Marine Biology (2000) 136: 1057–1073

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Importance of food quantity to structural growth rate and neutral lipid reserves accumulated in *Calanus finmarchicus*

Received: 28 May 1999 / Accepted: 10 February 2000

Abstract Growth and developmental rates were determined for copepodids of *Calanus finmarchicus* (Gunnerus) from experimental seawater mesocosms in a western Norwegian fjord. The instantaneous growth rates (g) from copepodid stage I (CI) to adult ranged from 0.08 to 0.10 d^{-1}. Daily per capita mortality rate of the cohorts was as low as 0.012 d^{-1} (1.2% d^{-1}). At local increasing temperatures (5.1 to 8.3 °C), development was equiproportional, and the cumulative median development time from egg to CV was approximately 65 d. CV moulted to males and females, and egg production was initiated. Enhancement of food resources by nutrient addition caused a 23.4% increase in growth rates from CI to adult. Additionally, copepodid stages showed a generally larger body size, carbon and nitrogen content and total storage lipid content (wax esters + triacylglycerols) in response to enhanced resources. Our data support an elsewhere proposed exponential-growth hypothesis; growth of the structural compartments and store lipids (mostly wax esters) was exponential during the copepodid stages. However, a sigmoidal pattern of growth best described growth of adult stages if reared at high resources, and depot lipid accumulation in late CVs and adults at high resources. Body nitrogen growth increased exponentially, however, no significant changes in nitrogen specific growth rates were found between individuals from low and high resources. CV and adults seem to have reached near-maximal weights at high resources, whereas structural weight continued to increase at low resources. Despite the differences in structural growth dynamics, cohort development was similar until the end of CV. During the onset of sexual differentiation, the male:female ratio and the adult:CV ratio were highest at high food resources, suggesting that the time used for the final moult depends on the feeding history of the copepods in relation to food quality and quantity. It appears that relatively small changes in food availability strongly influence the biochemical composition of *C. finmarchicus* copepodids.

Communicated by L. Hagerman, Helsingør

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Introduction

The calanoid copepods occupy a key position in the food chain in boral North Atlantic and North Sea waters, dominated by the herbivorous species *Calanus finmarchicus* and *C. helgolandicus* (Marshall and Orr 1955; Conover 1988). The dominant copepod in the North Sea and in the Norwegian fjord areas is *C. finmarchicus* (Williams and Lindley 1980; Tande 1982; Aksnes and Magnesen 1983; Backhaus et al. 1994) *C. finmarchicus* populations have an important impact on higher trophic levels in northern ecosystems in that eggs and nauplii are the principal food for the first-feeding larval stages of fish stocks (Marshall and Orr 1955; Runge 1988; Cushing 1990).

In order to understand the life cycle and production of *Calanus* spp. in the oceanic food web, a description of the quantitative relationship between phytoplankton and copepod production is of importance. In general, the growth rates of zooplankton are likely to be limited by the availability of food in open ocean environments, but not in coastal regions (Huntley and Boyd 1984). Food availability may fall below the critical concentration induced by marked seasonality of phytoplankton

community composition or senescent blooms reflecting diets of different size and nutritional quality (Sargent and Falk-Petersen 1988). *Calanus* spp. have developed the capability to accumulate extensive lipid stores in the form of wax esters (WE) and triacylglycerols (TAG) as a consequence of the strongly seasonal food availability in their habitat (e.g. Lee et al. 1970; Kattner and Krause 1987; Sargent and Falk-Petersen 1988; Miller et al. 1998; Hygum et al. 2000b). The amount of lipid deposited seems to be linearly related to the available food concentration (e.g. Lee et al. 1970, 1971b) and quality of food (Håkanson 1984). The lipid stores have several important metabolic functions in *Calanus* spp., e.g. fueling their metabolism during food-limitation in the growth season and especially during winter when the copepods are diapausing at great depth (reviewed by Hirche 1996b). Further, the depot lipids have a central role during the energy-demanding processes of gonadogenesis and oogenesis (reviewed by Hirche 1996a).

The functional relationship between growth of *Calanus* spp. and the supply of food has been investigated by laboratory studies (e.g. Paffenhöfer 1971; Vidal 1980; Thompson 1982; Peterson 1986; Pedersen and Tande 1992). It is relatively easy to measure the response by copepods to different conditions of food in the laboratory, but the relevance of these observations for describing conditions at sea is doubtful (Håkanson 1987). The quality of food and the feeding history of the copepod are important for the nutritional status of the copepods (Huntley 1988; Harris 1996), however, in nature, food is often patchily distributed, which makes it difficult to describe both in quantitative and qualitative terms. A complete understanding of the reasons underlying changes in biochemical composition of *Calanus* spp. requires knowledge about the time of recruitment of a particular developmental stage and how long it persists in the population (Hopkins et al. 1984). This requires frequent sampling of a particular population with a long generation time which is almost impossible to accomplish in the ocean. However, Hygum et al. (2000b) demonstrated that aspects of the development and the nutritional status of nauplii and copepodid stages of *C. finmarchicus* could be followed in mesocosms at excess food concentrations.

It has been suggested that when the development rate is at a maximum in *Calanus finmarchicus*, the "structural" growth rate is exponential (McLaren 1986). We here address this hypothesis by comparing and contrasting patterns of "structural" growth with the production of lipid for storage by *C. finmarchicus* copepodids reared in mesocosms at different natural plankton concentrations in a Norwegian fjord. Two separate papers describe the growth and development rate of nauplii (Hygum et al. 2000a), and the fecundity of the females reared in mesocosms (Rey et al. 1999). In the present study we examine how different levels of food availability influence the cohorts in relation to: (i) mortality rate, (ii) growth, development and sex ratios and (iii) biochemical changes in terms of carbon, nitrogen, and storage lipid (WE and TAG) deposited within each stage.

Materials and methods

Mesocosm set-up

Large scale incubations were conducted with artificial cohorts of *Calanus finmarchicus* at the Marine Biological Field Station, Espegrend, University of Bergen, Norway, 11 March to 12 May 1997. The experiment included four mesocosms made of polyethylene (90% penetration for PAR), with an enclosed volume of approximately 18 m^3 (diameter: 2 m, depth: 7 m). Further details of the design of the mesocosm experiments have been presented in Hygum et al. (2000b). The mesocosm water was screened while being filled using 50 μm Nitex, to remove potential predators and mesozooplankton competitors of the cultured cohorts. The mesocosms were manipulated by addition of inorganic nutrients simulating near-natural food concentrations for the fjord (termed L1, L2 for low resources) and enhanced food concentrations (termed H1, H2 for high resources). Growth dynamics of the copepods in terms of stage specific body carbon, nitrogen and lipid storage, and information on the diet (phytoplankton and microplankton) will be presented with a focus on mesocosms L1 and H1. To compare the biochemical composition of the copepods from the replicate mescosms, L2 and H2 and in situ, Copepodid Stage V (CV) males and females were measured at the end of the experimental period.

Female collection and egg production

Calanus finmarchicus females were collected in Raunefjord (60°17′N; 05°10′E) using a pelagic fish larval trawl, 4 m^2 opening and 600 μm mesh size, fitted with a non-filtering 40-litre plastic cod end. Hauls covered the depth strata from 0 to 100 m. The net was retrieved at low speed, and the contents of the cod end were gently emptied into a darkened container with 30 litre seawater. The copepods were transported to the laboratory within 2 to 3 h of collection and brought to a walk-in cold room at 4 to 5 °C. Large-scale egg production for the build-up of the artificial *C. finmarchicus* cohorts was conducted in the cold room with females kept in 100-litre cylinders with 400 μm mesh false bottoms suspended in 120-litre containers. The females were fed cultures of *Rhodomonas baltica* in excess concentrations. Every 24 h the produced eggs were decanted from the 120-litre containers. The eggs were then suspended in 10-litre glass cylinders containing filtered, aerated seawater. Numbers of nauplii were recorded every 24 h allowing an estimate of total numbers. Approximately 80 000 nauplii, 1 to 2 d old, were added to mesocosms L1 and L2 on 10 March, and H1 and H2 on 12 March.

Abiotic and biotic analyses

Temperature (°C) was measured continuously with a temperature data-logger, and salinity and oxygen were measured at regular time intervals with a Sea Cat Profiler (Sea Bird Instruments). Water sampling was conducted with a 3-litre heart-valve water-sampler every second day, representing the depth strata 0, 1, 2, 3, 4 and 5 m. Samples were pooled in a container, and subsamples were taken for chemical and biological analysis.

The concentrations of particulate organic carbon and nitrogen (POC, PON) were measured on precombusted GF/C (47 mm) filters and analysed with an EA 1110 CE Instruments CHNS analyser. Samples for chlorophyll *a* and phaeopigment were extracted with methanol and measured fluorometrically using a Turner fluorometer (methods of Holm-Hansen et al. 1965). On the first three sampling days in March, total plant pigment was spectrophotometrically determined in ethanol extract according to Jespersen and

Christoffersen (1987). The total plant pigment in that period was converted to chlorophyll *a* assuming that 30% of the pigments were phaeopigments. The C:chlorophyll *a* ratio was calculated following Banse (1977) from the slope of the POC versus chlorophyll *a* regressions.

Microscopic enumeration of samples was done by the Utermöhl (1931) technique, and samples were preserved in 2% acid Lugol fixative. Samples were counted at 400× (small *Phaeocystis*-like cells of approximately 5 μm) and 200× (diatoms, dionoflagellates and ciliates). Cells smaller than ~2 μm were not resolved at these magnifications. Cells were identified to genus or whatever possible to functional groups from the categories: diatoms, dinoflagellates and ciliates. However, the group of *Phaeocystis*-like cells could be a mixture of various small autototropic or heterotrophic cells or detritus, but spherical colonies of *Phaeocystis* sp. were very abundant during the post-bloom period, and the colonies were easily identified in the unpreserved net haul samples for zooplankton collection. Cell volume was calculated using simple geometrical formulas, and carbon was calculated by multiplying cell volume by a conversion factor of 0.13 pg C μm^{-3} for thecate dinoflagellates (Smetacek 1975) and 0.11 pg C μm^{-3} for diatoms (Strathmann 1967), athecate dinoflagellates, ciliates (Smetacek 1975) and *Phaeocystis*-like cells. Cell volume of diatoms was estimated using a constant thickness of 1 μm of the plasma, and 90% of the vacuole volume was subtracted from the plasma volume.

Copepods were collected every second or third day by towing a 25 cm diam., 200 μm mesh net vertically from 5 m depth up the centre of the mesocosm by hand. The contents of the codend were preserved in 5% buffered formalin. The copepods were enumerated, determined to developmental stage, and 40 individuals were measured (prosome length) from each stage using a dissecting microscope with eyepiece micrometer.

Potential sibling species of *Calanus finmarchicus* (Gunnerus), i.e. *C. helgolandicus* (Claus) and *C. glacialis* (Jaschnov), were determined from the formalin-preserved samples. A spot-test of 20 randomly selected, egg-producing females and 20 females originating from the cohort were classified to species. The females were dehydrated in 96% ethanol before the fifth swimming leg (P5) was removed from the specimens and gently flattened under a cover slip containing Euparal. Taxonomic characters of P5 were studied at 200× magnification on a Leica inverted microscope following the procedure described by Frost (1974) and Fleminger and Hulsemann (1977). Digitized images of the P5s were captured with a Photometrics SenSys 1400 CCD camera system. The image analysis system allowed a precise detection of length and curvature of the basipod and comparison of specimens. Additionally, five to ten individuals from each stage of CV, males and females representing all mesocosms, and five to ten females that produced eggs for the cohort were identified to species by PCR methods (Bucklin unpublished results).

Individual copepods for carbon and nitrogen analysis were grasped from freshly collected materials with fine-pointed forceps, rinsed in 0.2 μm filtered seawater, and then placed on precombusted aluminium boats, dried at 60 °C overnight and stored at −18 °C for later measurement. Individual carbon and nitrogen content were determined from late stages of CIV to adults, and measurements of CIII and early CIV were made on 15 to 30 individuals with an EA 1110 CE Instruments CHNS analyser. Individual carbon content of CI to early CIV was determined with an IRGA infrared gas analyser ADC 225 MK3. Measurements on stages CI and CII represented two or three individuals, from which a mean carbon value for the group was calculated.

Lipid analysis of individual copepods followed the method described above, but individuals were placed in glass vials containing chloroform:methanol (2:1 v/v), purged with N_2 gas and stored in liquid N_2. Hexadecane-3-one (ketone) was used as the internal standard, and lipids were analysed by thin layer chromatography/flame ionization detection (TLC-FID) using an Iatroscan^{TM} MK-5. Extraction and analysis were performed following standard procedures (Jónasdóttir 1999). A standard of native wax esters extracted from *Calanus finmarcicus* stage CV was used for calibration of the TLC-FID. Other standards for calibration were tristearine (triacylglycerol), palmitine acid (free fatty acid), cholesterol (sterol) and methyl manganate (phospholipid). Measurements on stages CIII and CIV represented 10 to 30 individuals, and CV and adults represented 3 to 6 individuals, from which a mean lipid value for the group was calculated.

Wax esters and triacylglycerols dominated the storage lipid of *Calanus finmarchicus*. WE was quantitatively the most important storage lipid, and the dominant fatty acids in WE were 16:0, 16:1(*n* - 7) or 18:4(*n* - 3), 20:5(*n* - 3) and 22:6(*n* - 3) polyunsaturated moieties (Hygum et al. in preparation). Thus when storage lipid was converted to carbon units, a lipid weight per C conversion of 0.8 was used.

Calculations and statistical analysis

Stage duration was estimated on cumulative frequencies of individuals sampled every second or third day using the method of median development time (MDT) of untransformed data. The method is described by Landry (1983) and Peterson and Painting (1990); briefly, the MDT is defined as the time at which 50% of the population has moulted to a specific stage. The initial time $t(0)$ was defined as the mean time of egg collection: $t_0 = \Sigma N \times t_i/(\Sigma N_i)n$, where N_i is the egg number released on Day i, t_i is the time of Day i and n is the total number of days during which eggs were collected.

The sex ratio of the cohort was determined from the onset of the final moult. The ratio between males and females and the ratio between adults and CVs were estimated.

Structural body carbon of the copepods were estimated according to $g = w - s$, where g is structural body C of the copepod, w is the total body C and s is the weight of the storage lipid converted to C. Growth rate was estimated from the observed carbon and nitrogen content, which was assumed to increase exponentially over time according to $W_t = W_0 e^{gt}$, where W_t is the weight (μg C or μg N) at time t, W_0 is the weight at time 0, g is the instantaneous growth rate (d^{-1}) and t is the elapsed time (e.g. Huntley and Lopez 1992). The slopes, g, of the linear regressions of ln W_t on t were used to test for differences between L1 and H2, using a Student's t-test. Differences between means of copepodid prosome length from L1 and H1 were analysed using a z-test.

Daily per capita mortality rates (m) for the cohorts in L1 and H1 were estimated by the relationship (see Aksnes et al. 1997): $m = \ln[n(t_1)] - \ln[n(t_2)]/t_2 - t_1$, where $n(t)$ is the total abundance (Σ all stages − individuals removed due to sampling) of the cohort at time t. The abundance of individuals was estimated from sixty 3-litre heart-valve water bottle catches, representing ten samples at each depth from 0, 1, 2, 3, 4 to 5 m of the mesocosm. This sampling technique is a better measure of the abundance of individuals in the mesocosm compared with net hauls or video techniques (unpublished data). This sampling program was carried out at seven different dates for each mesocosm, and a mean abundance of individuals was estimated for each specific date. In addition, the initial and final number of copepods was known. Lines were fitted to abundance of individuals versus time by simple exponential regression. As the population was closed, the slopes of the regression lines were interpreted as per capita mortality rates, and all decreases in population size were attributed to mortality. Percent daily mortality of the population was estimated by the equation: Percent daily mortality $= [1 - e^{-m}] \times 100$, where m is the daily per capita rate of mortality.

Results

Total plant pigment in mesocosms and in situ

The temperature rose from 5.1 °C in March to 8.3 °C by the end of the sampling period in May (Fig. 1a). An

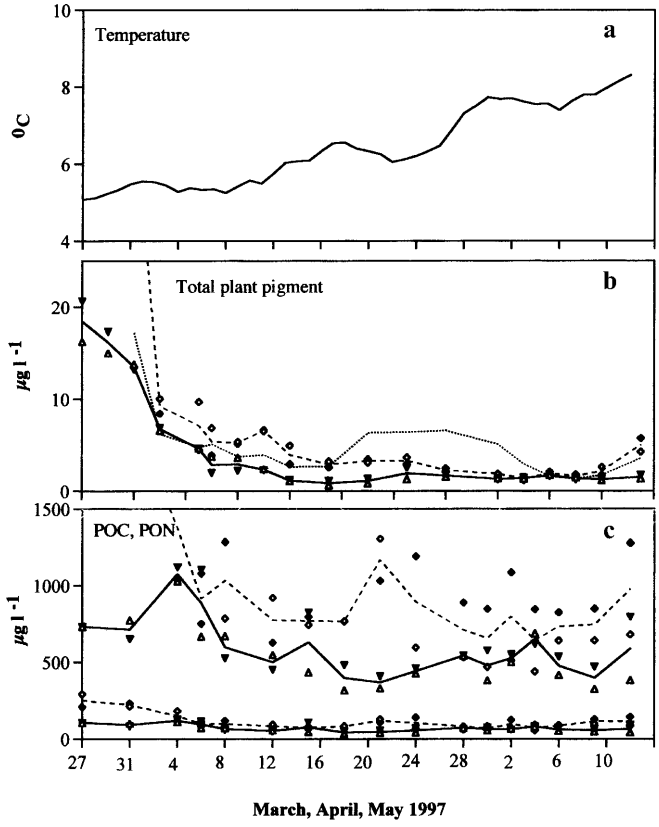


Fig. 1 **a** Time course of temperature, **b** total plant pigments and **c** POC and PON in mesocosms and in situ from 27 March to 9 May 1997 (Symbols in b: △, ▼, mesocosms L1, L2, *continuous line* mean values; ◇, ◆, mesocosms H1, H2, *dashed line* mean values; *fine dotted line* in situ; symbols in c: as in b, the curves below 250 µg l^{-1} represent PON values)

algal bloom occurred in the mesocosms and in situ by 23 March, peaked on 29 March, and the declining bloom could be followed until mid-April (Fig. 1b). During the bloom the plant pigment concentration varied significantly between mesocosms, with mean pigment values of 18.4 µg l^{-1} in L1 and L2 and 35.4 µg l^{-1} in H1 and H2 on 27 March. The pigment concentrations in situ where almost identical with L1 and L2 until 8 April; thereafter, the concentrations in situ exceeded L1 and L2 until 2 May (Fig. 1b). In H1 and H2 the pigment concentrations were higher than in situ, except in mid-April when concentrations were 1 to 2 µg l^{-1} lower. Mean values of chlorophyll *a* in L1 and H1 during the growth of the different copepodid stages are shown in Table 1. Significant changes were found between treatments during the growth of CIII to adult. The percentage of chlorophyll *a* < 10 µm increased from 5 to 10% during the bloom up to 80% at the end of April and was highest in L1 until 2 May, after which it was equal to H1 (Fig. 2a, b). The percentage of phaeopigments of chlorophyll *a* increased in both treatments from around 40% during the bloom up to 80–100% at the end of the sampling period (Fig. 2a, b).

POC and PON

POC concentrations in L1 and L2 decreased from a peak concentration of 1070 µg l^{-1} to 370–650 µg l^{-1}, and PON from 118 µg l^{-1} to 42–73 µg l^{-1} during April and May (Fig. 1c). Fluctuations of the POC concentrations were seen in H1 and H2, with an initial concentration of 2100 µg l^{-1} decreasing to 870 µg l^{-1}, and PON from 221 µg l^{-1} to 69–121 µg l^{-1} during April and May (Fig. 1c). The C:N ratios ranged from 5.6 to 10.9, with increasing C:N ratios during the declining bloom. The C:N ratio decreased by the end of April with a mean value of 7.8 and did not show any trend in relation to the different resources of the mesocosms. The C:chlorophyll *a* ratios ranged from 45 to 56 during the bloom and increased to a range of 250 to 400 throughout the post-bloom period. The N:chlorophyll *a* ranged from 5 to 9 during the bloom to 40–50 by the end of April. POC was related to chlorophyll *a* in L1 by POC = 417 ± 35 + 34 ± 8 chlorophyll *a* (r^2 = 0.58, p < 0.01) and in H1 by POC = 551 ± 41 + 50 ± 4 chlorophyll *a* (r^2 = 0.92, $p \leq 0.001$). Both equations are shown ± standard error. The intercept of both equations indicates a high proportion of carbon unrelated to phytoplankton.

Table 1 Mean values of total chlorophyll *a* and size-fractionated chlorophyll *a* < 10 µm (in parentheses) concentrations l^{-1} in mesocosms with low and high food resources during growth of the copepodid stages and adults of *Calanus finmarchicus*. Minimum and maximum values of total chlorophyll *a* and standard deviation are shown. Mann–Whitney *U*-test was used to determine significant differences between the medians of the two food resources (*ns* not significant)

Copepodid stage	Chlorophyll *a* (µg l^{-1})								Significance
	Low resources (L1)				High resources (H1)				
	Mean	Min.	Max.	SD	Mean	Min.	Max.	SD	
I–II	10.49 (9.22)	9.63	11.36	0.86	25.25 (23.7)	7.42	41.00	16.88	ns
II–III	5.72 (5.28)	3.25	9.63	3.42	6.67 (6.21)	5.58	7.42	0.96	ns
III–IV	1.99 (1.63)	0.94	2.70	0.82	4.45 (4.07)	3.52	5.58	0.89	p < 0.05
IV–V	0.62 (0.31)	0.38	0.94	0.29	2.68 (2.17)	2.16	3.52	0.73	p < 0.05
V–VI	0.88 (0.35)	0.53	1.21	0.22	1.59 (0.86)	0.65	2.97	0.74	p < 0.05

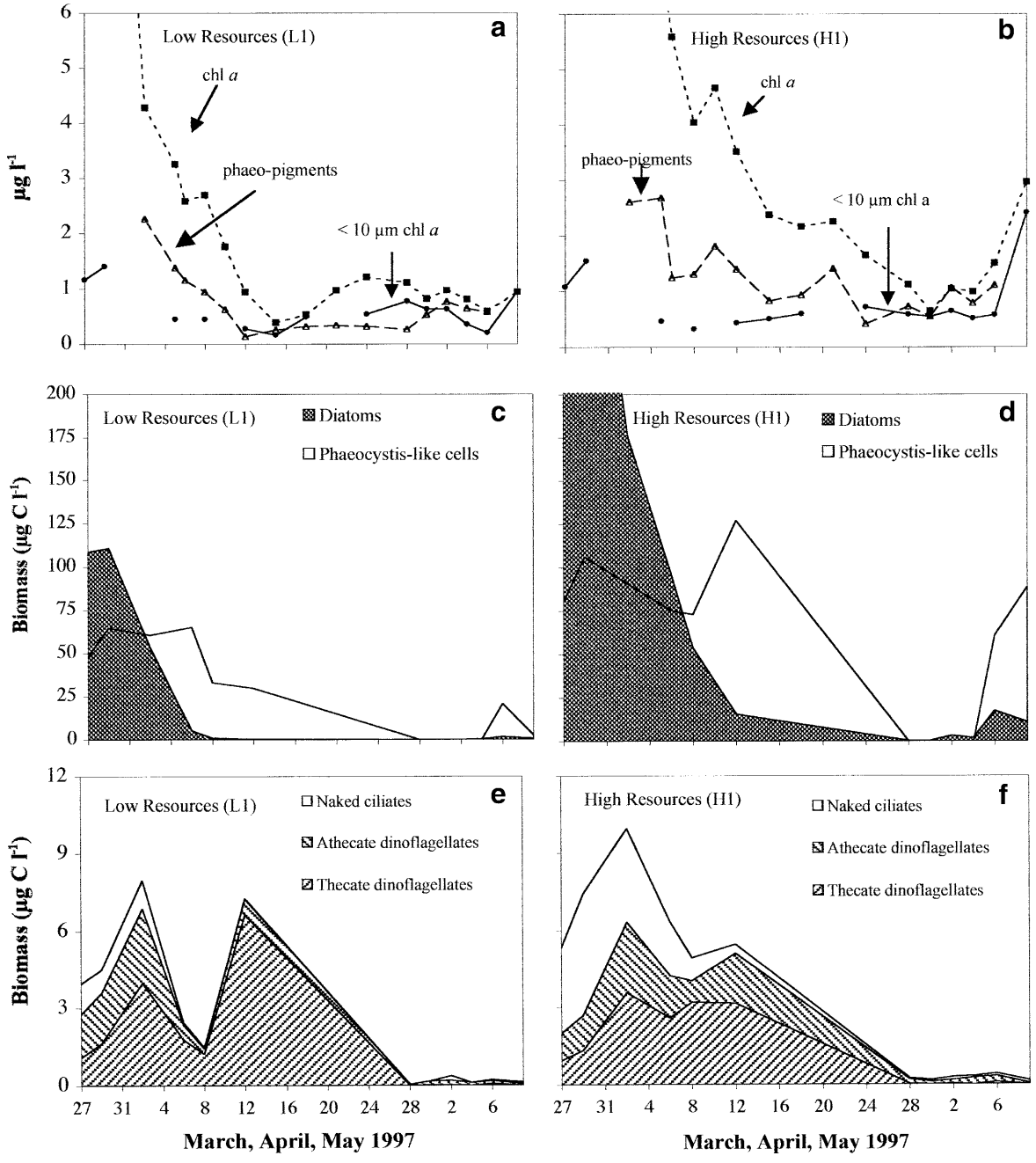


Fig. 2 Time course of chlorophyll *a* (■), phaeopigments (△) and fractionated (< 10 µm) chlorophyll *a* (●) in mesocosms L1 and H1 from 27 March to 9 May 1997 (**a**, **b**). Biomass of diatoms and *Phaeocystis*-like cells in mesocosms L1 and H1 (**c**, **d**). Biomass of ciliate and dinoflagellate biomass in mesocosms L1 and H1 (**e**, **f**)

Phytoplankton and microplankton

Diatoms were dominant during the bloom, represented by *Thalassionema* sp. (L1 = 23 cells ml^{-1}; H1 = 784 cells ml^{-1}) and *Thalassiosira* sp. (L1 = 1544 cells ml^{-1}; H1 = 2896 cells ml^{-1}). From 12 April, the bloom decreased to a mean diatom cell abundance of 4 cells ml^{-1} in L1 and 153 cells ml^{-1} in H1. After the bloom, *Phaeocystis* sp. became dominant. The estimated biomass in terms of carbon in the group of diatoms, *Phaeocystis* sp., dinoflagellates and ciliates is presented in Fig. 2c, d. Dinoflagellates peaked on 2 April in L1 and were dominated by the genera *Protoperidinium, Prorocentrum, Gyrodinium* and *Gymnodinium*, with a mean abundance of 20 cells ml^{-1}. On 12 April the mean abundance decreased to 13.3 cells ml^{-1} and by the end of April to 0.6 cells ml^{-1}. In H1, the genera *Gyrodinium* and *Gymnodinium* were most abundant and the mean concentration of dinoflagellates was 12 cells ml^{-1} on 2 April and 13 cells ml^{-1} on 12 April. At the end of April the concentrations decreased to 2 cells ml^{-1}. Only minor differences in dinoflagellate biomass were found between

L1 and H1 (Fig. 2e, f). Ciliates were only abundant during the bloom period and were dominated by *Strombidium* and *Lohmaniella oviformis* in both treatments and the obligate autotroph *Myrionecta* cf. *rubrum* in H1. The ciliate biomass declined from 1.1 μg C l^{-1} (1.3 to 0.9 cells ml^{-1}) during the diatom bloom in L1 to <0.1 μg C l^{-1} throughout the experimental period (Fig. 2e, f). Ciliates were most abundant in H1, peaked with a mean biomass of 4.7 μg C l^{-1} (1.4 to 3.4 cells ml^{-1}) during the bloom and decreased to 0.3 μg C l^{-1} (1.1 cells ml^{-1}) in mid-April and to <0.1 μg C l^{-1} by the end of April.

Species distribution, development, sex ratio and mortality

Calanus finmarchicus was the numerically dominant *Calanus* species among females collected in Raunefjord (Table 2). These females produced eggs for the build-up of the cohorts, thus *C. finmarchicus* was also numerically dominant in the mesocosms (Table 2). However, the sibling species *C. helgolandicus* and *C. glacialis* were represented, although in low numbers, and the former were only identified among the females collected in Raunefjord. Development was equiproportional, and enhancement of food resources did not affect the development rate (Table 3). Instar duration increased throughout the developmental stages (Fig. 3; Table 3). The estimated duration of CV was an underestimate because the experiments were terminated before $>50\%$ of the cohort developed to their final moult. The sex ratio between males and females showed that, when exposed to high resources (H1, H2), males dominated the adult stages (Table 4). During low resources (L1, L2) a more equal sex ratio was seen. The ratio of adults:CVs changed from <0.01 to 0.3–0.5 during 15 d in L1 and L2 (Table 4). In H1 the ratio changed from 0.03 to 0.35 during 12 d and, in H2, adults dominated at the end of the experimental period. The daily per capita mortality rates of the cohorts from L1 and H1 (Fig. 4) were based on abundance distributions from 3-litre heart-valve water bottle catches. Almost equal mortality rates were found between treatments, and the slopes of the exponential regressions showed a daily per capita mortality rate of 0.012 ($N_t = 5.717\ e^{-0.012t}$, $r^2 = 0.61$) for individuals in L1 and 0.011 ($N_t = 5.0945\ e^{-0.0113t}$, $r^2 = 0.80$) in H1. The percent daily mortality of the cohorts in L1 and H1 was estimated to be 1.2 and 1.1%, respectively.

Growth and lipid deposition

Food quantity (as μg chlorophyll *a* l^{-1}, Table 1) was a major determinant of nutritional status of the copepodids. The slope of pooled power regressions for the relationship between body weight in carbon and length relationships of *Calanus finmarchicus* copepodids changed by a factor of 1.22 between individuals grown at L1 and H1 (Fig. 5). Significant changes between treatments were also found for mean prosome length (μm) for all copepodid and adult stages (Table 5). The effect of food resources on individual growth of early copepodid stages was not clearly distinguished due to a natural nutrient pool in the enclosed water column resulting in a relatively high phytoplankton bloom in all mesocosms (Table 1). Carbon growth from CI to adult was greater than nitrogen growth (CIII to adult), and slopes of the carbon-specific growth rates were significantly different ($p < 0.01$) between L1 and H1 (Table 6; Fig. 6). No significant differences were recorded between nitrogen-specific growth rates from CIII to adult (Table 6). Growth of the oldest instars within Stage IV was negative in both treatments, which biased the exponential growth estimate for that particular development stage

Table 2 *Calanus* spp. Identification of sibling species developed during the growth of the cohorts in the mesocosms (*L1*, *L2*; *H1*, *H2*), identified by polymerase chain reaction (*PCR*) and by the fifth swimming leg (*P5*) characters. Females collected in Raunefjord (*R*), which produced eggs for the build-up of the artificial *C. finmarchiucs* cohorts, were also analysed (+, only *Calanus finmarchicus* identified in a spot-test; –, species not detected in a spot-test; ±, sibling species identified in a spot-test)

Stage	Date (d.mo.yr)	Method	Site	*C. finmarchicus*	*C. helgolandicus*	*C. glacialis*	*n*
CV	12.5.97	PCR	L1	+	–	–	5–10
	12.5.97	PCR	L2	+	–	–	5–10
	11.5.97	PCR	H1	+	–	–	5–10
	11.5.97	PCR	H2	+	–	–	5–10
Males	12.5.97	PCR	L1	±	1	–	5–10
	12.5.97	PCR	L2	±	–	1	5–10
	11.5.97	PCR	H1	+	–	–	5–10
	11.5.97	PCR	H2	+	–	–	5–10
Females	06.3.97	PCR	R	±	2		5–10
	06.3.97	P5	R	16	3	1	20
	12.5.97	PCR	L1	+	–	–	5–10
	12.5.97	PCR	L2	+	–	–	5–10
	11.5.97	PCR	H1	+	–	–	5–10
	11.5.97	PCR	H2	+	–	–	5–10
	12.5.97	P5	L1	18	–	2	20

Table 3 *Calanus finmarchicus*. Median development time (*MDT*) of copepodid stages in days and time of first appearance of the adult stages in cases where >5% of the cohort consisted of the adult stage. Cumulative MDT of the developmental stages and proportion of development time spent in each developmental stage until end of CV in mesocosm rearing of *C. finmarchicus* at low and high food resources at in situ temperature (5.1 to 8.3 °C). Time zero was considered the time at which 50% of the eggs had hatched; proportion was calculated as (cumulative MDT)/(total developmental time from eggs to end of CV)

	Copepodid Stage					Adults	
	I	II	III	IV	V	Male	Female
Low resources (L1)							
MDT (d)	4.6	4.9	5.2	9.6	>23	–	–
Time of first appearance (>5%)						63.4	60.4
Cumulative MDT (d)	23.6	28.5	33.7	43.4	66.4	–	–
Proportion on development time	0.35	0.42	0.51	0.65	1		
High resources (H1)							
MDT (d)	3.7	4.9	5.3	8.3	>23	–	–
Time of first appearance (>5%)						55.4	64.4
Cumulative MDT (d)	22.9	27.8	33.1	41.4	64.4	–	–
Proportion of development time	0.36	0.43	0.51	0.64	1		

(Fig. 6). Estimating the specific growth rate without the latest CIVs showed an effect of food resources; relatively low growth of 2.5% body C d^{-1} and 2.2% body N d^{-1} was obtained in L1 and 9.3% body C d^{-1} and 1.5% body N d^{-1} in H1. The low content of body carbon and nitrogen of the late instars of CIV corresponded with the relatively small body length of individuals (prosome length between 1750 and 1900 μm in H1 and 1850 and 2000 μm in L1); this indicates that the lower tail of the Gaussian distribution of recruits is slower to moult or may never succeed. There was no significant difference among slopes in carbon and nitrogen within stage V from different resources (Table 6), but significant (ANOVA, $p < 0.0001$) changes of biomass (C and N) during the instar development time were found. The biochemical composition of the copepods at the end of the experimental period and in situ is shown in Table 7. In general, no significant changes in mean body carbon and nitrogen were found between L1 and L2, except for body carbon in CVs (Table 7), indicating that growth was almost identical between mesocosm L1 and L2. Higher variability of the copepods was seen in H1 and H2; significant changes in mean carbon and nitrogen were found in females, and in nitrogen in males and in carbon in CVs (Table 7). The nutritional status of the in situ males, females and CV on 29 April (Table 7) were similar to individuals from L1 and L2, but different from H1 and H2, indicating that growth of *Calanus finmarchicus* in Raunefjord was not maximal.

The deposition of total storage lipids, WE and TAG, was different between animals reared at L1 or H1 (Fig. 7). In both treatments, WE accumulated from CIII to CV, but, in L1, adults continued accumulation of WE. In H1, depletion of WE occurred in males and females (Fig. 7). The TAG content of individuals from L1 was almost constant from CIII to CV, almost absent in males, and depleted in females. However, accumulation of TAG was found from CIII to CV in individuals from H1 and depleted in males and females (Fig. 7). The WE fraction constituted 82 to 99% of the total storage lipid (WE + TAG) in CIII to adults, and the lowest proportions were seen in CIII. The storage lipid proportion in carbon equivalents constituted on average 21% of total body carbon and ranged from 13 up to 30% in CIII to CV and adults. Highest percentage values were seen in late stages of CIII and CIV in L1. Relatively low variability of the proportion of total store as percentage of body carbon (18 to 26%) was seen in instars from H1. This indicates that structural and store weights increased by a constant proportion in the rich food environment. The C:N ratio increased from approximately 4 in CIII, to 10–12 in late CIVs, indicating lipid deposition. Decreasing or almost constant C:N ratios were seen in late CVs and adults (12 to 10), indicating catabolism of their major organic reserves during gonad growth and maturation. The effects of food resources on the increase in total body carbon weight and structural weight (total carbon − WE) and lipid content (WE) of the copepods are shown in Fig. 8.

Discussion

The taxonomic separation of *Calanus* sibling species showed that *C. finmarchicus* dominated both the standing stock of females in Raunefjord in early March 1997 and the cohorts in the mesocosms. Both morphological characteristics (P5) and PCR species analysis identified *C. helgolandicus* and *C. glacialis* among the females originating from Raunefjord, but the former species was not observed among CVs or adults in the mesocosms (Table 2). Different behavioural patterns among *C. finmarchicus* and *C. helgolandicus* have been reported from the shelf seas around the United Kingdom (Williams and Conway 1980), the eastern North Atlantic (Planque and Fromentin 1996) and in a Swedish fjord (Hirche 1984). Interspecific competition was minimised where the species have sympatric distributions, leading

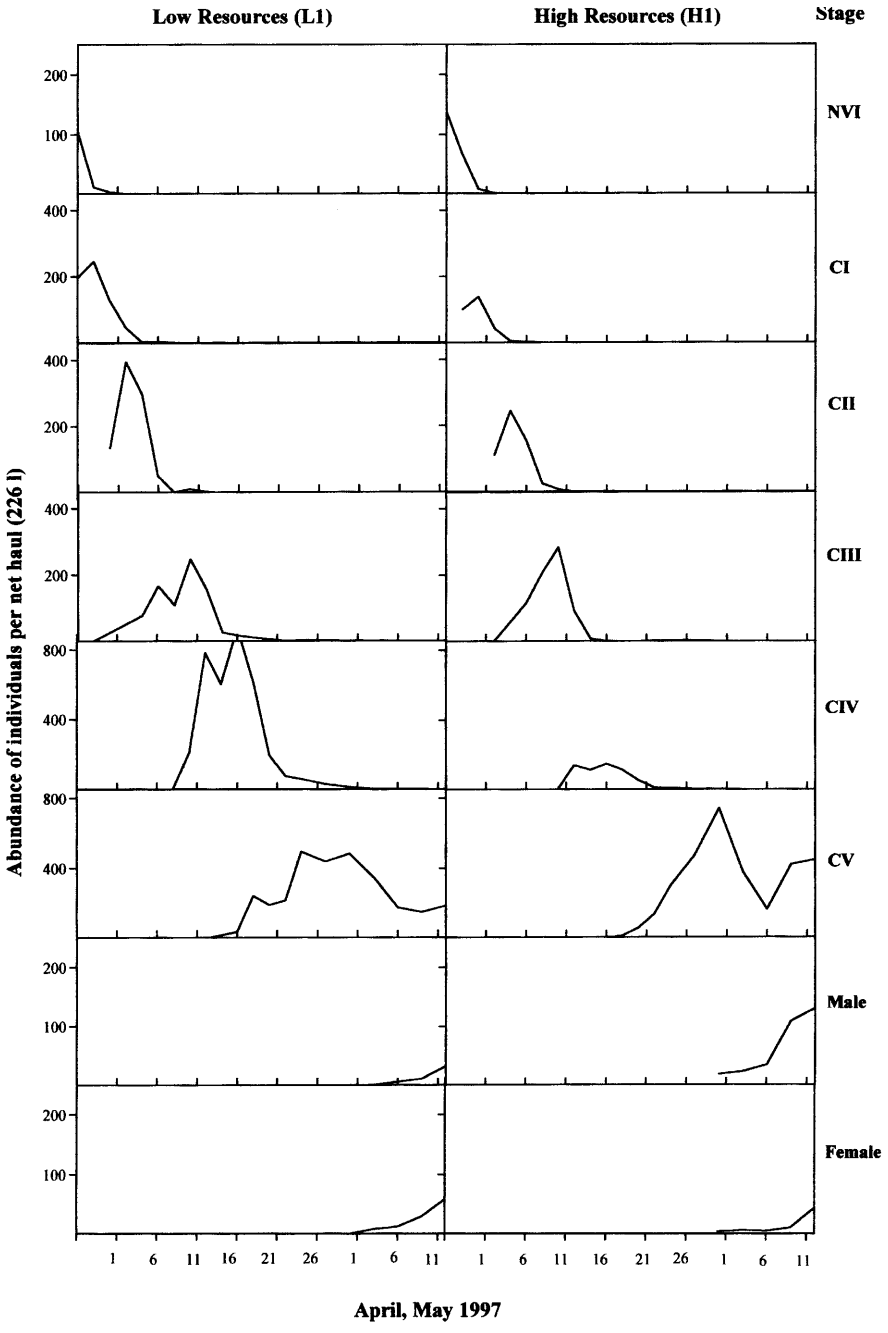


Fig. 3 *Calanus finmarchicus*. Abundance distribution of developmental stages reared in L1 and H1. Note scale change for ordinate axes

to different feeding regimes and strategies in the water column (Williams and Conway 1980). Our results indicate that competition between the two species favours the development of *C. finmarchicus* in the mesocosms. *C. glacialis* developed to adult stages in the mesocosms but with low abundance (Table 2). *Calanus glacialis* are normally somewhat larger in body size than *C. finmarchicus* in the northern part of Norway (Unstad and Tande 1991). However, there was no indication of bimodality of the prosome length of stage CV, males or females. Diagnostic taxonomic characters of P5 for species identification of the genus *Calanus* have been reported (e.g. Frost 1974; Fleminger and Hulsemann 1977). We offer our digital images as evidence of the validity of this method in the present study (Fig. 9).

The present study covers the range of oligotrophic to mesotrophic conditions with regard to food quantity during the growth of CII to adult, a food concentration typically represented in the Norwegian fjord areas and in the North Sea (Carlotti and Radach 1996). Therefore, the copepods generally exhibited good nutritional condition, also reflected by low mortality, despite rearing at different food quantities and, to some extent, also food qualities (Fig. 2). Development was equiproportional, and total development time from hatching to the end of CV was almost equal at low and high resources

Table 4 Sex ratio of males and females and the ratio between adults and CVs at the end of the experimental period in mesocosms with low (*L1*, *L2*) and high (*H1*, *H2*) food resources

Mesocosm	Date (d.mo)	Male:Female ratio	Adult:CV ratio
L1	27.4	–	0.002
	30.4	1	0.004
	03.5	0.2	0.03
	06.5	0.5	0.11
	09.5	0.4	0.28
	12.5	0.8	0.53
L2	27.4	–	0.002
	30.4	1.2	0.02
	03.5	1.5	0.07
	06.5	1.4	0.16
	09.5	0.7	0.27
	12.5	1.1	0.31
H1	30.4	4	0.03
	03.5	3.6	0.09
	06.5	6	0.25
	09.5	9.9	0.29
	12.5	3.6	0.35
H2	30.4	0.6	0.24
	03.5	1.8	0.64
	06.5	1.3	1.31
	09.5	1.4	0.74
	12.5	1.8	1.58

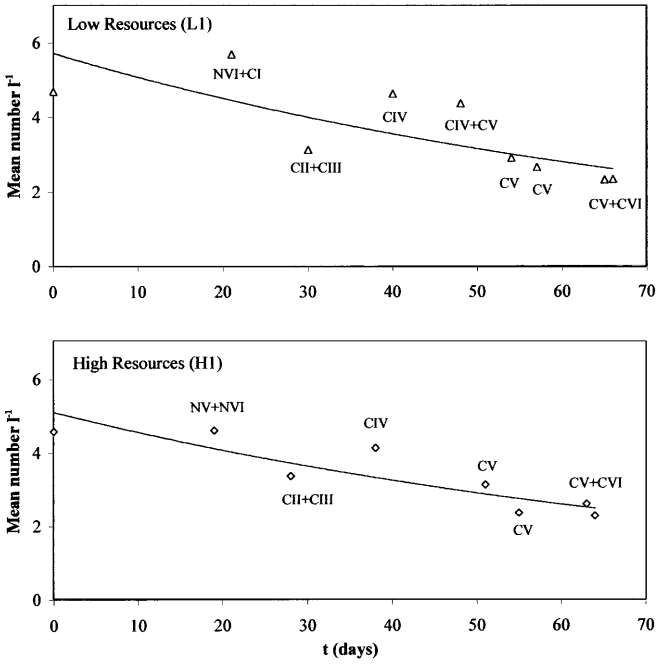


Fig. 4 *Calanus finmarchicus*. Time-dependent mortality rates of the cohort reared in mesocosms L1 and H1. Initial and final numbers of copepods in each mesocosm were estimated by counting. Curves are least-squares fits to the data, and the per capita mortality rate (m) is given by the slope of the regression: L1: $m = 0.012$ d^{-1} (1.2% d^{-1}) and H1: $m = 0.011$ d^{-1} (1.1% d^{-1})

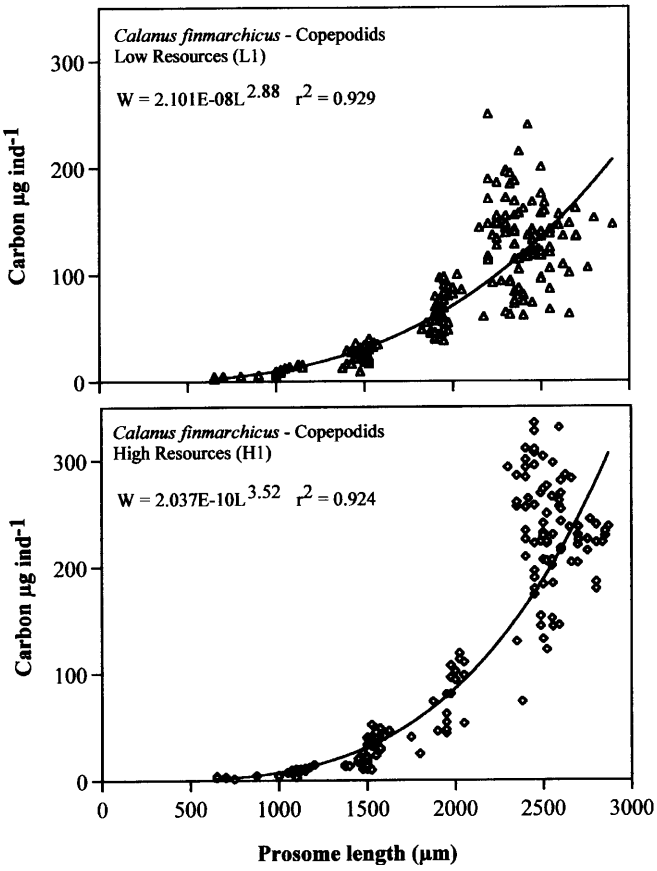


Fig. 5 *Calanus finmarchicus*. Length–weight relationships for copepodid stages reared in mesocosms L1 (*upper panel*, Δ) and H1 (*lower panel*, ◇)

(Table 3). The estimates of total development time are longer than reported by Thompson (1982). She reared *Calanus* sp. (probably *C. helgolandicus* and *C. finmarchicus*) in the laboratory at 7.5 °C and found a total development time to adult of 56.6 d. In the present study the temperature increased from 4.8 to 8.3 °C. The longer development time in our study can be explained by lower temperatures (reviewed by Carlotti et al. 1993). In the Gulf of Maine, Durbin et al. (1995) obtained estimated stage durations of 4.0 d for CIII and 6.6 d for CIV, at 6 °C. Our estimates were 5.2 to 5.3 d for CIII and 9.6 to 8.3 d for CIV in L1 and H1, respectively. These durations were slightly longer than reported by Durbin et al. (1995), but their data may have been biased by the confounding effect of spatial differences in the age of the cohort with the temporal pattern of sampling. Our estimates compare well with mesocosm rates reported by Hygum et al. (2000b) (5.1 d for CIII and 7.5 d for CIV) at excess food and in a similar temperature range, indicating that development rates in the present study were close to maximal.

Enhancement of food resources seems to have limited effect on the moult cycle in this study (Table 3). This indicates that tissue growth and related increases in body size resulting in a moult may proceed periodically independent of food concentration if the copepod has alternative energy sources such as TAG and WE deposited in the depot store. This energy store is dependent on the

Table 5 *Calanus finmarchicus*. Mean prosome length (μm) ± 95% confidence intervals for Copepodid Stages I to V and adults reared in mesocosms with low and high food resources. *Z*-test results for comparing the means of L1 and H1 are shown (*$p = 0.05$; **$p = 0.01$)

	Copepodid Stage					Adults	
	I	II	III	IV	V	Male	Female
Low resources (L1)							
Prosome length	770 ± 9.0	1123 ± 9.6	1538 ± 15.9	1983 ± 26.9	2429 ± 28.9	2574 ± 20.8	2779 ± 26.8
High resources (H1)							
Prosome length	791 ± 10.2	1145 ± 10.0	1563 ± 18.7	2064 ± 23.1	2636 ± 26.4	2773 ± 25.3	2915 ± 27.2
Z-test	**	**	*	**	**	**	**

Table 6 Parameters of least-squares linear regressions of the relationship between growth [ln(specific carbon or nitrogen weight)] and time in experiments with *Calanus finmarchicus* reared in mesocosms with low and high food resources. Significance levels of regressions are shown. Slopes (instantaneous growth rate, *g*) which differ significantly between treatments are shown in the r^2 column (only significant regressions are included) (*ns* not significant; *$p < 0.05$; **$p < 0.001$; ***$p < 0.0001$)

Copepodid Stages	Growth parameter	Intercept (ln) μg	Slope ± SE (d^{-1})	*n*	r^2
Low resources (L1)					
I	C	1.02	$0.144 \pm 0.019^{***}$	19	0.77
II	C	1.33	$0.138 \pm 0.017^{***}$	24	0.76
III	C	2.67	0.043 ± 0.027^{ns}	37	0.07
	N	−0.56	0.197 ± 0.133^{ns}	4	0.52
IV	C	3.94	0.007 ± 0.007^{ns}	47	0.02
	N	2.41	-0.022 ± 0.014^{ns}	25	0.09
V	C	3.44	$0.042 \pm 0.003^{***}$	80	0.67^{ns}
	N	1.08	$0.047 \pm 0.004^{***}$	80	0.66^{ns}
Male	C	4.03	$0.022 \pm 0.010^{*}$	21	0.20
	N	0.95	$0.046 \pm 0.009^{***}$	21	0.58
Female	C	3.03	$0.044 \pm 0.012^{**}$	28	0.33
	N	0.74	$0.052 \pm 0.013^{**}$	28	0.36
CI to adult	C	2.06	$0.077 \pm 0.002^{***}$	256	0.82^{**}
CIII to adult	N	0.90	$0.049 \pm 0.003^{***}$	158	0.64^{ns}
High resources (H1)					
I	C	1.13	0.052 ± 0.028^{ns}	19	0.17
II	C	0.45	0.209 ± 0.171^{ns}	16	0.10
III	C	1.20	$0.176 \pm 0.017^{***}$	37	0.76
	N	0.40	0.096 ± 0.028^{ns}	4	0.78
IV	C	4.28	0.007 ± 0.019^{ns}	36	0.00
	N	4.57	$-0.109 \pm 0.033^{*}$	21	0.37
V	C	4.28	$0.035 \pm 0.003^{***}$	85	0.62^{ns}
	N	1.69	$0.041 \pm 0.004^{***}$	85	0.60^{ns}
Male	C	5.41	-0.0002 ± 0.008^{ns}	29	0.00
	N	3.06	0.028 ± 0.007^{ns}	29	0.01
Female	C	5.66	-0.007 ± 0.014^{ns}	16	0.02
	N	1.91	0.029 ± 0.019^{ns}	16	0.14
CI to adult	C	1.97	$0.095 \pm 0.003^{***}$	238	0.81^{**}
CIII to adult	N	1.37	$0.046 \pm 0.004^{***}$	155	0.53^{ns}

feeding history of the copepod, in that earlier stages must have been grown at a food level that exceeds the minimum maintenance food concentration (Huntley and Boyd 1984); otherwise one could expect arrested growth and moulting. Studies on the importance of lipid catabolism fueling the moulting process during food-limited periods in the spring season are scarce. Depleted concentration of TAG was seen in newly moulted CVs from L1, indicating losses in stored energy during the moulting processes. This is consistent with a study of *Calanus helgolandicus* in which CVs utilised WE reserves during moulting and especially during female gonadogenesis (Gatten et al. 1980). During the onset of sexual differentiation, males first appeared in the mesocosms with high food resources (Fig. 3), and a more equal sex ratio was seen in the low resource mesocosms at the end of the experimental period (Table 4). The highest adult: CV ratio was seen in H2, and these animals were also the fattest (Table 7), suggesting that the time used for the final moult depends on the feeding history of the copepods in relation to food quality and quantity. The estimated sex ratios demonstrate that culturing *C. finmarchicus* in the present set-up does not affect the final moult. This is in contrast to other culture experi-

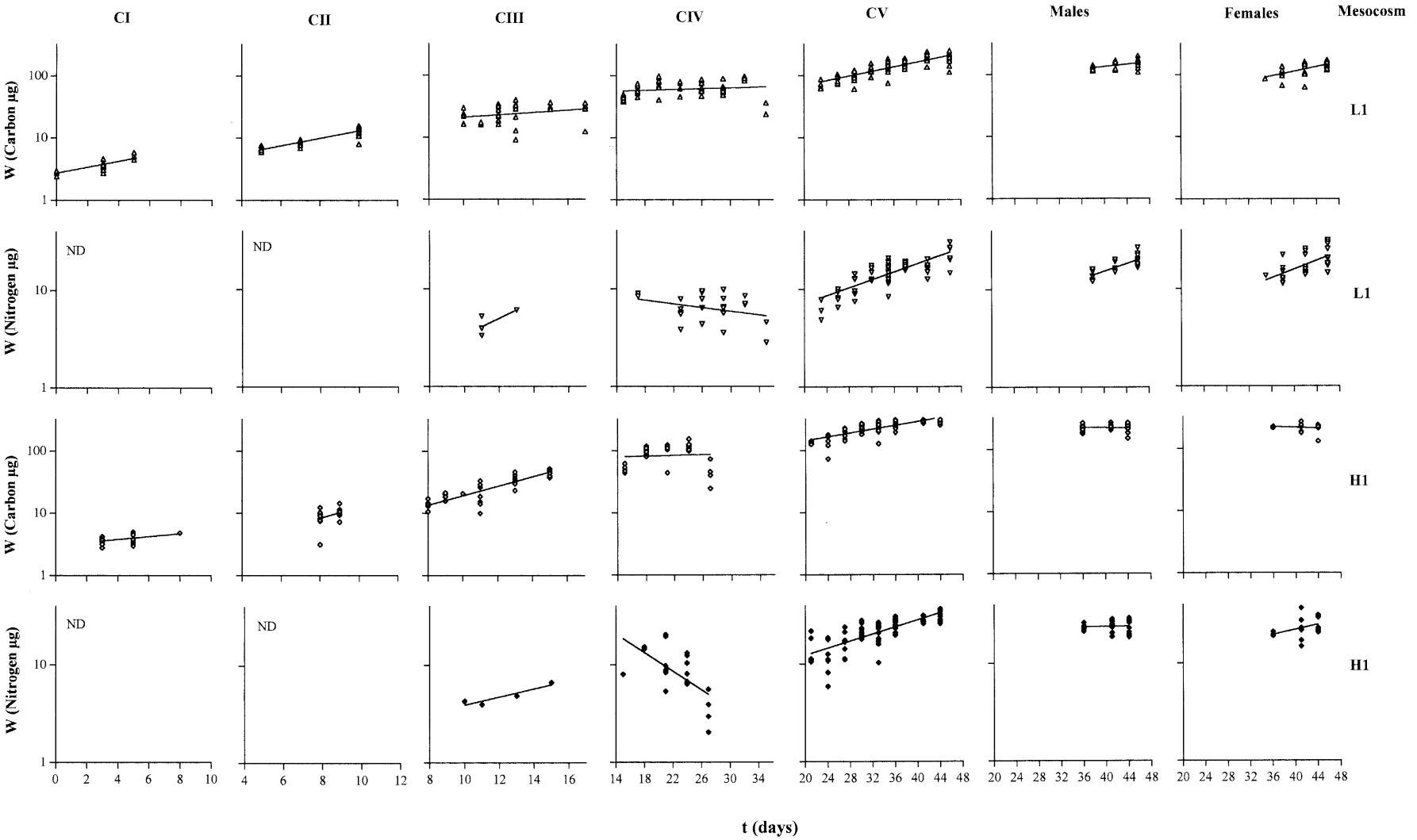


Fig. 6 *Calanus finmarchicus*. Relationship between body carbon weight and age (t) and nitrogen weight and age (t) within all stages of the copepodids and adults reared in mesocosms L1 (*upper panels*) and H1 (*lower panels*). Curves are least-squares fits to the data. Statistics of regression analyses are given in Table 6

Table 7 *Calanus finmarchicus*. Mean body carbon (C), nitrogen (N), wax ester (WE) and triacylglycerol (TAG) content ± standard deviation (SD) of copepodid stage V and adults determined on 10 and 11 May from the mesocosms L1 and L2 and H1 and H2 representing low and high food resources, respectively. Also in situ individuals from the Raunefjord determined on 29 April are shown. *n* represents numbers of samples analysed. Significant differences (*t*-test) of mean body C and N within the replicate mesocosms L1 and L2 and H1 and H2 are shown. In case of different variances of the two samples (indicated by #), a Mann–Whitney *U*-test was used (*ns* not significant; $*p < 0.05$; $**p < 0.01$)

Food resources	Stage	Carbon (μg ind^{-1})	Nitrogen (μg ind^{-1})	*n*	WE (μg ind^{-1}) mean ± SD	TAG (μg ind^{-1}) mean ± SD	*n*	*t*-test	
								Carbon	Nitrogen
L1	Male	153.1 ± 25.9	20.4 ± 3.2	10	41.2 ± 4.2	0.40 ± 0.17	4	ns	ns
L2	Male	154.7 ± 31.5	19.2 ± 2.8	8	49.63		1		
L1	Female	148.3 ± 18.6	22.5 ± 5.9	10	34.5 ± 6.3	0.58 ± 0.19	4	ns #	ns
L2	Female	163.4 ± 45.7	22.7 ± 7.7	8	30.3 ± 13.6	0.04 ± 0.06	2		
L1	CV	190.4 ± 40.7	23.7 ± 5.1	10	28.9 ± 9.2	1.28 ± 0.63	4	**	ns #
L2	CV	242.4 ± 25.8	24.7 ± 2.5	10	60.2 ± 9.6	1.91 ± 0.71	6		
H1	Male	218.3 ± 35.8	23.9 ± 4.1	10	53.4 ± 4.2	1.83 ± 0.77	4	ns	**
H2	Male	223.4 ± 31.1	30.7 ± 2.2	10	57.7 ± 13.9	1.19 ± 1.10	5		
H1	Female	214.2 ± 37.2	25.4 ± 4.7	7	55.9 ± 4.46	3.74 ± 0.91	4	**	**
H2	Female	287.3 ± 20.4	39.4 ± 2.4	10	68.5 ± 9.07	2.88 ± 0.97	3		
H1	CV	296.9 ± 37.2	30.7 ± 4.7	10	69.9 ± 8.90	10.31 ± 4.54	4	*	ns
H2	CV	331.7 ± 38.9	32.8 ± 3.8	10	77.03 ± 4.30	7.6 ± 4.39	4		
In situ	Male	142.2 ± 45.1	20.2 ± 5.29	5					
In situ	Female	101.9 ± 13.7	13.7 ± 4.99	5					
In situ	CV	133.4 ± 61.8	11.6 ± 4.62	6					
In situ	CIV	41.2 ± 17.1	3.5 ± 1.98	5					

ments in which males of the genus *Calanus* did not develop or were rare (e.g. Hirche 1980; Peterson 1986). However, in rearing experiments with *C. pacificus*, a sex ratio of 0.69 females was obtained (Landry 1983). The estimated daily per capita mortality rate was 0.012 d^{-1} (1.2% d^{-1}) in L1 and 0.011 d^{-1} (1.1% d^{-1}) in H1. The estimated rates cannot be related to top-down predation, because potential predators were removed during the initial screening of the mesocosm water. Top-down predation had a pronounced effect on the *C. finmarchicus* populations (mortality 0.02 to 0.4 d^{-1}) in Korsfjorden, western Norway (Matthews et al. 1978). However, a similar low mortality rate (0.8% d^{-1}) similar to that found in the present study was estimated for *Calanus* spp. in Lurefjorden, a western Norwegian fjord, which lacked mesopelagic fish (Bagøien 1999). The length–weight relationships determined for copepodid stages of *C. finmarchicus* at low and high food resources (Fig. 5) were consistent with length–weight relationships reported for similar species, location and experimental set-up in 1996 (Hygum et al. 2000b). In the present study, the effect of food resources on the length–weight determinations resulted in a 30% higher biomass of individuals from H1 compared to individuals from L1 (Fig. 5).

Growth of stages CI and CII continued during the declining bloom that was dominated by diatoms (Fig. 2c, d). In the post-bloom phase during the growth of CIII to adult, *Phaeocystis* sp. dominated the phytoplankton biomass in both treatments, but dinoflagellates were also present (Fig. 2e, f). Diatom fatty acids can easily be distinguished from those of dinoflagellates and *Phaeocystis* sp. The principal fatty acids of diatoms are 16:1(*n* - 7) and 20:5(*n* - 3) (e.g. Sargent and Falk-Petersen 1988; Graeve et al. 1994), and major fatty acids of *Phaeocystis pouchetti* and dinoflagellates are 18:4(*n* - 3) 22:6(*n* - 3) (Sargent et al. 1985; Sargent and Falk-Petersen 1988; Graeve et al. 1994). The 18:4(*n* - 3) fatty acid was one of the major acids during the growth of late copepodid stages from both L1 and H1, and 16:1(*n* - 7) was quantitatively most important during the early copepodid stages and in the adult stages (Hygum et al. in preparation). This demonstrates that diatoms, but also *Phaeocystis* sp. and dinoflagellates, were part of the diet, and the declining biomass of dinoflagellates during the growth of CVs suggests grazing control (Fig. 2). The characteristic *Phaeocystis* sp. fatty acids at the end of the experimental period verify that this prymnesiophyte is a part of the diet of *Calanus* spp. (as also confirmed by Kattner and Krause 1987; Tande and Båmstedt 1987; Estep et al. 1990; Hansen et al. 1990; Hygum et al. 2000b).

It is well established that calanoids are rich in depot lipid, predominantly WE (Lee et al. 1970; Sargent and Henderson 1986; Kattner and Krause 1987; Miller et al. 1998); the *Calanus finmarchicus* studied in the present work conform to this general pattern (Fig. 7). Variations in body carbon and nitrogen (C:N ratio) reflected the lipid deposition in the different developmental stages. The highest C:N ratio was seen in individuals from H1, corresponding with high lipid values. Highest lipid deposition (high C:N ratio) was seen in late CIVs. Decreasing C:N ratio in late CV and adults in H1 suggests that sexual maturation with its inherent requirements for gonad growth occurs at the cost of lipid deposition. The amount of lipid deposited by the copepods was related to the concentration and quality of food, and there seems to be an upper threshold for the amount of lipid that can be deposited (Fig. 8). This is consistent with reported laboratory studies demonstrating that the growth rate of *C. pacificus* increases

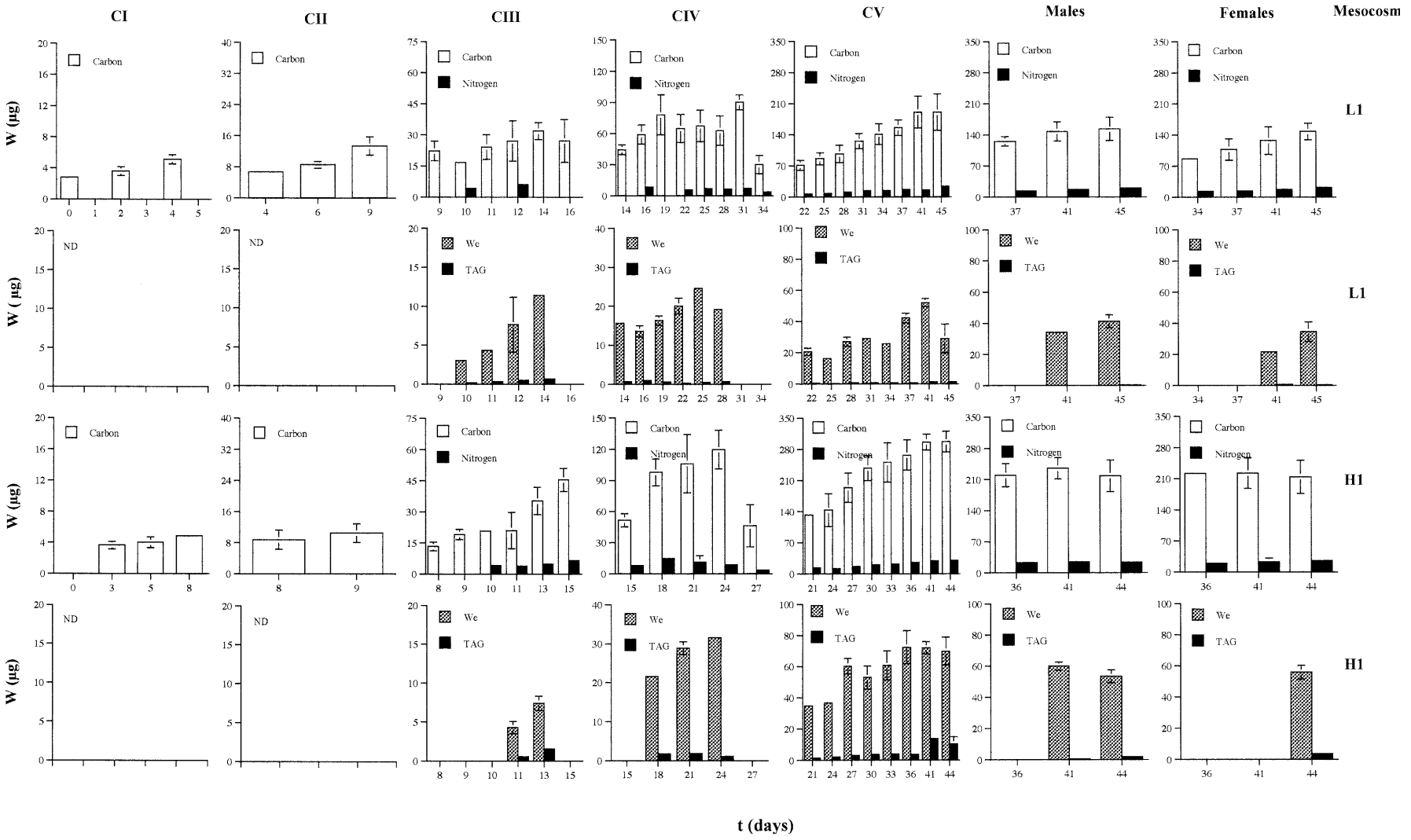


Fig. 7 *Calanus finmarchicus*. Relationship between mean body carbon weight and mean nitrogen weight (*w*) and age (*t*) within all stages of the copepodids and adults reared in mesocosms L1 and H1 (*first and third row of panels*). Relationship between mean wax ester (*WE*) content and mean triacylglycerol (*TAG*) content and age (*t*) within all stages of the copepodids and adults reared in mesocosms L1 and H1 (*second and fourth row of panels*). Error bars are standard deviations (*ND* not determined)

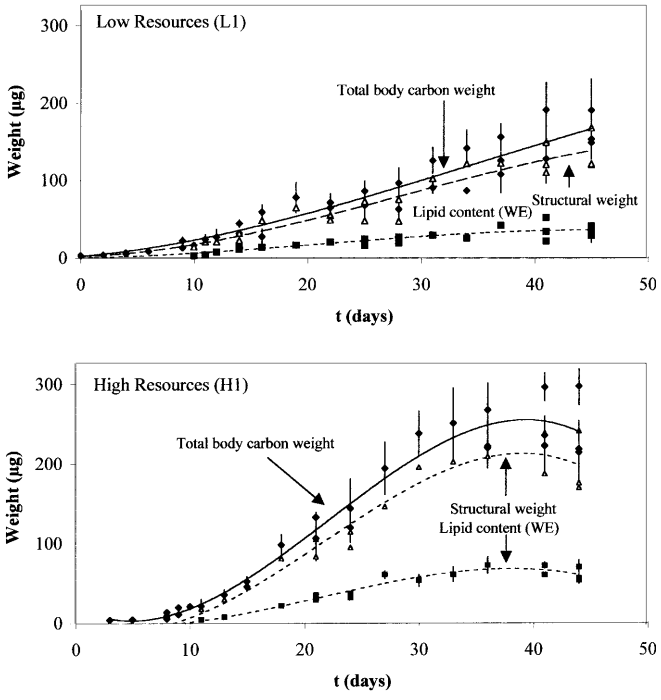
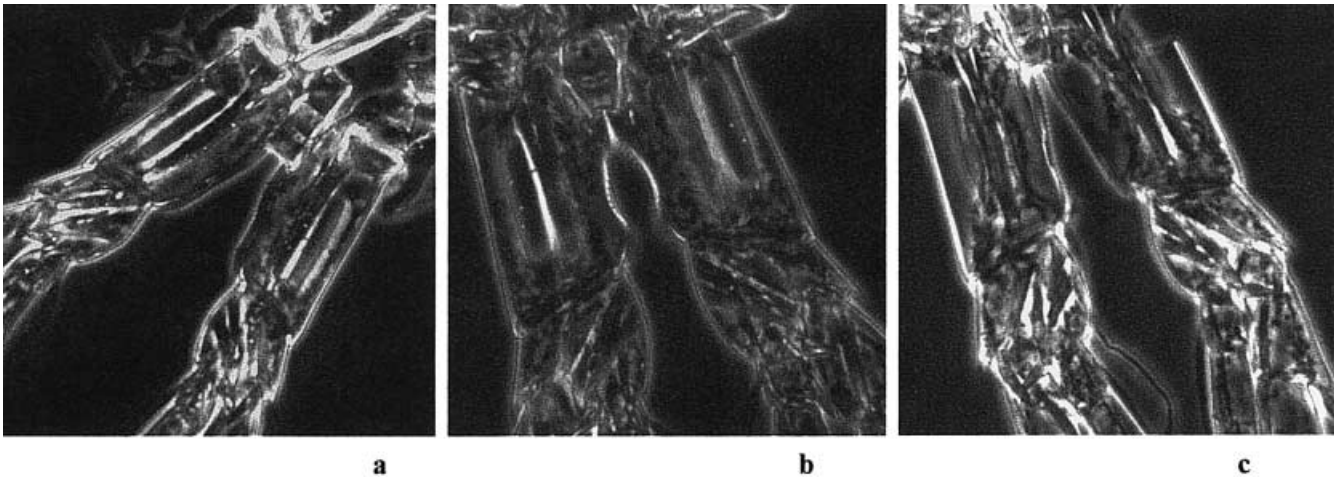


Fig. 8 *Calanus finmarchicus*. Relationship between total mean body carbon weight (sum of the structural weight and the lipid content), structural weight (total body C – WE) and lipid (*WE*) content and age (*t*) of copepodids and adults reared in mesocosms L1 and H1. A third order polynomial was used to describe data. Lipid content was plotted as µg WE mean ind^{-1} but converted to carbon when structural weight is described (see "Materials and methods"). Error bars are standard deviations

with food availability up to some "critical concentration", above which it remains constant (Vidal 1980).

Minor depot lipid accumulation has been found in *Calanus finmarchicus* from CI to CII (Kattner and Krause 1987; Hygum et al. 2000b). The present study shows that the ability to accumulate WE is prominent from stage III and absent in adult females and males in H1. However, adults fed in L1 continued lipid deposition (Fig. 7). The deposition of TAG in copepods from L1 or H1 was different. In the former, the TAG content was in general low, with a weak increase from CIII to CV; it was almost absent in males and reduced in females (Fig. 7). However, in the latter, TAG accumulated from stages III to V, and again was almost depleted in males and reduced in females. TAG is a more labile lipid reserve compared to WE (Lee et al. 1971a; Sargent et al. 1977; Håkonson 1984). Freshly caught *C. helgolandicus* were starved and re-fed on a diatom diet. Starving the copepods resulted in lipid depletion, and the animals utilised nearly 90% of their TAG but only 30% of their WE (Sargent et al. 1977). A similar experimental set-up was utilised by Ohman and Runge (1994), but with *C. finmarchicus*. Starvation for 3.5 d resulted in 54% depletion of TAG and 22.6% of WE. Re-feeding with diatoms resulted in increased TAG content, but no change in WE content. Thus, in terms of percentage loss, TAG was more labile than WE as a lipid reserve. In the present study, growth was limited by food availability from CIII/CIV to adult in L1. Compared to H1, diatoms constituted a lower proportion of the ration, and dinoflagellates, *Phaeocystis* and detritus constituted equally important prey categories in L1 during these stages. The low amount of TAG in the copepods from L1 is consistent with the interpretation that TAG varies in relation to recent nutritional conditions (Håkanson 1984). The almost total lack of TAG in males from both treatments may be related to energy costs needed to ripen the genital products, consistent with data on *C. finmarchicus* males from Balsfjorden in Norway (Tande 1982; Hopkins et al. 1984). Furthermore, spermatophores were found on females on 30 April; thus energy for spermatophore production and copulation could have been metabolised from the depot lipids.

Fig. 9 Morphological characters of the fifth swimming legs (P5) in adult females **a** *Calanus finmarchicus*, **b** *C. helgolandicus*, **c** *C. glacialis*. Curvature of the toothed border of the inner margin of the basipod of P5 easily separates *C. helgolandicus* from the two other species. However, taxonomic separation between *C. finmarchicus* and *C. glacialis* based on P5 morphology is not easy. Size difference of the basipod between the two species seems to be a valid parameter, but the curvature of the basipod is not a valid morphological character for *C. glacialis* in the present study



In our investigation we verified that relatively small changes in food quantity and quality (Fig. 2) have significant effects on the amount of storage lipid (WE + TAG) and structural components (proteins and carbohydrates) deposited during the growth of a *Calanus finmarchicus* population (Fig. 8). WE, accumulated during the later developmental stages, provides fuel for the developing eggs and embryos (Guisande and Harris 1995), and the amounts of WE present in mature animals are likely to be critical in determining the brood size and survival of the ensuing generation (Sargent et al. 1977). Thus, shortage in food quantity and quality may have important implications for *C. finmarchicus*' ability to provide offspring with large lipid reserves or to survive when diapausing at great depth. The lipid content may provide an estimate of total reproductive potential (Hirche and Kattner 1993). The total storage lipid content in females from L1 and H1 varied between 22.4–35.1 μg and 59.7 μg, respectively (Fig. 7). Given that the transformation of storage lipid content was exclusively towards egg production, that the carbon content of an egg is 0.191 μg (Hygum et al. 2000a), and a lipid weight per C conversion of 80%, then maximum potential fecundity of a female from L1 would have been between 93–147 eggs female^{-1} and 250 eggs female^{-1} in H1 until all lipids were exhausted. The calculation emphasises the importance of the feeding history of the females; relatively small changes of food quantity between L1 and H1 significantly affected the accumulation of lipids in stages CIII to CV, which may later influence the female egg production, especially if food availability falls below the critical concentration (Huntley and Boyd 1984).

This study provides the first quantitative and qualitative descriptions of the biochemical composition of all copepodid stages and adults of *Calanus finmarchicus* related to a natural, complex food composition experienced in Norwegian fjord areas. The study supports the exponential-growth hypothesis (McLaren 1986); growth of both structural and storage compartments was exponential. However, a sigmodal pattern of growth (Fig. 8) or deviation from exponential growth pattern best described: (i) growth of adult stages if reared at high food resources and (ii) depot lipid accumulation in late CVs and adults at high resources. Body nitrogen content, which might be a better measure of "structural compartments" (McLaren 1986), showed exponential growth; however, no significant changes in nitrogen-specific growth rates were found between individuals from low and high resources (Table 6). For comparison, the carbon-specific growth rate was 0.077 d^{-1} in low and 0.095 d^{-1} in high resources, or a 23% higher growth rate in individuals from high resources. Individuals from H1 were heavier, and CV and adults seemed to have reached near-maximal weights. However, structural weight continued to increase in L1 showing that the near-maximal weight has not been reached (Fig. 7). Despite the differences in structural growth dynamics, the cohort stage development was similar until the end of CV (Table 3). The estimates of the sex ratio, the mortality rate and the fecundity of the females (Rey et al. 1999) demonstrate that the mesocosm set-up is a valid ecological tool for investigating key aspects of *C. finmarchicus*' life cycle and will be closer to reality than laboratory systems.

Acknowledgements This study was financially supported by the Commission of the European Community through Contract MAS3-CT95-0039 (TASC) and by the TMR (Training and Mobility of Researchers) programme from the European Union through Contract No. ERBFMGECT950013. We thank M. Brozek, F. Jensen and P. Thor, Roskilde University; B. Vaaja, The Norwegian College of Fishery Science, University of Tromsø; M. Carlsen, Danish Fishery Institute; and M. Martinussen, Institute of Marine Research, University of Bergen for various forms of field, laboratory and logistical support. BHH acknowledges the NOVO Nordic Foundation for grants for the image analysis system. Valuable comments from V. Forbes and T. Kiørboe helped improve earlier versions of the manuscript.

References

Aksnes DL, Magnesen T (1983) Distribution, development, and production of *Calanus finmarchicus* (Gunnerus) in Lindåspollene, western Norway, 1979. Sarsia 68: 195–208

Aksnes DL, Miller CB, Ohman MD, Wood SN (1997) Estimation techniques used in studies of copepod population dynamics – A review of underlying assumptions. Sarsia 82: 272–296

Backhaus JO, Harms IH, Krause M, Heath MR (1994) An hypothesis concerning the space-time succession of *Calanus finmarchicus* in the northern North Sea. ICES J mar Sci 51: 169–180

Bagøien E (1999) Predatory impact of invertebrates and fish on overwintering *Calanus*. Dr. scient. thesis, Section of Marine Zoology and Marine Chemistry, Department of Biology, University of Oslo, Norway

Banse K (1977) Determining the carbon-to-chlorophyll ratio of natural phytoplankton. Mar Biol 41: 199–212

Carlotti F, Krause M, Radach G (1993) Growth and development of *Calanus finmarchicus* related to the influence of temperature: experimental results and conceptual model. Limnol Oceanogr 38: 1125–1134

Carlotti F, Radach G (1996) Seasonal dynamics of phytoplankton and *Calanus finmarchicus* in the North Sea as revealed by a coupled one-dimensional model. Limnol Oceanogr 41: 522–539

Conover RJ (1988) Comparative life histories in the genera *Calanus* and *Neocalanus* in high latitudes of the northern hemisphere. Hydrobiologia 167/168: 127–142

Cushing DH (1990) Plankton production and year-class strength in fish populations: an update of the match/mismatch hypothesis. Adv mar Biol 26: 249–293

Durbin EG, Gilman SL, Campbell RG, Durbin AG (1995) Abundance, biomass, vertical migration and estimated development rate of the copepod *Calanus finmarchicus* in the southern Gulf of Maine during late spring. Contin Shelf Res 15: 571–591

Estep KW, Nejstgaard JC, Skjoldal HR, Rey F (1990) Predation by copepods upon natural populations of *Phaeocystis pouchetii* as a function of the physiological state of the prey. Mar Ecol Prog Ser 67: 235–249

Fleminger A, Hulsemann K (1977) Geographical range and taxonomic divergence in North Atlantic *Calanus* (*C. helgolandicus, C. finmarchicus* and *C. glacialis*). Mar Biol 40: 233–248

Frost BW (1974) *Calanus marshallae*, a new species of calanoid copepods closely allied to the sibling species *C. finmarchicus* and *C. glacialis*. Mar Biol 26: 77–99

Gatten RR, Sargent JR, Forsberg TEV, O'Hara SMC, Corner EDS (1980) On the nutrition and metabolism of zooplankton.

XIV. Utilization of lipid by *Calanus helgolandicus* during maturation and reproduction. J mar Biol Ass UK 60: 391–399
Graeve M, Kattner G, Hagen W (1994) Diet-induced changes in the fatty acid composition of Arctic herbivorous copepods: experimental evidence of trophic markers. J exp mar Biol Ecol 182: 97–110
Guisande C, Harris R (1995) Effect of total organic content of eggs on hatching success and naupliar survival in the copepod *Calanus helgolandicus*. Limnol Oceanogr 40: 476–482
Hansen B, Tande KU, Berggreen UC (1990) On the trophic fate of *Phaeocystis pouchetii* (Hariot). 3. Functional responses in grazing demonstrated on juvenile stages of *Calanus finmarchicus* (Copepoda) fed diatoms and *Phaeocystis*. J Plankton Res 12: 1173–1187
Harris RP (1996) Feeding ecology of *Calanus*. Ophelia 44: 85–109
Hirche H-J (1980) The cultivation of *Calanoides carinatus* Krøyer (Copepoda: Calanoida) under different temperature and food conditions – with a description of eggs and nauplii. J mar Biol Ass UK 60: 115–125
Hirche H-J (1984) Seasonal distribution of *Calanus finmarchicus* (Gunnerus) and *C. helgolandicus* (Claus) in a Swedish fjord. Crustaceana 7(Suppl): 233–241
Hirche H-J (1990) Egg production of *Calanus finmarchicus* at low temperature. Mar Biol 106: 53–58
Hirche H-J (1996a) The reproductive biology of the marine copepod, *Calanus finmarchicus* – A review. Ophelia 44: 111–128
Hirche H-J (1996b) Diapause in the marine copepod *Calanus finmarchicus* – A review. Ophelia 44: 129–143
Hirche H-J, Kattner G (1993) Egg production and lipid content of *Calanus glacialis* in spring: indication of a food-dependent and food-independent reproductive mode. Mar Biol 117: 615–622
Holm-Hansen O, Lorenzen CJ, Holmes RW, Strickland JDH (1965) Fluorometric determination of chlorophyll. J Cons perm int Explor Mer 30: 3–15
Hopkins CCE, Tande KS, Grønvik S, Sargent JR (1984) Ecological investigations of the zooplankton community of Balsfjorden, northern Norway: an analysis of growth and overwintering tactics in relation to niche and environment in *Metridia longa* (Lubbock), *Calanus finmarchicus* (Gunnerus), *Thysanoessa inermis* (Krøyer) and *T. raschi* (M. Sars). J exp mar Biol Ecol 82: 77–99
Huntley M (1988) Feeding biology of *Calanus*: a new perspective. Hydrobiologia 167/168: 83–99
Huntley M, Boyd C (1984) Food-limited growth of marine zooplankton. Am Nat 124: 455–478
Huntley ME, Lopez MDG (1992) Temperature-dependent production of marine copepods: a global synthesis. Am Nat 140: 201–242
Hygum BH, Rey C, Hansen BW (2000a) Growth and development rates of *Calanus finmarchicus* nauplii during a diatom spring bloom. Mar Biol 136: 1075–1085
Hygum BH, Rey C, Hansen BW, Carlotti F (2000b) Rearing cohorts of *Calanus finmarchicus* (Gunnerus) in mesocosms. ICES J mar Sci (in press)
Håkanson JL (1984) The long and short term feeding condition in field-caught *Calanus pacificus*, as determined from the lipid content. Limnol Ocenogr 29: 794–804
Håkanson JL (1987) The feeding condition of *Calanus pacificus* and other zooplankton in relation to phytoplankton pigments in the California Current. Limnol Oceanogr 32: 881–894
Jespersen A-M, Christoffersen K (1987) Measurements of chlorophyll *a* from phytoplankton using ethanol as extraction solvent. Arch Hydrobiol 109: 445–454
Jónasdóttir SH (1999) Lipid content of *Calanus finmarchicus* during overwintering in the Faroe–Shetland channel. Fish Oceanogr 8(Suppl 1): 61–72
Kattner G, Krause M (1987) Changes in lipids during the development of *Calanus finmarchicus* s.l. from Copepodite I to adult. Mar Biol 96: 511–518
Landry MR (1983) The development of marine calanoid copepods with comments on the isochronal rule. Limnol Oceanogr 28(4): 614–624
Lee RF, Hirota J, Barnett AM (1971a) Distribution and importance of wax esters in marine copepods and other zooplankton. Deep-Sea Res 18: 1147–1165
Lee RF, Nevenzel JC, Paffenhöfer G-A (1970) Wax esters in marine copepods. Science 167: 1510–1511
Lee RF, Nevenzel JC, Paffenhöfer G-A (1971b) Importance of wax esters and other lipids in the marine food chain: phytoplankton and copepods. Mar Biol 9: 99–108
Marshall SM, Orr AP (1955) The biology of a marine copepod, *Calanus finmarchicus* (Gunnerus). Oliver and Boyd, Edinburgh
Matthews JBL, Hestad L, Bakke JLW (1978) Ecological studies in Korsfjorden, western Norway. The generations and stocks of *Calanus hyperboreus* and *C. finmarchicus* in 1971–1974. Oceanol Acta 1: 277–284
McLaren IA (1986) Is "structural" growth of *Calanus* potentially exponential? Limnol Oceanogr 31: 1342–1346
Miller CB, Morgan CA, Prahl FG, Sparrow MA (1998) Storage lipids of the copepod *Calanus finmarchicus* from Georges Bank and the Gulf of Maine. Limnol Oceanogr 43: 488–497
Ohman MD, Runge JA (1994) Sustained fecundity when phytoplankton resources are in short supply: omnivory by *Calanus finmarchicus* in the Gulf of St. Lawrence. Limnol Oceanogr 39(1): 21–36
Paffenhöfer G-A (1971) Grazing and ingestion rates of nauplii, copepodids and adults of the marine planktonic copepods *Calanus helgolandicus*. Mar Biol 11: 286–298
Pedersen G, Tande KS (1992) Physiological plasticity to temperature in *Calanus finmarchicus*. Reality or artefact? J exp mar Biol Ecol 155: 183–197
Peterson WT (1986) Development, growth, and survivorship of the copepod *Calanus marshallae* in the laboratory. Mar Ecol Prog Ser 29: 61–72
Peterson WT, Painting SJ (1990) Developmental rates of the copepods *Calanus australis* and *Calanoides carinatus* in the laboratory, with discussion of methods used for calculation of development time. J Plankton Res 12: 283–293
Planque B, Fromentin JM (1996) *Calanus* and environment in the eastern North Atlantic. I. Spatial and temporal patterns of *C. finmarchicus* and *C. helgolandicus*. Mar Ecol Prog Ser 134: 101–109
Rey C, Carlotti F, Tande K, Hygum BH (1999) Egg and faecal pellet production of *Calanus finmarchicus* females from controlled mesocosms and in situ populations: influence of age and feeding history. Mar Ecol Prog Ser 188: 133–148
Runge JA (1988) Should we expect a relationship between primary production and fisheries? The role of copepod dynamics as a filter of trophic variability. Hydrobiologia 167/168: 61–71
Sargent JR, Eilertsen HC, Falk-Petersen S, Taasen JP (1985) Carbon assimilation and lipid production in phytoplankton in northern Norwegian fjords. Mar Biol 85: 109–116
Sargent JR, Falk-Petersen S (1988) The lipid biochemistry of calanoid copepods. Hydrobiologia 167/168: 101–114
Sargent JR, Gatten RR, Corner EDS, Kilvington CC (1977) On the nutrition and metabolism of zooplankton. XI. Lipid in *Calanus helgolandicus* grazing *Biddulphia sinensis*. J mar biol Ass UK 57: 525–533
Sargent JR, Henderson RJ (1986) Lipids. In: Corner EDS, O'Hara SCM (eds) The biological chemistry of marine copepods. Clarendon Press, Oxford, pp 59–108
Smetacek V (1975) Die Sukzession des Phytoplankton in der westlichen Kieler Bucht. Dissertation, Universität Kiel, Kiel
Strathmann RR (1967) Estimating the organic carbon content of phytoplankton from cell volume or plasma volume. Limnol Oceanogr 12: 411–418
Tande KS (1982) Ecological investigations of the zooplankton community of Balsfjorden, northern Norway: generation cycles, and variations in body weight and body content of carbon and nitrogen related to overwintering and reproduction in the copepod *Calanus finmarchicus* (Gunnerus). J exp mar Biol Ecol 62: 129–142

Tande KS, Båmstedt U (1987) On the trophic fate of *Phaeocystis pouchetii*. 1. Copepod feeding rates on solitary cells and colonies of *P. pouchetii*. Sarsia 72: 3–4

Thompson BM (1982) Growth and development of *Pseudocalanus elongatus* and *Calanus* sp. in the laboratory. J mar biol Ass UK 62: 359–372

Unstad KM, Tande KS (1991) Depth distribution of *Calanus finmarchicus* and *C. glacialis* in relation to environmental conditions in the Barents Sea. In: Sakshaug E, Hopkins CCE, Øritsland NA (eds) Proceedings of the Pro Marc Symposium on Polar Marine Ecology. Tronheim, 12–16 May 1990. Polar Res 10(2): 409–420

Utermöhl H (1931) Neue Wege in der quantitativen Erfassung des Planktons. (Mit besonderer Berücksichtigung des Ultraplanktons). Verh int Verein theor angew Limnol 5: 567–596

Vidal J (1980) Physioecology of zooplankton. I. Effects of phytoplankton concentration, temperature, and body size on the growth rate of *Calanus pacificus* and *Pseudocalanus* sp. Mar Biol 56: 111–134

Williams R, Conway DVP (1980) Vertical distribution of *Calanus finmarchicus* and *C. helgolandicus* (Crustacea: Copepoda). Mar Biol 60: 57–61

Williams R, Lindley JA (1980) Plankton of the Fladen Ground during FLEX 76 III. Vertical distribution, population dynamics and production of *Calanus finmarchicus* (Crustacea: Copepoda). Mar Biol 60: 47–56