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# Correcting for underestimation of microzooplankton grazing in bottle incubation experiments with mesozooplankton

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ABSTRACT: Bottle incubation experiments are widely used in mesozooplankton grazing studies. However, we have shown here that traditional particle removal experiments with *Calanus finmarchicus* and *C. helgolandicus* as grazers on natural plankton may yield low or even statistically significant ($p < 0.05$) negative grazing estimates, even though negative grazing rates are impossible. Low grazing rates are often reported, especially on smaller prey types, despite abundant food and significant egg production. Microzooplankton, such as ciliates, show higher biomass-specific grazing rates on algae than do copepods and other mesozooplankton. Instead, copepods often selectively feed on the microzooplankton. Thus, apparent negative rates would be expected when the release of microzooplankton grazing pressure outweighs the copepod grazing rates on the same food items in the incubation bottle. We show that this potentially large bias increases with microzooplankton community grazing pressure in the control. A simplified general method to correct for this bias is presented and compared with the original method (Nejstgaard et al. 1997, Mar Ecol Prog Ser 147:197–217). Although complexity and the need for taxonomic accuracy are reduced in the general method, the results are not significantly different between the 2 methods. Both methods also show a good fit with ingestion rates estimated from faecal pellet production. We suggest that the general method be combined with automated sample treatment in future studies. In addition, we argue that carefully estimated faecal volume production provides a simple and quick overall feeding estimate with important advantages over the common gut pigment technique, and it may be used as an independent method in bottle incubation experiments.

KEY WORDS: *Calanus finmarchicus* · *Calanus helgolandicus* · Microzooplankton · Grazing methods · Clearance · Ingestion · Faecal pellet production · Natural plankton · Bottle effect



## INTRODUCTION

A number of methods have been developed to assess mesozooplankton grazing rates, ranging from fast and coarse bulk methods based on pigment analysis to detailed time-consuming microscopy studies of either gut content or the food medium (see Båmstedt et al. 2000).

Copepods are often omnivorous, and different species ingest food items ranging from small-sized algae of a few µm up to fish larva and mesozooplankton of a few mm (Turner et al. 1984, 1985, Landry & Fagerness 1988, Gifford 1991, Hansen et al. 1994, Nejstgaard et al. 1995). Many copepods feed selectively on the microzooplankton when available, and algal ingestion alone is often insufficient to meet metabolic costs in the sea (e.g. Stoecker & Capuzzo 1990, Kleppel 1993, Dam et al. 1994, Ohman & Runge 1994, Atkinson 1996,

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Peterson & Dam 1996, Verity & Paffenhöfer 1996, Nejstgaard et al. 1997, Roman et al. 2000, and this study). Quantification of copepod feeding rates in the field by methods based on gut pigment (Mackas & Bohrer 1976), $^{14}$C-labeled algae, or plant pigment analysis by HPLC (e.g. Kleppel & Pieper 1984, Meyer-Harms et al. 1999) is limited to herbivory, and may substantially underestimate total zooplankton ingestion rates and bias prey selectivity estimates. Such data must be treated with caution. The potential problem with food web interactions in incubation experiments as discussed here may further limit the value of phytoplankton-based methods, if not corrected.

The dual labelling technique (Roman & Rublee 1981, Roman & Gauzens 1997) yields data on zooplankton omnivory *in situ*. However, this method does not give detailed data on feeding selectivity and has a number of potential problems, including the fact that grazing on algae can only be measured during daytime (Roman & Rublee 1981). Many zooplankton show maximum feeding activity during night, even at high latitudes and at low food concentrations (Atkinson et al. 1996). Thus, this technique may result in significant underestimation of grazing rates, especially on larger algae and microzooplankton (cf. Roman & Rublee 1981). Therefore methods to quantify the overall daily grazing activity in natural plankton should ideally include the full 24 h period.

Microscopy of gut content and faecal pellets gives some information on omnivory. But important soft-bodied heterotrophic prey such as aloricate ciliates have not been possible to quantify by gut content analysis, although such attempts have been made (Ohman 1992, Juhl et al. 1996, M. D. Ohman pers. comm.).

Analysis of particle removal in bottle incubations is presently the only available method that allows direct feeding rate quantification of non-pigmented taxa (Båmstedt et al. 2000). Such experiments analysed according to the equations of Frost (1972), Omori & Ikeda (1984), or modifications thereof (Marin et al. 1986), are widely used for quantification of natural diets in copepods and other mesozooplankton (e.g. Gifford 1993).

A problem with bottle incubations is that prey suspensions containing several trophic levels, such as natural plankton, often yield low or in some cases even statistically significant negative mesozooplankton grazing estimates on some prey algae types and chlorophyll (chl) *a*. Although significant negative grazing rates per se are impossible, such rates are often ignored or assumed to simply reflect low feeding rates on those prey types if they cannot be explained by nutrient effects (Lehman 1980). But it is well known that microzooplankton, such as ciliates, show higher biomass-specific grazing rates on algae than do copepods and other mesozooplankton (Hansen et al. 1997). Copepods instead often feed selectively on the microzooplankton (e.g. Hansen et al. 1993, Kleppel 1993, Verity & Paffenhöfer 1996, Nejstgaard et al. 1997, Calbet & Landry 1999). Thus, negative rates would be expected when the release of microzooplankton grazing pressure outweighs the copepod grazing rates on the smaller food items in the incubation bottle. This has significant implications not only as a mechanism during development of blooms in the field, as pointed out by Hansen et al. (1993), but also for the validity of mesozooplankton grazing rates obtained from traditional bottle incubation studies (cf. Calbet & Landry 1999 and others).

We show that this artefact may have a substantial impact on grazing rate estimates in natural plankton, and that this artefact increases when microzooplankton community grazing pressure is high in the natural food suspension. A first method to correct for this bias was presented in Nejstgaard et al. (1997). Based on a larger data set, we present a simplified version of the method, compare it with the original method and with the classical faecal pellet production method, and then discuss the results of the methods further.

## MATERIALS AND METHODS

**Experimental materials.** The experiments were performed at the Marine Biological Field Station of the University of Bergen, Norway, on 7 occasions between 9 September 1996 and 2 May 1998 (Table 1). Natural plankton suspensions for the experiments were retrieved from 27 $m^3$ (2 m diameter, 9.25 m deep) transparent (90% penetration, PAR) polyethylene *in situ* sea water enclosures (mesocosms). In 1996 and 1997 the mesocosms were filled with natural water *in situ*, and stratification was established in the upper 4 to 5 m by adding ca 0.6 $m^3$ of fresh water and mixing the top layer with an air lift. After stratification was established, nutrients were added daily to the mesocosms, corresponding to final concentrations of 1.5 µmol $l^{-1}$ nitrate and 0.1 µmol $l^{-1}$ phosphate. Silicate was also added to the upper layer of half of the mesocosms to a final concentration of 1.5 µmol $l^{-1}$, in order to stimulate diatom blooms. The other mesocosms were expected to yield flagellate blooms. In 1998 the mesocosms were set up as described above, except that nutrients were added corresponding to final concentrations of 5 µmol $l^{-1}$ nitrate, 0.3 µmol $l^{-1}$ phosphate and 5 µmol $l^{-1}$ silicate on 2 occasions: at the start of the mesocosms (18 April) and on 30 April, which was a day before the grazing experiment was performed. Only water from the top mixed layers was used in grazing experiments. The

Table 1. Grazing experiments. Experiment abbreviation (Expt), incubation start date (Date), mesocosm nutrient addition type (Nutrient type: nitrate = N, phosphate = P, silicate = S). Initial nutrient concentrations measured in incubation water (after addition of nutrients in the 1997 a,b experiments). Terminal nutrient concentrations estimated from plankton accumulation in control bottles and compared to concentrations measured in mesocosms after termination of incubations (see text for explanation). Initial nutrient concentrations in Expts 97a,b measured after nutrients added, corresponding to 1.5 µmol $l^{-1}$ nitrate, 0.1 µmol $l^{-1}$ phosphate, and 1.5 µmol $l^{-1}$silicate (values in parentheses from untreated mesocosm water). nd = not detectable <0.02 µmol $l^{-1}$

| Expt | Date (d.mo.yr) | Nutrient type | Species | *Calanus* (no. $l^{-1}$) | (µg C $l^{-1}$) | Initial nutrient concentrations Nitrate (µmol $l^{-1}$) | Phosphate (µmol $l^{-1}$) | Silicate (µmol $l^{-1}$) | Terminal nutrients estimate from particle accumulation in control Nitrate (µmol $l^{-1}$) | Phosphate (µmol $l^{-1}$) | Silicate (µmol $l^{-1}$) | Terminal nutrients in mesocosms Nitrate (µmol $l^{-1}$) | Phosphate (µmol $l^{-1}$) | Silicate (µmol $l^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 96a | 09.09.96 | NP | *helgolandicus* | 4.8 | 330 | 11.3 | 1.0 | nd | 6.4 | 0.5 | nd | 12.6 | 1.2 | nd |
| 96b | 11.09.96 | NPS | *helgolandicus* | 5.2 | 373 | 11.1 | 0.4 | 1.4 | 11.1 | 0.4 | 1.4 | 7.2 | 0.3 | 1.3 |
| 96c | 17.09.96 | NP | *helgolandicus* | 4.8 | 366 | 15.5 | 0.9 | 0.2 | 11.7 | 0.7 | 0.2 | 14.6 | 0.8 | 0.2 |
| 96d | 19.09.96 | NPS | *helgolandicus* | 4.8 | 370 | 2.1 | 0.2 | 1.6 | 2.1 | 0.2 | 1.6 | 1.2 | 0.2 | 1.4 |
| 97a | 13.04.97 | NPS | *finmarchicus* | 5.7 | 730 | 3.3 (0.8) | 0.3 (0.2) | 2.3 (1.0) | 3.3 | 0.3 | 2.3 | 0.8 | 0.1 | 1.3 |
| 97b | 19.04.97 | NPS | *finmarchicus* | 5.7 | 767 | 1.5 (0.1) | 0.2 (0.1) | 3.0 (1.5) | –0.1 | 0.0 | –0.2 | 0.1 | 0.1 | 1.6 |
| 97c | 24.04.97 | NP | *finmarchicus* | 5.7 | 834 | 5.7 | 0.3 | 0.5 | 5.7 | 0.3 | 0.5 | 5.9 | 0.4 | 1.4 |
| 98 | 01.05.98 | NP | *finmarchicus* | 3.5 | 455 | 4.2 | 0.3 | 1.4 | 4.2 | 0.3 | 1.4 | 3.8 | 0.3 | 1.4 |

salinity varied between ca 30 PSU in 1996, and 32 in 1997 and 1998. The temperature varied from 6°C in 1997, 7.5°C in 1998, to 9–13°C in 1996. For further description of the mesocosm experimental design see Svensen et al. (2001); a general description of the mesocosm facility is available at: www.ifm.uib.no/LSF/inst2.html.

Females and stage V copepodites of *Calanus finmarchicus* and *C. helgolandicus* for the grazing experiments were collected from the surface water (0–20 m) of the nearby Raunefjorden, using a 1.2 m diameter 700 µm mesh net, with a 14 l non-filtering cod end. Actively swimming undamaged copepods were sorted out using wide mouth pipettes at *in situ* temperature shortly after collection.

**Grazing experiments and sampling.** Dates, abbreviated names, mesocosm water nutrient treatments and copepod species used in the experiments are summarized in Table 1. Both dilution experiments (Landry 1993) and bottle incubations with *Calanus* spp. as grazers were run simultaneously on each occasion. Water for the dilution experiments was collected in 25 l polycarbonate bottles and filtered through 0.2 µm sterile inline filters (500 $cm^2$, Supor™ DCF, Gelman) using tissue culture hoses and low pressure (ca 0.5 m water column). Filtration was done at *in situ* temperature immediately before set-up of the experiments. Target concentrations for the dilution series were triplicates of 10, 30 and 100% undiluted sea water. Undiluted sea water was screened through a submerged 200 µm net to avoid mesozooplankton. For determination of copepod feeding rates 8 to 14 copepods were added to each of a further 3 to 5 bottles (2.3 l polycarbonate) with 100% undiluted sea water. The 100% bottles from the dilution series also served as controls for the copepod bottles. Absolute dilutions were checked by the chl *a* concentrations at the start in all dilutions, and dilutions were corrected for change in total microzooplankton biomass during incubations by counts at the start in 100%-bottles and at the end in all bottles (cf. Landry 1993).

The amount of copepods added to the bottles may seem relatively high (Table 1). Note, however, that the total potential prey concentrations were also high (see Tables 4 & 5), and comparable to blooms in eutrophic fjords (see 'Results and discussion'). The average decrease in total prey C concentrations and chl *a* were only 11 and 6% respectively (see Table 6). This is much less than the ca 30 to 40% decrease needed to yield significant differences between cell counts from grazer and control bottles in traditionally performed incubation experiments (Gifford 1993, Båmstedt et al. 2000). Only the most selected prey items showed a decrease beyond 50% (see 'Results and discussion').

In order to facilitate comparison of algal growth in the bottles and in the fertilized mesocosms, excess nutrient addition to the incubation bottles was avoided. Extra nutrients were added to the bottles only when they were anticipated to become limiting during the incubations (cf. Landry 1993, Båmstedt et al. 2000), as in the 97a and b experiments (Table 1, also see 'Results and discussion').

Copepods were acclimated in the experimental water for 24 h prior to the incubations. All bottles were incubated *in situ*, outside the mesocosms overnight (22 to 25 h), hanging from a floating ring at 1.5 m depth in order to yield light conditions comparable to average conditions for the upper layer of the mesocosms, and to create gentle agitation, preventing sedimentation of plankton and yet minimising the disruption of faecal pellets. At the termination of the experiment the bottles were inverted and gently sampled by siphoning off samples for chl *a*, epifluorescence (not in 1996) and protist analysis. In 1996 copepods were thereafter screened on 200 µm submerged net and were immediately counted and examined for stage and viability under a dissecting microscope, before being fixed in 4% hexamine buffered formaldehyde. The remaining water was screened for particles >40 µm, fixed in formaldehyde. Both the >200 µm and >40 µm fractions were later enumerated for faecal pellets and less abundant larger-sized zooplankton. *Ceratium* spp. and other large dinoflagellates were checked for cell content (cf. Elbrächter 1973). In 1997 and 1998 copepods and larger items were collected on a 40 µm screen; copepods were checked for viability, and the samples were fixed in formaldehyde for later analysis.

**Analysis of the samples.** Nitrate, phosphate and silicate concentrations were determined on fresh samples using a Skalar SAN$^{plus}$ segmented flow analyzer. Replicate chl *a* samples were filtered onto 25 mm 0.45 µm cellulose nitrate filters. Samples were frozen (–22°C) until extraction overnight in 90% acetone and measured on a Turner Designs Model 10-AU Fluorometer according to the method of Welschmeyer (1994). Epifluorescence samples were filtered onto black 0.4 µm nucleopore filters, stained with primulin and fixed with 3.6% glutaraldehyde and 10% glycerine for determination of trophic status of the protists, according to our own modification of the method of Grebecki (1962), Hobbie et al. (1977) and Caron (1983). Phytoplankton samples (in 1996) and samples for all protists (in 1997/98) were preserved with a glutardialdehyde-lugol mix (35‰ final v/v) (Rousseau et al. 1990), settled in 2, 10 or 50 ml sediment chambers, and counted and measured at 200×, 400× and/or 600× magnification. In 1996 microzooplankton samples were fixed in acid Lugol's solution (final concentration 1%), settled in 50 ml sediment chambers, counted at 200× and measured at 400× magnification. Epifluorescence samples were analysed on Olympus or Zeiss Axioplan microscopes. Phytoplankton and microzooplankton were counted and measured on inverted Wild M40 or LUMAM-P8 microscopes. The 40 µm net samples, for faecal pellet and larger zooplankton, were examined under a Wild M10 dissecting microscope.

**Cell to carbon conversion.** Cell volume was calculated using simple geometrical formulae (Edler 1979, Ohman & Snyder 1991). Cell volume was converted into C according to the equations of Menden-Deuer & Lessard (2000): $\log C = (\log a) + (b \log V)$, where C is mass of carbon (pg) per cell, V is cell volume (µm$^3$), and $\log a = -0.541$ and $b = 0.811$ for diatoms, and $\log a = -0.665$ and $b = 0.939$ for all other protist plankton except aloricate ciliates. The volume to C conversion factor for aloricate ciliates strongly depends on fixative type and concentration (Stoecker et al. 1994). There was, however, little difference between aloricate species fixed in 1 or 2% acid Lugol's solution (Ohman & Snyder 1991). We therefore used the factor of 0.19 pg C µm$^{-3}$ experimentally derived for aloricate ciliates fixed in 2% acid Lugol's solution (Putt & Stoecker 1989). Metazooplankton abundance was converted into carbon by species and stage specific values from the literature (Båmstedt 1986, Blom et al. 1989, Båmstedt et al. 1990, Widdows 1991, Karlson & Båmstedt 1994), using the conversion factor of 0.547 C:DW for copepods from high latitudes (Båmstedt 1986). When species specific values were not found we used the general volume to C conversion factor of 0.126 g C cm$^{-3}$ from Hansen et al. (1997).

**Assigning trophic status to protists.** All algae were assumed to be mainly autotrophic, except when they did not contain chloroplasts in the epifluorescence samples. The more rare algal species not found in the epifluorescence samples in 1997 and 1998, and all algae in 1996 were assigned to trophic status according to the literature (Lessard & Swift 1986, Gaines & Elbrächter 1987, Hansen 1991, Bralewska & Witek 1995, Taylor et al. 1995, Tomas 1997, Tong et al. 1998). Except for the possible obligate autotrophic *Mesodinium rubrum* (Lohmann 1908) Jankowski 1976 (= *Myrionecta rubra*) (Gustafson et al. 2000), all ciliates were assumed to be mainly heterotrophic. Although many ciliates retain chloroplasts (e.g. Laval-Peuto & Rassoulzadegan 1988, Stoecker et al. 1989), they are here considered to be dependent on algal food such as the obligate mixotroph *Laboea strobila* (Stoecker et al. 1988), and are thus defined as heterotrophic in the following calculations.

**Calculations of growth and grazing rates.** ***Microzooplankton:*** Target dilutions were adjusted for changes in total microzooplankton body carbon density, and algae growth rates, microzooplankton grazing coefficients and daily grazing impact were calculated

according to Landry (1993). Due to the limited number of samples per experiment (n = 9) the microzooplankton grazing data were not tested for non-linearity, and may therefore be considered as minimum estimates (cf. Gallegos 1989). In order to evaluate whether the microzooplankton feeding rates obtained for each feeding guild (Table 2) seemed reasonable, we calculated microzooplankton body volume specific clearance ($C_{bv}$) and ingestion ($I_{bv}$) rates based on the microzooplankton grazing coefficient (Table 3) and average microzooplankton volume specific abundance as described in Hansen et al. (1997).

***Uncorrected copepod grazing rates:*** Copepod clearance and ingestion rates were first calculated according to the equation by Frost (1972), which is also used generally for natural plankton (e.g. Gifford 1993). Because this method does not account for the effect of feeding interactions in natural plankton discussed here, this method will hereafter be referred to as the 'uncorrected' method.

***The Guild method:*** We argue that when copepod predation significantly reduces microzooplankton grazing pressure in the copepod bottles, this leads to an underestimation of uncorrected copepod grazing rates on prey also grazed by the microzooplankton (see 'Results and discussion'). In order to correct for this bias we established a microzooplankton feeding guild table based on available literature (Table 2), and calculated corrected copepod feeding rates by the original method described in Nejstgaard et al. (1997). This method will be referred to as the 'Guild' method hereafter. The Guild method assumes that: (1) each microzooplankter only ingests prey from within a defined size spectrum (i.e. feeding guild, Table 2); (2) microzooplankton ingest all prey types within their feeding guild in direct proportion to the respective prey type

Table 2. Microzooplankton feeding guilds. Prey types assigned to each guild are within the size range expected to give at least 10% of maximum feeding rates. Size ranges: µm ESD (overlap in some overall size distributions are due to slight adjustments of different prey size groups in different experiments)

| Predator species | Prey type | | | | | | | | | | | | | Reference |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Chl *a* > 0.45 | Flagellates 2–5 | Flagellates 4–10 | Diatoms 6–15 | Flagellates 10–23 | Ciliates 10–30 | Dinoflagellates 10–30 | *Ebria tripartita* 15–30 | Diatoms > 16 | Flagellates > 31 | Ciliates > 31 | Dinoflagellates > 31 | *Cyclotrichium* sp. > 44 | |
| Copepodites[a] | X | X[b] | X | X | X | X | X | X | X | X | X | X | X | 1, 7, 15 |
| *Cyclotrichium* sp. > 44[c] | X | X | X | X | X | X | X | X | X | X | | | | 2, 3 |
| Nauplii[d] | X | X[b] | X | X | X | X | X | X | X | X | X | | | 7, 18 |
| Heterotrophic dinoflagellates > 31 | X | X | X | X | X | X | X | X | X | X | | | | 6, 7–11, 16, 19, 21 |
| Heterotrophic dinoflagellates 12–30 | X | X | X | X | X | X | X | X | X | | | | | 6, 7–11, 16, 19, 21 |
| Rotifers 50–200 | X | X | X | X | X | X | X | | | | | | | 6, 7 |
| *Ebria tripartita* 15–30[e] | X | | | X | | | | | | | | | | 4, 16, 22 |
| Ciliates > 31[f] | X | X | X | X | X | | | | | | | | | 2, 6–8, 12–14, 17, 20 |
| Ciliates 10–30[g] | X | X | X | | | | | | | | | | | 2, 6–8, 13, 14, 17, 20 |
| Heterotrophic flagellates[h] | X | X | | | | | | | | | | | | 7, 21 |

[a]Small calanoid copepodids and *Oithona* spp. [b]As the biomass of these prey were dominated by the 4–5 µm fraction they were expected to be successfully ingested also by copepods and nauplii. [c]A predatory ciliate. [d]Mostly copepod nauplii. [e]Heterotrophic silicoflagellate noted to feed on diatoms (*Skeletonema costatum*, Drebes 1974) in this study only diatoms were found in these cells (Naustvoll pers. obs.). [f]Non predatory species; mostly species of *Strombidium*, *Lohmaniella* and tintinnids. [g]Non-predatory species; mostly species of *Strombilidium*, *Balanion*, *Strombidium*, and *Urotrichia*. Although larger *Balanion* (32–34 µm ESD) has been shown to feed selectively on dinoflagellates (Stoecker et al. 1986) these found here were assumed to be too small (ca 15 µm ESD) to feed on the majority of the dinoflagellates. [h]Aplastidic flagellates (excluding E. tripartita), mainly 4–7 µm, were not included in the calculations due to uncertain microscopical counts, low biomass and assumed low grazing pressure from *Calanus*

1 (Atkinson 1994), 2 (Dolan 1991), 3 (Dolan & Coats 1991), 4 (Drebes 1974), 5 (Gaines & Elbrächter 1987), 6 (Godhantaraman & Krishnamurthy 1997), 7 (Hansen et al. 1994), 8 (Hansen 1991), 9 (Hansen & Calado 1999), 10 (Jacobson & Anderson 1986), 11 (Jeong 1994), 12 (Jonsson 1986), 13 (Kivi & Setälä 1995), 14 (Montagnes 1996), 15 (Nakamura & Turner 1997), 16 (Naustvoll pers. obs.), 17 (Sime-Ngando et al. 1995), 18 (Stoecker & Egloff 1987), 19 (Strom & Buskey 1993), 20 (Tamigneaux et al. 1997), 21 (Tomas 1997), 22 (http://www.marbot.gu.se/SSS/Ebria_tripartita.htm)

Table 3. Microzooplankton grazing experiments. Specific algal growth rate ($\mu$), microzooplankton grazing coefficient ($g$), grazing impact (percentage of average standing stock removed $d^{-1}$), body volume specific clearance ($C_{bv}$), and body volume specific ingestion ($I_{bv}$). $C_{bv}$ and $I_{bv}$ are based on total prey and grazer body volumes for the respective feeding guild (Table 2). I was not calculated for chl *a* due to uncertain conversion factor to algae volume. ±SE for the mean. $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ for $\mu$ or $g$ = 0, n = 9. Prey types are given with equivalent spherical diameter size ranges (µm). Dinoflagellates are only plastidic species. Only significant $g$-values ($p < 0.05$) were used for corrections of copepod grazing rates

| Prey type | Expt | $r^2$ | $\mu$ ($d^{-1}$) | $g$ ($d^{-1}$) | Grazing impact (% SS $d^{-1}$) | $C_{bv}$ ($10^2$ $d^{-1}$) | $I_{bv}$ ($d^{-1}$) |
|---|---|---|---|---|---|---|---|
| Chl *a* (>0.45) | 96a | 0.73 | 0.10 ± 0.04 | 0.34 ± 0.07** | 29 | 8 | |
| | 96b | 0.80 | 0.15 ± 0.05* | 0.47 ± 0.08*** | 37 | 15 | |
| | 96c | 0.75 | 0.58 ± 0.07*** | 0.55 ± 0.11** | 42 | 4 | |
| | 96d | 0.90 | 0.79 ± 0.07*** | 0.98 ± 0.12*** | 63 | 23 | |
| | 97a | 0.82 | 0.48 ± 0.05*** | 0.49 ± 0.08*** | 39 | 5 | |
| | 97b | 0.96 | 0.75 ± 0.05*** | 1.23 ± 0.09*** | 71 | 20 | |
| | 97c | 0.80 | 0.32 ± 0.06** | 0.67 ± 0.12*** | 49 | 14 | |
| | 98 | 0.59 | 0.66 ± 0.18** | 0.89 ± 0.28* | 59 | 7 | |
| Diatoms (8–15) | 96a | 0.71 | 1.10 ± 0.12*** | 0.84 ± 0.22** | 57 | 27 | 1 |
| | 96b | 0.99 | 1.23 ± 0.03*** | 1.12 ± 0.06*** | 67 | 49 | 3 |
| | 96c | 0.01 | −0.58 ± 0.06*** | −0.03 ± 0.12 | | | |
| | 96d | 0.94 | 0.57 ± 0.03*** | 0.47 ± 0.05*** | 37 | 11 | 8 |
| Diatoms (8–23) | 97c | 0.30 | 0.26 ± 0.09* | 0.27 ± 0.16 | 24 | 6 | 0.0 |
| Diatoms (8–29) | 97a | 0.44 | 0.50 ± 0.13** | 0.54 ± 0.23* | 42 | 6 | 1.5 |
| | 97b | 0.24 | 0.08 ± 0.02** | 0.05 ± 0.03 | 5 | 1 | 0.2 |
| | 98 | 0.28 | 1.70 ± 0.29*** | 0.74 ± 0.45 | 52 | 15 | 0.0 |
| Flagellates (4–9) | 96a | 0.51 | 0.10 ± 0.04 | 0.20 ± 0.08* | 18 | 5 | 1.4 |
| | 96b | 0.98 | 0.86 ± 0.03*** | 0.84 ± 0.05*** | 57 | 29 | 5 |
| | 96c | 0.72 | 0.43 ± 0.04*** | 0.30 ± 0.08** | 26 | 3 | 0.3 |
| | 96d | 0.45 | 0.05 ± 0.08 | 0.34 ± 0.15* | 29 | 13 | 3.8 |
| Flagellates (5–10) | 97a | 0.12 | 1.18 ± 0.10*** | 0.16 ± 0.16 | 15 | 2 | 0.0 |
| | 97b | 0.21 | 0.32 ± 0.03*** | 0.08 ± 0.06 | 8 | 1 | 0.0 |
| | 97c | 0.00 | 0.19 ± 0.13 | 0.04 ± 0.24 | 4 | 1 | 0.0 |
| | 98 | 0.23 | 0.72 ± 0.11*** | 0.24 ± 0.15 | 21 | 5 | 0.3 |
| Flagellates (10–18) | 96a | 0.01 | 0.28 ± 0.17 | 0.06 ± 0.31 | 6 | 3 | 0.6 |
| | 96b | 0.95 | 1.30 ± 0.07*** | 1.34 ± 0.13*** | 74 | 64 | 8 |
| | 96c | 0.31 | 0.68 ± 0.10*** | 0.31 ± 0.19 | 26 | 3 | 0.3 |
| | 96d | 0.00 | 0.00 ± 0.07 | 0.02 ± 0.14 | 2 | 1 | 0.1 |
| Flagellates (14–21) | 98 | 0.01 | 1.35 ± 0.16*** | 0.07 ± 0.24 | 7 | 1 | 0.1 |
| Dinoflagellates (12–20) | 96a | 0.03 | 0.36 ± 0.09** | 0.08 ± 0.17 | 7 | 3 | 0.1 |
| | 96b | 0.91 | 1.44 ± 0.09*** | 1.24 ± 0.16*** | 71 | 59 | 0.3 |
| | 96c | 0.81 | 0.34 ± 0.07** | 0.64 ± 0.13** | 47 | 6 | 0.4 |
| | 96d | 0.50 | 0.75 ± 0.16** | 0.72 ± 0.29* | 51 | 25 | 0.1 |
| Dinoflagellates (10–29) | 97c | 0.34 | 0.23 ± 0.07** | 0.22 ± 0.12 | 20 | 5 | 0.1 |
| | 98 | 0.67 | 0.66 ± 0.24* | 1.37 ± 0.36** | 75 | 28 | 0.1 |
| Dinoflagellates (22–42) | 97a | 0.01 | 0.10 ± 0.26 | 0.11 ± 0.45 | 11 | 4 | 0.6 |
| | 97b | 0.08 | 0.48 ± 0.06*** | 0.08 ± 0.10 | 7 | 3 | 0.2 |
| *Myrinecta rubra* (21–46) | 97a | 0.06 | 1.04 ± 0.15*** | 0.16 ± 0.25 | 15 | 3 | 0.0 |
| | 97b | 0.00 | 0.85 ± 0.25* | 0.04 ± 0.36 | 4 | 1 | 0.0 |
| | 98 | 0.51 | 0.35 ± 0.07** | 0.26 ± 0.11* | 23 | 41 | 0.0 |

concentration; and (3) microzooplankton grazing rates are proportional to body carbon for all microzooplankton species, for each prey type in each experiment. For equations and further description of the Guild method see Nejstgaard et al. (1997).

***The general method:*** The Guild method is rather complex (see 'Results and discussion'). Therefore we present and evaluate a simplified version of the method that does not include the use of guild tables. Instead it assumes that the underestimation of microzooplankton grazing for each prey type is proportional to the measured microzooplankton community grazing rate on this prey and the total loss of microzooplankton community biomass in the copepod bottle.

In this method the copepod grazing rates were corrected for each prey type ($p$), according to Eq. (1):

$$g_{corr,p} = g_{cop,p} + k_p \quad (1)$$

where $g_{corr,p}$ is the corrected copepod grazing coefficient ($d^{-1}$) for prey type $p$, $g_{cop,p}$ is the uncorrected copepod grazing coefficient for prey type $p$ according to Frost (1972), and $k_p$ is the correction for loss of microzooplankton grazing on prey type $p$ in the copepod bottle. $k_p$ is calculated according to Eqs (2) to (4):

$$k_p = g_{mic,p}\left(\frac{\bar{c} - \bar{c}^*}{\bar{c}}\right) \quad (2)$$

$$\bar{c} = (c_t - c_0)\ \ln(c_t/c_0)^{-1} \quad (3)$$

$$\bar{c}^* = (c_t^* - c_0)\ \ln(c_t^*/c_0)^{-1} \quad (4)$$

where $g_{mic,p}$ is the microzooplankton grazing coefficient for prey type $p$ ($d^{-1}$, obtained from simultaneously performed dilution experiments), while $\bar{c}$ and $\bar{c}^*$ is the average concentration of all microzooplankton (µg C $l^{-1}$) in the control and copepod bottle ($^*$) respectively, $c_0$ is the concentrations of all microzooplankton at the start of the incubation, while $c_t$ and $c_t^*$ are the concentrations of all microzooplankton at the end of the incubation in the control (average for all controls) and the copepod bottle ($^*$), respectively.

Because this correction method is based on the general loss of microzooplankton biomass in the grazing bottles, rather than specific feeding guilds, and because it makes a more general use of the method possible (see 'Discussion'), we will refer to this method as the 'general' correction method hereafter.

**Other calculations and statistical analysis.** Copepod prey preference was calculated as Manly's (1974) index for variable prey populations, normalized for copepod concentration as described in Nejstgaard et al. (1997). StatView5 (SAS Institute Inc., Cary, NC) was used for basic statistics, ANOVA and post hoc tests. Multiple comparisons among groups were tested using either Scheffé's multiple contrasts (Zar 1996, p. 222–225), or if n ≥ 6, the post hoc test described by Games & Howell (1976). Regressions were compared for differences using the Chow-test (Koutsoyiannis 1977).

## RESULTS AND DISCUSSION

### Microzooplankton grazing

The microzooplankton community showed a significant grazing impact on the phytoplankton, with grazing rates often surpassing the estimated specific phytoplankton growth rates (Table 3). The microzooplankton consumed 29 to 71% of the chl *a* standing stocks (SS) $d^{-1}$ (Table 3). Thus the phytoplankton appear to be controlled by microzooplankton grazing, as is often the case in the field, at least during the warmer seasons (Nielsen & Kiørboe 1994, Banse 1995, Putland 2000).

The cell counts indicated that the diatoms and some of the flagellates were exposed to the highest microzooplankton grazing pressure, although not all of these rates were statistically significant (Table 3). We argue that the increased errors associated with fixation, microscopic analysis and limited numbers of some protists may explain the lack of statistical significance ($p > 0.05$) in some cases of high grazing rates based on cell counts, compared to the chl *a* analysis (cf. Båmstedt et al. 2000). The fact that all grazing rates >0.16 $d^{-1}$ based on cell counts had p values <0.15 (not shown), and that the estimated microzooplankton grazing impact were within the same range for cell counts and chl *a* analysis (Table 3), supports this argument.

The microzooplankton body volume specific ingestion rates ($I_{bv}$ = 0 to 8 $d^{-1}$, Table 3) were only occasionally high and were always within published maximum values (Hansen et al. 1997); microzooplankton body volume specific clearance rates ($C_{bv}$ = 0 to 64 × $10^2$ $d^{-1}$, Table 3) were at least 2 orders of magnitude below reported maximum values (Hansen et al. 1997). Thus, we conclude that microzooplankton grazing rates based on cell counts were most likely conservative estimates. Nevertheless, as a precautionary measure only the copepod grazing rates corresponding to a significant ($p < 0.05$) microzooplankton grazing value were corrected.

### Selective copepod predation on microzooplankton and decrease of prey in the bottles

The females and copepodites of *Calanus finmarchicus* and *C. helgolandicus* always showed the highest clearance rates (up to 0.5 l $cop^{-1}$ $d^{-1}$) and prey preference (Manly's alpha generally > 12.5) for rotifers and ciliates >30 µm, regardless of whether or not grazing rates were corrected (Tables 4 & 5). This is in accordance with similar previous experiments (Nejstgaard et al. 1994, 1997) and other literature for late stages of *Calanus* from coastal waters (Fessenden & Cowles 1994, Ohman & Runge 1994).

The sum of all ciliates and metazoa made up only 2 to 30% of the available prey C but contributed to 17–93% of uncorrected, and 13 to 59% of corrected C ingestion rates, despite the presence of alternative algal prey, sometimes in very high concentrations. The copepods also ingested significant amounts of heterotrophic flagellates. Thus the copepod predation led to a substantial decrease in the biomass of the preferred prey items: rotifers, other mesozooplankton and ciliates >30 µm (37 to 93%, average 63%, Table 6).

Table 4. Copepod grazing experiments, cell counts. Average prey concentration in control bottles (Concentration) and uncorrected values are calculated according to Frost (1972). Other values are calculated according to the methods described in the text. ±SD for the mean (n = 4 in 1996, 5 in 1997 and 3 in 1998). Prey preference (Manly's alpha) values are multiplied by 100 for convenience; preferred food types are indicated by values >12.5. $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ (2-tailed *t*-test) for clearance and ingestion = 0, and prey preference = 12.5, respectively. Negative values were converted into zero before calculations of prey preference, total ingestion and daily carbon ratios. Total daily carbon ratios are shown in parentheses

| Expt | | Uncorrected values | | | Guild corrected values | | | General corrected values | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Prey type | Concentration (µg C $l^{-1}$) | Clearance (ml $d^{-1}$ $cop^{-1}$) | Ingestion (µg C $cop^{-1}$ $d^{-1}$) | Prey preference index (×100) | Clearance (ml $d^{-1}$ $cop^{-1}$) | Ingestion (µg C $cop^{-1}$ $d^{-1}$) | Prey preference index (×100) | Clearance (ml $d^{-1}$ $cop^{-1}$) | Ingestion (µg C $cop^{-1}$ $d^{-1}$) | Prey preference index (×100) |
| **96a** | | | | | | | | | | |
| Diatoms (9–15) | 47 | 17 ± 17 | 0.7 ± 0.7 | 4 ± 3** | 66 ± 24* | 3.0 ± 1.0** | 16 ± 2 | 54 ± 25* | 2.4 ± 1.0* | 13 ± 0*** |
| Flagellates (4–9) | 335 | −16 ± 0*** | −5.7 ± 0.2*** | 0 ± 0*** | −7 ± 1*** | −2.6 ± 0.4*** | 0 ± 0*** | −7 ± 2** | −2.6 ± 0.8** | 0 ± 0*** |
| Flagellates (10–18) | 287 | 47 ± 16* | 11.8 ± 3.7** | 12 ± 2 | 47 ± 16** | 11.8 ± 3.7** | 11 ± 2 | 47 ± 16** | 11.8 ± 3.7** | 11 ± 2 |
| *Ebria* (23–25) | 7 | 99 ± 123 | 0.5 ± 0.5 | 16 ± 16 | 99 ± 123 | 0.5 ± 0.5 | 14 ± 14 | 99 ± 123 | 0.5 ± 0.5 | 15 ± 14 |
| Dinoflagellates (12–24)[a] | 33 | 53 ± 27* | 1.5 ± 0.6* | 13 ± 2 | 53 ± 27* | 1.5 ± 0.6* | 11 ± 2 | 53 ± 27* | 1.5 ± 0.6* | 11 ± 2 |
| Ciliates (10–24) | 14 | 8 ± 17 | 0.1 ± 0.2 | 2 ± 1*** | 8 ± 17 | 0.1 ± 0.2 | 1 ± 4 | 8 ± 17 | 0.1 ± 0.2 | 2 ± 3** |
| Ciliates (31–84)[b] | 29 | 136 ± 16*** | 2.7 ± 0.1*** | 29 ± 12 | 136 ± 16*** | 2.7 ± 0.1*** | 26 ± 11 | 136 ± 16*** | 2.7 ± 0.1*** | 27 ± 11 |
| Metazoa (65–255) | 2 | 111 ± 54* | 0.2 ± 0.1* | 24 ± 2** | 111 ± 54* | 0.2 ± 0.1* | 21 ± 2* | 111 ± 54* | 0.2 ± 0.1* | 22 ± 2** |
| Total | 754 | | 17.4 (26.2) | | | 19.6 (29.6) | | | 19.1 (28.8) | |
| **96b** | | | | | | | | | | |
| Diatoms (9–15) | 90 | −32 ± 10** | −3.2 ± 1.0** | 0 ± 0*** | 35 ± 17* | 3.5 ± 1.6* | 7 ± 3* | 46 ± 41 | 4.5 ± 3.9 | 7 ± 5 |
| Flagellates (4–9) | 374 | −58 ± 1*** | −25.5 ± 0.4*** | 0 ± 0*** | −4 ± 6 | −1.6 ± 2.8 | 0 ± 0*** | 0 ± 22 | 0.1 ± 9.8 | 0 ± 0*** |
| Flagellates (10–18) | 149 | −115 ± 2*** | −23.8 ± 0.7*** | 0 ± 0*** | −28 ± 14* | −5.9 ± 2.9* | 0 ± 0*** | −22 ± 39 | −4.6 ± 8.1 | 0 ± 0*** |
| *Ebria* (23–25) | 3 | 26 ± 27 | 0.1 ± 0.1 | 7 ± 6 | 115 ± 16*** | 0.3 ± 0.0*** | 20 ± 2** | 113 ± 8*** | 0.3 ± 0.0*** | 17 ± 3** |
| Dinoflagellates (12–24)[a] | 5 | −66 ± 10*** | −0.4 ± 0.1** | 0 ± 0*** | 15 ± 1*** | 0.1 ± 0.0*** | 3 ± 0*** | 21 ± 25 | 0.1 ± 0.2 | 3 ± 4* |
| Ciliates (10–24) | 10 | 65 ± 29* | 0.5 ± 0.2* | 17 ± 6 | 149 ± 18*** | 1.3 ± 0.1*** | 24 ± 4** | 155 ± 7*** | 1.3 ± 0.1*** | 23 ± 6** |
| Ciliates (31–84)[b] | 20 | 160 ± 29** | 2.1 ± 0.2*** | 33 ± 5** | 160 ± 29** | 2.1 ± 0.2*** | 20 ± 1*** | 160 ± 29** | 2.1 ± 0.2** | 18 ± 4* |
| Metazoa (65–255) | 6 | 224 ± 32*** | 0.9 ± 0.1*** | 44 ± 8** | 224 ± 32*** | 0.9 ± 0.1*** | 26 ± 2*** | 224 ± 32*** | 1.2 ± 0.5*** | 31 ± 6** |
| Total | 658 | | 3.8 (4.5) | | | 8.1 (11.4) | | | 9.6 (13.2) | |
| **96c** | | | | | | | | | | |
| Diatoms (9–15) | 46 | 33 ± 35 | 1.3 ± 1.4 | 7 ± 8 | 33 ± 35 | 1.3 ± 1.4 | 5 ± 5 | 33 ± 35 | 1.3 ± 1.4 | 5 ± 5 |
| Flagellates (4–9) | 165 | 7 ± 36 | 0.9 ± 5.8 | 1 ± 2*** | 32 ± 35 | 5.0 ± 5.3 | 5 ± 6 | 31 ± 35 | 4.8 ± 5.3 | 5 ± 6 |
| Flagellates (10–18) | 120 | 1 ± 18 | 0.0 ± 2.2 | 0 ± 0*** | 1 ± 18* | 0.0 ± 2.2* | 0 ± 3** | 1 ± 18 | 0.0 ± 2.2 | 0 ± 3** |
| *Ebria* (23–25) | 8 | −10 ± 11 | −0.1 ± 0.1 | 0 ± 0*** | 8 ± 14 | 0.1 ± 0.1 | 2 ± 0*** | 41 ± 13** | 0.3 ± 0.1** | 5 ± 2* |
| Dinoflagellates (12–24)[a] | 81 | 13 ± 0*** | 1.0 ± 0.0*** | 3 ± 1*** | 69 ± 3*** | 5.4 ± 0.2*** | 13 ± 3 | 63 ± 2*** | 5.0 ± 0.2*** | 12 ± 3 |
| Ciliates (10–24) | 7 | 30 ± 32 | 0.2 ± 0.2 | 7 ± 7 | 86 ± 29* | 0.6 ± 0.2* | 15 ± 4 | 81 ± 30* | 0.5 ± 0.2* | 14 ± 4 |
| Ciliates (31–84)[b] | 88 | 395 ± 4*** | 13.5 ± 0.4*** | 42 ± 2*** | 395 ± 4*** | 13.5 ± 0.4*** | 31 ± 3** | 395 ± 4*** | 13.5 ± 0.4*** | 31 ± 3** |
| Metazoa (65–255) | 15 | 258 ± 93* | 2.1 ± 0.4** | 39 ± 13* | 258 ± 93* | 2.1 ± 0.4** | 29 ± 11 | 258 ± 93* | 2.1 ± 0.4** | 28 ± 11 |
| Total | 530 | | 18.9 (25.4) | | | 27.9 (37.6) | | | 27.5 (37.0) | |
| **96d** | | | | | | | | | | |
| Diatoms (9–15) | 918 | 6 ± 27 | 4.2 ± 23.9 | 1 ± 0*** | 23 ± 53 | 19.1 ± 46.9 | 4 ± 1*** | 37 ± 33 | 32.1 ± 28.2 | 6 ± 4* |
| Flagellates (4–9) | 375 | −48 ± 24* | −20.7 ± 11.2* | 0 ± 0*** | −19 ± 31 | −8.2 ± 13.6 | 0 ± 0*** | −26 ± 29 | −11.2 ± 12.7 | 0 ± 0*** |
| Flagellates (10–18) | 187 | 31 ± 53 | 4.8 ± 8.5 | 6 ± 2** | 31 ± 53 | 4.8 ± 8.5 | 4 ± 1*** | 31 ± 53 | 4.8 ± 8.5 | 5 ± 2** |
| *Ebria* (23–25) | 16 | 11 ± 6* | 0.2 ± 0.1* | 2 ± 1*** | 79 ± 6*** | 1.2 ± 0.1*** | 14 ± 2 | 59 ± 16** | 0.9 ± 0.2** | 10 ± 1* |
| Dinoflagellates (12–24)[a] | 7 | −79 ± 14** | −0.7 ± 0.1** | 0 ± 0*** | −10 ± 12 | −0.1 ± 0.1 | 0 ± 0*** | −31 ± 4*** | −0.3 ± 0.0*** | 0 ± 0*** |
| Ciliates (10–24) | 2 | 77 ± 117 | 0.1 ± 0.2 | 13 ± 21 | 146 ± 119 | 0.2 ± 0.1 | 22 ± 15 | 125 ± 128 | 0.2 ± 0.2 | 18 ± 16 |
| Ciliates (31–84)[b] | 30 | 335 ± 115* | 4.9 ± 0.8** | 41 ± 2*** | 335 ± 115* | 4.9 ± 0.8** | 30 ± 0*** | 335 ± 115* | 4.9 ± 0.8** | 32 ± 3*** |
| Metazoa (65–255) | 5 | 235 ± 29*** | 0.8 ± 0.1*** | 36 ± 17 | 235 ± 29*** | 0.8 ± 0.1*** | 27 ± 11 | 235 ± 29*** | 0.8 ± 0.1*** | 29 ± 14 |
| Total | 1540 | | 14.9 (19.8) | | | 30.9 (40.9) | | | 43.4 (57.7) | |

[a]All species, both aplastidic and plastidic; [b]including predatory species

Table 4 (continued)

| Expt / Prey type | Concentration (µg C $l^{-1}$) | Uncorrected values: Clearance (ml $d^{-1}$ $cop^{-1}$) | Uncorrected values: Ingestion (µg C $cop^{-1}$ $d^{-1}$) | Uncorrected values: Prey preference index (×100) | Guild corrected values: Clearance (ml $d^{-1}$ $cop^{-1}$) | Guild corrected values: Ingestion (µg C $cop^{-1}$ $d^{-1}$) | Guild corrected values: Prey preference index (×100) | General corrected values: Clearance (ml $d^{-1}$ $cop^{-1}$) | General corrected values: Ingestion (µg C $cop^{-1}$ $d^{-1}$) | General corrected values: Prey preference index (×100) |
|---|---|---|---|---|---|---|---|---|---|---|
| **97a** | | | | | | | | | | |
| Diatoms (8–29) | 334 | 25 ± 8** | 7.8 ± 2.2** | 2 ± 1*** | 50 ± 8*** | 15.6 ± 2.2*** | 4 ± 1*** | 50 ± 8*** | 15.6 ± 2.2*** | 4 ± 1*** |
| Flagellates (5–10) | 10 | 20 ± 12* | 0.2 ± 0.1* | 4 ± 2*** | 20 ± 12** | 0.2 ± 0.1* | 3 ± 2** | 20 ± 12** | 0.2 ± 0.1* | 3 ± 2** |
| Dinoflagellates (12–29)[a] | 102 | 37 ± 20* | 3.3 ± 1.5** | 7 ± 3** | 37 ± 20** | 3.0 ± 1.7* | 6 ± 3* | 37 ± 20** | 3.0 ± 1.7* | 6 ± 3* |
| Dinoflagellates (30–60)[a] | 129 | 40 ± 20* | 4.5 ± 2.1** | 8 ± 4* | 40 ± 20** | 4.1 ± 1.8** | 7 ± 3* | 40 ± 20** | 4.1 ± 1.8** | 7 ± 3* |
| Ciliates (7–29) | 7 | 8 ± 24 | 0.0 ± 0.2 | 1 ± 1*** | 8 ± 24 | 0.1 ± 0.1* | 4 ± 3** | 8 ± 24 | 0.1 ± 0.1* | 4 ± 3** |
| Ciliates (30–75)[b] | 24 | 86 ± 65* | 1.4 ± 1.0* | 13 ± 9 | 86 ± 65* | 1.4 ± 1.0* | 12 ± 8 | 86 ± 65* | 1.4 ± 1.0* | 12 ± 8 |
| Rotifers (80–120) | 19 | 466 ± 140** | 2.7 ± 0.1*** | 34 ± 5*** | 466 ± 140** | 2.7 ± 0.1*** | 33 ± 4*** | 466 ± 140** | 2.7 ± 0.1*** | 33 ± 4*** |
| Other metazoa (80–180)[c] | 9 | 291 ± 76** | 1.2 ± 0.1*** | 31 ± 4*** | 291 ± 76*** | 1.2 ± 0.1*** | 30 ± 5** | 291 ± 76*** | 1.2 ± 0.1*** | 30 ± 5** |
| Total | 633 | | 21.2 (17.2) | | | 28.4 (22.) | | | 27.8 (22.4) | |
| **97b** | | | | | | | | | | |
| Diatoms (8–29) | 365 | 11 ± 4** | 3.8 ± 1.3** | 2 ± 1*** | 11 ± 4** | 3.8 ± 1.3** | 2 ± 1*** | 11 ± 4** | 3.8 ± 1.3** | 2 ± 1*** |
| Flagellates (5–10) | 26 | 16 ± 11* | 0.4 ± 0.3* | 5 ± 3** | 16 ± 11* | 0.4 ± 0.3* | 5 ± 3** | 16 ± 11* | 0.4 ± 0.3* | 5 ± 3** |
| Dinoflagellates (12–29)[a] | 93 | 32 ± 21* | 2.6 ± 1.6* | 9 ± 6 | 32 ± 21* | 2.6 ± 1.6* | 9 ± 6 | 32 ± 21* | 2.6 ± 1.6* | 9 ± 6 |
| Dinoflagellates (30–60)[a] | 7 | 28 ± 37 | 0.2 ± 0.2 | 5 ± 5* | 28 ± 37 | 0.2 ± 0.2 | 5 ± 5* | 28 ± 37 | 0.2 ± 0.2 | 5 ± 5* |
| Ciliates (7–29) | 6 | 51 ± 21* | 0.2 ± 0.1** | 13 ± 5 | 51 ± 21** | 0.2 ± 0.1* | 13 ± 5 | 51 ± 21** | 0.2 ± 0.1* | 13 ± 5 |
| Ciliates (30–75)[b] | 15 | 79 ± 12*** | 0.9 ± 0.1*** | 19 ± 1*** | 79 ± 12*** | 0.9 ± 0.1*** | 19 ± 1*** | 79 ± 12*** | 0.9 ± 0.1*** | 19 ± 1*** |
| Rotifers (80–120) | 30 | 204 ± 50*** | 3.5 ± 0.4*** | 37 ± 6** | 204 ± 50*** | 3.5 ± 0.4*** | 37 ± 6** | 204 ± 50*** | 3.5 ± 0.4*** | 37 ± 6** |
| Other metazoa (80–180)[c] | 3 | 63 ± 95 | 0.1 ± 0.2 | 11 ± 11 | 63 ± 95 | 0.1 ± 0.2 | 11 ± 11 | 63 ± 95 | 0.1 ± 0.2 | 11 ± 11 |
| Total | 544 | | 11.8 (8.4) | | | 11.8 (11.4) | | | 11.8 (8.4) | |
| **97c** | | | | | | | | | | |
| Diatoms (8–23) | 5 | 32 ± 28 | 0.2 ± 0.1 | 2 ± 1*** | 32 ± 28 | 0.2 ± 0.1 | 2 ± 1*** | 32 ± 28 | 0.2 ± 0.1 | 2 ± 1*** |
| Flagellates (5–10) | 57 | 27 ± 16* | 1.4 ± 0.8* | 5 ± 3** | 27 ± 16* | 1.4 ± 0.8** | 5 ± 3** | 27 ± 16* | 1.4 ± 0.8** | 5 ± 3** |
| Dinoflagellates (12–29)[a] | 49 | 23 ± 18** | 1.0 ± 0.8** | 9 ± 6 | 23 ± 18* | 1.0 ± 0.8* | 9 ± 6 | 23 ± 18* | 1.0 ± 0.8* | 9 ± 6 |
| Dinoflagellates (30–60)[a] | 3 | 32 ± 41 | 0.1 ± 0.1 | 5 ± 5* | 32 ± 41 | 0.1 ± 0.1 | 5 ± 5* | 32 ± 41 | 0.1 ± 0.1 | 5 ± 5* |
| Ciliates (7–29) | 4 | 61 ± 22** | 0.2 ± 0.1** | 13 ± 5 | 61 ± 22** | 0.2 ± 0.1** | 13 ± 5 | 61 ± 22** | 0.2 ± 0.1** | 13 ± 5 |
| Ciliates (30–75)[b] | 3 | 254 ± 127* | 0.3 ± 0.1*** | 19 ± 1*** | 254 ± 127* | 0.3 ± 0.1*** | 19 ± 1*** | 254 ± 127* | 0.3 ± 0.1*** | 19 ± 1*** |
| Rotifers (80–120) | 1 | 462 ± 126** | 0.2 ± 0.0*** | 37 ± 6** | 462 ± 126** | 0.2 ± 0.0*** | 37 ± 6** | 462 ± 126** | 0.2 ± 0.0*** | 37 ± 6** |
| Other metazoa (80–180)[c] | 2 | 219 ± 192 | 0.2 ± 0.1* | 11 ± 11 | 219 ± 192 | 0.2 ± 0.1* | 11 ± 11 | 219 ± 192 | 0.2 ± 0.1* | 11 ± 11 |
| Total | 124 | | 3.5 (2.3) | | | 3.5 (2.3) | | | 3.5 (2.3) | |
| **98** | | | | | | | | | | |
| Diatoms (5–21) | 1 | 247 ± 44* | 0.2 ± 0.0** | 14 ± 2 | 247 ± 44* | 0.2 ± 0.0*** | 12 ± 2 | 247 ± 44* | 0.2 ± 0.0** | 12 ± 2 |
| Flagellates (5–10) | 77 | 89 ± 6** | 5.8 ± 0.3*** | 6 ± 0*** | 89 ± 6** | 5.8 ± 0.3*** | 5 ± 0*** | 89 ± 6** | 5.8 ± 0.3*** | 6 ± 0*** |
| Flagellates (14–57) | 95 | 205 ± 38* | 15.7 ± 2.3** | 15 ± 2 | 205 ± 38* | 15.7 ± 2.3** | 12 ± 2 | 205 ± 38* | 15.7 ± 2.3* | 13 ± 2 |
| Dino (10–29) | 3 | −21 ± 27 | −0.1 ± 0.1 | 0 ± 0*** | 167 ± 40* | 0.5 ± 0.1** | 13 ± 2 | 68 ± 22* | 0.2 ± 0.1* | 5 ± 1* |
| Dino (32–62) | 8 | 86 ± 43 | 0.6 ± 0.3 | 6 ± 3 | 126 ± 64 | 0.9 ± 0.4** | 8 ± 4 | 174 ± 50** | 1.2 ± 0.3*** | 12 ± 3 |
| Ciliates (14–29) | 4 | 269 ± 93* | 0.8 ± 0.2* | 16 ± 3 | 305 ± 96* | 0.9 ± 0.2** | 15 ± 2 | 286 ± 92* | 0.8 ± 0.2** | 15 ± 2 |
| Ciliates (32–135) | 37 | 498 ± 103* | 7.7 ± 0.3*** | 19 ± 1** | 498 ± 103* | 7.7 ± 0.3*** | 16 ± 1* | 498 ± 103* | 7.7 ± 0.3*** | 17 ± 1* |
| Rotifers (119–277)[d] | 16 | 529 ± 19*** | 3.9 ± 0.1*** | 23 ± 2** | 529 ± 19*** | 3.9 ± 0.1*** | 19 ± 2* | 529 ± 19*** | 3.9 ± 0.1*** | 20 ± 2* |
| Total | 241 | | 34.6 (26.6) | | | 35.5 (27.2) | | | 35.5 (27.3) | |

[a]All species, both aplastidic and plastidic; [b]including predatory species; [c]mainly small copepodites and nauplii; [d]including a small fraction of other metazoa such as nauplii

Table 5. Copepod grazing experiments, chl a >0.45 µm. As all microzooplankton are assumed to feed on chl *a*, there is no difference between Guild and General correction methods. Otherwise as Table 4

| Expt | Chl *a* concentration (µg $l^{-1}$) | Uncorrected values | | Corrected values | |
|---|---|---|---|---|---|
| | | Clearance (ml $d^{-1}$ $cop^{-1}$) | Ingestion (µg chl *a* $cop^{-1}$ $d^{-1}$) | Clearance (ml $d^{-1}$ $cop^{-1}$) | Ingestion (µg chl *a* $cop^{-1}$ $d^{-1}$) |
| 96a | 4.2 | −2 ± 9 | −0.01 ± 0.04 | 13 ± 8* | 0.05 ± 0.03* |
| 96b | 5.1 | 8 ± 32 | 0.03 ± 0.15 | 42 ± 37 | 0.20 ± 0.16 |
| 96c | 6.1 | 4 ± 6 | 0.02 ± 0.03 | 47 ± 6*** | 0.28 ± 0.03*** |
| 96d | 10.4 | 22 ± 3*** | 0.22 ± 0.02*** | 89 ± 11*** | 0.87 ± 0.11*** |
| 97a | 18.5 | 19 ± 3*** | 0.33 ± 0.05*** | 40 ± 5*** | 0.68 ± 0.08*** |
| 97b | 13.9 | −40 ± 19** | −0.64 ± 0.32* | 15 ± 18 | 0.31 ± 0.17* |
| 97c | 6.2 | 35 ± 14** | 0.20 ± 0.08** | 48 ± 9*** | 0.28 ± 0.03*** |
| 98 | 0.8 | 105 ± 92 | 0.07 ± 0.06 | 163 ± 86 | 0.11 ± 0.05* |

Table 6. Percentage loss of prey C and chl *a* in copepod incubation bottles relative to blank bottles. Metazoa are totally dominated by rotifers in the 1997 and 1998 experiments

| Expt | Chl *a* | All prey C | All autotroph C | Metazoa or rotifer C | Ciliates >30 µm C | Other microzooplankton C |
|---|---|---|---|---|---|---|
| 96a | −1 | 10 | 7 | 44 | 51 | 17 |
| 96b | 3 | −41 | −46 | 68 | 57 | 9 |
| 96c | 2 | 22 | 3 | 72 | 86 | 5 |
| 96d | 10 | −1 | −3 | 68 | 80 | 8 |
| 97a | 11 | 21 | 17 | 93 | 38 | 12 |
| 97b | −28 | 13 | 9 | 69 | 37 | 15 |
| 97c | 18 | 17 | 12 | 91 | 71 | 13 |
| 98 | 28 | 49 | 31 | 84 | 81 | 34 |
| Average | 6 | 11 | 4 | 74 | 63 | 14 |

Note however that the chl *a* and the majority of the potential prey biomass C decreased on average by only 4 to 14%, compared to the control bottles (Table 6). This is much less than the 30 to 40% decrease in prey abundance needed to yield statistically significant cell counts compared to control bottles in traditional uncorrected incubation experiments (Gifford 1993, Båmstedt et al. 2000).

### Negative uncorrected copepod grazing rates

A number of statistically significant ($p < 0.05$) negative clearance and ingestion rates were obtained from the uncorrected cell counts and chl *a* data (Tables 4 & 5). As negative rates per se are not possible, this strongly suggests a methodological bias in the uncorrected data. This could either be due to release of nutrients by the copepods favouring algal growth under nutrient-stressed conditions (Lehman 1980, Roman & Rublee 1980), and/or to grazing interactions between the microzooplankton and the copepods releasing the overall grazing pressure on the phytoplankton in the experimental bottles. In order to avoid the first problem, nutrients should be added when nutrient limitation is anticipated to become a problem (Roman & Rublee 1980, Landry 1993).

### Nutrient limitation?

Initial nutrient concentrations in the incubation bottles are shown in Table 1. Unfortunately nutrients were not re-sampled when the incubations were terminated. However, the nutrients were measured in the mesocosms after the incubations were terminated, but before the mesocoms were re-fertilized (Table 1). If we assume that the average light and nutrient environments were similar in the incubation bottles and the upper layers of the respective mesocosm (cf. 'Material and methods'), we may assume that the nutrients were depleted at comparable rates in these environments. Then nitrate and phosphate would not appear to be limiting in most experiments, perhaps with the exception of 97a,b. However 97a,b received extra nutrients (Table 1) and may not be directly compared in this way. The other experiments would probably not have been significantly nutrient limited

unless depletion rates were at least twice as high as in the mesocosms.

Alternatively, nutrient depletion rates in the control bottles may be estimated if we assume: (1) that the total accumulation of particulate (POC) and dissolved organic material (DOC) equals inorganic nutrients removed from the water in a Redfield atomic ratio (106 C : 16 N : 1 P, Falkowski 2000); and (2) that the total POC and DOC accumulation is 3× the measured net accumulation of total phyto- and zooplankton (cf. Banse 1974, Bronk & Ward 2000). If so, the phytoplankton would again only have become N-limited in Expt 97b (Table 1). Nitrate appeared to be potentially more limiting than phosphate, but none of these would probably be limiting in the other experiments.

Zooplankton grazing may decrease silicate concentrations (Sommer 1988). Silicate could have been limiting in the 96a and 96c experiments (Table 1). However, a negative uncorrected copepod grazing rate for diatoms was only found in 1 experiment with high silicate concentrations (96b). Instead a number of silicate-independent prey showed negative uncorrected copepod grazing rates in Expts 96a and c (Table 4). Thus, it is very unlikely that silicate limitation may have contributed to the negative uncorrected copepod grazing rates.

Copepod nutrient regeneration effects are complex. Copepods regenerate N in reduced forms, which may be more readily assimilated by the phytoplankton, but copepod predation on the microzooplankton may to some extent also control the ammonium regeneration (Glibert 1998). Note that microzooplankton grazing rates were high (Table 3), indicating high nutrient regeneration rates. Presence of reduced inorganic N sources at the start of the incubations would decrease the risk of nutrient limitation (not measured). Also, algal growth may show signs of significant external pools even at near-zero analytical values of dissolved nutrients (Andersen et al. 1991).

Nevertheless, if negative uncorrected copepod grazing rates were mainly caused by nutrient regeneration, we would expect uncorrected copepod grazing rates (Tables 4 & 5) to be correlated to terminal nutrient concentrations, and/or copepod concentrations (Table 1). However, no such correlation was found ($r^2 < 0.06$, $p = 0.12$ to 0.8, not shown). Thus we conclude that the main factor causing negative uncorrected copepod grazing rates was not nutrient limitation.

### Microzooplankton-copepod grazing interactions, evaluation of effects and corrections

Uncorrected instantaneous copepod grazing rates showed highly significant correlations ($p \leq 0.006$) to instantaneous microzooplankton grazing rates, regardless of whether or not the outlier was removed (Fig. 1). The outlier was a case of large (>32 µm) autotrophic ciliates (*Mesodinum rubrum*) occurring in high enough concentrations to be determined separately in the dilution experiments (Expt 98, Table 3). This outlier is most likely a result of strongly selective copepod feeding comparable to the other large ciliates (Experiment 98, Table 4). In contrast, corrected copepod grazing rates were not correlated to microzooplankton grazing rates, irrespective of whether or not the outlier was removed ($p = 0.56$ to 0.94, Fig. 1). This clearly suggests that food-web grazing interactions had a strong impact on the uncorrected copepod grazing rates that increased with microzooplankton community grazing pressure in the food suspension. But this bias was successfully corrected by both methods (Fig. 1).

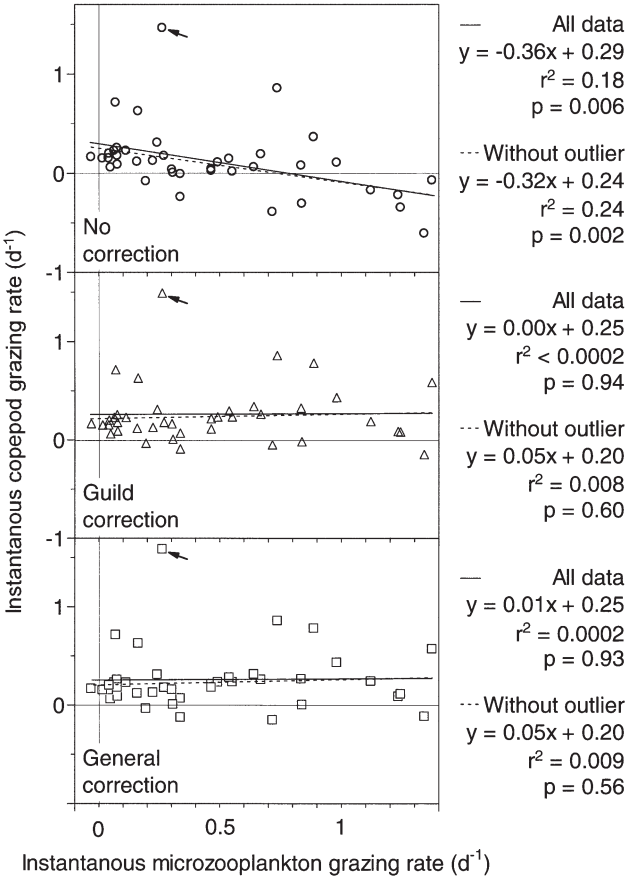


Fig. 1. Correlation between instantaneous microzooplankton and *Calanus* spp. grazing rates using either No, Guild, or General correction of the copepod grazing rates. Only autrophic food items for which microzooplankton grazing rates were measured are included. Flagellates <10 µm most likely contain some heterotrophic species. Regressions for all data (n = 46, solid lines), and with outlier (arrow) removed (n = 45, hatched lines); see text for comments

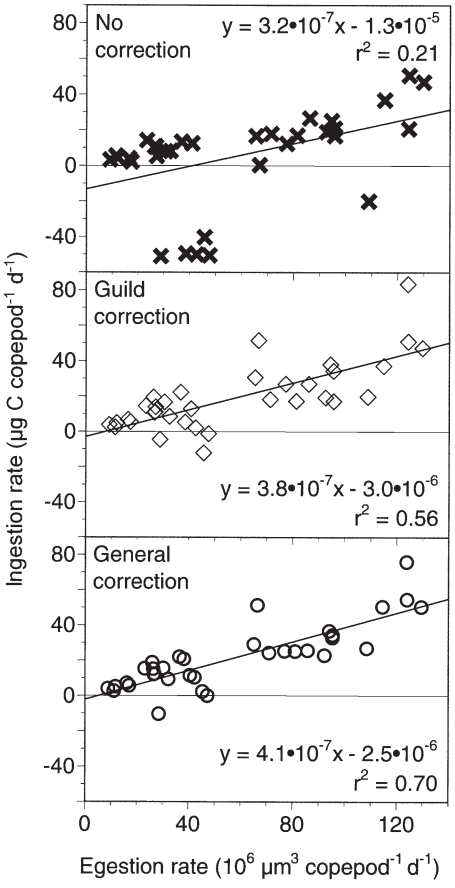


Fig. 2. *Calanus* spp. copepodites V and females. Relationship between volumetric egestion and ingestion rates, using either No, Guild, or General correction of the copepod ingestion rates (n = 34). The uncorrected regression showed 6 outliers below zero ingestion rates; see text for comments

Volumetric egestion rates, used as an independent measure of copepod feeding activity, showed strong linear correlations to corrected copepod ingestion rates ($r^2$ = 0.56 to 0.70, $p < 0.0001$), with *y*-axis intercepts that were not significantly different from zero ($p > 0.46$ to 0.47, Fig. 2). These data, especially from flagellate-dominated blooms (discussed below), also showed a striking similarity to ingestion-egestion data for *Calanus finmarchicus* stage CV feeding on monocultures of the naked flagellate *Rhodomonas baltica* recalculated from Båmstedt et al. (1999) (Fig. 3). As there are no food-web interactions in the monoculture data, this suggests that corrected copepod grazing rates apparently yield a very close estimate of the *in vitro* feeding rates.

The uncorrected copepod ingestion rates showed 6 clearly negative outliers in Fig. 2. These outliers were all obtained from experiments where high microzooplankton grazing rates coincided with high copepod predation on the microzooplankton. However, the outliers were successfully corrected (compare the lower panels). Because of the outliers, the uncorrected grazing data did not satisfy the criteria for a statistical analysis of the linear regression, but it indicated a substantial negative *y*-axis intercept in contrast to corrected (Fig. 2) and unbiased data (Fig. 3).

Corrected daily C rations were on average ca 30% (range 16 to 52%, Table 4), which is close to the upper range of *Calanus* spp. ingestion rates in the literature (Hansen et al. 1997). This was expected because plankton concentrations were above the critical food concentration (150 to 300 µg C $l^{-1}$) for *Calanus* spp. (Frost 1972, Gamble 1978), and were comparable to dense blooms in Scandinavian fjords and the North Sea (e.g. Braarud et al. 1958, Gamble 1978, Paasche & Østergren 1980, Andersen & Sørensen 1986, Heimdal & Reisegg 1996). Uncorrected rates averaged only 19% (range 5 to 34%, Table 4).

Also, corrected copepod clearance of prey <10 µm were low (Table 4), which is in accordance with the literature (e.g. Frost 1972, Nejstgaard et al. 1995, Hansen et al. 1997). This suggests that corrected rates yield precise estimates, and not maximum estimates as previously suggested for the Guild method (Perez et al. 1997).

### Comparison between guild and general corrected results

The Guild and General methods occasionally yielded somewhat different results (e.g. Expt 96d, Table 4), but in most cases the results were very similar (Table 4,

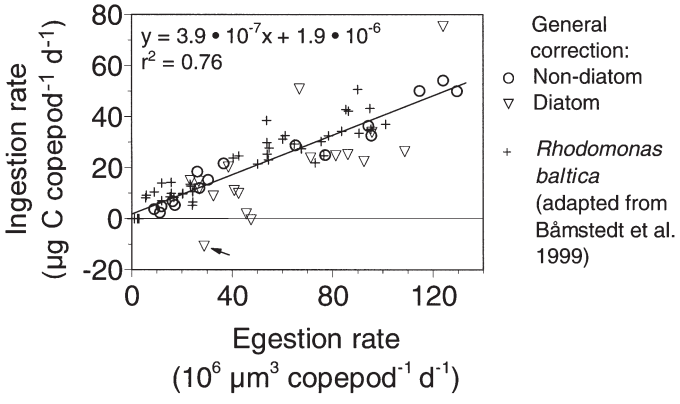


Fig. 3. *Calanus* spp. copepodites V and females. Relationship between ingestion rate and faecal volume production. Results from Expts 96a to 98 (○ and ▽) based on the General correction (Fig. 2), compared to results from laboratory experiments (+) with *C. finmarchicus* feeding on monospecific laboratory cultures of the cryptophyte *Rhodomonas baltica* (adapted from Båmstedt et al. 1999). Non-diatom food suspensions (▽) were dominated by flagellates, including dinoflagellates, of different size and taxa in the different experiments. Equation including all data except outlier, n = 77. Arrow denotes outlier; see text for further comments and equations

Figs 1 & 2, no significant differences between the regressions, $p \gg 0.50$, Chow-test).

The goal with the Guild correction method was to achieve precise corrections of copepod grazing rates in natural plankton (Nejstgaard et al. 1997). But this method is rather complex. In order to increase the applicability of the method we therefore omitted the use of different feeding guilds in the general method. This may theoretically lower the precision of the corrections. For instance, if both the copepod and its prey were highly selective feeders, and the selected microzooplankton made up only a fraction of the total microzooplankton, then the general method would tend to result in insufficient corrections. However, this would not be expected in our data, despite selective feeding by the copepods (cf. Table 6), because the selected microzooplankton were assumed to ingest a wide range of prey types (Table 2). Note also that the food medium was pre-screened to remove mesozooplankton. If this had not been done, copepods could also have fed upon carnivorous mesozooplankton. Then a more complex method may have been needed to resolve the feeding interactions.

On the other hand, the omission of feeding guilds may reduce sources of error associated both with assigning proper feeding guilds, and the taxonomical accuracy needed when analysing samples. It may also be argued that since both correction methods are based on the same microzooplankton community grazing rates there should be little difference between them. However, the total microzooplankton community grazing rate is a sum of all the individual rates. If the different microzooplankters were highly selective feeders, the rates calculated for each prey item would stem only from a fraction of the total microzooplankton community. Then, the Guild method would tend to yield a better (higher) estimate than the general method.

Reducing the complexity of the sample analysis in the General method also makes it suitable for computer-automated analysis. Computer analysis may handle pigment content and cell sizes properly, but typically not taxonomy (cf. Verity & Paffenhöfer 1996). Because the General method does not require manual microscopy (except for determination of the dominant taxa in each experiment) it should be ideal to combine with automated image analysis or flow-cytometric sample treatment in future grazing studies.

### Faecal pellet production as a simple measure of total feeding rate

Volumetric egestion rates were strongly correlated to corrected ingestion rates (Fig. 2), and matched previously published unbiased ingestion-egestion data for *Calanus* spp. (Båmstedt et al. 1999) (Fig. 3). In particular, the data from non-diatom food suspensions ($y = 3.9 \times 10^{-7}x + 1.0 \times 10^{-6}$, $r^2 = 0.95$, $p = 10^{-10}$, $n = 16$) closely matched ($p > 0.4$, Chow test) the regression for *Rhodomonas baltica* ($y = 4.0 \times 10^{-7}x + 3.0 \times 10^{-6}$, $r^2 = 0.87$, $p = 10^{-20}$, $n = 45$) (µgC µm$^{-3}$, Fig. 3). The combined equation for all non-diatom dominated data showed a very strong correlation ($y = 3.9 \times 10^{-7}x + 2.7 \times 10^{-6}$, $r^2 = 0.89$, slope $p = 10^{-30}$, intercept $p = 0.01$, $n = 61$), and should thus give the best estimates of total ingestion rates for late copepodites of *Calanus* spp. feeding on various natural non-diatom dominated diets.

The regression from diatom food suspensions showed larger variations in the data (Fig. 3), and was not significantly different from non-diatom data ($p \gg 0.5$, Chow-test), neither with nor without the outlier. However, the regression for *Rhodomonas baltica* was slightly different ($p = 0.05$, Chow-test) from the combined diatom and non-diatom regression. But with the outlier removed, the regression of the combined data ($y = 3.9 \times 10^{-7}x + 1.8 \times 10^{-6}$, $r^2 = 0.70$) was not significantly different from the *R. baltica* regression ($p > 0.1$, Chow-test). Thus all values (except the negative outlier) could be plotted as one regression ($y = 3.9 \times 10^{-7}x + 1.9 \times 10^{-6}$, $r^2 = 0.76$, slope $p \ll 0001$, intercept $p = 0.21$, $n = 77$), which may be used to estimate ingestion rates for *Calanus* spp. in natural food suspensions, including diatoms.

Faecal pellet numbers were also correlated to corrected ingestion rates, and were similar to previously published values for *Calanus* spp. (Fig. 4). However, pellet numbers show larger variation around the regression than pellet volume. This is expected from the literature because pellet number, size and density may all vary with feeding rates, as well as type, size and physiological status of both the predator and prey (e.g. Marshall & Orr 1955, Gaudy 1974, Ayukai & Nishizawa 1986, Urban et al. 1993, Butler & Dam 1994, Feinberg & Dam 1998, Båmstedt et al. 1999, and references therein).

There are also potential problems with pellet reworking by the copepods (Noji et al. 1991), disruption of pellets due to bottle agitation, loss and breakage during sampling (J.C.N. pers. obs). We believe that disruption, loss and measurement errors may explain a substantial part of the variation in previously published data (cf. the large size variation in fragile coccolith filled *Calanus* pellets, Harris 1994). However, these errors may be minimised by avoiding zooplankton specialised in pellet feeding, such as *Oithona* spp. (cf. González et al. 2000), use of egg production chambers with false mesh bottoms preventing contact between mesozooplankton and pel-

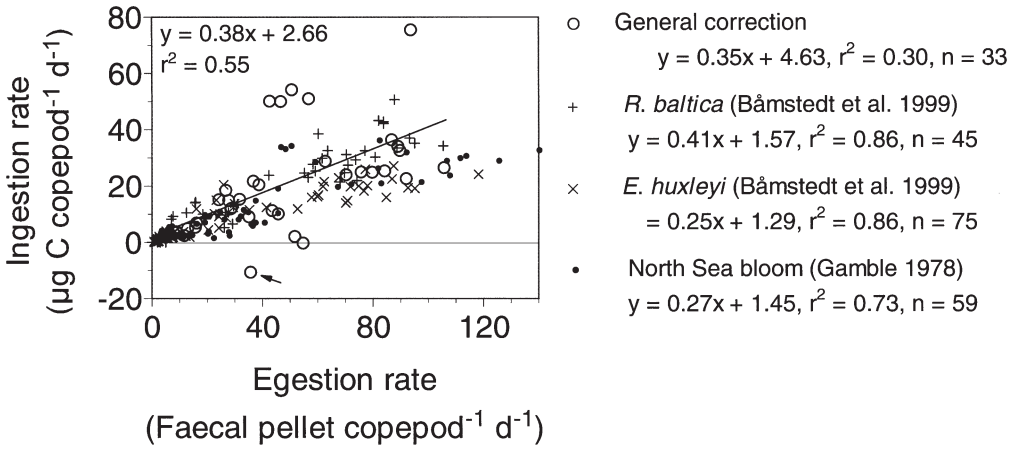


Fig. 4. *Calanus* spp. copepodites V and females. Relationship between ingestion rate and numbers of faecal pellets produced. Results from Expts 96a to 98 (○) based on the General correction (Fig. 2), compared to results from laboratory experiments (Båmstedt et al. 1999) with *C. finmarchicus* feeding on either the coccolithophorid *Emiliania huxleyi* (×) or the cryptophyte *Rhodomonas baltica* (+) as single food species, and field data (Gamble 1978) with *C. finmarchicus* feeding in a diatom dominated bloom in the North Sea (•). The negative outlier (arrow) was excluded from further analysis. The individual regressions were significantly different from each other ($p < 0.05$, Chow-test), except for the General correction that was not different from *R. baltica* ($p \gg 0.5$), and *E. huxleyi* versus the North Sea bloom ($p > 0.5$). The overall regression for the General correction and *R. baltica* is shown (slope $p \ll 0.0001$, intercept $p = 0.24$, $n = 78$)

lets (cf. Båmstedt et al. 1999), careful collection of the pellets (e.g. using reverse filtration) and application of image analysis to increase the analytical speed and precision of pellet volume determination in future studies.

Variation in assimilation efficiency, pellet packing and density will still limit the precision of volumetric pellet-based methods (cf. Butler & Dam 1994, Feinberg & Dam 1998). These errors appear to be relatively large for diatom dominated diets (cf. Fig. 3 and Butler & Dam 1994) but are still minor compared to the statistically significant negative rates that may be obtained by uncorrected bottle incubations. However, these errors may be reduced by calibration to the specific predator and prey types (as suggested in Båmstedt et al. 1999), and appear to be low for non-diatom diets. Furthermore, volumetric egestion rates have strong advantages compared to other simple methods, such as the gut pigment technique (Mackas & Bohrer 1976), because they include non-pigmented food (cf. Dam et al. 1994, Peterson & Dam 1996), and it may be used for repeated measurements of a single individual, e.g. in egg production studies.

Thus we suggest that the most species- and stage-specific equations available, such as those obtained for late stages of *Calanus* spp. in Fig. 3, may be used to estimate ingestion rates from faecal pellet volume. We also suggest that pellet production should be analysed in incubation experiments with natural plankton, as this provides a robust internal standard for total ingestion rates.

## SUMMARY AND CONCLUSIONS

We have shown that grazing rates based on the uncorrected traditional method were significantly underestimated in some cases due to selective loss of microzooplankton grazing pressure in the copepod bottles. This problem appears to increase when high microzooplankton grazing pressure coincides with high and selective copepod feeding on the microzooplankton.

In contrast, both correction methods appear to yield good copepod feeding estimates, because: (1) corrected copepod feeding rates were never significantly negative, but still low on sub-optimal sized prey, (2) there were no correlations between microzooplankton and corrected copepod feeding rates, but (3) very good fit with volumetric egestion data, and (4) ingestion rates were well within published values for comparable food situations.

We argue that the classical faecal production method provides a simple and quick overall feeding estimate with important advantages over the common gut pigment technique. But this requires that loss and estimation errors are minimised, and that volume egestion rates are adequately calibrated to food environment, stage and species specific ingestion rates. Thus we suggest to routinely include pellet production analysis as a robust internal standard for total ingestion rates in incubation experiments with natural plankton.

The complexity and need for taxonomical accuracy are reduced in the General method, while it still appears to yield estimates as accurate and precise as

those of the Guild method. Therefore we suggest that the General method of correction should be applied to control for the variable and potentially significant interaction of microzooplankton grazing in future experiments with trophically mixed prey and omnivorous predators. Although the General method of correction requires considerably more experimental effort than the classical uncorrected approach, it also adds information on microzooplankton grazing etc. with a minimal total number of additional experimental bottles. Thus, this is a minimum approach to gain detailed knowledge of the total phytoplankton-zooplankton interactions in natural plankton.

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