



Ingestion, faecal pellet and egg production rates of *Calanus helgolandicus* feeding coccolithophorid versus non-coccolithophorid diets

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Abstract

Ingestion rates, faecal pellet and egg production were obtained in laboratory experiments with females of the copepod *Calanus helgolandicus* collected from the English Channel in November 1994. Five different algal monocultures were used as food: *Prorocentrum micans* (30 μm ESD), *Thalassiosira weissflogii* (13 μm ESD), *Dunaliella tertiolecta* (7 μm ESD), *Emiliana huxleyi* (5 μm ESD) and *Coccolithus pelagicus* (14 μm ESD). Results obtained suggest the low ingestion efficiency of the copepod when feeding on coccolithophorids during late autumn–early winter. From the five species offered, only the largest non coccolithophorid *Prorocentrum micans* and *Thalassiosira weissflogii* supported efficient feeding and calculated respiratory demand for *C. helgolandicus*. Both coccolithophorids, irrespective of cell size, were ingested at very low rates even when offered at high concentrations (233–468 $\mu\text{g C l}^{-1}$). Besides low ingestion, no egg production was found in the copepods fed with *Emiliana huxleyi*, although unusual high gross efficiency (reaching 72%) was obtained in experiments performed with *Coccolithus pelagicus*. The late seasonal timing of the experiments (November) could explain the low ingestion and egg production rates. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

Coccolithophorids is a widely distributed phytoplankton group characterised by their

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calcium carbonate plates (coccoliths) surrounding the cell. The pelagic alga *Emiliania huxleyi* is the most widespread coccolithophore species and is considered to be the major producer of calcite in the biosphere. Extensive monospecific blooms of this alga have been described especially in mid-latitudes in both coastal and open oceanic waters. These blooms have an average annual area of 1.4×10^6 km² of the world's ocean (Brown and Yoder, 1994), and can persist for 3–6 weeks reaching densities higher than 10^6 cells l⁻¹.

Emiliania huxleyi has been largely studied in the last years in relation to the global carbon cycle, due to the influence of calcite synthesis on the CO₂ equilibrium in seawater and the production of the climate-related dimethylsulfide (DMS). Apart from that, it has also been suggested that coccoliths are important in carbon flux to the deep ocean, and the presence of coccoliths in sediment traps has been reported (Cadée, 1985). Sedimentation rates of individual coccoliths are very low (10 cm d⁻¹, Honjo, 1976), as well as intact cells (1 m d⁻¹, Smayda, 1971) so the formation of coccoliths or cell aggregates, such as zooplankton faecal pellets with higher sinking rates (> 100 m d⁻¹, Harris, 1994) may be one of the most important mechanisms contributing to this flux.

But the importance of this mechanism requires the existence of well-developed copepod populations, with animal abundance high enough to produce enough pellets to result in significant export. During monospecific *Emiliania* blooms, this alga may be the main source of food for zooplankton growth and reproduction. The present study analyses the capability of different coccolithophore diets (*Emiliania huxleyi* and *Coccolithus pelagicus*) to satisfy *Calanus helgolandicus* metabolic and reproductive requirements when offered as the only food, and also the capability to sustain high copepod abundance, when compared with non-coccolithophore diets such diatoms (*Thalassiosira weissflogii*), or flagellates (*Prorocentrum micans* and *Dunaliella tertiolecta*).

2. Material and methods

Copepods were collected from net tows made off Plymouth (English Channel) between 10th and 29th November 1994 using a WP2 net (60 cm diameter and 200 µm mesh). The cod end contents were transferred to the lab in surface seawater in less than 1 h. Ten *Calanus helgolandicus* females, actively swimming and apparently healthy (without broken appendices) were selected for each experiment and maintained for 24 h in filtered (0.2 µm) seawater to clean their gut contents. Each female was examined (dorsal view) under a Wild stereomicroscope (25×) to ensure that ova were visible in oviducts as opaque rows, extending to the last thoracic segment.

Phytoplankton monocultures were prepared with five algae species differing in cell-size and carbon content as determined with Coulter Multisizer (70 µm aperture) and CHN analysis. Phytoplankton characteristics and concentrations used in the experiments are given in Table 1. Unialgal cultures, obtained from the Plymouth Culture Collection, were grown at 15°C in 2.5 l Erlenmeyer flasks using f/2 medium (Guillard, 1975). Cultures were incubated in an 18:6 h light:dark cycle at a light intensity of 100–200 E m⁻² s⁻¹. Only cultures in exponential growth phase were used. Five replicates and two control bottles without copepods, in order to estimate phytoplankton growth, were used

Table 1
Phytoplankton cell size (Equivalent Spherical Diameter), C–N content and concentrations used in the experiments

Alga	Concentrations used (cells $\times 1000 \text{ ml}^{-1}$)	ng C cell $^{-1}$	ng N cell $^{-1}$	ESD (μm)
<i>Prorocentrum micans</i>	0.12 ; 0.25 ; 1	1.68	0.21	30
<i>Thalassiosira weissflogii</i>	0.75 ; 1.5 ; 3	0.16	0.037	13
<i>Coccolithus pelagicus</i>	0.25 ; 0.5 ; 1	0.23	0.04	14
<i>Dunaliella tertiolecta</i>	3 ; 6 ; 12 ; 24 ; 36	0.007	0.0014	7
<i>Emiliania huxleyi</i>	12 ; 24 ; 36	0.013	0.002	5

for each food concentration. Before each experiment, 18 l of algae culture at the desired concentration (Table 1) were prepared, thoroughly mixed and checked for initial concentration using Coulter Multisizer. Culture was poured into 2.3 l glass jars and ten *Calanus* females were added. Jars were attached to a wheel that rotated at 1 r.p.m for 24 h at 15°C to avoid phytoplankton sedimentation. Incubations were performed in light–dark cycles of 12:12 h.

After 24 h, each jar was removed from the wheel and contents were gently siphoned through a pipe into a vessel with a 30 μm mesh false bottom where animals, eggs and faecal pellets were retained. The mesh was maintained submerged in the water during all the procedure to avoid animal damage and pellet breakage. The filtrate was thoroughly stirred and three 20 ml subsamples were removed for Coulter Multisizer counts of final algal concentration. Animals were transferred to a beaker containing seawater, which facilitated easy removal of animals using a manual pipette. Animals were removed when swimming at the upper part of the beaker to avoid removing the pellets or eggs accumulated at the bottom. Animals were counted and transferred to fresh algal culture. Eggs and faecal pellets were retained on a 30 μm mesh and placed in 20 ml vials with 4 drops of Lugol's solution to preserve the samples.

The same procedure was repeated for four days for each food concentration, considering the first three days as an acclimation period to experimental conditions. Eggs and faecal pellets were counted and measured using an ocular micrometer at 400 \times magnification. Volume was calculated assuming eggs to be spheres and faecal pellets to be cylinders. Carbon content of eggs was estimated assuming values proposed by Kjørboe and Sabatini (1995) (0.32 $\mu\text{g C egg}^{-1}$).

Ingestion and clearance rates were calculated using the equation proposed by Marin et al. (1986) for experimental conditions where food concentration is lower than critical concentration, with phytoplankton growth in control bottles. We used this equation due to the difficulties to establish a proper critical concentration. In our experiments ingestion still increases above the critical concentration calculated using equations suggested by Huntley and Boyd (1984). Anyway, differences between the rates calculated using both equations were small.

$$F = \frac{V \left(\frac{\ln C_0 - \ln C_t}{t} + k \right)}{N}$$

$$I = F \times C_o$$

I =Ingestion rate (cells female⁻¹ h⁻¹); F =clearance rate (ml female⁻¹ h⁻¹); V =jar volume (ml); N =number of females; C_0 =initial phytoplankton concentration in experimental jar (cells ml⁻¹); C_t =final phytoplankton concentration in experimental jar (cells ml⁻¹); t =time (h); k =cell growth coefficient (h⁻¹).

Gross efficiency of egg production and percentage of body carbon ingested daily were calculated assuming 71.1 µg C copepod⁻¹ as proposed by Kiørboe and Sabatini (1995) for *Calanus helgolandicus* females.

3. Results

3.1. Ingestion rates

Ingestion and clearance rates obtained with the five algal species are plotted against food concentration in Figs. 1 and 2. Results suggest that *Calanus helgolandicus* only feed efficiently on two of the five algal species offered. Females fed the small coccolithophore *E. huxleyi* showed very low ingestion rates only apparent when offered more than 468 µg C l⁻¹. Rates with the other coccolithophore, *Coccolithus pelagicus*, and the small non-coccolithophore *Dunaliella tertiolecta* were also very low. Only the copepods fed the medium and large sized non-coccolithophore alga *Prorocentrum micans* and *Thalassiosira weissflogii* showed high ingestion and clearance rates, reaching values 9 and 4 times higher than those obtained with *Emiliana*.

Average values of ingestion and clearance rates for each concentration are given in Table 2. *Emiliana huxleyi* was grazed at maximum rates of 202 ng C animal⁻¹ h⁻¹ at a food concentration of 468 µg C l⁻¹. Lower maximum values were obtained for *D. tertiolecta* and *C. pelagicus* although food was also offered at lower concentrations: 187 ng C animal⁻¹ h⁻¹ at 94 µg C l⁻¹ for the small *Dunaliella*, and 158 ng C animal⁻¹ h⁻¹ at 233 µg C l⁻¹ for *Coccolithus*. On the other hand, the dinoflagellate *P. micans* gave the highest ingestion, with average maximum values of 1826 ng C animal⁻¹ h⁻¹ at 854 µg C l⁻¹. The diatom *T. weissflogii* showed also high ingestion values: 921 ng C animal⁻¹ h⁻¹ at 491 µg C l⁻¹.

Although the data is not sufficient to obtain an accurate functional response, there is no sign of ingestion saturation in the experiments performed with *E. huxleyi* and *P. micans*. With these two algae the copepod could not achieve a maximum rate. It seems that saturation of ingestion is observed in *T. weissflogii*, *D. tertiolecta* and *C. pelagicus*, at food levels of 237, 94 and 58 µg C l⁻¹ respectively.

Emiliana huxleyi was cleared at the lowest rates, with maximum values of 0.40 ml animal⁻¹ h⁻¹ at 468 µg C l⁻¹. Rates obtained for *Coccolithus* and *Dunaliella* were respectively 1.25 ml animal⁻¹ h⁻¹ (at 58 µg C l⁻¹) and 1.05 ml animal⁻¹ h⁻¹ (at 24 µg C l⁻¹). As found for ingestion rates, maximum clearance rates were obtained in experiments performed with *Prorocentrum* and *Thalassiosira*: 3.45 ml animal⁻¹ h⁻¹ (at 220 µg C l⁻¹) for the dinoflagellate and 4.17 ml animal⁻¹ h⁻¹ (at 123 µg C l⁻¹) for the diatom.

Results obtained (Table 2) represent very low percentages of body carbon ingested

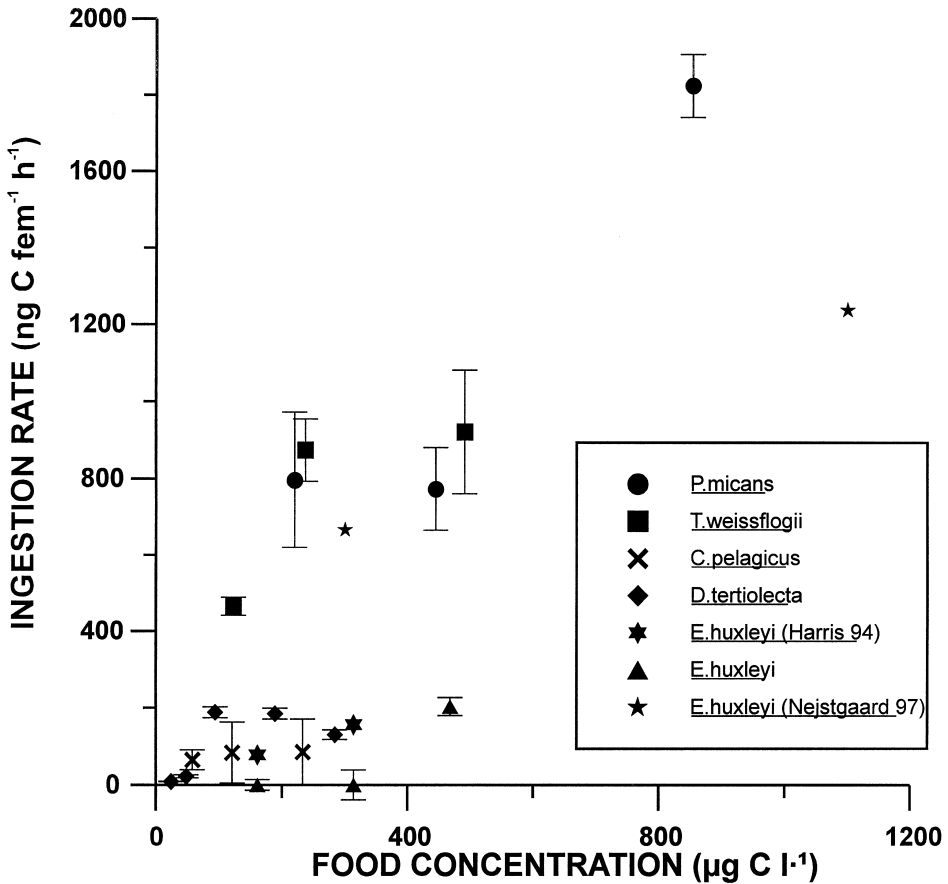


Fig. 1. *Calanus helgolandicus* ingestion rate (average and standard error) versus food concentration, with different monospecific algal diets.

daily by the animals fed with *Emiliana huxleyi*. Average values with this alga ranged between 0 and 6.8%. Low values were also found with the other coccolithophore and *Dunaliella*, 5.3% for the former and 0.3–6.3% for the latter. With *Prorocentrum*, ingestion represented 26–61% of body carbon while values of 15–31% were obtained with *T. weissflogii*.

3.2. Faecal pellet production

Relationships between number and total volume of faecal pellets egested and ingestion rate are shown in Figs. 3 and 4. Not enough pellets were produced in experiments performed with *E. huxleyi*. We only obtained pellets in one of the replicates

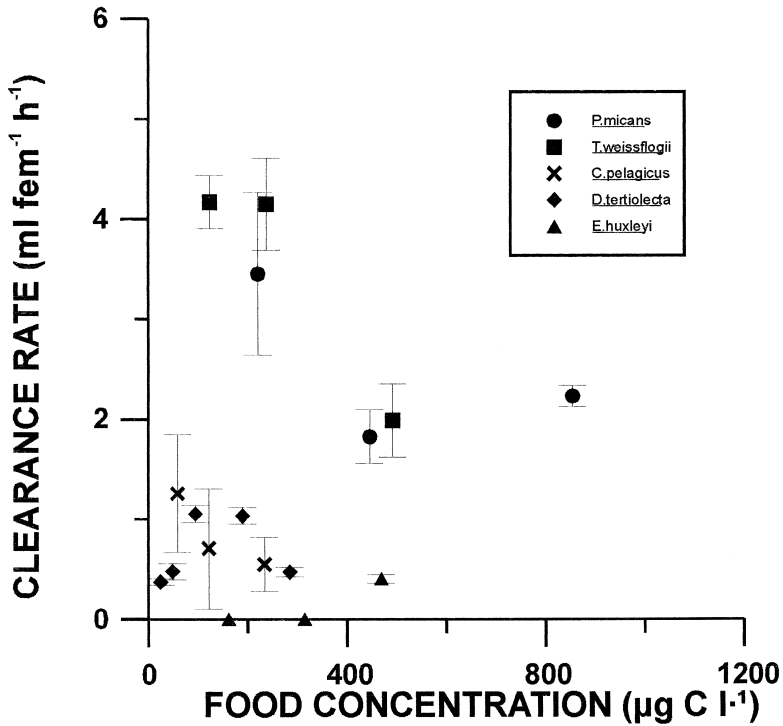


Fig. 2. *Calanus helgolandicus* clearance rate (average and standard error) versus food concentration, with different monospecific algal diets.

from an experiment at 314 $\mu\text{g C l}^{-1}$ so only faecal pellets from *P. micans*, *T. weissflogii*, *C. pelagicus* and *D. tertiolecta*, were measured and plotted in the graphs.

Linear relationships were found between number of pellets produced and ingestion rate, but better fits were found if we use total volume egested. Good fits were found in the experiments performed with *T. weissflogii* ($r^2=0.69$, $P=0.003$), *D. tertiolecta* ($r^2=0.73$, $P=0.002$) and *P. micans* ($r^2=0.73$, $P=0.00004$) and no trend is found with *C. pelagicus*, maybe due to the low number of data available. Regressions were tested (ANCOVA) to determine statistical differences between them. No differences ($P>0.1$) were found between linear regressions obtained with *P. micans* and *T. weissflogii*, neither considering total pellets volume, nor number of pellets. Pooled regression equations are shown in Figs. 4 and 5. Statistical differences were found between both algae and *D. tertiolecta*.

Maximum average values found (Table 2) are 66.8 pellets animal⁻¹ d⁻¹ for *P. micans*, 47.8 pellets animal⁻¹ d⁻¹ for *T. weissflogii*, 4.6 pellets animal⁻¹ d⁻¹ for *C. pelagicus*, and 3.7 pellets animal⁻¹ d⁻¹ for *D. tertiolecta*.

A logarithmic relationship ($r^2=0.94$, $P=0.00004$) was found between individual faecal pellet volume and ingestion rates (Fig. 5) if we used pooled data obtained from all

Table 2

Average values (standard error in brackets) of ingestion rate, clearance rate, egg and faecal pellets production, gross efficiency and % of body carbon ingested daily for each food concentration used

Alga	Food concentration ($\mu\text{g C l}^{-1}$)	Ingestion rate ($\text{ng C female}^{-1} \text{h}^{-1}$)	Clearance rate ($\text{ml female}^{-1} \text{h}^{-1}$)	Egg production ($\text{n}^\circ \text{female}^{-1} \text{d}^{-1}$)	FP production ($\text{n}^\circ \text{female}^{-1} \text{d}^{-1}$)	Gross eff. (in C)	% Body carbon ingested daily
<i>P. micans</i>	854	1826 (82)	2.23	22.7 (2.11)	66.8 (5.8)	16.6	61.7
<i>P. micans</i>	445	772 (107)	1.82	19.83 (1.29)	37.36 (3.3)	34.2	26.1
<i>P. micans</i>	220	795 (176)	3.45	11.37 (2.55)	39.55 (8.05)	19.1	26.9
<i>T. weissflogii</i>	491	921 (160)	1.99	8.86 (1.3)	25.28 (3.11)	12.8	31.1
<i>T. weissflogii</i>	237	874 (80)	4.15	11.64 (0.56)	48.76 (3.43)	17.8	29.5
<i>T. weissflogii</i>	123	464 (23)	4.17	3.37 (0.68)	19.77 (1.6)	9.7	15.7
<i>D. tertiolecta</i>	284	130 (12)	0.47	–	–	–	4.4
<i>D. tertiolecta</i>	189	184 (14)	0.37	–	–	–	6.2
<i>D. tertiolecta</i>	94	187 (14)	0.47	–	–	–	6.3
<i>D. tertiolecta</i>	48	22 (3,8)	1.03	0.28 (0.12)	3.69 (0.86)	17.0	0.7
<i>D. tertiolecta</i>	24	8.9 (0.6)	1.05	0.13 (0.1)	1.06 (0.23)	19.5	0.3
<i>C. pelagicus</i>	233	158 (133)	0.38	–	–	–	5.3
<i>C. pelagicus</i>	121	158 (64)	0.7	8.54 (0.9)	4.64 (0.4)	72.1	5.3
<i>C. pelagicus</i>	58	65 (25)	1.25	–	–	–	2.2
<i>E. huxleyi</i>	468	202 (23)	0.4	–	–	–	6.8
<i>E. huxleyi</i>	314	8.31	–	0.65 (0.52)	0.27 (0.04)	104.3	0.3
<i>E. huxleyi</i>	161	9 (7)	–	0	0	0	0.3

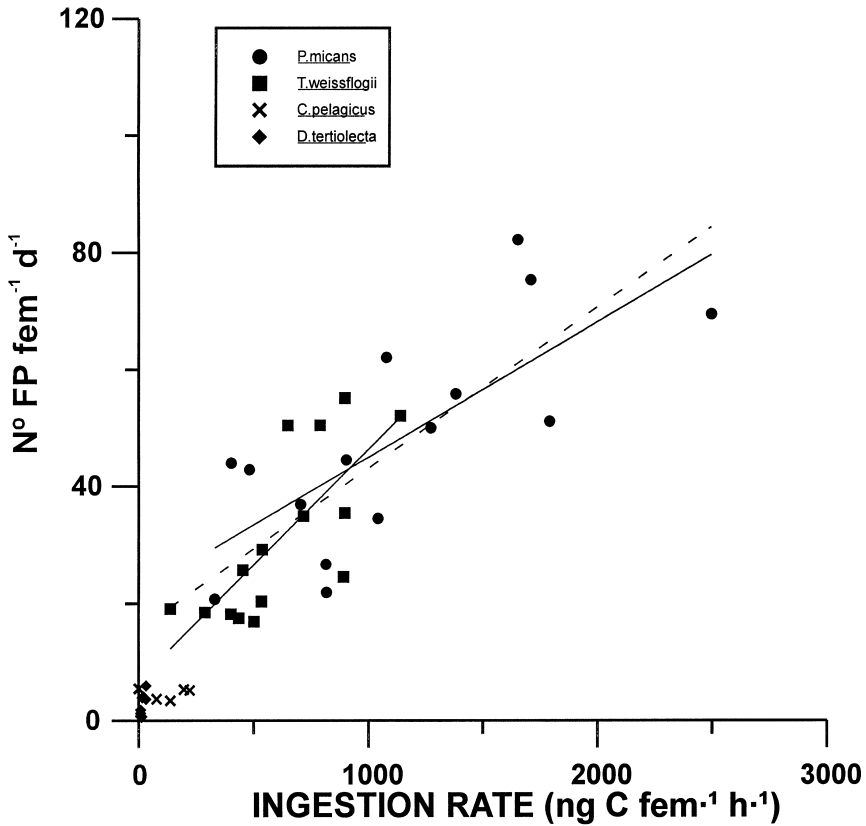


Fig. 3. Number of faecal pellets produced by *Calanus helgolandicus* fed with different algal diet and concentrations. Fits: *P. micans*: $y = 0.023x + 21.866$; $n = 15$; $r^2 = 0.55$; $P = 0.00015$; *T. weissflogii*: $y = 0.0395x + 6.845$; $n = 15$; $r^2 = 0.55$; $P = 0.0025$; *D. tertiolecta*: $y = 0.173x - 0.331$; $n = 10$; $r^2 = 0.64$; $P = 0.003$; *P. micans* and *T. weissflogii* pooled regression (—) $y = 0.027x + 15.56$; $n = 30$; $r^2 = 0.76$; $P = 0.0001$.

the experiments with the different algae and concentrations. Saturation volume was 0.0025 mm^3 .

3.3. Egg production

The relationship between egg production and ingestion rate is shown in Fig. 6. No evaluation could be obtained with data from experiments performed with *Emiliana* due to the extremely low number of eggs produced. Maximum average values with this alga were $0.65 \text{ eggs animal}^{-1} \text{ d}^{-1}$ at a $314 \mu\text{g C l}^{-1}$ concentration. We also found very low egg production (even lower than with *Emiliana*) for copepods fed with *Dunaliella*. With this algae production was nearly zero: $0.28 \text{ eggs animal}^{-1} \text{ d}^{-1}$, so only enough data are available in experiments with *Prorocentrum*, *Thalassiosira* and *Coccolithus*. *Calanus helgolandicus* fed with *Coccolithus* produced an appreciable number of eggs, but only

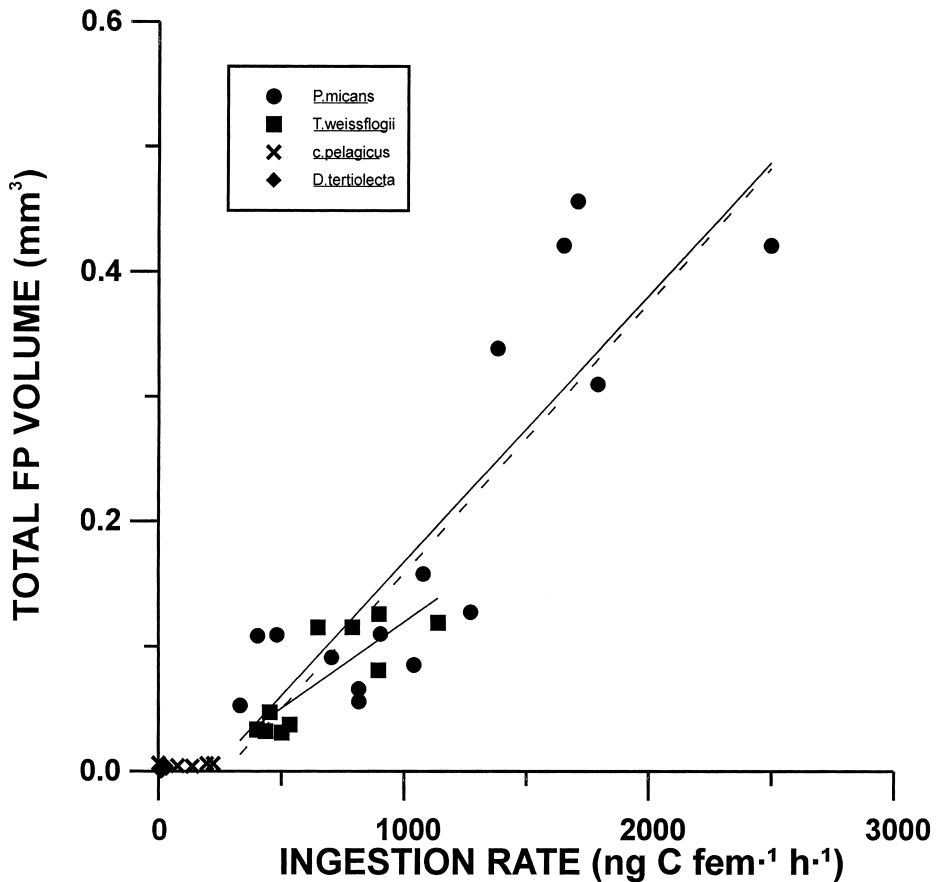


Fig. 4. Total volume of faecal pellets egested by *Calanus helgolandicus* fed with different algal diets and concentrations. Fits: *P. micans*: $y = 0.00021x - 0.0046$; $n = 15$; $r^2 = 0.73$; $P = 0.00004$; *T. weissflogii*: $y = 0.00013x - 0.0185$; $n = 10$; $r^2 = 0.69$; $P = 0.003$; *D. tertiolecta*: $y = 0.17x - 0.33$; $n = 10$; $r^2 = 0.73$; $P = 0.002$; *P. micans* and *T. weissflogii* pooled regression (---) $y = 0.0002x - 0.0582$; $n = 25$; $r^2 = 0.76$; $P = 0.00001$.

data from an experiment performed at $233 \mu\text{g C l}^{-1}$ are available, so we only show (Fig. 6) fits obtained with *Prorocentrum* and *Thalassiosira*. Good fits to a linear model were found when plotting egg production against ingestion rates in the experiments performed with *P. micans* ($r^2 = 0.33$, $P = 0.023$) and *T. weissflogii* ($r^2 = 0.49$, $P = 0.004$), although the P value obtained for *P. micans* makes this relationship to be considered carefully. Both regressions were statistically different (ANCOVA, $P < 0.001$).

Only copepods fed with *P. micans*, *T. weissflogii* and *C. pelagicus* produced an appreciable number of eggs. The average values obtained for these algae (at food concentration with maximum ingestion rates) are $22.7 \text{ eggs animal}^{-1} \text{ d}^{-1}$ with *P. micans*, $11.6 \text{ eggs animal}^{-1} \text{ d}^{-1}$ with *T. weissflogii* and $8.5 \text{ eggs animal}^{-1} \text{ d}^{-1}$ with *C. pelagicus*. These results translate (Table 2) into values of gross-efficiency (expressed as

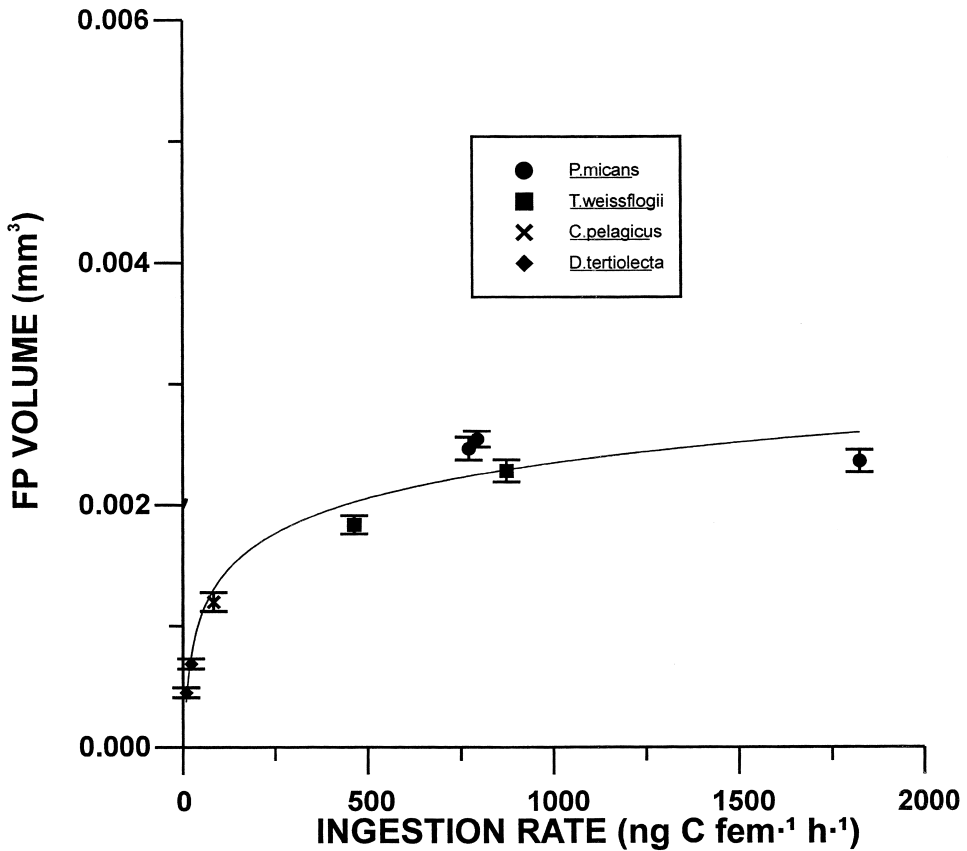


Fig. 5. *Calanus helgolandicus* faecal pellet individual volume (average and standard error) versus ingestion rate for four different algal diets. Fit: $y = 0.00041 \log x - 0.00054$; $n = 8$; $r^2 = 0.94$; $P = 0.00004$.

Carbon in eggs/Carbon ingested) between 0.16 and 0.34 for *P. micans*, 0.09 and 0.12 for *T. weissflogii*, and 0.72 for *C. pelagicus* (Table 2).

4. Discussion

To estimate ingestion rates in copepods using the disappearance of cells in incubation bottles has some experimental problems. Roman and Rublee (1980), Peters and Downing (1984) and others discuss the most important problems associated with this method. These problems include: behavioural effects, enhanced growth of algae in experimental bottles caused by ammonium excretion by copepods, loss of faecal pellets due to breakage, ingestion of pellets (coprophagy) and eggs (cannibalism) and particle generation by grazing copepods which can interfere with Coulter Multisizer counts. The problem of limiting normal copepod behaviour (bottle effects) is always present, but in

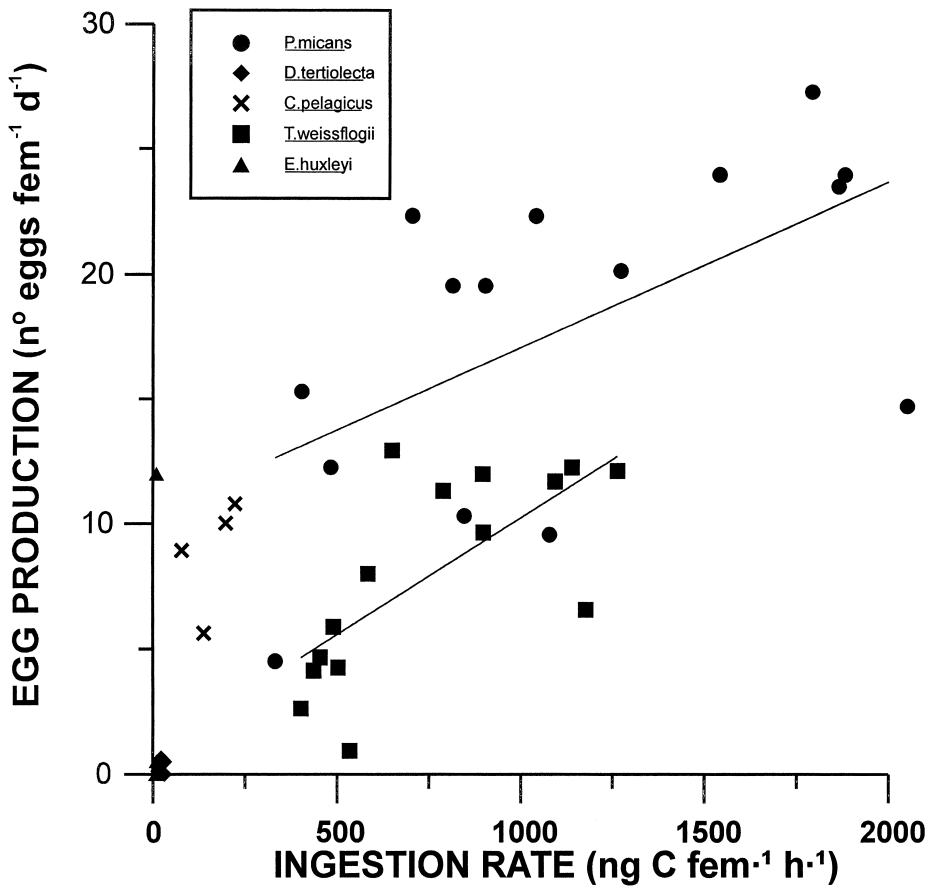


Fig. 6. Egg production obtained for *Calanus helgolandicus* feeding at different concentrations of five monospecific algal cultures. Fits: *P. micans*: $y = 0.00663x + 10.447$; $n = 15$; $r^2 = 0.33$; $P = 0.023$; *T. weissflogii*: $y = 0.00933x + 0.913$; $n = 15$; $r^2 = 0.49$; $P = 0.004$.

this kind of grazing experiment it is suggested not to be a limiting factor (Kjørboe et al., 1985).

Results obtained from this work suggest low ingestion efficiency of *Calanus helgolandicus* when feeding on coccolithophorids. From the five algal species offered, copepods only fed efficiently on the non coccolithophorids *Prorocentrum micans* and *Thalassiosira weissflogii*. Ingestion rates obtained with *Dunaliella tertiolecta* and with both coccolithophore species *Coccolithus pelagicus* and *Emiliania huxleyi* are very low, 4–5 times lower than with *Prorocentrum* and *Thalassiosira* at similar carbon concentrations. Few publications concerning copepod feeding on coccolithophorids are available, but some authors have previously found similar low efficiency of *Calanus* feeding on coccolithophorids, in particular *Emiliania huxleyi*. Nejtgaard (1997) reported maximum clearance rates for *Calanus finmarchicus* fed with *Emiliania huxleyi* of 15–41

ml cleared daily in a 3–47 000 cells ml⁻¹ suspension. Harris (1994), in experiments performed with *Calanus helgolandicus* and *Emiliana huxleyi*, found maximum values of 16 000 cells animal⁻¹ h⁻¹ at 24 000 cells ml⁻¹. Our results with *Emiliana* are even lower: 15 000 cells animal⁻¹ h⁻¹ at 36 000 cells ml⁻¹. We didn't find any previous data for *Coccolithus*, but our results are also very low. Maximum values obtained with this alga are 158 ng C animal⁻¹ h⁻¹ at 1000 cells ml⁻¹. The effect of food concentration on ingestion rates has been demonstrated for many phytoplankton and copepod species. We did not observe any ingestion at food concentrations below 36 000 cells ml⁻¹, however Harris (1994) and Nejstgaard (1997) both found linear and exponential relationships between both parameters, with measurable ingestion below this concentration. Nejstgaard found saturation at food concentrations around 750 µg C l⁻¹ while Harris did not find any saturation, but the maximum food concentration used was 24 000 cells ml⁻¹. Anyway carbon concentrations offered are higher than the maximum ones that the copepod could find in the sea during most bloom situations. Usually natural coccolithophorid concentrations are between 0.1 and 100 cells ml⁻¹ while in bloom situations they can reach 1000–6000 cells ml⁻¹ (Head et al., 1998) although extremely high values of 115 000 cells ml⁻¹ have been reported by Berge (1962) for Norwegian coastal waters and fjords. We offered a maximum food concentration 6 times higher than usual concentrations in the sea, so higher rates found in the literature for this food concentrations (or even higher) should be considered carefully when compared to the sea, because copepods usually do not experience such concentrations.

There are many papers demonstrating the importance of food cell size in determining ingestion rates (Frost, 1972, 1977; Lam and Frost, 1976; Richman et al., 1977; Cowles, 1979; Paffenhöfer, 1984; Berggreen et al., 1988; Støttrup and Jensen, 1990; Nejstgaard et al., 1995). In the genus *Calanus*, Frost (1977) has suggested a lower threshold of 4–11 µm for the food to be efficiently ingested and Nival and Nival (1976) found copepods unable to feed on particles smaller than 10 µm. Although not efficiently ingested, particles smaller than 10 µm have been found in FP produced by *Calanus finmarchicus*, so despite the small size, cells are ingested when abundant. Our results seem to confirm these previous findings. *Calanus helgolandicus* only fed efficiently when we offered medium-large algae. However, cell size is not the only factor determining low ingestion as *Coccolithus pelagicus* is larger than *Thalassiosira*, but is ingested at lower rates when offered at the same concentrations. Food quality has also been suggested to determine ingestion or rejection by copepods. Young (1994) suggests that coccoliths can inhibit copepod ingestion. Wolfe and Steinke (1996) suggest production of anti-predator metabolites related to DMS and Keller (1989) also suggested DMS as an agent which results in food rejection.

In the present work, *Calanus* actively ingested *Emiliana* and *Coccolithus*, but at very low rates, lower than those found in the literature. Our lower rates compared with previous publications are probably explained by the different season in which the experiments were performed. Nejstgaard (1997) suggests the strong influence of season on copepod ingestion and the present study seems to confirm that suggestion making our results to be considered as minimum estimates. Our experiments were performed in November, while Nejstgaard's and Harris' ones were performed in spring and summer, when natural coccolithophorid blooms occur in the sea and the copepods could be

adapted to the available food. Nejstgaard et al. (1995) also found that ingestion by *Calanus finmarchicus* grazing on *Emiliana huxleyi* in laboratory experiments does not occur when experiments were performed during winter, at food concentrations similar or even higher than the ones we used. According to this importance of season, the highest feeding (and reproduction) rates was achieved on the dinoflagellate diet. Although this could simply be explained solely by the large size, this could be also an effect of a different seasonal copepod adaptation to this type of algae, which sometimes form blooms during fall and even later in the season in temperate waters.

Besides the low ingestion, we also found no egg production with *Emiliana huxleyi*, not even when offered at high concentrations. Despite the large number of published studies on the influence of diet on egg production by copepods, there are not so many using coccolithophorids as food. Even considering the extremely low ingestion found with this food, egg production is lower than expected from the high values found by Nejstgaard et al. (1997) for *Calanus finmarchicus* (45 eggs animal⁻¹ d⁻¹ in laboratory and 67 eggs animal⁻¹ d⁻¹ in mesocosm experiments). Probably, the late time of the season, with the copepods approaching the end of their reproductive period is the origin of this low production. This could be also the explanation of why the egg production rates obtained with non-coccolithophore foods are lower than the ones found in literature: 50 eggs animal⁻¹ d⁻¹ for *Calanus pacificus* fed with *Thalassiosira weissflogii* (Runge, 1984), 50 eggs animal⁻¹ d⁻¹ for *Calanus pacificus* fed with *Prorocentrum* and 75 eggs animal⁻¹ d⁻¹ with *Thalassiosira* (Uye, 1996), 39 eggs animal⁻¹ d⁻¹ for *Calanus pacificus* with *Prorocentrum micans* and 23 eggs animal⁻¹ d⁻¹ with *Thalassiosira weissflogii* (Peterson, 1988). The risk of egg cannibalism in bottles without meshes must be considered, especially in the presence of less preferred food like coccolithophorids. The methodology used could have contributed to the low apparent egg production rates obtained with this alga.

On the other hand, egg production found with *Coccolithus* is very surprising. Values obtained are very high if we consider that ingestion rates, in terms of carbon, were even lower than the ones found with *Emiliana*. No previous data have been found in literature with this alga, but probably chemical composition makes *Coccolithus* a very nutritional food in order to maintain secondary production. It has been suggested that high levels of n-3 PUFA and other lipids found in coccolithophorids (Pond and Harris, 1996) could represent a high nutritional value for reproduction (Nejstgaard et al., 1997). Anyway, discussion of results obtained with *C. pelagicus* may be taken carefully, due to the small number of experiments performed with this alga.

If we consider the ingestion rates observed in relation to the respiration requirements (5.9 mg C d⁻¹) calculated using formulae proposed by Ikeda (1985) for a copepod of 71 µg C and a temperature of 15°C, coccolithophorids do not supply enough carbon to maintain respiratory requirements (Table 3). During this late season, grazing on *Emiliana* only supplies 8.2% of the requirements (and only when offered at very high concentrations), and *Coccolithus* only supplies 26–64% of carbon requested. For these calculations we have to make the unrealistic assumption that all the carbon ingested when feeding on *Emiliana* is assimilated, because no eggs and no FP are produced when feeding on this alga. This could be the explanation of why no eggs are produced with *Emiliana* and *Dunaliella*, and makes more surprising the value of 85% gross

Table 3
Carbon balance for the different algal diets offered

Alga	Food concentration ($\mu\text{g C l}^{-1}$)	IR (ng C d^{-1})	ng C in Respiration ing-resp-eggs eggs d^{-1} (ng C d^{-1})			% non assimilated
<i>P. micans</i>	854	43 824	7264	5887	30 673	69.99
<i>P. micans</i>	445	18 528	6345.6	5887	6295.4	33.98
<i>P. micans</i>	220	19 080	3638.4	5887	9554.6	50.08
<i>T. weissflogii</i>	491	22 104	2835.2	5887	13 381.8	60.54
<i>T. weissflogii</i>	237	20 976	3724.8	5887	11 364.2	54.18
<i>T. weissflogii</i>	123	11 136	1078.4	5887	4170.6	37.45
<i>D. tertiolecta</i>	284	3120	0	5887	–	–
<i>D. tertiolecta</i>	189	4416	0	5887	–	–
<i>D. tertiolecta</i>	94	4488	0	5887	–	–
<i>D. tertiolecta</i>	48	528	89.6	5887	–5448.6	–
<i>D. tertiolecta</i>	24	213.6	41.6	5887	–5715	–
<i>C. pelagicus</i>	233	3792	0	5887	–	–
<i>C. pelagicus</i>	121	3792	2732.8	5887	–4827.8	–
<i>C. pelagicus</i>	58	1560	0	5887	–	–
<i>E. huxleyi</i>	468	4848	0	5887	–	–
<i>E. huxleyi</i>	314	199.44	208	5887	–5895.56	–
<i>E. huxleyi</i>	161	216	0	5887	–	–

efficiency found with *Coccolithus*. This value is much higher than that found in the literature not only for *Calanus*, but for other copepods fed with different diets as well. Stored lipids must sustain this high egg production; no egg production could be maintained with the carbon ingested when offered this alga as the only food. Further investigations will be required to understand the high nutritional value of *Coccolithus* related to secondary production. Only copepods fed with *Prorocentrum* and *Thalassiosira* have ingestion high enough to compensate respiration and to maintain the observed egg production.

Once respiratory requirements and carbon invested in eggs are discounted, we observed a positive balance in the experiments performed with *Thalassiosira* and *Prorocentrum*, but not with the other algae. If we assume that the copepod does not accumulate stores (it seems reasonable if it is producing eggs) and does not grow (we have selected adult females), the rest of the ingested carbon not respired and not converted in eggs, is not assimilated carbon and should be egested as FP. Considering the number and volume of FP egested in our experiments we obtain values of 0.07–0.09 mg C mm^{-3} for pellets produced with *Prorocentrum*, and 0.1–0.13 mm^{-3} for pellets produced with *Thalassiosira*. These results are similar to those found by Andreassen et al. (1996) in *Calanus* pellets fed with natural suspensions and translates into values of 34–72% of non-assimilated carbon for *Prorocentrum* and 37–63% for *Thalassiosira*. For both diets higher assimilation is found at lower food concentrations suggesting copepod ingestion is more efficient when food is scarce. When abundant, it is ingested at higher rates but with lower assimilation.

As a conclusion of the present work, the two coccolithophore diets are not adequate to maintain *Calanus helgolandicus* metabolic, respiratory or reproductive requirements during this late season. Ingestion rates may be high enough at higher food concentrations

early in the season, Nejstgaard (1997) found much higher ingestion (comparable to the ones we found with *Thalassiosira* or *Prorocentrum*) at double the concentrations of the maximum ones we used. During late autumn, *Calanus helgolandicus* should complement its diet with non-coccolithophorid algae or with non algal food. Kleppel (1993) suggests the importance of diet diversity in copepod feeding. This effect of coccolithophorid diets on *Calanus helgolandicus* feeding and reproduction suggests difficulties in the existence of well developed *Calanus helgolandicus* populations during monospecific blooms in this late season, so the potential role of this genera in carbon flux during such situations may be restricted.

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