 0.075$), and the analysis of Anderson and Pond (2000) reworked without changing any of the other variables (Table 7.3, Figure 7.2B), N is predicted to limit over a wide range of prey mixtures. Whilst DHA limitation is still predicted when only diatoms are ingested, it is apparent that if the N:C ratio in the diatoms were to decrease below 0.075, N would soon become limiting. Considering that the diatoms Kiorboe (1989) offered as prey had N:C ratios as low as 0.03, the low gross growth efficiencies for N displayed by the copepods may well have been representative of K^*_N, rather than being caused by DHA limitation. This suggests that over the spectrum of diatom N:C ratios offered to copepods by Kiorboe (1989), the low K_N could have been brought about by limitation of N at low ratios and DHA at high ratios.

If the analysis is reworked under the assumption that K^*_N is 0.4 (Figure 7.2C), it is apparent that N limitation is predicted to occur over most diatom-dinoflagellate prey mixtures, including when solely diatoms are ingested. However, EPA still limits when only dinoflagellates are offered as prey, and the realised K_N falls to 0.2. Thus if K^*_N really is as low as 0.4 because of N requirements for maintenance, then this is sufficient to override limitation by EPA and DHA except for when dinoflagellates constitute the vast majority of the diet.

Copepods are typically observed to have a gross growth efficiency for C of between 0.2 and 0.3 (Straile 1997). This suggests that using a K^*_C of 0.7, which although theoretically possible, may actually be too high. Using the initial parameter set (Figure 7.2A), K_C never reaches 0.7, and is generally < 0.5 (data not shown), illustrating that poor food quality (e.g. low quantities of PUFAs) may be the cause of the low observed gross growth efficiencies. Alternatively, if copepods have a high respiratory demand for C, K^*_C cannot be as high as the theoretically derived 0.7. If this value is halved and K^*_N is returned to 0.9, the limiting potential of C relative to the other substrates is doubled (Figure 7.2D). Interestingly, although C limitation is

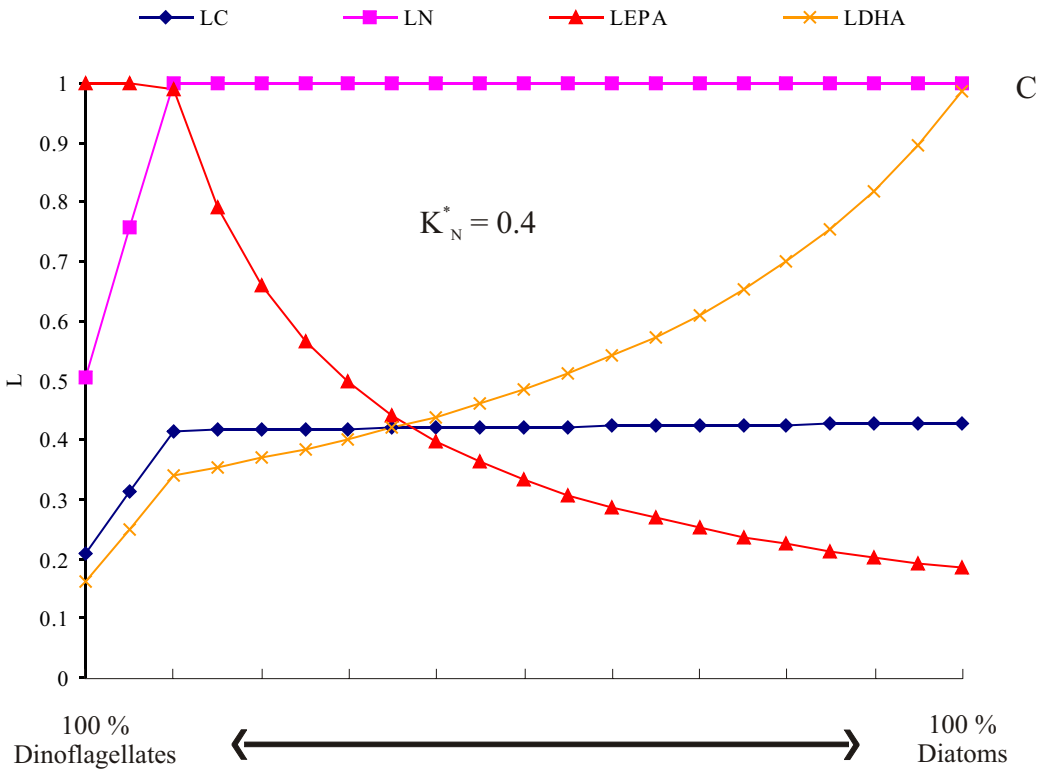


Figure 7.2 C. How the predicted limiting potentials of each dietary component vary when K^*_N is reduced to 0.4. The limiting potential of the component predicted to limit is always 1.0. Other parameters as defined by Anderson and Pond (2000): see Table 7.4.

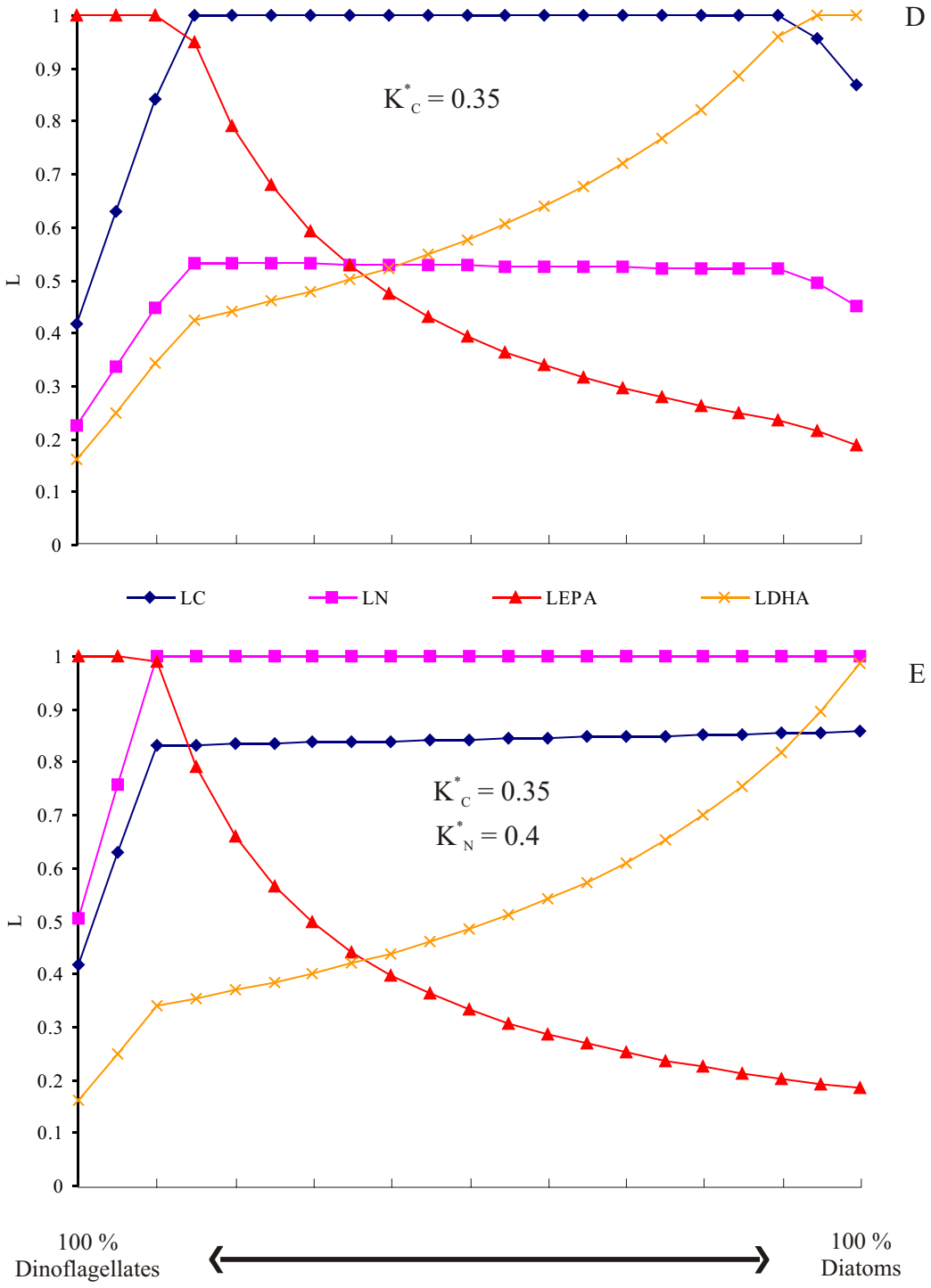


Figure 7.2 D and E. Predicted limiting potentials of each dietary component and how it is influenced by the relative contribution of diatoms and dinoflagellates in the diet. The limiting potential of the component predicted to limit is always 1.0. Parameters as defined by Anderson and Pond (2000): see Table 7.4.

predicted when the copepods receive a mixed diet, if only dinoflagellates or diatoms are offered, EPA and DHA are still predicted to limit production. In a final reworking of the parameters, K^*_N and K^*_C are set to 0.4 and 0.35 respectively (Figure 7.2E). N limitation predominates at all prey mixtures until the dinoflagellate-diatom ratio of the diet is > 90:10, where EPA limitation occurs. Although C is not predicted to limit, it is used relatively efficiently, with a typical K_C of 0.3 (data not shown).

An important conclusion of this work is that zooplankton production is not necessarily limited by bulk C or N. Specific micronutrients such as PUFAs are of potential importance, depending on the compositions of consumers and their prey. Studies addressing the limitation of copepods must therefore consider such compounds to avoid erroneous conclusions (Anderson and Pond 2000). In the analysis presented here, it is assumed that copepods derive all their PUFAs solely from the diet. If body reserves or biosynthesis of EPA or DHA provide these compounds in significant quantities, their limiting potentials will therefore decrease. This may be of particular importance in polar copepods, which are known to store large quantities of lipid (e.g. Kattner and Hagen 1995). However, strong correlation between production and dietary quantities of PUFAs suggests that they have an important role to play in copepod reproduction (Jonasdottir 1994, Jonasdottir et al. 2002). Maintaining dietary diversity would appear to be an important strategy to ensure that the nutritional demands of the copepods are met (Kleppel 1993), which in turn should promote higher overall gross growth efficiencies.

7.3. RESULTS

The following section brings together the experimental data presented in the preceding chapters on the sources (ingestion, I_i, and biomass, B_i) and sinks (egg production and accumulation of biomass, collectively, G_i) of dietary substrates used by *C. finmarchicus*. These data are used to construct the physiological budgets (see equations 9 and 10) for C, N, EPA and DHA in April and July/August. By assuming that all substrates are assimilated with an efficiency of 0.9 (see Section 7.2.6.), the quantities of material egested, W_i, can be determined. The only components missing from the physiological budgets are then respiration, R_C, and excretion, E_i, which can subsequently be estimated by mass balance. Comparing these values to literature derived estimates of respiration and excretion provides a means by which the quality of the experimental data can be independently assessed.

7.3.1. Physiological budgets. Daily determinations of the biochemical composition of the experimental animals, and thus the daily contributions of bodily reserves, were not possible. To overcome this, all data were totalled over the 5-day period. The budgets compiled for the females in April and July/August are presented in Figures 7.3 and 7.4, and also Tables 7.5 and 7.6 respectively. Molar ratios of N:C, EPA:C and DHA:C in the food, biomass and eggs for the two seasons are presented in Table 7.7.

7.3.1.1. April. It is immediately apparent that the majority (82 %) of the C available to *C. finmarchicus* during the incubations in April was derived from the females' biomass (Figure 7.3). Indeed, this was also the case for the other dietary substrates (in all cases, B_i > 60 %). It can be seen that the experimentally determined quantities of C supplied by ingestion (I_C) and biomass (B_C) were in approximate balance with the amounts expended (production of eggs, G_C, respiration, R_C, and egestion, W_C). This balance is reflected in the close agreement between the respiration rates determined by mass balance and estimated using the regression of Ikeda et al. (2001), based on the incubation temperature and average N biomass of the females (Table 7.5). In contrast, the N excretion rate calculated by mass balance is approximately twice as great as that estimated using temperature and N biomass (Ikeda et al. 2001). This indicates that either the food ingested had a C:N ratio greater than that of Redfield, or that other important N-sinks exist. The EPA and DHA budgets were similar to N in that excretion rates calculated by mass balance also suggest that a large proportion (> 60 %) of the available PUFAs were excreted. Unfortunately, experimentally determined excretion rates of EPA and DHA for *Calanus* do not exist and it is therefore difficult to assess how realistic the values determined by mass balance actually are. All dietary substrates were utilised for egg production with a gross utilisation efficiency of approximately 30 % (Table 7.5).

7.3.1.2. July/August. In addition to producing eggs, the females in July/August also gained biomass (Section 6.3.1.2). Consequently, all material must have been supplied via ingestion. The gross utilisation efficiencies for EPA and DHA were both low (<15 %, Table 7.6) and consequently their excretion rates calculated by mass balance were very high (>75 %), as found in April (Figures 7.3 and 7.4). Unfortunately, it is evident that the C and N budgets do not balance (Figure 7.4). The observed quantities of C and N allocated to growth both exceeded the quantities of

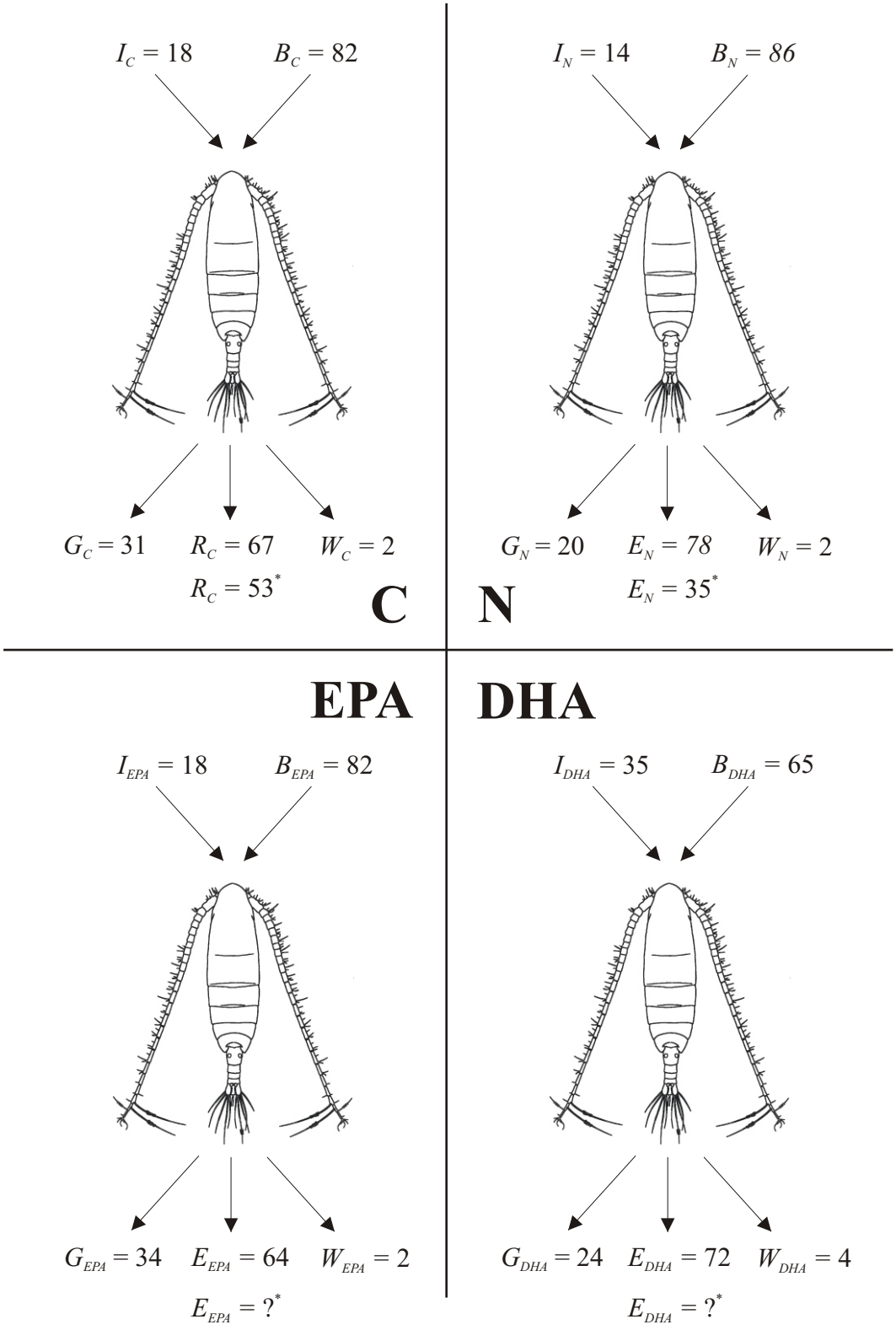


Figure 7.3. The physiological budgets for *Calanus finmarchicus* in April. All values are expressed as a percentage of the total input. Parameters are explained in section 7.2.1. * values are derived from the literature - see Table 7.5 for details.

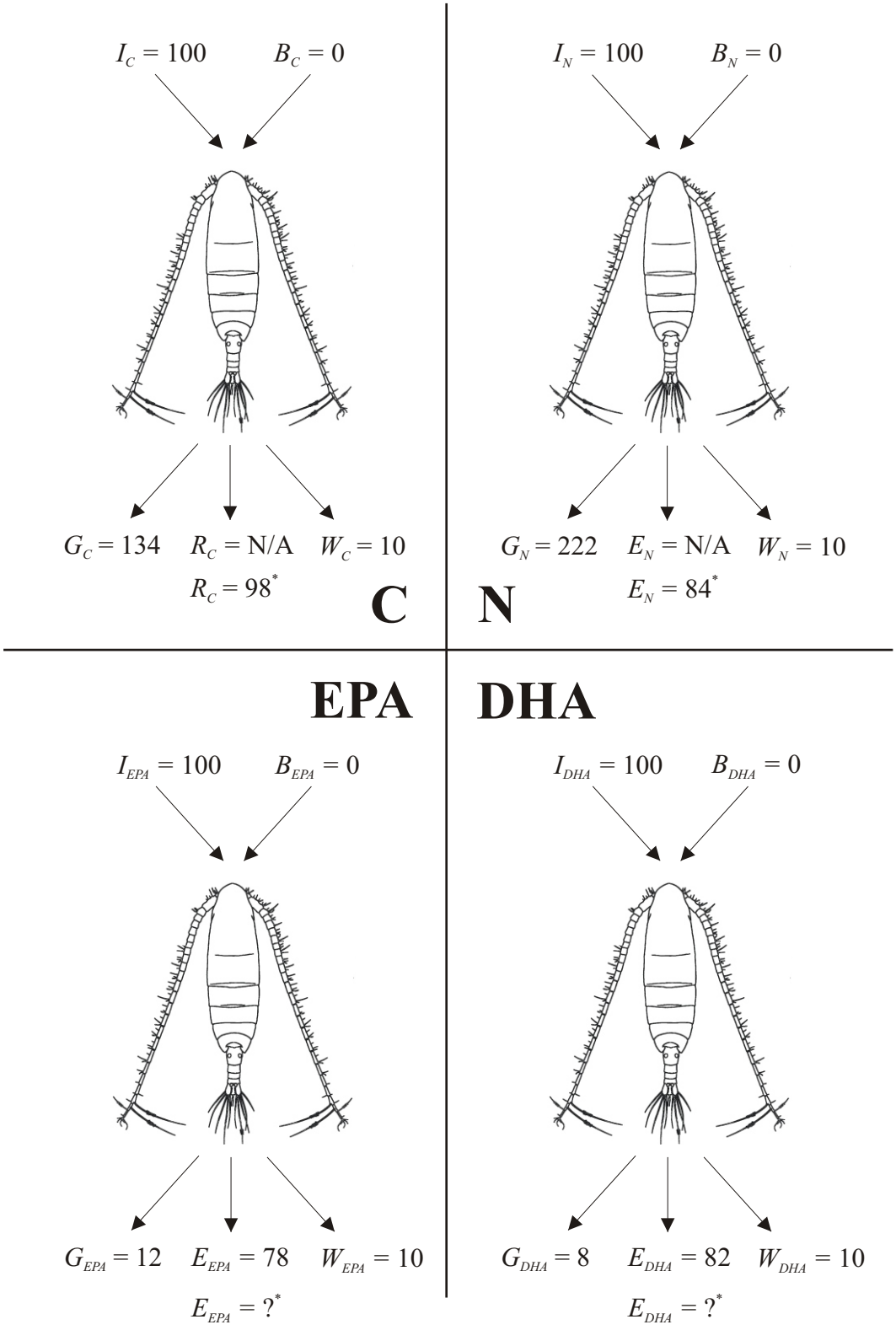


Figure 7.4. The physiological budgets for *Calanus finmarchicus* in July/August. All values are expressed as a percentage of the total input. Parameters are explained in section 7.2.1. * values are derived from the literature - see Table 7.6 for details.

Table 7.5. Elemental and essential fatty acid budgets (equations 9 and 10) for *C. finmarchicus* over the 5 day egg production experiment conducted in April. [a]Assuming 90 % assimilation efficiency (see text for details). [b]Oxygen consumption estimated using the equation; ln O_2 consumption (µl O_2 ind^{-1} hr^{-1}) = 1.640 + 0.843 * ln N-biomass (mg ind^{-1}) + 0.068 * Temp (ºC) (Ikeda et al. 2001). Respiration rates determined assuming protein metabolism (RQ of 0.8, Prosser 1961). [c]Estimated by mass balance. Values in parentheses represent losses of C and N to respiration and excretion as a percent of the body content of each item. [d]Excretion rates estimated using the equation; ln Ammonia excretion (µg N ind^{-1} hr^{-1}) = -1.386 + 0.772 * ln N-biomass (mg ind^{-1}) + 0.070 * Temp (ºC) (Ikeda et al. 2001).

	C	N	EPA	DHA
Avg. biomass (mol cop^{-1})	6.26E-06	1.49E-06	2.82E-09	3.22E-09
I_i (mol cop^{-1} exp^{-1})	4.77E-07	7.20E-08	2.93E-10	6.01E-10
B_i (mol cop^{-1} exp^{-1})	2.11E-06	4.35E-07	1.32E-09	1.11E-09
G_i (mol cop^{-1} exp^{-1})	8.09E-07	1.04E-07	5.51E-10	4.09E-10
W_i (mol cop^{-1} exp^{-1}) [a]	4.77E-08	7.2E-09	2.93E-11	6.01E-11
R_i (mol cop^{-1} exp^{-1})[b]	1.36E-06 (4.3 %)			
R_i (mol cop^{-1} exp^{-1})[c]	1.73E-06 (5.5 %)			
E_i (mol cop^{-1} exp^{-1})[d]		1.76E-07 (2.4 %)		
E_i (mol cop^{-1} exp^{-1})[c]		3.97E-07 (5.3 %)	1.03E-09	1.24E-09
U_i	0.31	0.20	0.34	0.24
u_i	0.32	0.21	0.35	0.25

Table 7.6. Elemental and essential fatty acid budgets (equations 9 and 10) for *C. finmarchicus* over the 5 day egg production experiment conducted in July/August. [*]includes gain in biomass. [a]Assuming 90 % assimilation efficiency (see text for details). [b]Oxygen consumption estimated using the equation; ln O_2 consumption (µl O_2 ind^{-1} hr^{-1}) = 1.640 + 0.843 * ln N-biomass (mg ind^{-1}) + 0.068 * Temp (ºC) (Ikeda et al. 2001). Respiration rates determined assuming protein metabolism (RQ of 0.8, Prosser 1961). [c]Estimated by mass balance. Values in parentheses represent losses of C and N to respiration and excretion as a percent of the body content of each item. [d]Excretion rates estimated using the equation; ln Ammonia excretion (µg N ind^{-1} hr^{-1}) = -1.386 + 0.772 * ln N-biomass (mg ind^{-1}) + 0.070 * Temp (ºC) (Ikeda et al. 2001).

	C	N	EPA	DHA
Avg. biomass (mol cop^{-1})	8.21E-06	1.77E-06	3.46E-09	4.28E-09
I_i (mol cop^{-1} exp^{-1})	2.1E-06	3.17E-07	5.79E-09	1.04E-08
B_i (mol cop^{-1} exp^{-1})	0	0	0	0
G_i (mol cop^{-1} exp^{-1}) [*]	2.82E-06	7.03E-07	6.88E-10	8.51E-10
W_i (mol cop^{-1} exp^{-1}) [a]	2.10E-07	3.17E-08	5.79E-10	1.04E-09
R_i (mol cop^{-1} exp^{-1})[b]	2.06E-06 (5.0 %)			
R_i (mol cop^{-1} exp^{-1})[c]	-9.30E-07			
E_i (mol cop^{-1} exp^{-1})[b]		2.66E-07 (3.0 %)		
E_i (mol cop^{-1} exp^{-1})[c]		-4.17E-07	4.53E-09	8.50E-09
U_i	1.34	2.22	0.12	0.08
u_i	1.49	2.46	0.13	0.09
Shortfall	3.04E-06	7.22E-07		
No. eggs cannibalised to meet shortfall d^{-1}	26	40		

Table 7.7. Experimentally determined molar ratios in April (A) and July/August (B).

A	N:C	EPA:C	DHA:C
Ingested food	0.1509	0.000615	0.00125
Biomass utilised	0.2063	0.000626	0.000527
Eggs	0.1319	0.000602	0.000451

B	N:C	EPA:C	DHA:C
Ingested food	0.1509	0.0028	0.0049
Biomass gained	0.3183	N/A	N/A
Eggs	0.1495	0.000646	0.000784
All new biomass	0.2494	0.000244	0.000302

these substrates ingested, and therefore respiration and excretion rates calculated by mass balance were negative (Table 7.6). Gross utilisation efficiencies for C and N of > 1 are physically impossible, indicating that one or more components of the budget were incorrectly determined.

7.3.2. Stoichiometric analysis of the experimental data. The stoichiometric analysis of Anderson and Pond (2000) is applied here to the experimental data collected in April. In keeping with the previous analyses, all ratios are expressed relative to dietary C. All the variable and parameter values are presented in Table 7.8. Unfortunately, because the loss and gain terms of the budget determined in July/August do not balance (Table 7.6), stoichiometric analysis of this data set was not possible.

7.3.2.1. Predicting the dietary element or compound that limited production in April based solely on ingested material. Values of the K^*_i parameters are initially set to equal those used by Anderson and Pond (2000; Table 7.8). It is assumed that ingested matter is the sole source of all material available to the copepods, and therefore δ for all compounds is equal to 1. The turnover (i.e. maintenance) of structural biomass is assumed to be zero.

Of all the K^*_i parameters, K^*_C is the most crucial because, in addition to other requirements, C is also needed for respiration. This parameter is difficult to define, therefore the limiting potentials of each substrate are initially plotted against a variable K^*_C (Figure 7.5A). It is quite apparent that C is predicted to strongly limit egg production rates in April over the entire range of potential C gross growth efficiencies. However, recent modelling work has suggested that there is a maintenance demand for N associated with the turnover of structural biomass (Kuijper et al. 2004, Anderson et al. 2005). Maximum net production efficiency for N, k^*_N, cannot then equal 1 because a fraction of the ingested N will be required to maintain the animals structure. A K^*_N of 0.9 is therefore unrealistically high. Indeed, experimental evidence suggests that a K^*_N of 0.4 may be more realistic (Checkley 1980, Kiorboe 1989). If K^*_N in the initial parameter set is decreased to 0.4, the effects of a large maintenance demand for N can be examined (Figure 7.5B). The limiting potential of N is now predicted to increase rapidly until it limits when $K^*_C > 0.45$. Conversely, when K^*_C is low (< 0.45), production is then predicted to be limited by

Table 7.8 Initial and alternative parameters used for the stoichiometric analysis of the April data set. *after Anderson and Pond (2000) #experimentally derived.

Parameters	April initial	Alternative
K^*_C	0.70*	0.35
K^*_N	0.90*	0.4
K^*_{EPA}	0.90*	0.90*
K^*_{DHA}	0.90*	0.90*
Variables		
δ_C	1*	0.169#
δ_N	1*	0.130#
δ_{EPA}	1*	0.167#
δ_{DHA}	1*	0.327#
$\theta_{ZN:C}$	0.1320#	0.1320#
$\theta_{ZEPA:C}$	0.000615#	0.000615#
$\theta_{ZDHA:C}$	0.000451#	0.000451#
$\theta_{fN:C}$	0.1510#	0.1510#
$\theta_{fEPA:C}$	0.0006#	0.0006#
$\theta_{fDHA:C}$	0.0013#	0.0013#

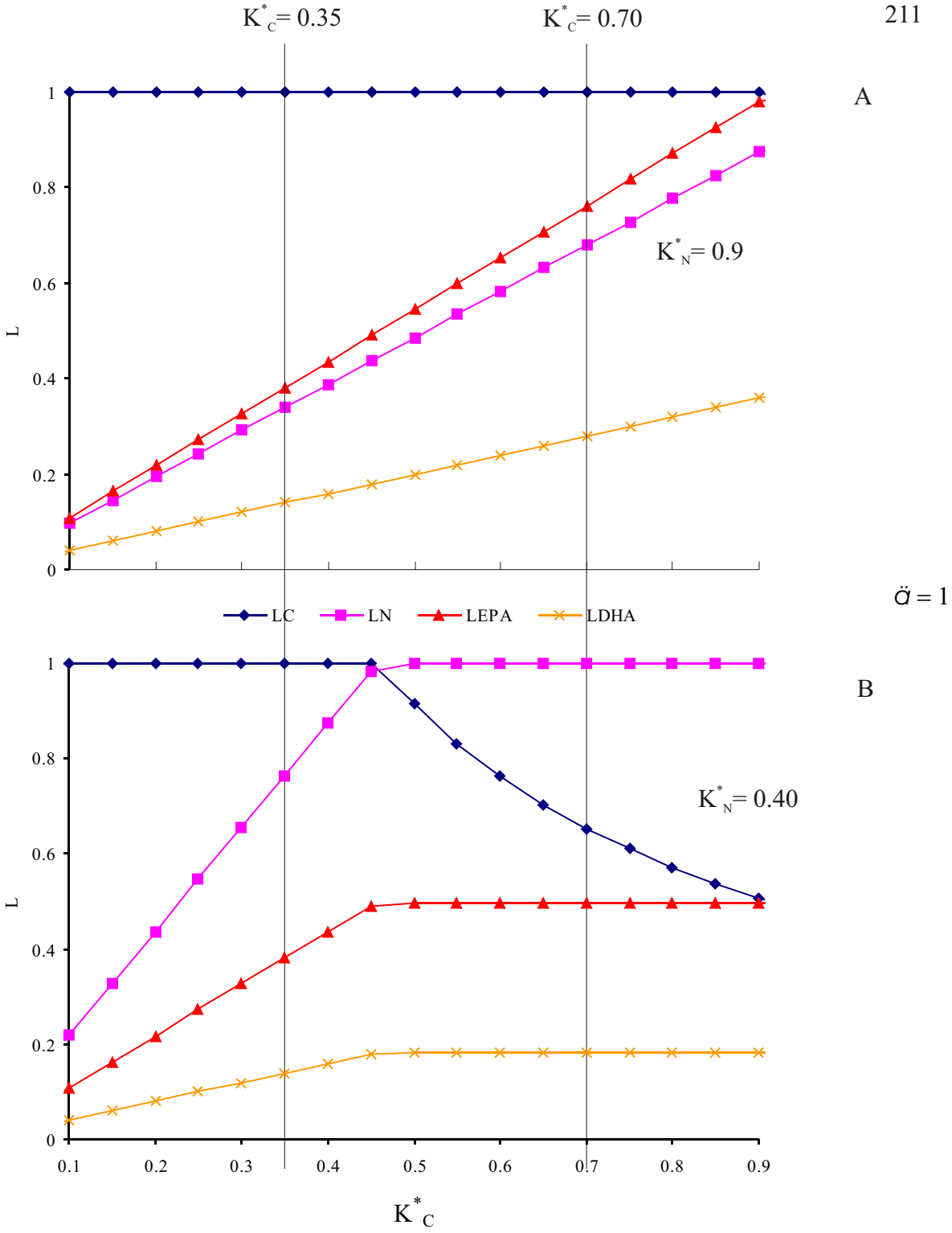


Figure 7.5. Illustration of how the predicted limiting potential of each dietary component in spring changes with variable K^*_C for given values of K^*_N (0.90 and 0.40, figures A and B). $\ddot{\alpha}$ for each parameter is set to 1.

C. Regardless of K^*_C, under this scenario the limiting potentials of EPA and DHA are predicted to remain relatively low, with realised gross growth efficiencies of < 0.5.

By varying K^*_N, the following analysis examines how the maintenance demand for N affects the limiting potentials of each substrate under a constant K^*_C. To begin with, K^*_C is set to 0.7 (initial parameter set, Table 7.8, Figure 7.5C). Nitrogen is predicted to limit production until $K^*_N > 0.6$, after which C then becomes limiting. However, this particular analysis is based on the assumption that the copepods are using C with the maximum theoretically achievable gross growth efficiency (0.7). In reality the energetic demands for obligatory processes such as osmoregulation and locomotion mitigate against the achievement of this maximum efficiency (Calow 1977). As discussed above, experimentally determined values of K^*_C in copepods are typically between 0.2 and 0.3 (Straile 1997). These values are much lower than a theoretical maximum of between 0.7 and 0.8 (Calow 1977), suggesting that there may be large respiratory/maintenance demands for C, or that C was typically not limiting in these experimental studies (Straile 1997 and refs within). Reworking the data with a K^*_C of 0.35 (Figure 7.5D) illustrates that under the conditions encountered in April, C is expected to limit whenever $K^*_N > 0.3$.

The results thus far highlight the fact that using stoichiometric theory to understand the limitation of copepods is restricted by the accuracy with which the parameters can be defined. Considering that a K^*_N of 0.4 has been derived both experimentally (Checkley 1980, Kiorboe 1989) and theoretically (Kuiper et al. 2004, Anderson et al. 2005), this value appears to be a reasonable starting point. Examining Figure 7.5B illustrates that either C or N are poised to limit, depending on the value of ascribed to K^*_C. Interestingly, the limiting potentials of both EPA and DHA remain relatively low, regardless of K^*_C and K^*_N.

7.3.2.2. Predicting the dietary element or compound that limits production based on material derived from both ingestion and biomass. The analysis above assumes that substrates in the diet are the sole source of material for growth. During the experiments in April, daily mass specific ingestion rates were only ~ 1.5 % (Table 5.1), and significant quantities of all substrates were derived from copepod biomass, rather than the diet, over the duration of the incubations (Figure 7.3). Indeed, the material derived from the animals' biomass constituted the majority (e.g. 82 % for C) of the material utilised, thus rendering the above analysis based solely on dietary substrates (section 7.3.2.1) of limited merit. The analysis is

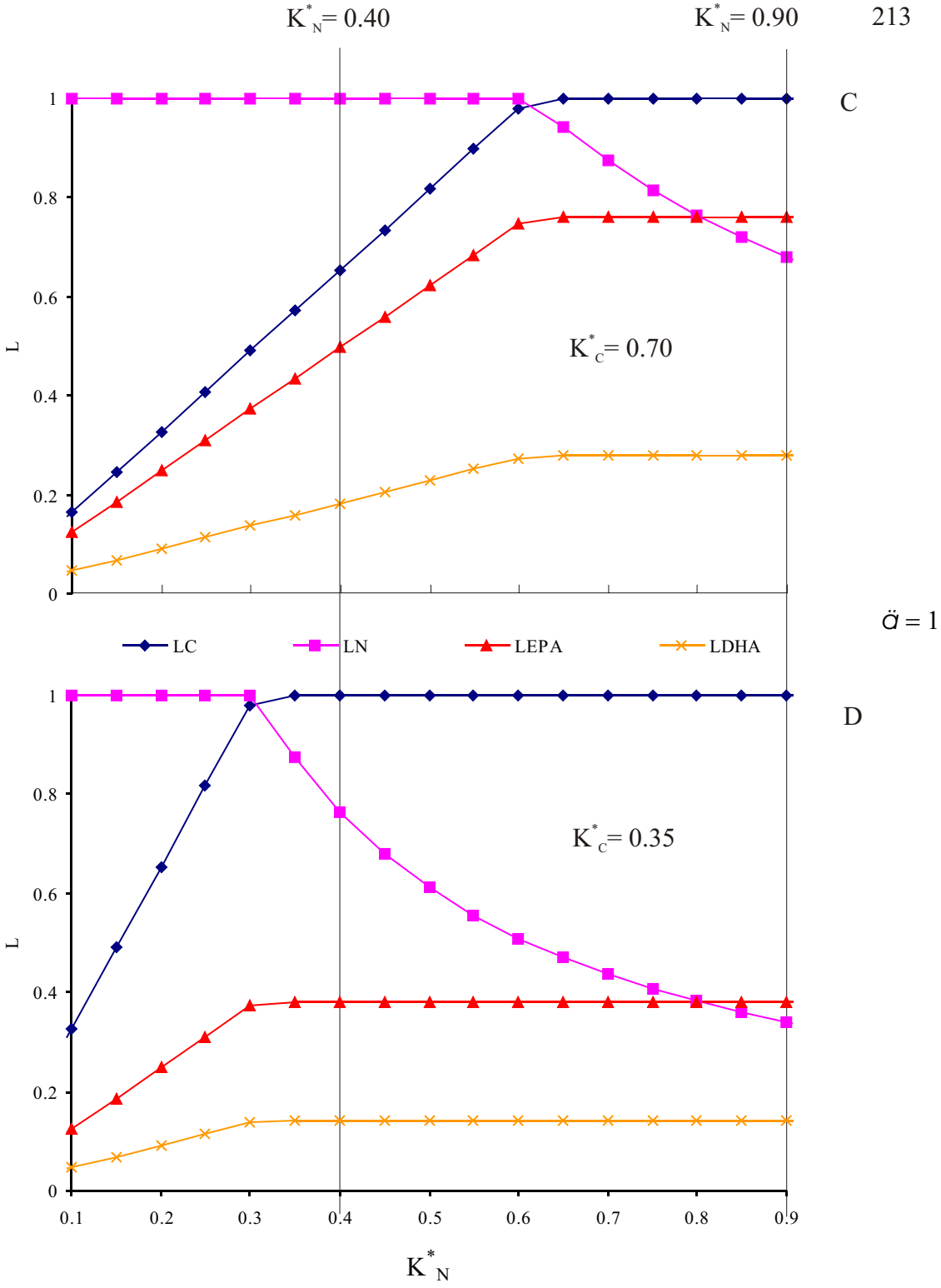


Figure 7.5. Illustration of how the calculated limiting potential of each dietary component, L, in spring changes with variable K^*_N for given values of K^*_C (0.70 and 0.35, figures C and D). $\ddot{a}$ is set to 1.

now extended to include substrates derived from biomass (fraction 1-δ being the fraction of total available substrates originating from biomass, fraction δ from the diet). Synthesis is again assumed to be zero. The fraction of the demand supplied by ingestion (δ) for each element and compound was constantly < 0.33 (Table 7.8), demonstrating that ingestion alone was not sufficient to meet the metabolic demands. Interestingly, the scarcity of storage fatty acids in the copepods indicated that lipid reserves were essentially depleted (Figure 6.3). Furthermore, the biomass lost in April had a low C:N ratio (~4), suggesting that protein was being catabolised. The above analysis is now repeated using values of δ_i derived from the spring experiments (Table 7.8). Results may now be expected to differ from those in section 7.3.2.1 given that values of δ_i were less than 1 and the composition of the biomass utilised was different to that of the diet (Table 7.7). The substrate with the greatest limiting potential, L_W, now corresponds to the substrate with the lowest gross utilisation efficiency, U_i, rather than that with the lowest K_i (equation 22).

This analysis begins by setting the maximum gross utilisation efficiency parameters, U^*_i, to equal the corresponding maximum gross growth efficiencies, K^*_i, used previously (Table 7.8). In other words, it is assumed that substrates of either dietary of biomass origin can potentially be used with the same maximum efficiencies. When the analysis is expressed relative to U^*_C (Figure 7.6A), and U^*_N was 0.9, C is again predicted to limit production throughout the range of theoretically achievable values of U^*_C (0 to 0.8). The limiting potentials of C and EPA displayed in this analysis are similar to those previously predicted when using the same K^*_i parameters but where values of δ_i were set to 1 (Figure 7.5A). Limitation by C is still predicted because the biomass used during the incubations was deplete in C relative to other substrates. Indeed, the predicted limiting potential of N now decreases relative to the earlier analysis because the N:C ratio in the biomass utilised was greater than that of the ingested food (Table 7.7). The limiting potential for EPA remains much the same because EPA:C ratios in the food and biomass utilised are the same. Finally, the limiting potential for DHA increases because it is relatively deplete in the biomass utilised, but not enough for DHA to be predicted to be limiting overall.

If U^*_N is now decreased to 0.4 (Figure 7.6B), C limitation is predicted to occur until $U^*_C > 0.6$, after which N limitation is expected. Previously, when K^*_N was set to 0.4 but values of δ_i were set to 1, the switch from C to N limitation was

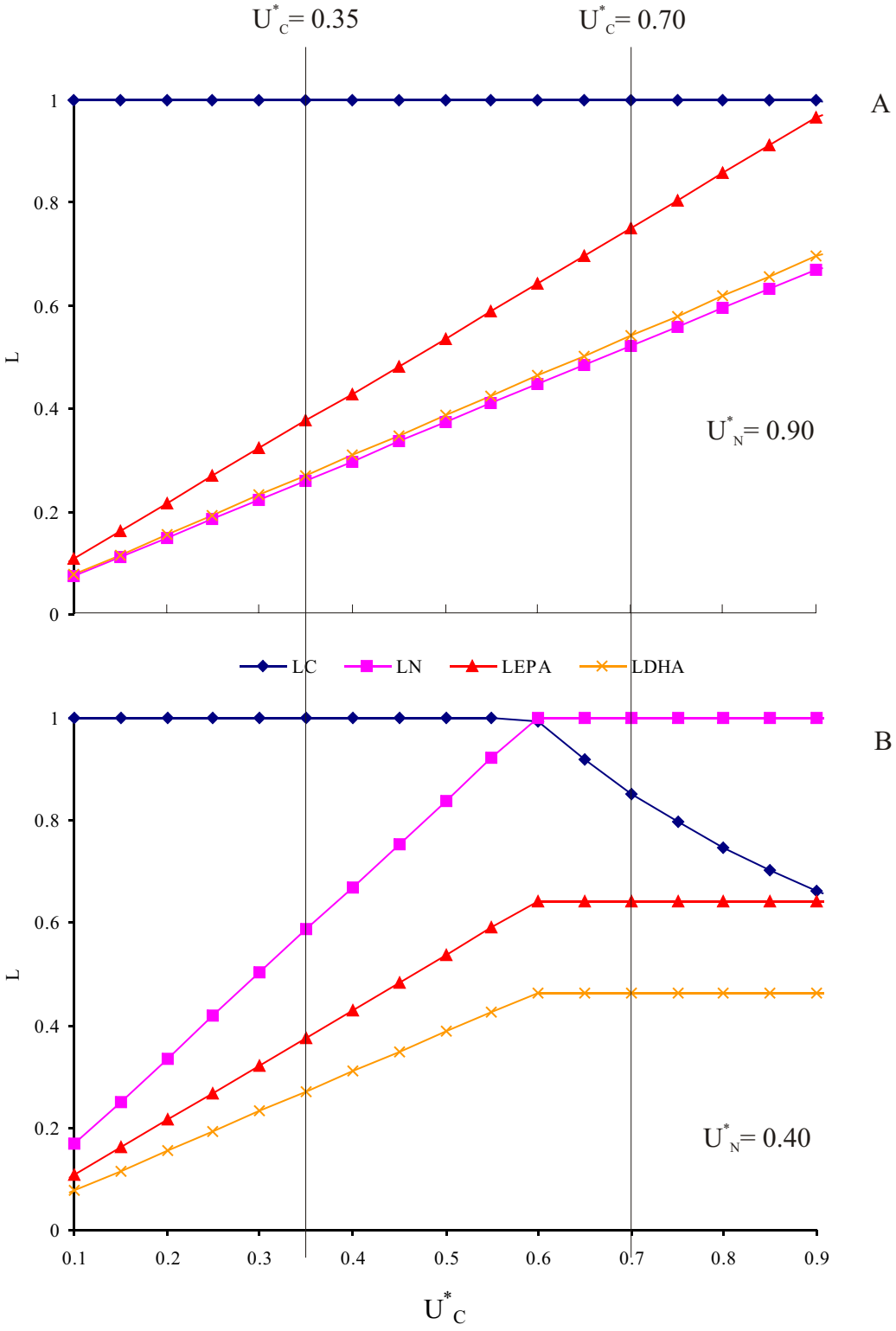


Figure 7.6. Illustration of how the predicted limiting potential of each dietary component, L, in spring changes with variable U^*_C for given values of U^*_N (0.90 and 0.40, figures A and B). $\ddot{\alpha}$ is calculated using experimental data.

predicted to occur where K^*_C was > 0.45 (Figure 7.5B). Again, although decreasing U^*_N essentially increases the demand for N, the range of U^*_C values over which C limitation is predicted still increases when internal sources of substrates are considered because the biomass utilised is rich in N relative to C (Table 7.7). Interestingly, by including biomass utilisation in the analysis, although the limiting potentials for EPA and DHA follow the same trends as before, the values at which they plateau out are both predicted to increase (Figures 7.5B and 7.6B). Nitrogen is less limiting when biomass utilisation is included in the analysis (because of the high N:C in biomass), and so the limiting potential of other substrates, C, EPA and DHA, increases. The predicted limiting potential of DHA in Figure 7.6B is higher than in Figure 7.5B because the DHA:C in the biomass is less than half what it is in the food (Table 7.7).

When the U^*_i parameters are returned to the initial K^*_i values (Table 7.8) and the analysis is re-plotted against maximum gross utilisation efficiency for N on the X-axis (using U^*_C = 0.7), the effect of deriving substrates from both ingestion and biomass relative to a variable U^*_N can be compared and contrasted with the previous analysis in which ingestion was assumed to be the sole source of material i.e. δ_i = 1 (Figures 7.5C and 7.6C). The imbalance between the N:C ratios in the biomass utilised and the food is once again apparent. That is to say, even though in this analysis U^*_C is set to its theoretical maximum (0.7), the relative demand for C still increases because the N:C ratio supplied from the biomass is significantly higher than that in the ingested food. The predicted range of U^*_N over which C limitation occurs now increases, beginning whenever U^*_N > 0.45 (Figure 7.6C), whereas previously, the switch between N and C limitation did not occur until K^*_N > 0.6 (Figure 7.5C). The predicted limiting potential of DHA is again greater than when food is assumed to be the sole source of substrates (Figure 7.5C) because of the low DHA:C in the biomass relative to the food (Table 7.7). In a final alteration to the parameter set, the maximum gross utilisation efficiency for C is decreased to 0.35. Not surprisingly, the effect of reducing U^*_C causes the range over which C limitation is predicted to increase. Indeed, N limitation is only expected where N is used with a very low efficiency (U^*_N < 0.25, Figures 7.5D and 7.6D).

In conclusion, the potential for C limitation increased markedly when biomass utilisation is included in the analysis because biomass is relatively rich in N compared to food. When C is utilised with the theoretical maximum efficiency of 0.7

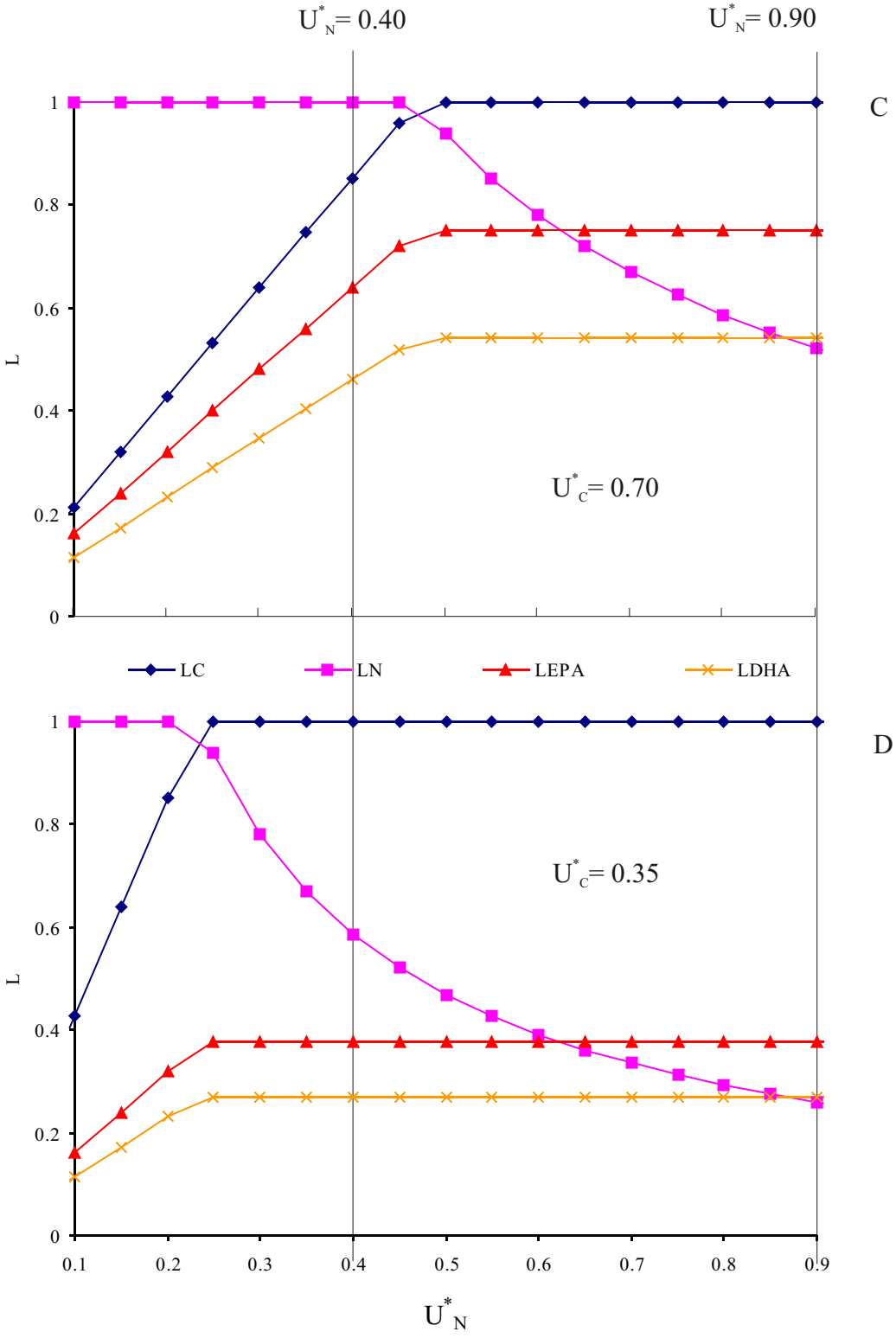


Figure 7.6. Illustration of how the predicted limiting potential of each dietary component, L, in spring changes with variable U^*_N for given values of U^*_C (0.70 and 0.35, figures C and D) .$\ddot{\alpha}$ is calculated using experimental data.

(Calow 1977), C limitation in April is predicted when $U^*_N > 0.45$. If U^*_C is decreased to 0.35 then C limitation occurs when $U^*_N > 0.25$. If marine copepods have an apparently low K^*_N of 0.4, e.g. as suggested by the experimental evidence of Kiorboe (1989), this is nevertheless only sufficient to cause limitation by N rather than C if U^*_C is given the theoretically maximum value of 0.7 (Calow 1977), based solely on biosynthesis costs. Decreasing U^*_C to 0.35 causes production to be limited by C, even when K^*_N is 0.4. It therefore seems likely that C was the substrate limiting production of *C. finmarchicus* in April.

7.4. DISCUSSION

7.4.1. Physiological budgets. The data presented here support the observation that the metabolic demands of *C. finmarchicus* relative to requirements for growth vary over the seasonal cycle (Conover and Corner 1968, Butler et al. 1969, Butler et al. 1970, Tande 1982). Before the spring bloom, the females were heavily dependent upon internal sources of C, N, EPA and DHA, whereas in July/August, they were both producing eggs and increasing their structural biomass.

7.4.1.1. April. Significant quantities of C, N, EPA and DHA were lost from the animal's biomass during the incubations in April (Sections 6.3.1.1. and 6.3.2.1). The data presented here demonstrate that during the incubations in April, > 80 % of the C and N utilised by the females was derived from their biomass (Figure 7.3, Table 7.5), suggesting that the ingested food was not sufficient to meet the animals' metabolic demands. Data collected previously suggest that in April, each female would have respired a total of ~ 1.92×10^{-6} mol C cop^{-1}, and excreted up to 2.11×10^{-7} mol N cop^{-1} over the 5-day experimental period at the incubation temperature of 7 ºC. (Marshall and Orr 1958, Butler et al. 1970, Ikeda and Skjoldal 1989). Although the values determined by mass balance were similar (1.73E-06 moles C and 3.97E-07 moles N), exact matches may not be expected because respiration and excretion rates are known to be strongly influenced by body mass (Ikeda 1985). A comparison of biomass-specific rates is therefore more revealing. The regressions of Ikeda et al. (2001), based on incubation temperature and copepod N content, estimated that the experimental animals in April would have excreted N at a mass-specific rate of 2.4 % d^{-1}. This is approximately half that estimated by mass balance (5.3 %, Table 7.5), but considering that female *C. finmarchicus* in April have previously been reported to excrete between 3.7 and 9.8 % d^{-1} (Butler et al. 1970, data correct to 7 ºC using a Q_{10} of 2.1), the value estimated by mass balance does not seem unreasonable.

Mass-specific respiration rates estimated by mass balance and using the N-specific regression (see Table 7.5 for equations; Ikeda et al. 2001) were in excellent agreement (5.5 and 4.3 % respectively, Table 7.5). Such close agreement with previously determined respiration and excretion rates suggests that the experimentally determined values of ingestion, biomass utilisation and production here are indeed representative of their true values. Furthermore, the females produced eggs with a gross C utilisation efficiency of 31 % (Table 7.5, $U_i \times 100$),

well within the range (29 – 38 %) of gross growth efficiencies previously determined for other species of marine copepods (Checkley 1980, Berggreen et al. 1988, Peterson 1988, Kiorboe 1989). Unfortunately, similar data for the excretion of PUFAs do not exist in the literature therefore this type of analysis cannot be used to assess the quality of these data.

7.4.1.2. July/August. The physiological requirements of the females in July/August were quite different to those in April (Figures 7.3 and 7.4, Tables 7.5 and 7.6). During these incubations, the females both produced eggs and also increased their biomass. Net utilisation of biomass could not therefore have been a source of substrates for production in this instance and so ingestion must be the net source of substrates. However, the C and N budgets in July/August did not balance, and there were considerable shortfalls in the estimated amounts of C and N ingested relative to the observed growth (Figure 7.4, Table 7.6). When the estimated respiration and excretion rates (Ikeda et al. 2001, see above) are incorporated into the budget, it is apparent that these processes only represent a relatively small proportion of the overall C and N budgets in July/August (Table 7.6). However, less than 50 % of the C and N demand are fulfilled by the experimentally determined ingestion rates. In contrast, the estimated quantities of PUFAs ingested are in excess relative to their demands for growth, and are utilised with only low efficiency (Table 7.6). Such low efficiencies would suggest that these compounds were not limiting, although this does assume that the maintenance demands for EPA and DHA are low.

The large discrepancy between the supply and demand of C and N in July/August suggested at least one error had occurred during the analysis of the experimental samples. This was somewhat surprising because the approximate balance of the budget compiled in April suggested that the experimentally derived data were representative of their real values. Considering the attention paid to maintaining the analytical precision of the elemental analyser (Section 2.5.3), the C and N biomass determinations were considered to be reliable, making the estimation of ingestion the most likely source of error. Microzooplankton grazing artefacts were taken into consideration (Chapter 3), as was their contribution to the diet of *C. finmarchicus*. However, one possible source of error not considered was that associated with the cannibalism of eggs. Previously recorded post-bloom egg production rates are variable, but typically greater than those determined here (Tables 6.1 to 6.3 and refs their in), suggesting that this may have been apparent.

Using the estimated values of respiration and excretion (Ikeda et al. 2001, Table 7.6), the physiological budgets for C and N can be used to estimate the quantities of cannibalised eggs required to meet the shortfalls. In July/August, the average egg contained 2.5x10-8 moles of C and 3.8x10-9 moles of N (Section 6.3.6.1), therefore 26 and 40 eggs would have had to be consumed by each female daily in order to balance the C and N budgets respectively (Figure 7.7). In addition to the measured egg production rate, this would suggest that the egg production rate in July/August was actually ~40 eggs female^{-1} day^{-1}, or ~12 % of their C biomass daily. The concentration of eggs in the incubations would therefore have reached up to a maximum of ~180 eggs litre^{-1}. At such high concentrations, *Calanus* is predicted to ingest 24 eggs female^{-1} day^{-1} (see regression in Figure 1 A in Bonnet et al. 2004). Whilst this does not provide conclusive evidence, the suggestion that cannibalism contributed a significant proportion to the daily budgets does therefore seem feasible. Indeed, it has been shown that egg mortality in the field is positively correlated with the abundance of female and CV copepodites (Ohman and Hirche 2001). However, the extent to which cannibalism occurs in the open ocean remains difficult to assess because the vertical distribution of the eggs of *C. finmarchicus* remains poorly defined (Ohman and Hirche 2001, Bonnet et al. 2004).

The higher egg production rates inferred by this analysis are similar to the maximum rates previously determined for *C. finmarchicus* in the waters above the Reykjanes Ridge in summer (Gislason and Astthorsson 2000), and are well within the upper limit of rates determined under post-bloom conditions (Tables 6.2 and 6.3 and refs their in). Furthermore, the biomass-specific egg production rates are then similar to the 14 % reported under post-bloom conditions in the Norwegian sea (Irigoien et al. 1998), and well below maximum values reported elsewhere (Table 6.3). Unfortunately, because individual egg production experiments were not conducted, this suggestion cannot be verified.

Other possible sources of error associated with the ingestion rates may have arisen through loss of cells due to preservation. Acidified Lugol's is known to cause a fraction of cells, particularly flagellates, to burst due to osmotic stresses (Klein Breteler 1985). Furthermore, a loss of almost 70 % of ciliates is reported for Lugols preserved samples after 9 months of storage (Ngando and Groliere 1991). Considering the importance of both flagellates and ciliates in the summer microplankton samples (Figures 4.1 and 4.3), it is quite possible that significant

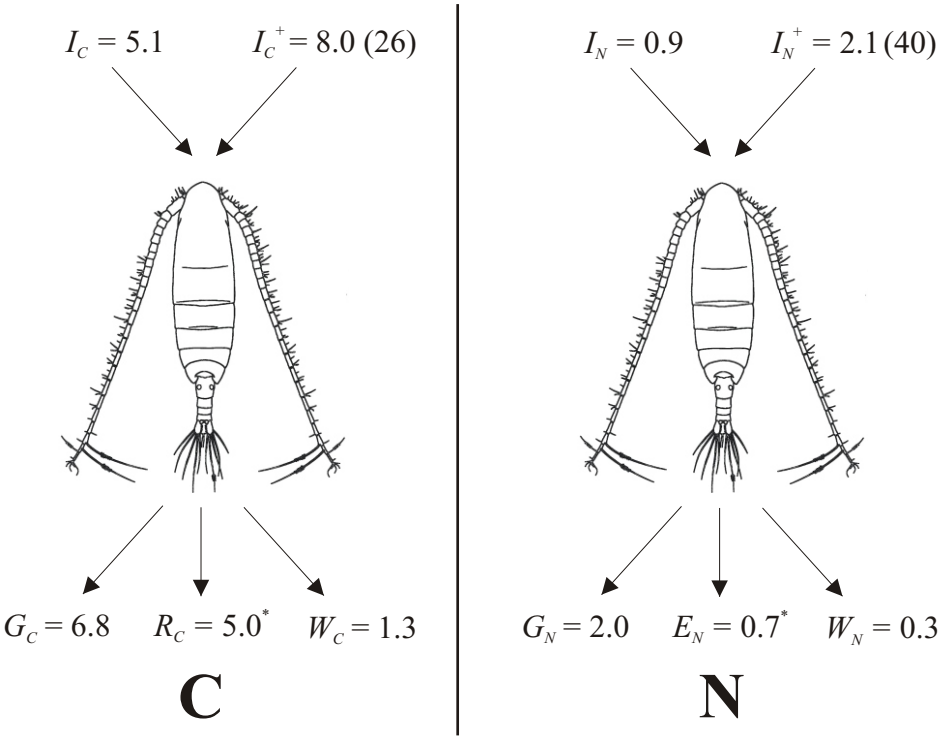


Figure 7.7. The C and N budgets for *Calanus finmarchicus* in July/August. All values are expressed as µg copepod^{-1} day^{-1}. Parameters are explained in section 7.2.1. $^+$ the quantities of C and N from cannibalised eggs required to meet the respective shortfalls (values in parentheses are the number of cannibalised eggs day^{-1}). * values derived from the literature - see Table 7.6 for details.

cellular losses due to preservation were apparent. Although great care was taken to consider the shrinkage effects of preservation, the cell count data were not corrected for losses associated with preservation and storage. The post-bloom samples also contained a much higher quantity of cells per unit volume, increasing the fraction of cells that were likely to have been obscured by others settling on top of them during the sedimentation process. This artefact is reported to reduce cell counts by up to 20 % (Dale and Burkhill 1982). Problems associated with cellular losses in the pre-bloom samples are likely to be much less pronounced because > 80 % of the C and N utilised by the females during the incubations was derived from their biomass. The physiological budget in April is therefore much less sensitive to errors associated with the estimation of grazing rates.

7.4.2. Stoichiometric analysis of *C. finmarchicus* in April. Discerning which dietary substrate was limiting the production of *C. finmarchicus* in April using the stoichiometric theory of Anderson and Pond (2000) was difficult. Although the substrates derived internally and from ingestion were both determined experimentally, defining the U^*_i parameters was complicated because empirical data on the maintenance demands for individual substrates remain absent. Various alternate parameterisations were therefore investigated, taking into account both theoretical (e.g. Calow 1977) and empirical studies (Checkley 1980, Kiorboe 1989). Experimental work has shown that marine crustaceans can assimilate EPA and DHA with an efficiency > 0.9 (e.g. Pond et al. 1995), and considering that copepods cannot synthesise these compounds (Nanton and Castell 1999, Mike Bell pers. comm.), it would appear reasonable to expect that all the assimilated EPA and DHA can therefore be utilised for growth (U^*_{EPA} and U^*_{DHA} = 0.9; section 7.2.6.). In contrast, marine copepods are observed to use N with a gross growth efficiency of 0.4 (Checkley 1980, Kiorboe 1989), therefore setting U^*_N to 0.4 is a justifiable starting point. When these parameters are used and the contribution of material from biomass is considered, neither EPA nor DHA were predicted to limit over the entire range of theoretically possible values of U^*_C (Figure 7.6B). Carbon was predicted to limit when $U^*C < 0.6$, and the switch from C to N limited growth was predicted to occur when $U^*_C > 0.6$ (Figure 7.6B). This is because the supply of substrates had a high N:C ratio relative to the demands for growth (Table 7.7). This N-rich supply of substrates is thought to have arisen because the females in April were respiring

protein rather than lipids, as suggested by the lack of storage fatty acids (Figure 6.3) and also the high N:C ratio of the biomass lost over the duration of the experiments (Table 7.7).

In the experiments of Kiorboe (1989), copepods were fed diatom monocultures with variable N:C ratios and produced eggs with a gross growth efficiency of 0.4. Carbon was apparently in excess i.e. the N:C ratio of the food was considerably lower than that of the new biomass produced (eggs), yet K_N remained constant. This strongly suggests that N was limiting throughout and that the observed values of K_N are representative of the maximum achievable gross growth efficiency for N (K^*_N). Carbon can theoretically be used with a K^*_C of 0.7 (Calow 1977). However, this utilisation efficiency only accounts for the energetic requirements of the synthesis of new tissues, and not other costs such as basal metabolism, osmoregulation, feeding, etc. It is likely that the actual K^*_C will be considerably lower than 0.7, but not necessarily as low as the 0.2 to 0.3 commonly observed for K_C of marine copepods (Straile 1997). If the female *C. finmarchicus* in April used N with K^*_N of 0.4, the stoichiometric analysis predicts that when K^*_C is less than 0.6 then C is limiting. I therefore conclude that C limitation is the likely limiting factor in this instance. Given that the N:C, EPA:C and DHA:C ratios in the food and biomass utilised (Table 7.7) were all greater than those found in eggs, limitation by C may well have been expected in April. Considering that C is required for both growth and maintenance costs, the low food concentrations in April suggest that U^*_C must be low, and therefore strengthening the case for limitation by C. However, it should be noted that if maintenance demands for nutritive substances are high then it is quite possible that these can become limiting even at low food concentrations (Boersma and Kreutzer 2002, Anderson et al. 2005).

If the experiments had been conducted with females that had plentiful lipid reserves, it is highly probable that ingestion would have supplied a much smaller fraction of the total C utilised i.e. δ_C would be closer to zero as much of the C would have been derived from the lipid stores. In this case N limitation would be more probable because the N:C ratio of the biomass utilised would have been significantly lower. Evidently, the development of parameter ϕ_i (equation 21) to incorporate the contribution of substrates supplied from the animals' biomass (δ_I; equation 22) is of

great importance when undertaking a stoichiometric analysis of polar copepods, or indeed any other organism that stores significant quantities of any substrate.

Predicted C limitation is in direct contrast to previous experimental work, which has suggested that copepod reproduction is limited by N (Checkley 1980, Kiorboe 1989). Theoretical stoichiometry has demonstrated that micronutrients such as fatty acids are also capable of limiting production at times (Anderson and Pond 2000, Anderson et al. 2004), particularly when fed algal monocultures. Furthermore, numerous observational studies have documented significant positive relationships between the quantities of PUFAs in the seston and copepod egg production rates (Stottrup and Jensen 1990, Jonasdottir 1994, Jonasdottir et al. 1995, Jonasdottir and Kiorboe 1996, Pond et al. 1996, Jonasdottir et al. 2002, Hazzard and Kleppel 2003, Shin et al. 2003). However, the majority of these studies were carried out with *Acartia* spp., a much smaller calanoid copepod that does not sequester lipid reserves, and therefore responds rapidly to changes in the food environment (Dagg 1977, Kiorboe et al. 1985a). Nonetheless, positive correlations between egg production rates and quantities of PUFAs in the seston have also been found for field populations of *Calanus* (Pond et al. 1996, Jonasdottir et al. 2002), although stronger correlations with other particulate descriptors such as chlorophyll *a* and total fatty acids were present in both of these studies. Egg production rates in *Calanus* are known to be closely related to food concentrations (Marshall and Orr 1952, Hirche 1990, Hirche et al. 1997). It is therefore possible that like chlorophyll *a* and fatty acids, positive correlations with EPA and DHA simply reflect increases in food concentration, rather than a biochemical dependence upon them. Furthermore, in cases where *Calanus* produces eggs from internal reserves, observed egg production rates are decoupled from any qualitative aspect of the seston (Jonasdottir et al. 2002). Considering that the majority of material was derived from the animals' biomass, finding PUFA limitation of egg production in April was thus unlikely.

7.4.3. Stoichiometric theory. The stoichiometric approach of Anderson and Pond (2000) provides a relatively simple theoretical framework with which the limitation of zooplankton can be assessed. However, this method is dependant upon a knowledge of maximum gross growth and net production efficiencies (K^*_i and k^*_i parameters), or, when contributions from biomass are considered, maximum gross and net utilisation efficiencies (U^*_i and u^*_i). Unfortunately, defining these parameters

is problematic. Experimental data are available for the gross growth efficiencies of C and N (e.g. Calow 1977, Checkley 1980, Kiorboe 1989), yet the extent to which either element was limiting in these cited experimental works cannot be deduced. As a result, whether the observed rates are realised (K_i) or maximum (K^*_i) remains impossible to know. The observation that egg production rates increase proportionally with the quantity of N in the diet (Kiorboe 1989) does indicate that this element can limit the production of marine copepods, at least when they are fed diatom monocultures. Furthermore, the constant K_N of 0.4 does suggest that this value is representative of K^*_N. But why is this value only 0.4?

In addition to the quality of the diet, the K^*_i and k^*_i parameters are influenced by the availability of food and also maintenance demands. As food concentration decreases, the fraction of ingested food required for structural maintenance eventually increases to the point where positive growth is no longer possible. Unlike N, EPA and DHA, C is required for both structural maintenance and basal metabolism. Therefore, K^*_C may be expected to decrease faster relative to the K^*_i parameters for other substrates. In this instance, the probability of C limitation increases as food concentration decreases. However, maintenance demands for N and other nutrients mean that the quality of the food is also of potential importance (Boersma and Kreutzer 2002, Anderson et al. 2005). Experimentally derived maintenance demands are scarce, which raises the question as to how representative the K^*_i and k^*_i parameters used here really are. For example, in the analysis presented here it is assumed that once EPA and DHA have been assimilated, they are used with 100 % efficiency (k^*_{EPA} and k^*_{DHA} = 1) i.e. there is no turn over of these substrates, and they are therefore only required for the production of new biomass. Experimentally determining the quantities of these PUFAs excreted/egested by starved copepods should theoretically provide us with estimates of these maintenance costs. Unfortunately, this is difficult because the amounts in question are likely to be beyond the resolution of current analytical techniques.

Alternative models that examine how food composition influences the production of consumers by explicitly addressing terms in the metabolic budget such as respiration and excretion, i.e. without recourse to using the K^*_i parameters, have been developed. One such method is the Dynamic Energy Budget (DEB) approach. This type of model distinguishes between structural and reserve components of the animal's biomass, and emphasises the need to consider the maintenance demands for

all elements and nutrients by requiring all assimilated substrates to be used to meet maintenance costs before being allocated for production (Kooijman 1995, Kooijman 2000, Kuijper et al. 2004). However, although this type of model has moved away from the K^*_i parameters, they remain difficult to parameterise because of the increased complexity of the biochemical processes that they represent. Some of these relate to biochemical processes that remain difficult to experimentally define.

The most recent stoichiometric development (Anderson et al. 2005) incorporates all the separate terms of the metabolic budget. In this model, the K^*_i coefficients are replaced by a sequence of parameters that explicitly account for assimilation of ingested substrates and associated costs, protein turnover, other basal costs (e.g. osmoregulation and locomotion) and finally, growth. Again, this approach is confounded by a number of parameters that have yet to be experimentally constrained, such as the costs of osmoregulation, protein turnover rate and the reclamation of substrates lost in turnover. The key advantages with this type of model over the relatively simple empirical stoichiometric approaches such as that used here are that it provides a unified parameter set that is independent of food quantity (whereas K^*_i parameters vary with food quantity) and that parameters represent real processes that can be determined experimentally.

7.5 SUMMARY

Data from the previous chapters were used to construct balanced physiological budgets for *C. finmarchicus* in April and July/August. The input terms of these budgets consisted of ingestion and the use of biomass, and the outputs were comprised of growth (including reproduction), respiration, excretion and egestion. Respiration and excretion were not determined experimentally, and were therefore estimated by mass-balance. In April, females were heavily dependant upon their biomass for fuelling metabolic costs, with more than 80 % of the C utilised being derived internally. Values of respiration and excretion determined by mass balance were in good agreement with those derived from the literature, suggesting that the experimentally determined data were accurate. In contrast, the estimated ingestion rates determined in July/August were not sufficient to support the observed growth. Indeed, when literature-derived estimates of respiration and excretion were considered, less than 50 % of the observed metabolic demands were fulfilled by the experimentally determined ingestion rates. Shortfalls in the budgets indicated that

one or more of the components were determined incorrectly. The estimated ingestion rates did not consider egg cannibalism during the incubations, and it is possible that this discrepancy could have explained why the estimated ingestion rates fell short of the observed demands.

The stoichiometric theory of Anderson and Pond (2000) was developed here to allow consumers to use material from their own biomass for growth. Importantly, the ϕ_i parameter, which defines the fraction of demand for a substrate that is met by ingestion (the remainder being synthesised internally), was redefined as δ_i, incorporating material from ingestion, synthesis and biomass. Parameterisation of the model was difficult because realised utilisation efficiencies, U, only equal U^* when the substrate in question is limiting, and so one can only use observed U to estimate U^* with caution. As a result, various alternate parameterisations were investigated. Essential fatty acids were assumed to be utilised with high efficiency (0.9) because they are efficiently assimilated and not synthesised by copepods. In contrast, experimental evidence (Kiorboe 1989), in combination with modelling studies (Kuijper et al. 2004), suggests that N is used with a relatively low efficiency (0.4). Using these parameter values, the stoichiometric analysis of the April data set predicted that C is limiting for typical values (<0.6) of maximum C utilisation efficiency, U^*_C. It is therefore concluded that C was the substrate most likely to have been limiting *C. finmarchicus* in April. This result is in contrast with the previous experimental work that found correlations between egg production and food N. The result here arose primarily because the material supplied from the biomass was rich in N, EPA and DHA relative to the demand for C. Interestingly, neither EPA nor DHA were predicted to limit. Unfortunately the physiological budget in July/August did not balance and therefore a stoichiometric analysis was not feasible.

Chapter 8

General discussion

8.1. Trophic interactions. For years pelagic food chains have been considered as a linear progression from the large primary producers (diatoms), though the predominant secondary producers (zooplankton) and ultimately to fish. As a result, a wealth of information on the grazing response of *Calanus* to varying species and concentrations of diatoms has been derived from laboratory experiments. However, as the true diversity of the microplankton has become known, the classical diatom-copepod link has been replaced by a myriad of trophic pathways, with the heterotrophic fraction of the microplankton playing a key role (e.g. Azam et al. 1983). Indeed, microzooplankton are now widely acknowledged as the primary grazers in the global ocean (Calbet and Landry 2004, Landry and Calbet 2004), and they are thought to represent a considerable proportion of the matter ingested by many copepods (e.g. Sherr et al. 1986, Stoecker and Capuzzo 1990, Gifford 1991, Kleppel 1993). Extrapolating the results of diatom monoculture feeding trials to the 'real world' is therefore problematic.

The realisation that copepod diets are diverse has led to their grazing rates being determined using food removal experiments in which natural seawater assemblages are offered as prey. However, it is somewhat ironic to observe that the theoretical framework that underpins these experiments is potentially undermined by the very presence of the microzooplankton (Nejstgaard et al. 1997, 2001b). Nonetheless, the complex trophic cascades that are unleashed when incubating natural plankton are typically overlooked. It is hoped that the method for correcting macrozooplankton grazing rates for microzooplankton grazing artefacts proposed here (Chapter 4, Mayor et al. submitted) will emphasize the necessity to consider microzooplankton grazing in zooplankton feeding studies and ultimately provide a robust and useful means by which copepod grazing rates can be estimated.

It is acknowledged that this method does not resolve the fine-scale interactions that undoubtedly occur in natural seawater assemblages. For example, it is known that zooplankton excretion can stimulate phyto- and bacterio-plankton growth (Roman and Rublee 1980, Zubkov and Lopez-Urrutia 2003), which in turn, may effect the growth and grazing rates of the microzooplankton. If the nutrient dynamics differ between the control and experimental bottles, the equations of both Frost (1972) and those presented here (Mayor et al. submitted) are subject to error because gross algal growth cannot then be assumed to be the same in the two bottles (see Cushing and Horwood 1998). No attempt was made to quantify the

remineralisation of nutrients by the microzooplankton or *Calanus* during the experiments presented here, primarily because the need to improve upon Frost's (1972) method was not anticipated until after experimentation. However, in both seasons, nutrients were probably non-limiting because of their high concentrations in the study area, and therefore excretion artefacts were assumed to be insignificant. Nonetheless, where excretion and remineralisation effects do cause the nutrient dynamics to differ significantly between experimental and control bottles e.g. in oligotrophic waters, specific gross growth rates would have to be calculated separately for the different treatments, i.e. giving separate values for $r_{(C)}$ and $r_{(E)}$. These problems may be alleviated by the addition of nutrients (Landry and Hassett 1982, Landry 1993).

8.2. Determining the quantity and quality of food consumed when presented with a natural diet. The complex interactions that occur between the various components of the microplankton are highlighted by the discrepancies between the patterns of POC, PON, cell biomass and total fatty acids observed in this study (Figures 5.1 and 5.2). Furthermore, these inconsistencies also illustrate that if only one of these analyses is undertaken, an incomplete, and possibly incorrect understanding of the quantity and quality of the food ingested by a non-detritus feeding copepod, such as *C. finmarchicus*, may be gained. This is particularly evident in the case of the POC and PON data from the seston samples.

8.2.1. POC/PON. The quantities of C determined by elemental analysis of the seston were at least 300 % greater than the microplankton biomass, as determined using inverted microscopy and cell volume:C conversions. These discrepancies were attributed to the presence of detritus, as suggested by its abundance in the settled microplankton samples and the quantities of the detrital biomarker, 18:0 (Leveille et al. 1997, Hama 1999). It is also possible that bacteria and other microorganisms that are beyond the resolution of the light microscope were present and contributed to the POC. Whilst it has been suggested that copepod nauplii may feed on bacteria (Turner and Tester 1992, Roff et al. 1995), it is thought that they are too small to be efficiently ingested by adult *Calanus*. Therefore, POC and PON data did not provide useful information about the available food or feeding dynamics during the experiments. Any changes that occurred in the biomass of the cells ingested by the

copepods were not reflected in the POC/PON data because the majority of the POC was associated with material unavailable to *C. finmarchicus*.

In order to analyse the 25 mm GF/F POC/PON samples to within 0.5 % of the theoretical maximum, constant recalibration of the Carlo Erba elemental analyser was required. This is because ash from the filters soon blocked the combustion column, rendering it unusable. Considering the time, effort and resources required to maintain this machine, the usefulness of following patterns in POC/PON in copepod bottle incubations is questionable, particularly when natural plankton assemblages are offered as prey. Perhaps the only useful information to be provided by these data was that *C. finmarchicus* did not ingest any noticeable quantity of detritus (see section 8.3 below).

8.2.2. Fatty acids. By contrast, in addition to providing information about relative abundance of detritus, the fatty acid data also provided reliable information on the relative contribution of certain microplankton cell groups, as revealed by the significant correlations between the biomass of individual cell groups and their respective biomarkers. This result confirms the usefulness of specific fatty acids as general biomarkers that can be used to provide qualitative information about the phytoplankton community (Kattner et al. 1983, Skerratt et al. 1995, Reuss and Poulsen 2002). However, the resolution of these data are not ideal as they cannot be used to provide information about the presence or relative abundance of ciliates, nor can they be used to distinguish between auto- and heterotrophic flagellates (collectively the microzooplankton). Microzooplankton are an important group of protists as they are currently thought to be quantitatively and qualitatively important for copepods (Stoecker and Capuzzo 1990). Furthermore, fatty acid data are difficult to translate in to C biomass because the biochemical composition of any particular class of algae is to an extent, determined by the conditions under which it grew (Ackman et al. 1968, Chuecas and Riley 1969, Dunstan et al. 1993).

Although EPA and DHA can be used to assess the quality of the available food (e.g. Jonasdottir et al. 2002, Hazzard and Kleppel 2003, Shin et al. 2003), determining the quantities of these PUFAs ingested by copepods when feeding on natural microplankton communities is problematic. In the experiments presented here, this was achieved by using the cell biomass:PUFA ratios in the seston at the beginning of each daily incubation. Such an approach was justifiable because PUFAs are primarily associated with viable cells (Hama 1991, 1999; see Particulate chapter

discussion) and *Calanus* typically consumed prey in direct proportion to their availability. However, in cases where copepods show strong feeding selectivity, this method cannot be justified. Even if the feeding preferences are known, determining the quantities of EPA and DHA ingested remains difficult because of the intra-specific biochemical variability of algae (see above). Determining the ingested quantities of mono and saturated fatty acids is even more problematic because they are found in both microplankton and detritus, and to differentiate between the two sources is difficult. It is evident that whilst fatty acid data can provide qualitative and quantitative information about the microplankton in general, it is difficult to use these data to provide quantitative information about the material ingested by copepods during bottle incubation experiments. Furthermore, the value of fatty acid data is greatly increased when collected in conjunction with inverted microscopy cell counts.

8.2.3. Cell counts. In contrast to POC and fatty acid data, the cell counts provided a means by which the amount of food (carbon) available and ingested could be quantified without the need for parallel measurements. These data also enabled patterns of selection towards both auto- and hetero-trophic organisms to be determined, and the relative contribution of detritus to be examined. Furthermore, although cell counts cannot be used to directly quantify the quality of the food in terms of essential fatty acids, generalisations can be made by examining typical fatty acid compositions of the dominant microplankters from the literature. However, the inverted microscope technique is not without criticism. Cell counts are subject to observer bias, and are limited to the resolution of the light microscope. There are also shrinkage artefacts due to preservation (e.g. Montagnes et al. 1994), and problems associated with the conversion of cell volume into cell carbon (Menden-Deuer and Lessard 2000).

High performance liquid chromatography (HPLC) determinations of phytoplankton pigments combined with CHEMTAX analysis, a computer application that undertakes the necessary class pigment:Chlorophyll *a* and Chlorophyll *a*:carbon conversions (Mackey et al. 1996), could have been used to provide a qualitative and quantitative understanding of the phytoplankton communities in the experiments. This method is relatively quick, but again, has several shortfalls. The relationship between biomass determined by inverted microscopy and HPLC-CHEMTAX is not clear (Llewellyn et al. 2005), although this may also reflect problems with cell

volume:C conversions. Ascribing the class pigment:Chlorophyll *a* and chlorophyll *a*:carbon ratios necessary for the CHEMTAX analysis is complicated because these ratios are influenced by the conditions under which the cells grew (e.g. Goericke and Montoya 1998). Furthermore, when pigment based indices are used to examine copepod feeding rates on natural plankton assemblages, the estimations often fall short of the animals expected metabolic demands, presumably because non-pigmented microzooplankton provide the shortfall (Dagg and Walser 1987, Gifford and Dagg 1991, White and Roman 1992, Atkinson 1996, Razouls et al. 1998, Mayzaud et al. 2002a, b). Clearly the heterotrophic component of the diet can represent a significant proportion of the daily ration, as found here. Therefore, when natural microplankton assemblages are offered to copepods as prey, counting individual cells using the inverted microscope technique remains a useful means by which the entire diet and feeding dynamics of copepods can be determined. Despite the time consuming nature of this method, interpreting the results of the bottle incubation experiments presented here would have been very difficult without the cell count data.

8.3. Feeding behaviour.

8.3.1. Detritus. Cell biomass determinations typically constituted << 40 % of the POC, suggesting that detritus and possibly bacteria (Section 8.2.1.) comprised the majority of the POC. Significant reductions in the quantities of POC in the experimental bottles relative to the controls would therefore have indicated that non-microplankton C i.e. detritus was ingested over the duration of the experiments. However, such a reduction only occurred at a single station in July/August. Considering that the POC data were determined to within 0.5 % of the theoretical maximum, and the standard errors of these determinations were generally < 10 % of the average, even small differences between the quantities of POC in the experimental and control bottles should have been statistically discernable. It is therefore suggested that *C. finmarchicus* does not ingest detritus in any significant quantity.

8.3.2. Food selection. Positive selection towards ciliates was found in both April and July/August, regardless of whether feeding was determined using the traditional approach (Frost 1972) or the new method. This confirms that *C. finmarchicus* shows strong positive selection towards motile prey (e.g. Nejstgaard et

al. 2001b). However, despite this apparent selective feeding behaviour, the diet was dominated by flagellates < 10 µm equivalent spherical diameter (ESD) in both seasons. This was also confirmed by the presence of flagellate biomarkers (biomarker Table) in the animals. Upon closer inspection of the composition of the diet it is apparent that non-motile prey items were generally consumed in proportion to their abundance in the seston. A 'fixed' feeding behaviour (Greene 1985) agrees well with previous studies which have shown that the diet of *C. finmarchicus* reflects that of the food environment (Huntley 1981, Meyer-Harms et al. 1999, Levinsen et al. 2000b). The fact that detritus was apparently selected against remains an inconsistency that is difficult to explain. A possible hypothesis is that whatever dominated the POC was either too large (detritus) or too small (bacteria) for *Calanus* to effectively filter (Section 1.4), although this cannot be tested because the nature of the POC remains unknown.

The size-limit of cells below which *Calanus* cannot effectively retain has been the subject of study for over half a century (Harvey 1937, Ussing 1938 c.f. Marshall and Orr 1955b). Traditionally, cells < 10 µm ESD were considered to be beyond the limit of efficient filtration (Marshall and Orr 1955b). Small cells have only recently been acknowledged as being potentially important in the diets of copepods (Huntley 1981, Hansen et al. 1994b, Nejstgaard et al. 1997, Irigoien et al. 1998, Bamstedt et al. 1999, Levinsen et al. 2000b). The data presented here support the view that even small flagellates can represent an important component in the diet of *C. finmarchicus* when larger cells are scarce. From an evolutionary standpoint, considering that the biomass of protists in the North Atlantic is dominated by cells 2 - 20 µm i.e. nanoplankton (Sieburth et al. 1978) throughout much of the year (Sieracki et al. 1993, Verity et al. 1993a, b, Stoecker et al. 1994, Gifford et al. 1995), it is intuitive that a planktivore such as *C. finmarhchicus* will have evolved feeding appendages suited to harvesting these cells, whilst retaining the ability to take advantage of the episodic blooms of larger cells.

8.4. Measuring growth in adult female copepods. Until now, concurrent data on egg production, ingestion and changes in body weight have been lacking for high-latitude copepods. The unique data set presented here provides a critical test of the assumption that egg production represents net growth in adult female copepods (Poulet et al. 1995, Runge and Roff 2000). It is evident from the pre-bloom data

collected in April that the majority of the C utilised over the duration of the 5-day incubation period came from the biomass of *Calanus*. Indeed, approximately 80 % was derived internally (Figure 7.3). This finding clearly demonstrates that egg production does not always equal net growth, and in cases where biomass contributes substantially to the observed reproductive output, net copepod secondary production (growth) may be grossly overestimated. Conversely, during the post-bloom incubations in July/August, more than 50 % of the observed growth was that associated with the production of new biomass in the females. In this case, the secondary production of *C. finmarchicus* would be underestimated if growth was considered to be solely in the form of eggs. Together, these data highlight the need for estimates of growth in polar copepods to consider changes in parental biomass. However, to do so may require an incubation period greater than the 24 hrs typically used (Runge and Roff 2000). This is because changes that occur in the animals' biomass over such a short duration may not be statistically distinguishable against the natural variability in the C and N content of *C. finmarchicus*.

8.5. The elemental budgets of *C. finmarchicus*. The quantities of C and N ingested, derived from biomass and allocated to growth (eggs and biomass) were experimentally determined (Grazing and Animals chapters) and used to compile the physiological budgets (Section 7.2.1. equations 9 and 10, Figures 7.3 and 7.4) for *C. finmarchicus*. By assuming constant assimilation efficiencies, it was possible to estimate the quantities of C respired and N excreted by mass balance, thereby completing the budgets. Although the budgetary approach has scope for error with each individual determination (Bamstedt et al. 2000), comparing the estimated respiration and excretion rates to literature values has proven to be a useful technique for assessing the validity of the experimental data. It is interesting to note that the majority of existing respiration and excretion rates for *C. finmarchicus* are typically published as 'per copepod' rates and are presented alongside the dry weights of the experimental animals (e.g. Butler et al. 1970, Marshall 1973). To compare mass-specific rates, assumptions about the C:N ratio of the experimental animals must then be made. This is problematic because the C:N ratio of *C. finmarchicus* is known to change significantly over the annual cycle (Tande 1982). However, the regressions of Ikeda et al. (2001) enabled mass-specific respiration and excretion rates to be

estimated without the need to make any such assumptions. It was therefore possible to compare both absolute and specific rates.

8.5.1. April. Absolute and mass-specific respiration and excretion rates determined by mass balance in April agreed well with experimental values derived from the literature (Marshall and Orr 1958, Butler et al. 1970, Ikeda and Skjoldal 1989, Ikeda et al. 2001). This suggests that the components of the budget determined in the experiments presented here were accurate. As mentioned earlier, the predominant feature of the budget of *C. finmarchicus* in April was that the majority of the C utilised over the duration of the incubations was derived internally, as opposed to being derived from the diet. Interestingly, the storage fatty acids 20:1(n-9) and 22:1(n-11) were essentially absent from the females at the beginning of the experiment, and < 10 % of the C lost from the animals' biomass during these experiments was attributable to the loss of fatty acids. Together, these data suggest that the lipid reserves were essentially depleted, and therefore that the majority of the C utilised was derived from an alternative source. The biomass lost during these incubations had a C:N ratio very similar to that of protein (Vollenweider 1985), suggesting *C. finmarchicus* were reproducing by catabolising their structural biomass. Such an action is presumably detrimental to the fitness of the animals i.e. this process results in impaired locomotion and prey capture etc., and it would appear that once initiated, egg production continues until the animals have literally starved themselves to death. A minimum biomass must exist, below which the biological machinery and resources are not sufficient to meet the demands of respiration and egg production. To date, this 'critical biomass' has yet to be examined, and consequently it is not possible to determine the maximum number of eggs that may have potentially been produced by the experimental copepods if the experiments had continued until the animals were completely spent. However, the observed loss rates of C and fatty acids suggest that the females would have been completely exhausted after approximately 10 days. It is quite possible that at least some of the incubated animals may have already been close to, or even beyond the 'critical biomass' during the experiments, possibly explaining why the average egg production rates were low.

This reproductive strategy resembles a semelparous one, in which animals have a single reproductive period in their lifetime and typically die shortly afterwards. Such a reproductive strategy is not uncommon in invertebrates, and is also observed in some fish. For example, octopod and decapod cephalopods

generally die following their reproductive effort (Arnold and Williams-Arnold 1977, Wells and Wells 1977), as do various salmonid fishes (see Crespi and Teo 2002 and references therein). Semelparity explains why 'spent' females (c.f. Conover 1967) are observed in the surface waters from April (e.g. Pasternak et al. 2001).

8.5.2. July/August. In contrast to April, the females gained significant quantities of C and N over the duration of the experiments in July/August and also produced eggs. As noted above, more than 50 % of the observed growth was associated with the production of new biomass in the females. The low C:N ratio of the biomass gained suggests that the animals had increased their protein content during the incubations. The reason behind this apparent gain in protein is not immediately clear. Considering that females do not contribute significantly to the overwintering population of *C. finmarchicus* (Hirche 1983, Heath and Jonasdottir 1999, Gislason and Astthorsson 2000), it may be reasonable to expect the females to allocate all the available material to reproduction. However, newly moulted females have previously been observed to increase their protein content (Hygum et al. 2000, Campbell et al. 2001, Helland et al. 2003a), therefore suggesting that the majority of the females incubated in July/August were thus. Nonetheless, it is difficult to accurately deduce the origin of the experimental females because during July and August, the population of *C. finmarchicus* in the North Atlantic is comprised of generation 0 (G_0) females that have successfully overwintered, and also generation 1 and 2 (G_1 and G_2) females, that are the product of G_0 and G_1's reproductive effort earlier in the year (Durbin et al. 2000, Gislason et al. 2000, Pedersen et al. 2000, Arashkevich et al. 2004). It follows that the experimental animals may have been part of a late arriving cohort of G_0 females, or any of the subsequent generations. However, *Calanus* is thought to catabolise up to 70 % of its lipid reserves between the onset of diapause and the arrival of mature females in surface waters (Gatten et al. 1980, Hopkins et al. 1984). Considering that these reserves are not sequestered after the animals have matured (Hygum et al. 2000), any late arriving G_0 females would be expected to contain negligible reserves. The presence of large quantities of both 20:1(n-9) and 22:1(n-9) in the experimental animals therefore indicates that the females were most probably G_1 or G_2 animals that had fed well as immature copepodites during the preceding weeks.

It was evident that the estimated ingestion rates were not sufficient to balance the observed growth and expected respiration/excretion and egestion rates (Figures

7.4 and 7.7). The success of the budget in April suggested that all samples were correctly processed and the resulting data were reliable. Therefore something other than analytical error may have been responsible for the observed discrepancies. *Calanus* is known to cannibalise the eggs of conspecifics (Bonnet et al. 2004), yet this was not considered when ingestion rates were estimated. Using the C and N budgets to examine whether or not this may have occurred in these experiments, it was apparent that they would have balanced if the females had ingested between 25 and 40 eggs day^{-1} (Figure 7.7). Although this would suggest that the actual egg production rates were significantly greater than those observed (Table 6.1), the resulting rates of between 35 and 49 eggs $female^{-1}$ day^{-1} were very close to previously reported maximum rates for *C. finmarchicus* above the Reykjanes Ridge in June (46 eggs $female^{-1}$ day^{-1}, Gislason and Astthorsson 2000) and less than half of the maximum rates reported elsewhere (Tables 6.2 and 6.3 and refs therein). Furthermore, the inferred mass-specific rates of egg production were very close to those previously reported for *C. finmarchicus* under post-bloom conditions (Irigoien et al. 1998). Although not conclusive, the suggestion of cannibalism cannot be ruled out. This again raises the question as to how justifiable is it to extrapolate results from bottle experiments to the 'real world'? *Calanus* rarely, if ever, reaches densities of 10 l^{-1} (the concentration in the experimental bottles) in their natural environment, and it is unlikely that they would encounter such high densities of eggs. Therefore, presumably eggs do not contribute such a large proportion to the daily ration in the real world. To what extent then, do the budgets determined in July/August truly reflect the physiological demands of the *in situ* population of *C. finmarchicus*? This question is difficult to answer, and serves more to highlight the methodological limitations of the experiments presented here. Parallel egg production experiments where individual females are maintained above a mesh to exclude them from their eggs (Runge and Roff 2000) would have been useful. These would have provided comparative egg production rates without the complication of cannibalism, enabling the extent of cannibalism in the feeding incubations to be determined. Unfortunately, time and resources did not permit such measurements.

8.6. The relationship between the biochemical composition of copepod eggs and that of ingested food. This study has demonstrated that the overall fatty acid compositions of *C. finmarchicus* and their eggs both show significant inter-

seasonal variation. The quantities of individual fatty acids in the eggs correlated with their concentrations in the females in both April and July/August, suggesting that in general, the fatty acid composition of the eggs is controlled by the composition of the parents. This agrees well with previous investigations that have shown the diet to influence the composition of non-essential compounds in the eggs of *Calanus* (Laabir et al. 1999, Lacoste et al. 2001, Helland et al. 2003b). The fatty acids primarily responsible for the differences between April and July/August females were also responsible for a large proportion of the seasonal differences between the eggs. In particular, the storage fatty acids 20:1(n-9) and 22:1(n-11), which were present in greater quantities in July/August, explained much of the inter-seasonal differences in both the females and the eggs. Both these moieties are essentially absent in algae (e.g. Viso and Marty 1993), and primarily biosynthesised by copepods (Sargent and Henderson 1986, Kattner and Hagen 1995), suggesting that the quantities of these fatty acids in the eggs is determined by their availability in the females.

In contrast, absolute quantities of C, N, EPA and DHA in the eggs did not differ significantly between April and July/August, and thus appeared at fixed ratios (Figure 6.13b). This suggests essential PUFAs in the eggs of *C. finmarchicus* are homeostatic relative to C, as previously suggested (Anderson and Pond 2000). However, these data remain inconclusive because homeostasis can only be assumed if the absolute quantities of C differ, but the ratios remain the same. Therefore the true extent to which the eggs of *C. finmarchicus* are homeostatic remains unknown and requires further experimental investigation.

8.7. The efficiencies with which C, N, EPA and DHA are used for egg production. Stoichiometric theory states that the limiting substrate will be used with maximum efficiency, U^*_i, whereas all other substrates are used with efficiencies lower than their theoretical maximum. Considering that heterotrophs can theoretically achieve efficiencies of ~ 70 % (Calow 1977), it was at first sight somewhat surprising to find that all substrates were used with a low and relatively constant utilisation efficiency in April (Table 7.5). Nonetheless, maximum utilisation efficiencies decrease proportionally with food concentration because an ever increasing fraction of the material ingested will be required to meet maintenance costs. Indeed, there must be a point at which the utilisation efficiency reaches zero

because all the ingested material is required for turnover processes. Beyond this point, the animal enters starvation. The scarcity of food in April could therefore have been responsible for the low observed utilisation efficiencies. However, this is unlikely because the majority of C (82 %) came from the copepods' biomass. If this C was derived from internal reserves e.g. lipid stores, a higher U^*_C might have been expected. After all, it would seem reasonable to assume that animals store C in a form that can be efficiently catabolised when required. However, it must be remembered that in reality, biomass is composed of both structural and storage components (e.g. Kuijper et al. 2004). Indeed, the relative scarcity of the storage fatty acids 20:1(n-9) and 22:1(n-11) in the experimental animals indicated that their lipid reserves were essentially exhausted. Furthermore, the low C:N of the material derived from the biomass (Section 6.3.1.1) suggested that the animals were respiring structural protein (Section 6.4.1.1), rather than lipid stores (high C:N). It can be hypothesised that using structural biomass may be less efficient i.e. more energetically demanding, that using designated reserves as a source of C because this process requires the production of enzymes that are not normally expressed in copepods. Unfortunately, from the data collected in this study it is not possible to conclude whether material lost from the animals' biomass was of structural or storage origin. However, the strong similarities between the EPA:C and DHA:C ratios in the biomass lost from the animals and those in the eggs (Table 7.7) support the idea that structural biomass was being catabolised. This would appear to be a final reproductive strategy, since it is well known that polar copepods such as *C. finmarchicus* store energy in the form of lipids (e.g. Sargent and Falk-Petersen 1988).

8.8. The roles of C, N, EPA and DHA in limiting egg production of *C. finmarchicus*. The limitation of marine copepod production has previously been studied experimentally by examining egg production over a gradient of food C:N (Kiorboe 1989). Copepods were observed to use N with a remarkably constant gross growth efficiency (K_N) of 0.4, even when N was apparently limiting i.e. egg production was strongly correlated with food N content. This suggests that the maximum efficiency with which copepods can utilise N (K^*_N) is 0.4. These observations have fostered the understanding that copepods are limited by this element in the marine environment (Checkley 1980, Kiorboe 1989).

In contrast, it is commonly assumed in simple theoretical studies that N is potentially used with a high efficiency on the basis that nutrient elements fulfil primarily structural roles, e.g. K^*_N = 0.68 (Anderson and Hessen 1995). If the C:N ratio of marine seston is close to that of the consumers, and the maximum utilisation efficiency for C is much lower than that for N (Anderson and Hessen 1995), then C is predicted to be limiting. But C limitation is not consistent with the constant N utilisation efficiency observed experimentally (Checkley 1980, Kiorboe 1989). If N is limiting, why is it used with a low efficiency of 0.4? One possibility is that, rather than being limited by bulk N, the copepods in the experiments of Checkley (1980) and Kiorboe (1989) were limited by something that covaries with food N. Subsequently, dietary imbalances of N-rich essential amino acids have been shown to adversely effect the growth of copepods (Kleppel et al. 1998b, Guisande et al. 2000, Anderson et al. 2004). Although devoid of N, certain essential fatty acids have also been shown to correlate strongly with egg production rates (Jonasdottir 1994, Pond et al. 1996, Jonasdottir et al. 1995, Jonasdottir et al. 2002). By extending the stoichiometric analysis to include these 'micronutrients', the possibility for limitation by minor dietary compounds has also been proven theoretically (Anderson and Pond 2000).

Considering the paucity of EPA and DHA and the high C:N ratio of the seston sampled in April, either N or PUFA limitation may have reasonably been expected. However, when internal sources were taken into account, the stoichiometric analysis presented here strongly suggests that *C. finmarchicus* in April were not limited by EPA or DHA (Section 7.3.2.2.). Assuming that these PUFAs are used with high maximum efficiency (U^*_{EPA} and U^*_{DHA} = 0.9), then they are predicted to be non-limiting regardless of the parameter settings for utilisation of C and N. The remaining analysis of limiting factors thus focussed on C and N. When N is utilised with a maximum efficiency of 0.4 (see above), C is predicted to limit unless it is used with an efficiency greater than 0.6 (Figure 8.1). Theoretically, C can be used to produce new biomass with a maximum efficiency of ~ 0.7 (Calow 1977). However, this efficiency only accounts for the energetic costs associated with the synthesis of new tissue, and the costs of basal metabolism, osmoregulation and feeding etc. mitigate against the copepods achieving this theoretical maximum. These additional costs would suggest that utilising C with an efficiency of 0.6 is not feasible, and

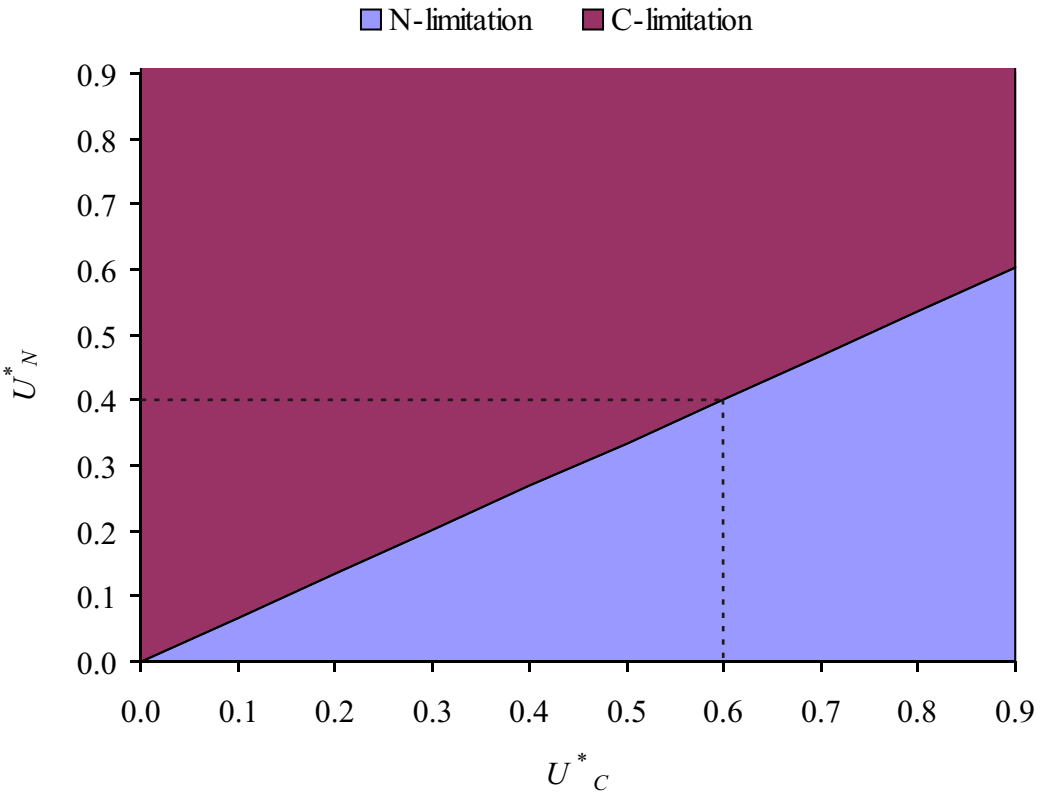


Figure 8.1. The predicted switch between C and N limited growth for *C. finmarchicus* in April using variable utilisation efficiencies (see Section 7.2.5, equation 23). Dashed lines illustrate the efficiency with which C must be utilised to cause N to limit when U^*_N is set to the experimentally observed value of 0.4 (Kiorboe 1989). Variables are presented in Table 7.9.

therefore it is concluded that in April, *C. finmarchicus* was most probably limited by C.

A U^*_N setting of 0.4 is in principle justified on the basis that significant amounts of N may be required for maintenance (Kuijper et al. 2004, Anderson et al. 2005). However experimental evidence to support this apparently large maintenance demand is lacking. Nitrogen is not required for energetic pathways, therefore it can by hypothesized that theoretically, it should be possible to utilise this element with a maximum net efficiency of 0.9, assuming that it is assimilated with an efficiency of 0.9 (Marshall and Orr 1955a, Corner et al. 1976, Landry et al. 1984). Setting U^*_N to equal 0.4 would thus appear to be low. If this parameter is assigned a higher value then the probability of N limitation becomes less, further strengthening the case for limitation by C (Figure 8.1). Indeed, if N were utilised with a maximum efficiency of 0.6, C is predicted to limit regardless of how efficiently it is utilised.

Whilst the finding of C limitation apparently contradicts earlier work (Checkley 1980, Kiorboe 1989), it must be remembered that the circumstances of the experiments presented here were quite different to those previously conducted. Kiorboe (1989) worked with *Acartia tonsa*, a small calanoid copepod that does not sequester lipid reserves, whereas these experiments were conducted with the much larger, polar copepod, *C. finmarchicus*. Even more importantly, the experimental copepods used by Kiorboe (1989) were reproducing from ingested material. This is in contrast to the data presented here, which demonstrated that the majority of the material utilised by *C. finmarchicus* in April was derived from their biomass. It is quite possible that if *C. finmarchicus* had been utilising lipid reserves to reproduce, the stoichiometric analysis would have predicted limitation by N. Using biomass to fuel reproduction was the surprise finding of this study, particularly as the low C:N ratio of the biomass utilised suggested that the internally derived material appeared to be protein, rather than storage lipids. Nonetheless, the observation of 'spent' females (c.f. Conover 1967) in the surface waters from April (e.g. Pasternak et al. 2001) suggests that this may be a normal occurrence in the life-history of *C. finmarchicus*.

8.9. Conclusions. The experimental data collected in April demonstrated that *C. finmarchicus* was able to reproduce in advance of the spring bloom by utilising their own biomass. Surprisingly, it appeared that they had catabolised structural

protein in order to maintain a reproductive output. The essential fatty acids EPA and DHA were not predicted to limit production because the material supplied from their biomass was rich in these compounds relative to the demand for C. Discerning between C and N limitation was slightly more complicated because it was dependant upon the U^*_i parameters used. However, in light of experimental and theoretical evidence, C was concluded to have limited the production of *C. finmarchicus* in April, even when the maximum utilisation efficiency for N was ascribed the relatively low value of 0.4. It would appear that this situation exemplifies the reproductive strategy that female *C. finmarchicus* adopt when they have exhausted their lipid reserves and food is scarce. Considering that these animals do not diapause as adults, semelparity is not entirely surprising.

It is understandable that animals are liable to face limitation by a range of dietary substrates, depending on the quantity and quality of the available food that they encounter over their life-time. The prevalence of lipid stores in polar copepods (Sargent and Henderson 1986, Sargent and Falk-Petersen 1988) strongly suggests that these have evolved in order to minimise limitation by C. Similarly, it can be argued that the apparent absence of the necessary biochemical enzymes or symbiotic intestinal bacteria to synthesise EPA and DHA (Dave Pond, Pers. Comm..) demonstrates that PUFA limitation has been uncommon over their evolutionary history. Why then, have egg production rates repeatedly been found to correlate with the content of EPA and DHA in the food, rather than with the C content of the food? Clearly, our current understanding of copepod nutrition is still in its infancy, and more constrained experimental work is required to span the gap between our understanding of the substrates limiting when copepods are fed diatom monocultures and when they are offered natural microplankton assemblages.

8.10. SUMMARY

- Microzooplankton grazing artefacts in copepod bottle incubation experiments can lead to significant underestimations of their daily rations. A method to correct for these artefacts is presented.
- The microplankton communities above the Reykjanes Ridge were dominated by flagellates before and after the spring bloom. Ciliates and dinoflagellates

were also important components of the microplankton in both seasons, whereas diatoms were typically scarce, particularly before the bloom in April.

- Individual fatty acid biomarkers correlated significantly with particular algal classes and 18:0 provided useful information about the relative abundance of detritus.
- The trends observed in the cell biomass, total fatty acid, PUFAs, POC and PON data over each 24 hr incubation generally contradicted each other. This illustrates that each one of these individual measurements provides different information about the complex nature of the dynamics that operate within natural seawater assemblages.
- Considering the effort required to maintain the elemental analyser, it is doubtful whether the POC and PON data were worthwhile as they provided little information about the trophic dynamics of the incubations. Conversely, inverted microscopy cell count data provided extremely useful information about the food and feeding habits of *C. finmarchicus*. Although this technique is extremely time consuming, the data provided were crucial to the success of this study.
- The composition of the material ingested by *C. finmarchicus* closely reflected that of the available food in both seasons, demonstrating that prey were consumed in direct proportion to their abundance in the plankton. Only ciliates were consistently selected for.
- Average biomass-specific ingestion rates for *C. finmarchicus* were 1.5 and 5.1 % day^{-1} (µg C ingested [µg C copepod]$^{-1}$ day^{-1} * 100) in April and July/August respectively. The first ever ingestion rates for *C. finmarchicus* consuming EPA and DHA are also presented.
- In April, the storage fatty acids 20:1(n-9) and 22:1(n-11) were essentially absent in *C. finmarchicus*, yet the animals lost significant quantities of C, N, EPA and DHA over the duration of the incubations. The C:N ratio of this biomass lost was low, suggesting that female *C. finmarchicus* adopt a selemparous reproductive strategy in which they continue to produce eggs until their biomass is completely spent. This is supported by the fact that females are rarely found in diapause.

- During the incubations in July/August, the experimental animals produced eggs and also gained biomass. It is possible that the low C:N of the biomass gained was because the animals had only recently moulted and were still in the process of maturing their gonads.
- The composition of non-essential fatty acids in the animals and eggs displayed significant inter-seasonal variation, illustrating that these compounds are not homeostatic. In contrast, the quantities of C, N, EPA and DHA in the eggs remained constant between seasons, suggesting that essential fatty acids are homeostatic relative to C.
- Physiological budgets for *C. finmarchicus*, comprised of ingestion, the use of biomass, growth, respiration, excretion and egestion, proved to be a useful means to assess the quality of the experimental data. Respiration and excretion were not determined experimentally, and were thus calculated by mass balance and compared to literature-derived estimates.
- Respiration and excretion values determined by mass balance in April were in good agreement with values from the literature, suggesting that the experimentally determined components of the budget were accurate.
- In contrast, the observed growth of *C. finmarchicus* in July/August exceed the quantities of material provided by ingestion. It is possible that these shortfalls were provided by cannibalising eggs, a process that was not considered when ingestion rates were estimated.
- The stoichiometric analysis of the experimental data in April predicted that EPA and DHA were always in excess, and therefore were non-limiting components of the diet, regardless of how efficiently C and N were utilised. C was predicted to limit production unless it is utilised with an efficiency close to the theoretical maximum of 0.7. This limitation by C occurred even when N is utilised with the low efficiency of 0.4 seen in experimental studies.

Appendix 1

Estimating the live volume of marine protists from acidified Lugol's preserved cells

A1.1. INTRODUCTION

Traditionally, low concentrations of acid Lugol's solution have been used to preserve phytoplankton samples (Throndsen 1978). This preservative appears to out perform similar fixatives (Ohman and Snyder 1991), with abundances of ciliates determined from acid Lugol's samples being the most representative of their live abundances, relative to other preservatives (Sime-Ngando et al. 1990, El Serehy and Sleigh 1993, Leakey et al. 1994). As a result of the Marine Productivity II external review, acid Lugol's (10%) was recommended as the most suitable preservative for microplankton samples (David Montagnes, pers. comm.).

Fixation has repeatedly been shown to affect the cell volume of both marine and freshwater protists (Klein Breteler 1985, Borsheim and Bratbak 1987, Choi and Stoecker 1989, Putt and Stoecker 1989, Ohman and Snyder 1991, Verity et al. 1992, Jerome et al. 1993, Leakey et al. 1994, Stoecker et al. 1994, Montagnes et al. 1994, Wiackowski et al. 1994, Menden-Deuer et al. 2001, Chaput and Carrias 2002). If such effects are not corrected for, biovolume and thus biomass are vulnerable to gross under or over-estimation. Various data demonstrate that the biomass of protists may be grossly underestimated (20 to 50 %) if cell volumes are based on fixed samples but carbon biomass conversions were based on live cells (e.g. Choi and Stoecker 1989, Stoecker et al. 1994).

The effect of fixative concentration on cell volume appears to vary depending on the group of organisms under investigation. Both Montagnes et al. (1994) and Menden-Deuer et al. (2001) concluded that the concentration of Lugol's iodine is not a significant factor in the extent of cell volume changes for diatoms, dinoflagellates and flagellates. Changes in cell volume due to preservation with 2 % acid Lugol's are thought to be representative of changes for cells fixed with 1 to 10% Lugol's (Montagnes et al. 1994). Ciliates appear to be more sensitive to changes in preservative concentration, with significant differences existing between the volumes of cells preserved with 2, 5 and 10 % acid Lugol's (Stoecker et al. 1994).

Despite several studies investigating the extent of cellular shrinkage caused by a variety of fixatives on several taxa, inter-comparison between studies is difficult because of methodological differences. Live cell volumes are not always measured (e.g. Leakey et al. 1994, Stoecker et al. 1994), and shrinkage is expressed only as a percentage of formaldehyde preserved volume (which typically causes less shrinkage than other preservatives; Leakey et al. 1994).

A1.2 METHODS

A1.2.1. Data collection and preparation. Key authors that have published data on live and acid Lugol's preserved protists (Susanne Menden-Deuer, Diane Stoecker, David Montagnes, Knut Yngve Borsheim and Mark Ohman) were contacted via email and their original data sets requested. Unfortunately, many of the original data sets were no longer available. Data on live and Lugol's preserved cell volumes were subsequently extracted from the literature (Table A1.1), regardless of the concentration of Lugol's used. Because only one data point was available for shrinkage at concentrations > 2 %, this datum (Borsheim and Bratbak 1987) was excluded. Montagnes et al. (1994) presented cell volume data determined by inverted microscopy and Coulter Counter. To avoid biasing the outcome of these analyses towards this data set, only cell volumes determined by microscopy were included. Because these data were subject to rounding errors during publication (David Montagnes pers. comm.), the original data set was acquired and used.

In cases where final concentration of Lugol's were not explicitly stated (Booth 1987, Choi and Stoecker 1989), it was assumed to be 1%; the average concentration of Lugol's when it is added to produce the 'weak tea' colour suggested by Throndsen (1978) (see Montagnes et al. 1994). Montages et al. (1994) indicated that there are significant differences between cell volumes determined microscopically (Section 2.4.3) and by Coulter Counter. Despite differing in absolute volume, it was assumed that cellular shrinkage is constant over all cellular dimensions, i.e. the aspect ratio remains constant, and thus the calculated percentage of shrinkage will be the same, regardless of how volume is determined, as long as both live and preserved measurements are made using the same technique. Consequently, data from Putt and Stoecker (1989) were excluded from this analysis because live and preserved volumes were determined by particle analyser and microscopically, respectively.

Choi and Stoecker (1989) and Ohman and Snyder (1991) both present a range of live cell volumes for a given species, yet they express shrinkage only as a single percentage of live volume (live and preserved volumes are not both reported). In both cases, live cell volume is averaged and the preserved volume back calculated i.e. it is assumed that the reported '% of live volume' is calculated in such a manner.

A1.2.2. Mathematical considerations. Choosing the appropriate regression (Model I or II) analysis to apply to this data set is complicated. Laws and Archie (1981) strongly argue that because both the variables measured (in this case, live and fixed cell volume) are subject to error (natural variability and measurement error), a Model II (functional regression; Ricker 1973) regression should be used. Despite violating a vital assumption for a Model I regression, Sokal and Rohlf (1995) concede (p543) that if the primary intention of fitting the regression line is to be able to predict *Y* from *X*, a Model I regression is permissible. Although a Model II regression relates *Y* to *X*, they are less suitable for predicting *Y* from *X* because essentially they best describe the joint variation of the two variables (determining *Y* from *X* would be biased), and thus only determine the functional relationship between the two variables. Because the objective of this analysis was to develop a relationship to predict live volume (*Y*) from preserved volume (*X*), a Model I regression was used.

If two variables, *X* and *Y*, are logarithmically transformed (base 10), the linear regression equation will be of the form:

$$LogY = a + (b\ LogX) \qquad (1)$$

To remove the transformation, the following logarithmic rules apply:

$$X^{a+b} = XaXb$$

$$aLogX = Log(X^a)$$

Thus, the Log_{10} linear regression equation becomes:

$$Y = 10^a X^b \qquad (2)$$

If the slope of the Log_{10} transformed data (constant b; Equation 1) is not significantly different from 1, the relationship between *X* and *Y* is linear on non-log_{10} transformed axes i.e. equation 2 becomes: $Y = 10^a * X$. Where this holds true, assuming that the data pass through the origin, 10^a gives the proportionality between *X* and *Y*. If the constant *a* (equation 1) is not significantly different from zero, the constant 10^a is not

significantly different from 1 (i.e. $10^0 = 1$ *or* $Log_{10}\ 1 = 0$) and thus $Y = X$. If a is significantly bigger than zero, X will be less than the corresponding Y value, and vice versa.

A1.2.3. Effect of concentration of Lugol's. The first step of this study was to test the assumption that the extent of cell volume shrinkage is independent of Lugol's concentration, as described above (Montagnes et al., 1994, Stoecker et al. 1994). The volume of cells preserved with 0.6 (Jerome et al. 1993), 1 (Booth 1987, Choi and Stoecker 1989, Ohman and Snyder 1991) and 2% (Ohman and Snyder 1991, Montagnes et al. 1994, Menden-Deuer et al. 2001) Lugol's, and their respective live volumes, were used to calculate the extent of shrinkage (% of live volume: Table 1). These data were examined to assess if Lugol's concentration significantly affects the extent of shrinkage (one-way ANOVA, n: 0.6 % = 6, 1 % = 17, 2 % = 50). Before analysis, the data were tested for normality and homoscedasticity.

A1.2.4. Predicting live from preserved volume. As concentration does not affect the extent of shrinkage (Montagnes et al. 1994; Menden-Deuer et al. 2001; see Results), all live and preserved volume data were pooled (Table 1). When regressed on arithmetic (linear) axes, the data were non-normal (Kolmogorov-Smirnov test, $p<0.001$); data were thus Log_{10} transformed to achieve normality (Sokal and Rohlf 1995). To examine if the relationship between Log_{10} preserved and log-live cell volume was linear (i.e. non-allometric), the data were regressed. A two-tailed t-test was employed to assess if the slope (constant b; Equation 1) differed significantly from 1. The relationship was not allometric (see results); thus, to test if the slope of the data on normal axes was different from 1 (i.e. that preservation did have an effect on cell volume), a two-tailed t-test was used to test if the log_{10} intercept was different from zero.

A1.3. RESULTS AND DISCUSSION

A1.3.1. Effect of concentration. Log-transformed data were normally distributed (Kolmogorov-Smirnov test, $p > 0.20$) and homoscedastic ($p = 0.186$). The concentration of Lugol's did not significantly affect the extent of shrinkage (one-way ANOVA, $p>0.05$). This confirms the previous findings (Ohman and Snyder 1991,

Montagnes et al. 1994, Menden-Deuer et al. 2001). However, more recent experimental work suggests that the concentration of Lugol's does in fact effect the extent of cellular shrinkage (David Montagnes, pers. comm.), as found for ciliates (Stoecker et al. 1994). The result of the current analysis may arise due to insufficient data for cells preserved with concentrations other than 2 % Lugol's. Clearly a thorough and rigorous experimental approach using a variety of marine protists and concentrations of Lugol's is required to clarify this matter.

A1.3.2. Predicting live from preserved volume. Log-transformed data were normally distributed (Kolmogorov-Smirnov test, $p = 0.794$) and homoscedastic ($p = 0.333$). The correlation between log-preserved and log-live cell volume was highly significant (ANOVA, $n = 73$, $p < 0.001$; Figure 1). The slope of the regression was not significantly different from 1 ($p > 0.1$). Thus, on normal axes, the relationship is linear (i.e. cell volume does not affect the extent of shrinkage) and the constant 10^a gives the proportionality between X and Y. The constant a is significantly different from zero ($p<0.001$), indicating that preservation with Lugol's does effect cell volume. The constant 10^a (= 1.384) can thus be used to predict live volume from preserved volume according to the equation:

$$L_v = 1.384P_v \qquad (3)$$

where L_v and P_v are live and preserved cell volumes respectively (μm^3).

On average, ciliates preserved with 10 % acid Lugols are 77.5 % of their volume when preserved at 2 % (Stoecker *et al*. 1994). Because the regression equation of Putt and Stoecker (1989) was determined using 2 % acid Lugol's preserved ciliates, before calculating ciliate cell carbon their volume was adjusted according to:

$$Cv_{2\%} = 1.29Cv_{10\%} \qquad (4)$$

where Cv is preserved ciliate volume (μm^3) and the numerical underscore ($_{2\%}$ and $_{10\%}$) denotes concentration of Lugol's.

Table A1.1. Live and preserved cell volume (μm^3) data extracted from the literature. Data of Borsheim and Bratbak (1987) and Putt and Stoecker (1989) were excluded (see text). * Lugol's concentration not stated so assumed to be 1 % (see Montagnes et al. 1994). O, oligotrichous ciliate, A, athecate dinoflagellate, T, thecate dinoflagellate, M, inverted microscope, CC, coulter counter.

Group	Species	% acid Lugol's used	Live analysis	Preserved analysis	Preserved vol. (μm^3)	Live vol. (μm^3)	% of live volume	Reference
Ciliate	*Euplotes* sp.	0.6	M	M	26700	36600	73.0	Jerome et al. 1993
Ciliate (O)	*Strombidium actum*	0.6	M	M	2700	4500	60.0	Jerome et al., 1993
Ciliate (O)	*Strobilidium spiralis* a	0.6	M	M	71800	121100	59.3	Jerome et al. 1993
Ciliate (O)	*Strobilidium spiralis* b	0.6	M	M	60800	109400	55.6	Jerome et al. 1993
Tintinnid	*Eutintinnus* sp.	0.6	M	M	7700	11500	67.0	Jerome et al. 1993
Dinoflagellate (A)	*Gymnodinium sanguineum*	0.6	M	M	14500	17600	82.4	Jerome et al. 1993
Ciliate (O)	*Strombidium* sp.	1	CC	CC	15640	22666	69	Ohman & Snyder 1991
Ciliate (O)	*Strombidium actum*	1*	M	M	72489	97300	74.5	Choi & Stoecker 1989

Group	Species	% acid Lugol's used	Live analysis	Preserved analysis	Preserved vol. (μm^3)	Live vol. (μm^3)	% of live volume	Reference
Ciliate (O)	*Strobilidium spiralis*	1*	M	M	89849	140608	63.9	Choi & Stoecker 1989
Tintinnid	*Favella* sp.	1*	M	M	354664	504500	70.3	Choi & Stoecker 1989
Flagellate	*Paraphysomonas imperforata*	1*	M	M	298	481	62	Choi & Stoecker 1989
Flagellate	HM-2	1*	M	M	80	118	67.7	Choi & Stoecker 1989
Flagellate	*Chrysochromulina* cf. *parva*	1*	M	M	19	20	97.1	Booth 1987
Flagellate	*Ch. cymbium*	1*	M	M	160	164	97.2	Booth 1987
Flagellate	*Ch. ericinna*	1*	M	M	79	100	79.0	Booth 1987
Flagellate	*Corymbellus aureus*	1*	M	M	218	258	84.4	Booth 1987
Flagellate	*Phaeocystis* sp.	1*	M	M	12	54	21.1	Booth 1987

Group	Species	% acid Lugol's used	Live analysis	Preserved analysis	Preserved vol. (μm^3)	Live vol. (μm^3)	% of live volume	Reference
Flagellate	*Imantonia rotunda*	1*	M	M	25	27	92.1	Booth 1987
Coccolithophorid	*Emiliania huxleyi*	1*	M	M	66	62	106.2	Booth 1987
Flagellate	*Ochromonas* sp.	1*	M	M	55	51	108.0	Booth 1987
Flagellate	Chrysophyte sp. 184	1*	M	M	119	157	75.7	Booth 1987
Flagellate	*Olisthodiscus luteus*	1*	M	M	1082	1084	99.9	Booth 1987
Flagellate	*Micromonas pusilla*	1*	M	M	3	4	72.9	Booth 1987
Ciliate (O)	*Strombidium* sp.	2	CC	CC	14506	22666	64	Ohman & Snyder 1991
Diatom	*Thalassiosira pseudonana*	2	M	M	30	42	71.9	Montagnes *et al*. 1994
Diatom	*Thalassiosira weissflogii*	2	M	M	522	552	94.5	Montagnes *et al*. 1994
Diatom	*Detonula pumila*	2	M	M	6360	7395	86.0	Montagnes *et al*. 1994

Group	Species	% acid Lugol's used	Live analysis	Preserved analysis	Preserved vol. (μm^3)	Live vol. (μm^3)	% of live volume	Reference
Flagellate	*Apendinella spinifera*	2	M	M	251	464	54.0	Montagnes *et al*. 1994
Flagellate	*Pseudopedinella pyiformis*	2	M	M	201	365	55.1	Montagnes *et al*. 1994
Flagellate	*Pelagococcus* sp. 1	2	M	M	38	59	63.7	Montagnes *et al*. 1994
Flagellate	*Pelagococcus* sp. 2	2	M	M	37	63	58.5	Montagnes *et al*. 1994
Flagellate	*Dunaliella tertioleta*	2	M	M	201	285	70.3	Montagnes *et al*. 1994
Flagellate	*Nannochloropsis oculata*	2	M	M	6	9	68.5	Montagnes *et al*. 1994
Flagellate	*Chlamydomonas* sp.	2	M	M	3939	3392	116.1	Montagnes *et al*. 1994
Flagellate	*Cryptomonas* sp.	2	M	M	543	766	71.0	Montagnes *et al*. 1994
Flagellate	*Chroomonas salina*	2	M	M	222	245	90.3	Montagnes *et al*. 1994
Flagellate	*Chroomonas* sp.	2	M	M	424	621	68.3	Montagnes *et al*. 1994

Group	Species	% acid Lugol's used	Live analysis	Preserved analysis	Preserved vol. (μm^3)	Live vol. (μm^3)	% of live volume	Reference
Flagellate	*Rhodomonas lens*	2	M	M	223	272	81.7	Montagnes *et al*. 1994
Flagellate	*Cryptomonas profunda* 1	2	M	M	560	863	64.9	Montagnes *et al*. 1994
Flagellate	*Cryptomonas profunda* 2	2	M	M	529	670	78.9	Montagnes *et al*. 1994
Flagellate	*Pyrenomonas salina*	2	M	M	258	379	68.0	Montagnes *et al*. 1994
Dinoflagellate (A)	*Gymnodinium simplex*	2	M	M	300	379	79.1	Montagnes *et al*. 1994
Dinoflagellate (A)	*Gymnodinium vitiligo*	2	M	M	859	885	97.0	Montagnes *et al*. 1994
Dinoflagellate (A)	*Gymnodinium sanguineum*	2	M	M	30167	34663	87.0	Montagnes *et al*. 1994
Dinoflagellate (A)	*Gyrodinium uncatenum*	2	M	M	10370	12226	84.8	Montagnes *et al*. 1994
Dinoflagellate (A)	*Gyrodinium aureolum*	2	M	M	4128	3362	122.8	Montagnes *et al*. 1994
Flagellate	*Micromonas pusilla* 1	2	M	M	2	1	127.4	Montagnes *et al*. 1994

Group	Species	% acid Lugol's used	Live analysis	Preserved analysis	Preserved vol. (μm^3)	Live vol. (μm^3)	% of live volume	Reference
Flagellate	*Micromonas pusilla* 2	2	M	M	3	2	124.5	Montagnes *et al*. 1994
Flagellate	*Mantoniella squamata*	2	M	M	33	37	90.4	Montagnes *et al*. 1994
Flagellate	*Chrysochromulina herdlensis*	2	M	M	117	221	53.1	Montagnes *et al*. 1994
Flagellate	*Phaeocystis pouchetii*	2	M	M	70	80	87.3	Montagnes *et al*. 1994
Flagellate	*Pavlova lutheri*	2	M	M	55	74	74.1	Montagnes *et al*. 1994
Flagellate	*Isochrysis galbana*	2	M	M	60	85	70.1	Montagnes *et al*. 1994
Coccolithophorid	*Emiliana huxleyi*	2	M	M	53	69	76.7	Montagnes *et al*. 1994
Flagellate	*Prymnesium parvum*	2	M	M	88	141	62.5	Montagnes *et al*. 1994
Coccolithophorid	*Coccolithus pelagicus*	2	M	M	471	790	59.7	Montagnes *et al*. 1994
Raphidophyceae	*Heterosigma carterae*	2	M	M	559	1096	51.0	Montagnes *et al*. 1994

Group	Species	% acid Lugol's used	Live analysis	Preserved analysis	Preserved vol. (μm^3)	Live vol. (μm^3)	% of live volume	Reference
Dinoflagellate (A)	*Amphidinium carterae*	2	M	M	1072	1018	105.3	Menden-Deuer *et al*. 2001
Dinoflagellate (A)	*Gymnodinium sanguineum*	2	M	M	55045	71859	76.6	Menden-Deuer *et al*. 2001
Dinoflagellate (A)	*Gymnodinium simplex*	2	M	M	218	209	104.3	Menden-Deuer *et al*. 2001
Dinoflagellate (T)	*Ceratium fusus*	2	M	M	54697	44619	122.6	Menden-Deuer *et al*. 2001
Dinoflagellate (T)	*Glenodinium foliaceum*	2	M	M	5605	5275	106.3	Menden-Deuer *et al*. 2001
Dinoflagellate (T)	*Prorocentrum micans*	2	M	M	1914	1795	106.6	Menden-Deuer *et al*. 2001
Dinoflagellate (T)	*Protoperidinium depressum*	2	M	M	578364	454451	127.3	Menden-Deuer *et al*. 2001
Dinoflagellate (T)	*Scrippsiella trochoidea*	2	M	M	4873	4408	110.5	Menden-Deuer *et al*. 2001
Diatoms	*Chaetoceros didymus*	2	M	M	1091	1062	102.7	Menden-Deuer *et al*. 2001
Diatoms	*Coscinodiscus* sp.	2	M	M	282077	280697	100.5	Menden-Deuer *et al*. 2001

Group	Species	% acid Lugol's used	Live analysis	Preserved analysis	Preserved vol. (μm^3)	Live vol. (μm^3)	% of live volume	Reference
Diatoms	*Ditylum brightwellii*	2	M	M	7291	9713	75.1	Menden-Deuer *et al*. 2001
Diatoms	*Leptocylindrus danicus*	2	M	M	25	37	67.6	Menden-Deuer *et al*. 2001
Diatoms	*Lithodesmium undulatum*	2	M	M	16367	16917	96.7	Menden-Deuer *et al*. 2001
Diatoms	*Stephanopyxis palmeriana*	2	M	M	79832	69517	114.8	Menden-Deuer *et al*. 2001
Diatoms	*Thalassiosira rotula*	2	M	M	13170	14273	92.3	Menden-Deuer *et al*. 2001
Diatoms	*Thalassiosira* sp.	2	M	M	53	66	80.3	Menden-Deuer *et al*. 2001

Appendix 2

The importance of microzooplankton in the global ocean

A2.1. INTRODUCTION

Microzooplankton, defined here as the heterotrophic fraction of the plankton < 200 μm ("Protozooplankton" as discussed by Sieburth et al. 1978), are typically abundant (> 1000 l^{-1}) in the marine environment (cf. Lessard 1991). They are a taxonomically diverse group that includes flagellates, dinoflagellates, sarcodines, actinopods and small metazoans (Capriulo et al. 1991). The proposition of the "Microbial loop" (Azam et al. 1983) acknowledged microzooplankton as a major functional component of pelagic food webs. Concurrent advances in experimental (e.g. the "Dilution technique": Landry and Hassett 1982; The "Dual-label radioisotope technique": Lessard and Swift 1985) and analytical techniques (e.g. Flow cytometry: Burkill 1987; epifluorescent microscopy: Watson et al. 1977) have enabled the importance of microzooplankton in marine ecosystem dynamics to be more thoroughly examined.

Up to 50 % of photosynthetically fixed C in the marine environment is estimated to be exuded by the phytoplankton as dissolved organic matter (Larsson and Hagstrom, 1982), and subsequently utilised by bacteria (Linley et al. 1983). This secondary production represents a significant fraction of the total productivity in the oceans (see Ducklow 2003). A substantial proportion of bacterial and primary production are grazed by a suite of heterotrophic organisms that constitute the microzooplankton (see figure 3 from Azam et al. 1983, Calbet and Landry 2004). Because of their low growth efficiencies (Straile 1997), this 'microbial loop' is relatively inefficient, at least in terms of C transferral. However, a fraction of the production that they consume is ultimately 'repackaged' and potentially 'nutritionally upgraded' (see Klein Breteler et al. 1999) by the microzooplankton, where it eventually becomes accessible to the mesozooplankton (200 to 2000 μm).

The purpose of this review is to highlight the importance of microzooplankton grazing in marine ecosystems. In particular, this work focuses on heterotrophic dinoflagellates and ciliates, whose grazing is most likely to impact upon the cells typically enumerated in natural seawater assemblage incubation experiments, such as those commonly used to quantify copepod grazing (see Bamstedt et al. 2000).

A2.2. MICROZOOPLANKTON GRAZING

A2.2.1. Heterotrophic dinoflagellates. Heterotrophic dinoflagellates have been recorded in all the world's oceans (Taylor 1987), and are capable of reaching > 400 cells ml^{-1} in the North Atlantic (Shapiro et al. 1989, Verity et al. 1993a). Described as 'eclectic' feeders (for a review on dinoflagellate heterotrophic feeding mechanisms, see Hansen and Caldo 1999), they are capable of consuming prey ranging in size from bacteria to protists and metazoans larger than themselves (Lessard 1991 and references therein), including a wide range of phytoplankton foods (Strom and Buskey 1993).

While their specific growth rates are lower than similar sized phytoplankton (Strom and Buskey 1993, Tang 1995) and ciliates (Strom and Morello 1998), they are able to achieve maximal cell-specific clearance and ingestion rates similar to those of ciliates (Table A2.1). Considering that the biomass of heterotrophic dinoflagellates is often equal to, or in excess of, ciliate biomass in pelagic marine ecosystems (e.g. Table 1 from Lessard 1991: Table 1 from Burhill et al. 1993, Levinsen et al. 1999), it is not surprising that they are thought to be one of the key grazers of bacterial and phytoplankton populations (Lessard and Swift 1985). Interestingly, during periods where food concentration falls below a threshold value, heterotrophic dinoflagellates have been shown capable of reducing their metabolism (Hansen 1992) and can survive starvation for up to 30 minimum-generation times (Strom 1991).

Data on their seasonal cycles in oceanic regions are sparse, though it is thought that in the North Atlantic, they are present throughout the year, with a seasonal maximum coinciding with that of the diatoms (Lessard 1984 cf. Lessard 1991). Hansen (1991) reported a similar pattern in the Kattegat, and the association with diatoms has also been noted in arctic and coastal waters (Smetacek 1981, Levinsen et al. 1999). It is possible that this response reflects the ability of heterotrophic dinoflagellates to ingest prey at least as large as themselves. Microflagellates have been observed consuming diatoms up to six times longer than their diameter (Suttle et al. 1986). Naked dinoflagellates are also known to efficiently ingest prey much larger than themselves (> 5 times their own body volume; Hansen 1992). In a recent review of size ratios between predators and their prey it was reported that on average dinoflagellates maintain a linear size ratio of 1:1 (Hansen et al. 1994a). For the larger dinoflagellates (> 20 μm), this suggests that

rather than with other microzooplankters, they are in direct competition with copepods. Indeed, recent work in Disko Bay, Greenland, has shown that relative to the *Calanus* spp. dominated copepod community, heterotrophic dinoflagellates exert a comparable or greater grazing impact (Nielsen and Hansen 1995, Hansen et al. 1999).

Heterotrophic dinoflagellates may play another important role, particularly in coastal waters. Jeong et al. (2001) recently demonstrated that *Oxyrrhis marina* grew well on the toxic, bloom forming dinoflagellate, *Amphidinium carterae*, and was capable of either detoxification or excretion of the toxin. In turn, *O. marina* is readily ingested by copepods, thus serving as a trophic intermediate and permitting the use of a potentially large and otherwise inaccessible nutritional resource.

A2.2.2. Heterotrophic ciliates. Like the dinoflagellates, heterotrophic ciliates are distributed almost ubiquitously in the marine plankton. They achieve their nutritional ration through ingesting a broad spectrum of food particles (see Pierce and Turner 1992). However, they have an 8:1 linear size ratio with their optimal prey (Hansen et al. 1994a), determined in part by diameter of the oral cavity (Heinbokel 1978, Jonsson 1986). This effectively limits their maximum prey size.

Although little is known about the global distribution of the naked oligotrich ciliates because of their fragile nature (Pierce and Turner 1992), they have been observed to reach densities > 2.0 x 10^5 l^{-1} (Landry and Hassett 1982, Setala and Kivi 2003). A combination of high growth and clearance rates (Table A.2.1B) would suggest that when abundant, they could be important grazers. This importance is becoming increasingly recognised, and many cases are documented where ciliates are implicated as being responsible for the control of algal population growth (Pierce and Turner 1992, Calbet et al. 2003, Setala and Kivi 2003). Heterotrophic ciliates in the North Atlantic measure between < 10 to > 20 μm in length (Gifford et al. 1995). Using the predator:prey size ratio for ciliates (Hansen et al. 1994a), it follows that their prey will consist of nanoplankton (2 to 20 μm; Sieburth et al. 1978), and an association between the two may be expected. Observational data support this theory. Verity (1987) found that ciliates reached peak abundances at the end of spring, and significant correlations between ciliate and nanoplankton abundance have been reported for various regions (Verity 1986, Stoecker et al. 1994, Setala and

Kivi 2003). Similarly, Nielsen and Kiorboe (1994) found that the seasonal distribution of ciliates in the southern Kattegat (Denmark) typically followed that of the phytoplankton.

Due to their rapid growth rates (Table A2.1B), ciliates can quickly respond to ephemeral periods of elevated food supply, and their production has been calculated to exceed that of copepods in several areas (Verity 1987, Capriulo and Carpenter 1980, Leakey et al. 1992, Nielsen and Kiorboe 1994). However, rapid fluctuations of ciliate population densities are reported for various regions, with causal factors ranging from temperature to unfavourable or insufficient food (Pierce and Turner 1992 and refs therein). Various species of planktonic ciliates have been observed to die rapidly at sub-threshold food concentrations, surviving < 4 minimum-generation times when starved (Montagnes 1996, Jakobsen and Hansen 1997).

A2.2.3. Heterotrophic dinoflagellates vs. ciliates. Assessments of the relative importance of these two microzooplankton groups are not common in the literature. However, it is apparent that either individually or together, they are responsible for the removal of a substantial proportion of daily primary production (e.g. Gifford et al. 1995, Hansen et al. 1999) and may represent a large quantity of secondary production (Levinsen et al. 1999). In a cross-latitude comparison of the trophic roles of ciliates and heterotrophic dinoflagellates in coastal ecosystems, the two groups were of great importance (Levinsen and Nielsen 2002). Because their specific growth and ingestion capacities are an order of magnitude greater than those of copepods (Hansen et al. 1997), both were potentially individually responsible for the removal of > 50 % of the annual primary production in the arctic and temperate ecosystems investigated.

By comparison, mesozooplankton (200 to 2000 μm; Sieburth et al. 1978) can be expected to graze only ~20 % of the primary production, assuming that the waters are 'moderately productive' (250 to 1000 mg C m^{-2} d^{-1}; Calbet 2001). Similarly, both Gifford et al. (1995) and Hansen et al. (1999) concluded that microzooplankton (predominantly ciliates and heterotrophic dinoflagellates) were potentially more important for C flow than copepods at high latitudes.

A2.2.3. Microzooplankton in the North Atlantic. Several dilution experiments in the North Atlantic and adjacent waters, by which both algal growth rates (μ, d^{-1}) and microzooplankton grazing rates (g, d^{-1}) can be estimated, have been carried out. Although taxon-specific (e.g. heterotrophic dinoflagellates, ciliates etc.) ingestion rates are not always estimated (see Burkill et al. 1987), they allow the ingestion rates of the microzooplankton community to be compared to those of the mesozooplankton community where they are available.

Between 70 and 80 % of the heterotrophic dinoflagellate biomass (μg C l^{-1}) in the upper 200 m during spring is represented by nano-dinoflagellates (cells < 20 μm) (Verity et al. 1993a). Gross approximations of their grazing using literature based growth and conversion rates imply that they alone may be responsible for the removal of up to 25 % of the primary production, dominated by small diatoms (Verity et al. 1993a) and phytoflagellates (Sieracki et al. 1993). Throughout the same period, Verity et al. (1993b) demonstrated that in general, heterotrophic nanoplankton were the dominant herbivores. Even when estimates of ingestion by ciliates, nauplii and dinoflagellates were combined, the estimated community ingestion rates of the nanoplankton were often greater. Combined microzooplankton grazing removed between 37 and 100 % of the estimated daily primary production, while the mesozooplankton were only capable of removing 0.6 to 5.2 % daily (Dam et al. 1993; see also Gifford et al. 1995).

By mid-summer (avg. 13 ºC), over 80 % of the microzooplankton standing stock is represented by protists (typically between 7000 and 10,000 cells l^{-1}), with equal contributions from aloricate ciliates and dinoflagellates (both thecate and athecate) (Burkill et al. 1993). At 60 ºN, microzooplankton herbivory was found to account for 39 % of the primary production, and its importance increased southwards to a maximum of 115 % (Burkill et al. 1993).

A2.2.4. Biomass specific ingestion rates. By combining spring bloom estimates of microzooplankton abundance in the North Atlantic (47 ºN) with literature data on ingestion and growth rates (Table 8 in Verity et al 1993b), it has been demonstrated that as a community they could potentially ingest between 2.4 and 3.1μg C [μg C microzoo]$^{-1}$day^{-1}, or more simply 240 to 310 % of their body C day^{-1}. These values appear typically conservative, representing 30 to 115 % (avg. 67

%) of the grazing determined by dilution experiments (Verity et al. 1993b). High grazing rates persist into the summer, where between 27 and 45 % of the phytoplankton is turned over each day, representing a daily ration of between 100 and 800 % body C day^{-1} (Burkill et al. 1993). Equally high mid-summer daily biomass specific microzooplankton ingestion rates (0.11 to 5.5 µg C [µg C microzoo]$^{-1}day^{-1}$) have been reported elsewhere in the North Atlantic (Gaul and Antia 2001).

Such observations are not limited to the North Atlantic. Landry et al. (1984) showed that phagotrophic microflagellates in Kaneohe Bay, Hawaii, were ingesting ~4.7 times their body C each day. In the equatorial Pacific, the microheterotrophic community is estimated to consume between 70 and 100 % of body C day^{-1}, increasing to between 800 and 900 % during an iron-induced diatom bloom (Landry et al. 2000). Furthermore, Rassoulzadegan (1982) demonstrated that the naked oligotrichous ciliate, *Lohmanniella spiralis*, from the Mediterranean grazed between 156 and 581 % of its body volume day^{-1}.

A2.2.5. Regulation of microplankton. Recently, in coastal waters of the NW Mediterranean, Calbet et al. (2003) illustrated that despite considerable ingestion rates, copepods were ineffective at removing a harmful algal bloom. Conversely, a tight coupling between the bloom growth (0.79 d^{-1}) and microzooplankton grazing (0.84 d^{-1}) rates was observed. In coastal North Pacific waters, Strom et al. (2001) showed a similar coupling between algal growth and microzooplankton grazing, tightening as phytoplankton biomass increased. In their experiments, microzooplankton grazing was approximately equivalent to two thirds (64 %) of phytoplankton growth, close to the 71 % average for data pooled from the literature (Strom et al. 2001).

Using the g : µ * 100 ratio (see section 2.4 for description of terms), which is assumed to be a reasonable proxy for the percentage of ^{14}C primary production consumed by microzooplankton (Calbet and Landry 2004), other recent data (Stelfox-Widdicombe et al. 2000, Gaul and Antia 2001) show good agreement (23 to 119 % of primary production removed by the microzooplankton). By applying the same analysis to data sets from latitudes between 70 ºN to 70 ºS, the potential of microzooplankton to control production in the global ocean is very apparent (Table

A2.2). The efficient recycling and remineralisation of nutrients by the microzooplankton enables the phytoplankton to sustain relatively high growth rates, thus maintaining healthy, steady-state communities (Landry et al. 1997, Landry et al. 2000).

A2.2.6. Microzooplankton as a dietary component. The magnitude of microzooplankton grazing and production in marine waters is indisputable (e.g. Lynn and Montagnes 1991). Their ability to consume and repackage primary and secondary production, particularly the fraction below the effective limits of copepod ingestion, providing an essential dietary component for copepods, is becoming increasingly apparent in the literature.

When ingestion of only autotrophic (defined by the presence of chlorophyll) prey is examined in copepods (e.g. using the gut-fluorescence method), it is clear that in many cases, not even demands for basal metabolic processes are met (e.g. Dagg and Walser 1987, Gifford and Dagg 1987, Gifford and Dagg 1991, White and Roman 1992, Atkinson 1996, Razouls et al. 1998, Mayzaud et al. 2002a, b). Thus, considering the importance of microzooplankton in ecosystem functioning, it is not surprising that when considered as prey items, they are often i) cleared at higher rates than autotrophic cells (Stoecker and Egloff 1987, Gifford and Dagg 1988, Wiadnyana and Rassoulzadegan 1989, Gifford and Dagg 1991, Atkinson 1994, 1995, 1996, Verity and Paffenhofer 1996, Meyer-Harms et al. 1999, Levinsen et al. 2000b, Nejstgaard et al. 1997, 2001a, b, Zeldis et al. 2002, Bollens and Penry 2003), and ii) typically constitute a large proportion of copepod dietary carbon (Table A2.3; Kleppel et al. 1988, Gifford and Dagg 1991, White and Roman 1992, Kleppel et al. 1996). The predominance of microzooplankton lipid biomarkers in copepods further suggests the dietary importance of microzooplankton (Hygum et al. 2000, Stevens et al. 2004).

A3. CONCLUSIONS

Whilst little attention has been paid to the smaller components of the microzooplankton here, the above information highlights the complex community- and tropho-dynamics of cells < 200 μm, and that the interactions between the different groups within this community are still poorly understood. Close coupling

between growth rates of the auto- and heterotrophic components, the size-relationships between these groups and high biomass specific grazing rates of microzooplankton strongly suggest that they, rather than copepods, are ideally situated to regulate both autotrophic and smaller heterotrophic cells. This becomes most apparent in systems where much of the production is derived from cells < 20 µm, as typically found in oceanic areas. Indeed, average values suggest that between 59 and 75 % of primary production is consumed by the microzooplankton daily throughout the marine realm (Calbet and Landry 2004), compared to annual average of ~12 % consumed by mesozooplankton (Calbet 2001). It is quite conceivable that to an extent, the microzooplankton also regulate themselves (Stoecker and Evans 1985, Dolan and Coats 1991, Frost 1993), at least when metazooplankton are scarce (Paffenhofer 1998).

Considering the abundance of literature demonstrating that microzooplankton are prolific grazers, and most likely responsible for the removal of the majority of primary production, it is surprising that their grazing potential remains to be acknowledged in contemporary experimental protocols for investigating the grazing rates of copepods feeding on natural seawater assemblages.

Table A2.1. Maximal growth rates (GR_{max}), clearance rates (CR) and ingestion rates (IR) determined for heterotrophic dinoflagellates (A) and ciliates (B) from various regions. ND = not determined, *potential underestimation.

A	Group	Location and Temp. (ºC)	GR_{max} (d-1)	CR (µl cell^{-1} hr^{-1})	IR_{max} (pg C cell^{-1} hr^{-1})	Reference
Heterotrophic Dinoflagellates	Oxyrrhis marina	Elait, Israel. 20ºC	1.3	0.4	ND	Goldman et al. 1989
	Gymnodinium sp.	Subarctic Pacific. 13ºC	0.7	0.8	30	Strom 1991
	Gymnodinium spirale	Kattegat, Denmark. 15ºC	0.5	0.3	ND	Hansen 1992
	Oblea rotunda	Texas. 20ºC	0.7	0.7	54	Strom and Buskey 1993
	Oblea rotunda	Germany. 20ºC	0.5	0.4	ND	Tillmann and Reckermann 2002
	Gymnodinium sp.	Weddell Sea. –1ºC	0.3*	0.12*	6*	Bjornsen and Kuparinen 1991

B	Group	Location and Temp. (ºC)	GR_{max} (d^{-1})	CR (µl cell^{-1} hr^{-1})	IR_{max} (pg C cell^{-1} hr^{-1})	Reference
Heterotrophic Ciliates	*Lohmanniella spiralis*	Mediterranean. 20ºC	1.0	5.0	ND	Rassoulzadegan 1982
	Strombidium eticulatum	Kosterfjord, Sweden. 12ºC	0.9	3.5	ND	Jonsson 1986
	Lohmanniella spiralis	Kosterfjord, Sweden. 12ºC	1.1	26	ND	Jonsson 1986
	Tintinnopsis vasculum	Narragansett Bay. 15ºC	1.1	7.5	811	Verity 1985
	Tintinnopsis acuminata	Narragansett Bay. 25ºC	2.0	2.6	261	Verity 1985

Table A2.2. Latitudinal variation in the potential percentage of ^{14}C primary production removed daily by microzooplankton grazing (%PP), as estimated using the g/μ * 100 ratio (Calbet and Landry 2004).

Location	Lattitude	Temp. ºC	% P.P.	Reference
Fjords, Northern Norway	69-70ºN	1.6-4.1	47-95	Archer et al. 2000
NE Atlantic	59-60ºN	10.4	83-119	Stelfox-Widdicombe et al. 2000
SE Bering Sea	55-58ºN	1.5-10.5	10-202	Olson and Strom 2002
Subarctic Pacific	50-53ºN		7-318	Landry et al. 1993
Coastal Pacific, USA (Oregon)	44ºN	10	4-133	Neuer and Cowles 1994
NE Atlantic	37ºN	20.4	23-81	Stelfox-Widdicombe et al. 2000
NE Atlantic	28-36ºN	17.4-26.2	44-120	Quevedo and Anadon 2001
S Atlantic – Southern Ocean	36-65ºS	1-22	0-55	Froneman and Perissinotto 1996
Antarctic Polar Front, 170ºW	55-70ºS	-1.1-3.7	60-83	Landry et al. 2002
Bellingshausen Sea	67-71ºS	-1.8- -1	21-271	Burkill et al. 1995

Table A2.3. Feeding data for copepods offered a natural plankton assemblage. Dominant prey groups in the diet (Diet: ciliates (C), dinoflagellates (D) and protists (P)). Clearance rate (CR), % of total carbon ingested derived from microzooplankton (%). NS, not stated, *assuming all ciliates and dinoflagellates are heterotrophic and all flagellates are autotrophic.

Location Temp. (ºC)	Copepod	Diet	CR	%	Reference
Northern Greenland Sea. –1ºC	*Calanus finmarchicus*	C	NS	85	Barthel 1988
Open Gulf of St. Lawrence. 4-9ºC	*C. finmarchicus*	C, D	1-30	75	Ohman and Runge 1994
West Greenland. 3ºC. Post bloom.	*C. hyperboreus*	P	~17	100	Levinsen et al. 2000b
Subarctic Pacific. June	*Neocalanus plumchrus* CV	C, D	7-39	26-80	Gifford 1993
Subarctic Pacific. June	*N. cristatus* CV	C, D	33-43	51-81	Gifford 1993
Oregon, USA. 10ºC. Pre upwelling	*C. pacificus*	C	25	100	Fessenden and Cowles 1994
Oregon, USA. 10ºC. Pre upwelling	*Pseudocalanus sp.*	C	6	48	Fessenden and Cowles 1994
Oregon, USA. 10ºC. Pre upwelling	*Centropages abdominalis*	C	7	100	Fessenden and Cowles 1994
Bergen, Norway. 10ºC. (L1b)	*C. helgolandicus* ?	C	17	78*	Nejstgaard et al. 2001a

Appendix 3

Particulate fatty acid tables

Table A3.1. Fatty acid content (µg fatty acid l^{-1}) ±SE of the particulate environment at water stations sampled in April. *Single value.

	Station				
Component	1	2	3*	4	5
14:0	1.07 0.11	1.34 0.06	0.69	1.76 0.22	0.93 0.06
14:1	0.20±0.03	0.17±0.01	0.04	0.24±0.03	0.11±0.01
15:0	0.65±0.08	0.85±0.02	0.32	1.05±0.21	0.57±0.04
16:0	2.41±0.23	2.99±0.23	2.29	3.81±0.44	2.10±0.12
16:1 (n-9)	0.71±0.16	0.92±0.05	0.28	1.26±0.23	0.64±0.07
16:1 (n-7)	0.56±0.07	0.65±0.03	0.16	1.00±0.06	0.50±0.03
16:1 (n-5)	0.25±0.04	0.44±0.02	0.05	0.45±0.06	0.15±0.00
17:0	0.07±0.03	0.08±0.01	0.02	0.11±0.03	0.04±0.01
17:1	0.37±0.05	0.43±0.02	0.16	0.55±0.12	0.29±0.02
16:4 (n-1)	0.02±0.01	0.04±0.01	0.01	0.05±0.00	0.01±0.00
18:0	2.32±0.23	2.45±0.03	1.72	3.10±0.44	1.70±0.15
18:1 (n-9)	2.28±0.49	3.13±0.10	0.85	2.90±0.39	1.75±0.09
18:1 (n-7)	0.43±0.04	0.57±0.03	0.10	0.72±0.09	0.36±0.02
18:2 (n-6)	0.85±0.15	1.23±0.09	0.32	1.98±0.28	0.87±0.06
18:3 (n-6)	0.06±0.01	0.08±0.01	0.03	0.09±0.03	0.02±0.01
18:3 (n-3)	0.20±0.10	0.39±0.05	0.06	0.60±0.05	0.13±0.06
18:4 (n-3)	0.24±0.22	0.78±0.09	0.14	1.11±0.06	0.13±0.12
20:0	0.28±0.02	0.31±0.02	0.14	0.44±0.06	0.21±0.01
20:1 (n-9)	0.01±0.00	0.01±0.00	0.00	0.02±0.01	0.01±0.00
20:1 (n-7)	0.03±0.01	0.06±0.00	0.01	0.06±0.01	0.03±0.00
20:4 (n-6)	0.00±0.00	0.00±0.00	0.00	0.01±0.01	0.00±0.00
20:4 (n-3)	0.00±0.00	0.01±0.00	0.00	0.01±0.01	0.00±0.00
20:5 (n-3)	0.12±0.12	0.46±0.07	0.09	0.76±0.05	0.08±0.07
22:0	0.16±0.02	0.18±0.02	0.09	0.25±0.06	0.11±0.01
22:1 (n-11)	0.00±0.00	0.01±0.01	0.00	0.01±0.00	0.00±0.00
22:5 (n-3)	0.00±0.00	0.01±0.00	0.00	0.00±0.00	0.00±0.00
22:6 (n-3)	0.42±0.16	0.90±0.09	0.32	1.36±0.15	0.32±0.13
Total	13.73±2.24	18.47±0.65	7.92	23.73±2.61	11.07±1.03
SAFA	6.97±0.69	8.19±0.26	5.27	10.53±1.45	5.68 0.38
MUFA	4.84±0.85	6.39±0.21	1.67	7.21±0.95	3.83±0.23
PUFA	1.92±0.75	3.88±0.39	0.97	5.99±0.26	1.56±0.44
16:1 (n-7)/16:0	0.23±0.01	0.22±0.01	0.07	0.27±0.01	0.24±0.00
n-3/n-6	0.95±0.45	1.93±0.11	1.77	1.93±0.32	0.71 0.36
20:5(n-3)/22:6(n-3)	0.16±0.16	0.50±0.03	0.28	0.56±0.04	0.15±0.12

Table A3.2. Fatty acid content (µg fatty acid l^{-1}) ±SE of the particulate environment at water stations sampled in July/August.

	Station				
Component	1	2	3	4	5
14:0	5.46± 0.34	3.95± 0.16	4.83± 0.14	4.84± 0.26	5.28± 0.82
14:1	0.76± 0.07	0.63± 0.05	0.74± 0.00	0.80± 0.08	0.65± 0.04
15:0	1.93± 0.07	2.03± 0.32	1.79± 0.07	2.21± 0.10	1.95± 0.17
16:0	8.33± 0.45	7.44± 1.24	6.97± 0.33	7.69± 0.62	7.99± 1.20
16:1 (n-9)	2.59± 0.23	2.92± 0.42	2.22± 0.18	2.31± 0.17	2.53± 0.11
16:1 (n-7)	4.59± 0.29	3.17± 0.25	3.17± 0.21	2.97± 0.16	3.50± 0.41
16:1 (n-5)	0.56± 0.07	0.59± 0.08	0.50± 0.04	0.48± 0.03	0.67± 0.06
17:0	0.18± 0.08	0.25± 0.01	0.18± 0.03	0.15± 0.02	0.16± 0.03
17:1	0.96± 0.08	1.01± 0.11	0.83± 0.08	1.02± 0.07	0.87± 0.11
16:4 (n-1)	0.37± 0.03	0.29± 0.03	0.31± 0.01	0.14± 0.01	0.78± 0.04
18:0	5.15± 0.61	4.75± 0.84	4.20± 0.37	4.74± 0.22	4.59± 0.57
18:1 (n-9)	6.02± 0.05	5.03± 0.78	4.38± 0.28	5.02± 0.25	5.03± 0.35
18:1 (n-7)	2.28± 0.22	1.82± 0.16	1.87± 0.14	1.93± 0.18	1.92± 0.17
18:2 (n-6)	3.85± 0.12	2.47± 0.33	1.98± 0.15	1.87± 0.12	2.28± 0.24
18:3 (n-6)	0.25± 0.04	0.13± 0.02	0.14± 0.00	0.12± 0.01	0.12± 0.01
18:3 (n-3)	1.08± 0.11	1.55± 0.12	1.42± 0.03	1.17± 0.11	1.56± 0.13
18:4 (n-3)	2.00± 0.10	2.77± 0.32	2.58± 0.06	2.19± 0.13	2.61± 0.14
20:0	0.75± 0.05	0.62± 0.08	0.46± 0.03	0.58± 0.04	0.47± 0.05
20:1 (n-9)	0.02± 0.01	0.01± 0.01	0.01± 0.00	0.03± 0.00	0.03± 0.01
20:1 (n-7)	0.31± 0.11	0.44± 0.05	0.49± 0.01	0.35± 0.12	0.40± 0.12
20:4 (n-6)	7.31± 0.99	7.90± 0.82	7.27± 0.31	7.82± 0.47	6.05± 0.55
20:4 (n-3)	0.13± 0.01	0.14± 0.01	0.15± 0.00	0.11± 0.01	0.24± 0.03
20:5 (n-3)	3.47± 0.14	3.31± 0.44	3.60± 0.09	3.31± 0.14	4.91± 0.21
22:0	0.53± 0.04	0.43± 0.06	0.35± 0.03	0.43± 0.03	0.38± 0.05
22:1 (n-11)	0.00± 0.00	0.01± 0.00	0.01± 0.00	0.00± 0.00	0.02± 0.00
22:5 (n-3)	0.13± 0.01	0.12± 0.01	0.13± 0.00	0.10± 0.00	0.13± 0.01
22:6 (n-3)	7.40± 0.30	7.43± 1.26	7.19± 0.22	7.14± 0.11	7.35± 0.33
Totals	66.41± 0.76	61.21± 7.48	57.76± 2.01	59.49± 0.67	62.48± 4.34
SAFA	22.33± 0.91	19.46± 2.42	18.77± 0.98	20.62± 1.03	20.83± 2.55
MUFA	18.09± 0.85	15.62± 1.76	14.22± 0.93	14.91± 0.85	15.62± 0.94
PUFA	25.99± 0.89	26.12± 3.30	24.78± 0.22	23.96± 0.03	26.03± 1.02
16:1 (n-7)/16:0	0.55 ±0.04	0.44 ±0.04	0.45 ±0.01	0.39 ±0.05	0.45 ±0.06
n-3/n-6	1.27 ±0.14	1.45 ±0.07	1.61 ±0.07	1.43 ±0.09	2.01 ±0.12
20:5(n-3)/22:6(n-3)	0.47 ±0.02	0.45 ±0.02	0.50 ±0.00	0.46 ±0.02	0.67 ±0.04

Table A3.3. Fatty acid composition (mol %) ±SE of the particulate environment at the beginning (initial) of each experimental day in April. *Single value.

	Station				
Component	1	2	3*	4	5
14:0	9.32± 0.50	8.55± 0.10	10.22	8.77± 0.15	9.90± 0.28
14:1	1.75± 0.26	1.13± 0.05	0.60	1.22± 0.03	1.23± 0.05
15:0	5.35± 0.23	5.13± 0.13	4.46	4.87± 0.42	5.75± 0.17
16:0	18.69± 1.09	17.03± 1.10	30.16	16.94± 0.49	19.95± 0.90
16:1 (n-9)	5.37± 0.34	5.27± 0.20	3.71	5.57± 0.57	6.02± 0.18
16:1 (n-7)	4.34± 0.23	3.75± 0.10	2.16	4.53± 0.23	4.78± 0.24
16:1 (n-5)	1.97± 0.12	2.53± 0.09	0.70	1.99± 0.16	1.49± 0.10
17:0	0.48± 0.14	0.43± 0.05	0.25	0.45± 0.06	0.39± 0.03
17:1	2.75± 0.09	2.37± 0.15	2.08	2.27± 0.23	2.60± 0.08
16:4 (n-1)	0.17± 0.07	0.21± 0.02	0.13	0.23± 0.03	0.05± 0.02
18:0	16.37± 1.74	12.62± 0.32	20.40	12.35± 0.48	14.47± 0.26
18:1 (n-9)	15.53± 1.78	16.23± 0.31	10.19	11.65± 0.53	15.05± 0.55
18:1 (n-7)	3.01± 0.22	2.93± 0.15	1.25	2.90± 0.11	3.08± 0.16
18:2 (n-6)	5.87± 0.30	6.41± 0.28	3.84	8.17± 1.31	7.49± 0.14
18:3 (n-6)	0.46± 0.02	0.40± 0.03	0.36	0.37± 0.07	0.18± 0.12
18:3 (n-3)	1.30± 0.46	2.05± 0.22	0.75	2.51± 0.28	1.08± 0.38
18:4 (n-3)	1.30± 1.17	4.09± 0.37	1.69	4.66± 0.45	0.98± 0.87
20:0	1.82± 0.16	1.43± 0.12	1.55	1.61± 0.09	1.61± 0.04
20:1 (n-9)	0.05± 0.02	0.04± 0.02	0.05	0.07± 0.02	0.06± 0.00
20:1 (n-7)	0.16± 0.02	0.27± 0.01	0.16	0.23± 0.05	0.22± 0.01
20:4 (n-6)	0.00± 0.00	0.00± 0.00	0.00	0.05± 0.03	0.00± 0.00
20:4 (n-3)	0.01± 0.01	0.06± 0.00	0.00	0.06± 0.03	0.00± 0.00
20:5 (n-3)	0.60± 0.60	2.19± 0.28	1.02	2.93± 0.38	0.55± 0.51
22:0	0.96± 0.06	0.79± 0.09	0.91	0.80± 0.10	0.81± 0.02
22:1 (n-11)	0.01± 0.01	0.03± 0.02	0.00	0.02± 0.01	0.00± 0.00
22:5 (n-3)	0.00± 0.00	0.03± 0.01	0.00	0.01± 0.01	0.00± 0.00
22:6 (n-3)	2.34± 0.52	4.00± 0.38	3.34	4.77± 0.41	2.26± 0.70
SAFA	52.98± 3.40	45.98± 0.84	67.97	45.79± 1.45	52.88± 1.29
MUFA	34.95± 1.78	34.57± 0.71	20.89	30.46± 0.81	34.53± 1.00
PUFA	12.06± 2.72	19.44± 1.55	11.14	23.76± 1.67	12.59± 2.28
16:1 (n-7)/16:0	0.23± 0.01	0.22± 0.01	0.07	0.27± 0.01	0.24± 0.00
n-3/n-6	0.88 ±0.43	1.81 ±0.10	1.62	1.81 ±0.30	0.65 ±0.34
20:5(n-3)/22:6(n-3)	0.18 ±0.18	0.55 ±0.03	0.31	0.61 ±0.05	0.16 ±0.13

Table A3.4. Fatty acid composition (mol %) ±SE of the particulate environment at the beginning (initial) of each experimental day in July/August.

	Station				
Component	1	2	3	4	5
14:0	9.91± 0.56	8.11± 1.10	10.14± 0.12	9.84± 0.52	10.08± 0.89
14:1	1.40± 0.14	1.27± 0.07	1.57± 0.06	1.65± 0.18	1.28± 0.13
15:0	3.30± 0.10	3.78± 0.16	3.53± 0.04	4.23± 0.14	3.55± 0.21
16:0	13.48± 0.64	13.02± 0.57	13.01± 0.18	13.93± 1.06	13.59± 1.17
16:1 (n-9)	4.23± 0.41	5.19± 0.12	4.17± 0.19	4.23± 0.31	4.41± 0.15
16:1 (n-7)	7.49± 0.50	5.71± 0.27	5.95± 0.18	5.43± 0.33	6.07± 0.51
16:1 (n-5)	0.92± 0.12	1.05± 0.02	0.94± 0.04	0.88± 0.04	1.17± 0.09
17:0	0.28± 0.13	0.42± 0.04	0.32± 0.04	0.25± 0.04	0.28± 0.07
17: 1	1.49± 0.13	1.70± 0.03	1.48± 0.10	1.76± 0.09	1.44± 0.16
16:4 (n-1)	0.62± 0.05	0.53± 0.01	0.60± 0.01	0.26± 0.02	1.38± 0.06
18:0	7.51± 0.82	7.48± 0.41	7.05± 0.36	7.74± 0.28	7.10± 0.60
18:1 (n-9)	8.85± 0.12	8.02± 0.28	7.42± 0.20	8.25± 0.36	7.86± 0.05
18:1 (n-7)	3.36± 0.35	2.94± 0.14	3.16± 0.13	3.18± 0.30	3.01± 0.29
18:2 (n-6)	5.70± 0.12	3.99± 0.11	3.37± 0.14	3.10± 0.20	3.58± 0.22
18:3 (n-6)	0.37± 0.06	0.22± 0.01	0.25± 0.01	0.21± 0.02	0.20± 0.04
18:3 (n-3)	1.62± 0.18	2.56± 0.14	2.45± 0.05	1.95± 0.20	2.51± 0.32
18:4 (n-3)	3.00± 0.18	4.55± 0.08	4.48± 0.10	3.69± 0.25	4.20± 0.23
20:0	1.00± 0.07	0.89± 0.03	0.70± 0.02	0.86± 0.06	0.67± 0.07
20:1 (n-9)	0.02± 0.01	0.02± 0.01	0.02± 0.00	0.04± 0.01	0.04± 0.01
20:1 (n-7)	0.42± 0.15	0.67± 0.14	0.75± 0.02	0.52± 0.18	0.58± 0.19
20:4 (n-6)	9.94± 1.24	11.82± 0.41	11.51± 0.85	11.93± 0.63	8.76± 0.47
20:4 (n-3)	0.18± 0.02	0.21± 0.01	0.24± 0.01	0.16± 0.02	0.36± 0.06
20:5 (n-3)	4.77± 0.24	4.96± 0.14	5.71± 0.18	5.10± 0.26	7.21± 0.34
22:0	0.65± 0.06	0.57± 0.02	0.49± 0.02	0.58± 0.03	0.50± 0.06
22:1 (n-11)	0.00± 0.00	0.01± 0.00	0.01± 0.00	0.00± 0.00	0.03± 0.01
22:5 (n-3)	0.17± 0.01	0.16± 0.01	0.18± 0.01	0.13± 0.00	0.18± 0.02
22:6 (n-3)	9.35± 0.41	10.15± 0.52	10.49± 0.33	10.10± 0.23	9.96± 0.65
SFA	36.11± 1.12	34.27± 0.08	35.24± 0.59	37.43± 1.65	35.77± 2.08
MUFA	28.18± 1.59	26.58± 0.26	25.47± 0.71	25.95± 1.49	25.90± 0.94
PUFA	35.71± 0.96	39.15± 0.33	39.29± 1.21	36.63± 0.44	38.33± 1.35
16:1 (n-7)/16:0	0.56± 0.04	0.44± 0.04	0.46± 0.01	0.40± 0.05	0.46± 0.06
n-3/n-6	1.21± 0.14	1.41± 0.07	1.56± 0.07	1.39± 0.09	1.95± 0.12
20:5(n-3)/22:6(n-3)	0.51± 0.02	0.49± 0.02	0.54± 0.00	0.50± 0.02	0.73± 0.05

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