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# Feeding rates of *Calanoides carinatus*: a comparison of five methods including evaluation of the gut fluorescence method

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ABSTRACT: A comparison was made of ingestion rates of the copepod *Calanoides carinatus* estimated from the disappearance of chlorophyll (by fluorometry and HPLC), disappearance of cells, egg production, and from the gut fluorescence technique. Three measures of gut turnover time were also compared. Our primary goal was to determine whether the gut fluorescence method underestimated ingestion and if so, to determine if the problem was due (i) to pigment digestion or (ii) to incorrect estimates of gut passage time. Experiments used as food the diatom *Thalassiosira weissflogii* at 3 concentrations. The first 4 methods yielded the same result: grand means were 2.7 µg chl $d^{-1}$ at 8000 cells $ml^{-1}$, 1.5 µg chl $d^{-1}$ at 3000 cells $ml^{-1}$ and 0.84 µg chl $d^{-1}$ at 1500 cells $ml^{-1}$. The gut fluorescence technique underestimated ingestion, yielding rates of 1.0, 0.92 and 0.58 µg chl $d^{-1}$ when calculated following the traditional approach of multiplying gut pigment content by gut evacuation rate (GER). The underestimate was not due to pigment digestion because pigment budgets showed that all pigment was accounted for, 106 % on average. Thus we suggest that the problem was associated with using GER as a measure of gut passage time (GPT). GPT estimated from the GER was 16.7 min, but when estimated from (i) measurements of fecal pellet poduction rates and from (ii) the ratio, pigment egestion rate/mean gut pigment content, results averaged 10.2 min. Using this value, ingestion rates were equal to the other techniques at the intermediate and low food concentrations, and 80 % of the other techniques at the high concentration.

## INTRODUCTION

Since its re-discovery in the 1980's, the gut fluorescence technique (Nemoto 1968, Mackas & Bohrer 1976, Dagg & Wyman 1983) has been used widely to study feeding rates of herbivorous zooplankton both in the laboratory and in the sea. The technique has been particularly useful for the study of feeding rates in situ. With it, one avoids artifacts associated with lengthy incubations (Roman & Rublee 1980) and with the use of electronic particle counters (Harbison & McAlister 1980). The technique permits easy collection of data needed to calculate feeding rates of several species of herbivorous zooplankton collected simultaneously, thus allowing evaluation of food niche separation. Studies of diel variations in feeding rate are simple to conduct as is investigation of the feeding impact of herbivorous zooplankton on a phytoplankton assemblage (e. g. Peterson et al. 1990).

Recently it has been suggested that the technique may not produce unbiased results. The method involves measurement of 2 parameters, gut pigment content (G) and gut clearance rate constant (k). The two are combined to yield an estimate of ingestion rate (I) as follows:

$$I = k\,G$$

with units of I, pigment ind.$^{-1}$ time$^{-1}$; k, time$^{-1}$; and G, pigment ind.$^{-1}$. It has been suggested that there may, at times, be significant amounts of pigment digestion such that any measurement of gut pigment content underestimates the actual gut pigment content (Conover et al. 1986, Lopez et al. 1988). Furthermore it is possible that k is underestimated by the conventional method of calculating the decline in gut pigment content G, with time for specimens held in filtered sea-

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water (Huntley et al. 1987). Thus, if either k or G (or both) are underestimated, ingestion rate will be underestimated.

In this study we set out firstly to measure copepod feeding rates using 5 different techniques, including the gut fluorescence method, in order to determine if measurements of gut pigment content, G, lead to underestimates of grazing rates. Secondly, we evaluated the problem of pigment digestion by constructing pigment budgets for the grazing experiments. Finally, we investigated the possibility that the gut evacuation rate constant, k, is a source of error by comparing gut passage times measured by 3 independent methods.

## METHODS

Zooplankton was collected at a station 2 km offshore of Hout Bay, South Africa, by suspending a plankton net at a depth of 20 m and sampling for 5 min while the ship drifted. Copepods were returned to the laboratory in plastic buckets and adult female *Calanoides carinatus* sorted with wide-bore pipettes. The elapsed time between capture and sorting was 2 or 3 h. After sorting, the copepods were fed the diatom *Thalassiosira weissflogii* at a concentration corresponding to the planned experimental concentration. Specimens were maintained at that concentration for at least 24 h before being used in an experiment.

**Disappearance of chlorophyll and cells.** For each experiment measurements of the disappearance of chlorophyll and cells were initiated as follows: a small amount of a culture of *Thalassiosira weissflogii* was added to a 20 l bucket filled with 0.45 µm filtered seawater, and the cell concentration was measured. Successive additions were made until the desired cell concentration was achieved. Then, triplicate measurements of cell concentration were made with the Coulter Multisizer and triplicate subsamples (50 and 250 ml) were filtered through GF/F filters for later determination of chlorophyll concentration by fluorometry and HPLC, respectively. Four or five 1.1 l plastic bottles were filled with the food suspension and 4 or 5 female *Calanoides carinatus* were added to each bottle. The bottles were attached to a plankton wheel (1 rpm) and incubated in a 15 °C room on a 13:11 h light-dark cycle (10 µE $m^{-2}$ $s^{-1}$) for 24 h. Two control bottles without copepods were set up for measurement of phytoplankton growth rates.

At the end of the incubation period, the water in each bottle was filtered through a 40 µm Nitex nylon screen (to remove eggs, females and fecal pellets). Triplicate determinations were then made of chlorophyll *a* and phaeopigments by fluorometry (50 ml subsamples), of chlorophyll *a* by HPLC (200 ml subsamples) and of cell numbers in the filtrate, from each 'grazed' and 'control' bottle. Ingestion rates in terms of chlorophyll and cells were calculated following Frost's (1972) equations. Ingestion rates based on cell counts were converted to chlorophyll units by multiplying by the factor, chlorophyll $cell^{-1}$, determined by dividing chlorophyll concentration (as measured by HPLC) by cell concentration (as measured by the Coulter Counter). The factor used was the mean of the t = 0 and t = 24 h values of chlorophyll $cell^{-1}$ (see Table 1).

A pigment budget based on the disappearance of chlorophyll measurements was calculated as follows: the amount of pigment (chlorophyll + phaeopigment) in the fecal pellets was compared to the amount of pigment ingested. Fecal pellets retained on the 40 µm Nitex screen were rinsed into a small glass dish with filtered seawater, then filtered onto a GF/F filter. Pigment content was measured by fluorometry.

For all fluorometric chlorophyll determinations, filters were extracted in 10 ml of 90 % AR acetone in centrifuge tubes, stored in the dark at −20 °C for 24 h, then homogenized. The glass fiber/acetone slurry was centrifuged at 800 × *g* for 10 min. Fluorescence of the supernatant was measured using a Turner Designs Fluorometer before and after acidification with 2 drops of 10 % HCl. Pigment concentrations were calculated as described in Dagg & Walser (1987). This yields an estimate of the amount of pigment (chlorophyll + phaeopigment) in units of µg chlorophyll $ind.^{-1}$, and does not include any correction for pigment digestion.

For chlorophyll analysis by HPLC, filtered algal samples were extracted immediately after filtration in 100 % AR acetone by homogenisation, to minimise chlorophyllase activity (see Jeffrey & Hallegraeff 1987). Chlorophyll *a* was separated from other pigments by reverse-phase HPLC on a Varian 5000 chromatograph using a modification of the method of Mantoura & Llewellyn (1983): Solvent A was similar to that used by Mantoura & Llewellyn while Solvent B consisted of 60 % methanol and 40 % acetone. Pigments were separated on a Beckmann Ultrasphere XL-C18, 3 µm column (4.6 × 700 mm) at a flow rate of 2 ml $min^{-1}$ by gradient elution from 75 % A:25 % B, to 100 % B in 5 min, followed by an isocratic hold of 100 % B for 4 min. Pigments were detected by absorbance at 440 nm; chlorophyll *a* standard was purchased from Sigma (USA).

**Egg production measurements.** Five 1.1 l plastic bottles were filled with the seawater-diatom mixture and 2 female *Calanoides carinatus* added. The bottles were incubated on a plankton wheel as described above. After 24 h the water (containing eggs and females) in each bottle was filtered through a 60 µm Nitex screen and the eggs counted immediately. Egg production measurements were converted to ingestion

rates in terms of chlorophyll ingested using the following formula:

$$I = [(E)\ (C_E)]/[(C{:}chl)\ (K_1)]$$

where E = egg production rate (eggs female$^{-1}$ d$^{-1}$); $C_E$ = carbon content of an egg; (C:chl) = carbon:chlorophyll ratio; and $K_1$ = gross efficiency of egg production. The carbon content of an egg was estimated by measuring egg diameter (160 µm) then applying a carbon to volume conversion factor of 0.14 × $10^{-6}$ µg C µm$^{-3}$ to egg volume (Kiørboe et al. 1985). Carbon:chlorophyll ratios were obtained for each of the experiments by dividing carbon cell$^{-1}$ by chlorophyll cell$^{-1}$. Carbon cell$^{-1}$ (= 230 pg C cell$^{-1}$) was determined by measuring the protein, carbohydrate and lipid contents of *Thalassiosira weissflogii*, then converting each to carbon using conversion factors in Omori & Ikeda (1984, p. 97). Methods for determination of biochemical composition were Lowry (for protein), phenol-sulfuric acid (for carbohydrates) and Bligh-Dyer (for lipid); additional details will be presented elsewhere (Peterson, Barlow & Huggett unpubl.).

In converting the egg production rate to chlorophyll ingestion rate, the gross efficiency of egg production ($K_1$) was assumed to be 0.33 (from Kiørboe et al. 1985, Peterson 1988). Though our measurements of ingestion (of phytoplankton cells) permitted calculation of the true $K_1$, we used a constant factor of 0.33 so that the egg production rate represented an independent estimate of ingestion. As shown in Table 2, the true (= measured) $K_1$ was 0.35, thus corroborating our assumption.

**Gut fluorescence measurements.** Female *Calanoides carinatus* were placed into 1 l beakers or 1.1 l bottles filled with the seawater-diatom mixture at a concentration of 4 or 5 individuals per container. Copepods in one beaker or bottle were sacrificed approximately every 2 h over a 24 h period, and females placed into a centrifuge tube with 10 ml of 90 % acetone. Tubes were stored in the dark at −20 °C for 24 to 48 h, then centrifuged at 800 × *g*. Fluorescence before and after acidification was measured with the aid of a Turner Designs fluorometer. The amount of pigment ingested (I) over a 24 h period was calculated from:

$$I = k\ G \qquad (1)$$

where G = mean gut pigment content per individual averaged for all observations at a given food concentration; k = gut evacuation rate in units of d$^{-1}$.

Gut evacuation rate was measured by first allowing 20 to 30 females to feed for several hours, then removing them with a 500 µm Nitex screen and rinsing them into a 5 l beaker filled with 0.45 µm filtered seawater. Two to 4 individuals were removed at 10 min intervals, over a 40 min experimental period, placed into centrifuge tubes with 10 % acetone and processed as above. Gut evacuation rate (k) was calculated from the relationship.

$$G_t = G_o\ e^{-kt} \qquad (2)$$

where G = gut pigment content; t = time. Measurements were made at 1500 and 8000 cells ml$^{-1}$.

Gut passage time was estimated in 2 ways. One, in a single experiment (at 3300 cells ml$^{-1}$ of *Thalassiosira weissflogii*), 5 individual female *Calanoides carinatus* were incubated for 24 h. The number of fecal pellets produced by each individual was determined by direct count and the mean time interval between production of fecal pellets calculated. Assuming that 2 pellets occur in the gut simultaneously, the gut passage time is then twice the time interval between the production of a single pellet. The other method (of Dagg & Walser 1987) was to divide mean gut pigment content by hourly pigment egestion rate. Hourly pigment egestion rates were obtained by dividing the total amount of pigment found in the fecal pellets after 24 h (shown in Table 4), by 24.

## RESULTS

Ingestion rates measured by the methods of disappearance of chlorophyll (by fluorometry and HPLC) and disappearance of cells are listed in Table 1. Within experiments (i. e. at a given food concentration), standard errors of the mean were usually between 5 and 20 % of the mean and coefficients of variations ranged from 20 to 40 %. Agreement between techniques was excellent; mean ingestion rates calculated for each technique were within 4 to 13 % of the mean of the 3 techniques combined.

Egg production rate data (Table 2) ranged from 75 eggs female$^{-1}$ d$^{-1}$ at 8000 cells ml$^{-1}$ to 32 eggs d$^{-1}$ at 1500 cells ml$^{-1}$. In units of carbon, egg production ranged from 22.4 µg C produced female$^{-1}$ d$^{-1}$ at the highest food concentration (= 45 % of female body weight) to 9.5 µg C female$^{-1}$ d$^{-1}$ (= 19 % of body weight) at the lowest food concentration. The gross efficiency of egg production averaged 0.35.

Changes in gut pigment content (GPC) vs time at each of the 3 cell concentrations are shown in Fig. 1. As expected GPC was highest at the highest food concentrations. There was little evidence of diel variation in GPC. Measurements of the gut evacuation rate constant averaged 0.0598 min$^{-1}$ ($R^2$ = 0.95 and 0.97, n = 5 for both experiments). Ingestion rates calculated from the gut pigment content and evacuation rates were 1.11 µg chl female$^{-1}$ d$^{-1}$ at 8000 cells ml$^{-1}$,0.84 at 3000 cells ml$^{-1}$ and 0.64 at 1500 cells ml$^{-1}$.

Results of all experiments are listed in Table 3. Ingestion rates estimated from disappearance of chlorophyll and cells were the same as rates estimated from egg

Table 1. *Calanoides carinatus*. Ingestion rates (ng chl *a* female$^{-1}$ h$^{-1}$), of copepods at 3 food concentrations, calculated from disappearance of chlorophyll and phytoplankton cells during 24 h laboratory incubations. Chlorophyll concentrations were measured by fluorometry and HPLC, and cells were counted on a Coulter Multisizer. Particle counts were converted from cells h$^{-1}$ to ng chl *a* h$^{-1}$ using factors (pg chl *a* cell$^{-1}$) calculated for each experiment: 9.7 at 8000 cells ml$^{-1}$, 8.9 at 3000 cells ml$^{-1}$ and 6.5 at 1500 cells ml$^{-1}$. $S_{\bar{x}}$: standard error of the mean; CV: coefficient of variation (= standard deviation/mean)

| Bottle | 8000 cells ml$^{-1}$ | | | 3000 cells ml$^{-1}$ | | | 1500 cells ml$^{-1}$ | | |
|---|---|---|---|---|---|---|---|---|---|
| | Fluorometry | HPLC | Cell counts | Fluorometry | HPLC | Cell counts | Fluorometry | HPLC | Cell counts |
| 1 | 165.6 | 232.5 | 126.3 | 78.3 | 57.2 | 49.4 | 49.2 | 44.8 | 48.5 |
| 2 | 80.9 | 45.6 | 82.6 | 54.0 | 48.1 | 111.4 | 22.9 | 19.9 | 17.6 |
| 3 | 87.1 | 142.8 | 127.6 | 51.9 | 48.5 | 44.7 | 22.9 | 40.7 | 20.1 |
| 4 | 81.6 | 71.9 | 85.8 | 46.2 | 50.1 | 63.4 | 35.0 | 39.0 | 36.0 |
| 5 | | | | | | | 47.7 | 39.0 | 43.1 |
| Mean | 103.8 | 123.2 | 105.6 | 57.6 | 51.0 | 67.2 | 35.5 | 36.7 | 33.1 |
| $S_{\bar{x}}$ | 20.65 | 41.82 | 12.36 | 7.09 | 2.12 | 15.25 | 5.72 | 4.33 | 6.14 |
| CV | 0.40 | 0.68 | 0.23 | 0.25 | 0.08 | 0.45 | 0.36 | 0.26 | 0.42 |

Table 2. *Calanoides carinatus*. Summary of egg production data at the 3 experimental food concentrations. Units are eggs female$^{-1}$ d$^{-1}$. Egg counts were converted to carbon units using a factor of 0.30 µg C egg$^{-1}$. Carbon content of a female *C. carinatus* (50 µg carbon female$^{-1}$) was calculated as 40 % of dry weight

| Bottle | 8000 cells ml$^{-1}$ | 3000 cells ml$^{-1}$ | 1500 cells ml$^{-1}$ |
|---|---|---|---|
| 1 | 77 | 52 | 37 |
| 2 | 82 | 30 | 26 |
| 3 | 79 | 47.3 | 0 |
| 4 | 60 | 46 | 46 |
| 5 | – | 23 | 50 |
| 6 | – | 72.5 | – |
| 7 | – | 66.5 | – |
| 8 | – | 23 | – |
| n (females) | 8 | 16 | 10 |
| Mean (eggs fem.$^{-1}$ d$^{-1}$) | 74.5 | 45.0 | 31.8 |
| $S_{\bar{x}}$ | 4.94 | 6.64 | 8.96 |
| Egg carbon (µg C fem.$^{-1}$ d$^{-1}$) | 22.4 | 13.5 | 9.5 |
| Ingested carbon[a] (µg C fem.$^{-1}$ d$^{-1}$) | 63.3 | 36.2 | 29.6 |
| Carbon:chlorophyll | 23.7 | 25.7 | 35.2 |
| Gross efficiency of egg production ($K_1$) | 0.35 | 0.37 | 0.32 |

[a] Calculated using the data on chlorophyll ingested per day averaged from the measurements of disappearance of chlorophyll and disappearance of cells (from Table 1), converted to carbon ingested using the carbon:chlorophyll ratios shown here

production measurements. Rates derived from gut fluorescence data were less than the other techniques. Differences were 41 % of the grand mean at 8000 cells ml$^{-1}$, 58 % at 3000 cells ml$^{-1}$ and 76 % at 1500 cells ml$^{-1}$.

Pigment budgets (Table 4) showed that all of the pigment ingested during the grazing experiments was accounted for. When pigment egested was compared to pigment ingested on a per individual basis, 85 % of the pigment ingested was accounted for at 8000 cells ml$^{-1}$, 100 % at 3000 cells ml$^{-1}$ and 133 % at 1500 cells ml$^{-1}$, an average of 106 %. When pigment in the grazed bottles (i.e. pigment in all remaining cells + pigment in fecal pellets) was compared to pigment in the control bottles after 24 h, the result was 98, 95 and 94 % respectively (Table 4). Thus we conclude that chlorophyll *a* is not digested nor degraded to non-fluorescing molecules in the guts of *Calanoides carinatus*.

The shortfall between ingestion rates calculated from gut fluorescence data and ingestion rates estimated from the other 4 methods must result from incorrect estimates of gut passage time rather than pigment digestion. The gut clearance rate constant used in our calculations, 0.0598 min$^{-1}$, is equivalent to a gut passage time of 16.7 min (= 1/k). Based on the other 2 methods used to estimate gut passage time (discussed below), it would seem that this rate is too slow, by about 40 %.

By direct observation of defecation, we found that a

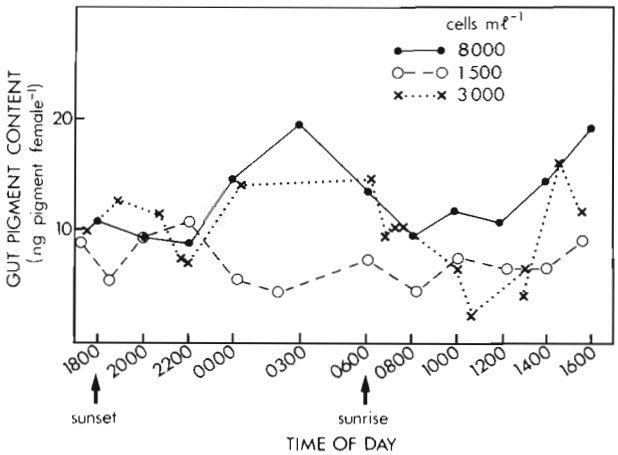


Fig. 1. *Calanoides carinatus*. Changes in gut pigment content as a function of time, for females feeding on the diatom *Thalassiosira weissflogii* at concentrations of 1500, 3000 and 8000 cells $ml^{-1}$

tent agreed better with those estimated from the other methods: 1.8 vs 2.7 µg chl copepod$^{-1}$ d$^{-1}$ at 8000 cells $ml^{-1}$, 1.4 vs 1.5 at 3000 cells $ml^{-1}$ and 1.0 vs 0.84 at 1500 cells $ml^{-1}$. A shortfall remained only for the highest food concentration. In that experiment there could have been as much as a 15 % pigment loss (Table 4) which would account for all but 19 % of the pigment.

## DISCUSSION

Excellent agreement was found between ingestion rate measured by disappearance of chlorophyll, disappearance of cells and from egg production data. Thus any of these techniques is suitable for estimation of daily ingestion rate for copepods in the laboratory as well as in the sea, as noted by Kiørboe et al. (1985) in their comparison of the same techniques. With respect

Table 3. *Calanoides carinatus*. Results of all ingestion rate measurements. Data are means of 4 or 5 experimental bottles including their corresponding standard errors ($S_{\bar{x}}$). For most of the data listed, units of ingestion rate are µg chl *a* female$^{-1}$ d$^{-1}$ For gut pigment data, units are: ng chl *a* female$^{-1}$ (mean gut pigment content, GPC),and µg chl *a* female$^{-1}$ d$^{-1}$ (pigment ingested). $K_1$ = gross efficiency of egg production; a constant 0.33 was used in the calculations (see 'Methods')

| Method | Ingestion rates (µg chl *a* female$^{-1}$ d$^{-1}$) | | | | | |
|---|---|---|---|---|---|---|
| | 8000 cells $ml^{-1}$ | | 3000 cells $ml^{-1}$ | | 1500 cells $ml^{-1}$ | |
| | Mean | $S_{\bar{x}}$ | Mean | $S_{\bar{x}}$ | Mean | $S_{\bar{x}}$ |
| Disappearance of chl *a* | | | | | | |
| Fluorometry | 2.5 | 0.50 | 1.4 | 0.17 | 0.85 | 0.14 |
| HPLC | 3.0 | 1.00 | 1.2 | 0.05 | 0.88 | 0.10 |
| Disappearance of cells[a] | 2.5 | 0.30 | 1.6 | 0.37 | 0.79 | 0.15 |
| Egg production[b] | 2.9 | 0.17 | 1.6 | 0.23 | 0.82 | 0.23 |
| Grand mean | 2.73 | | 1.45 | | 0.84 | |
| Gut pigment data | | | | | | |
| Mean GPC | 12.9 | 1.13 | 9.6 | 0.99 | 7.3 | 0.62 |
| Pigment ingested | 1.11 | | 0.84 | | 0.64 | |
| Percent underestimate: | | | | | | |
| (Pigment ingested/grand mean) | 41 % | | 58 % | | 76 % | |

[a] I = (cells d$^{-1}$) (chl *a* cell$^{-1}$)
[b] I = (eggs fem.$^{-1}$ d$^{-1}$) (µg C egg$^{-1}$)/(C [chl *a*]$^{-1}$) ($K_1$)

pellet appeared on average every 6.4 min. If we assume that only 2 pellets are present in the gut at any time (pers. obs.; confirmed by Bautista et al. 1988) then the gut passage time for a pellet is twice 6.4 min, or 12.8 min. Using Dagg & Walser's (1987) method (in which the average gut pigment content is divided by the hourly egestion rates), gut passage times on the order of 9 min were calculated (Table 5). The overall mean of both methods was 10.2 min.

When the gut passage time of 10.2 min (rather than 16.7 min) was used as an estimate of gut evacuation rate, ingestion rates calculated from gut pigment content to measurements of disappearance of chlorophyll, there was no reason to prefer use of an HPLC over fluorometry. Except for the experiment at 8000 cells $ml^{-1}$, the standard errors associated with both instruments were small and about equal. In fact none of the 4 techniques showed a tendency to be more or less prone to error in our experiments.

The use of egg production data as a means of estimating ingestion rate is probably the most likely method to produce erroneous results. Egg production may not necessarily be an exact multiple of ingestion, especially if feeding and egg production are not in

Table 4. *Calanoides carinatus*. (a) Comparison of pigment (chlorophyll + phaeopigment) ingestion (I) and egestion (E) rates for females. Units are µg pigment female$^{-1}$ d$^{-1}$. $S_{\bar{x}}$: standard error of the mean; CV: coefficient of variation. (b) Comparison of pigment in 'grazed' bottles (pigment in phytoplankton cells + pigment in fecal pellets) and 'control' bottles (pigment in phytoplankton cells only) at the end of each 24 h experiment. The small amount of phaeopigment in the 'control' bottles was taken into account in the calculations. Units are µg pigment l$^{-1}$

| (a) | 8000 cells ml$^{-1}$ | | 3000 cells ml$^{-1}$ | | 1500 cells ml$^{-1}$ | |
|---|---|---|---|---|---|---|
| | I | E | I | E | I | E |
| Mean | 2.49 | 2.11 | 1.39 | 1.39 | 0.85 | 1.13 |
| $S_{\bar{x}}$ | 0.49 | 0.18 | 0.17 | 0.07 | 0.14 | 0.09 |
| CV | 0.40 | 0.17 | 0.24 | 0.10 | 0.36 | 0.18 |
| E/I | 85 % | | 100 % | | 133 % | |
| (b) | 8000 cells ml$^{-1}$ | | 3000 cells ml$^{-1}$ | | 1500 cells ml$^{-1}$ | |
| | Grazed | Control | Grazed | Control | Grazed | Control |
| Mean | 81.0 | 79.5 | 32.30 | 33.98 | 10.69 | 10.63 |
| $S_{\bar{x}}$ | 1.03 | 0.77 | 0.51 | 0.68 | 0.12 | 0.08 |
| CV | 0.03 | 0.02 | 0.03 | 0.05 | 0.03 | 0.02 |
| G/C | 102 % | | 95 % | | 100 % | |

Table 5. Estimates of gut passage time, calculated from mean gut pigment content (GPC) divided by hourly egestion rate (Dagg & Walser 1987). GPC values are listed in Table 3; hourly egestion rates are from data in Table 4

| Food conc. (cells ml$^{-1}$) | Mean GPC (ng female$^{-1}$) | Egestion rate (ng female$^{-1}$ h$^{-1}$) | Gut passage time (h) | (min) |
|---|---|---|---|---|
| 8000 | 12.9 | 87.9 | 0.147 | 8.8 |
| 3000 | 9.6 | 57.9 | 0.166 | 9.9 |
| 1500 | 7.3 | 47.1 | 0.155 | 9.3 |

equilibrium. For example, for several species of *Calanus* which had been previously starved for a few days there was a several-day time lag between renewed feeding and resumption of egg production (Runge 1984, Peterson 1988, Attwood & Peterson 1989). In our experiments females were fed *Thalassiosira weissflogii* at the experimental concentration for several days before being used in the experiments. The fact that the gross efficiency of egg production averaged 35 %, the 'expected' efficiency, suggests that the females were fully acclimated to the food supply.

Our suggestion that chlorophyll is not digested or reduced to non-fluorescing molecules in copepod guts is in agreement with some workers but in stark contrast to others. Trivial losses were reported by Dagg & Walser (1987), Kiørboe & Tiselius (1987) and Pasternak & Drits (1988): no more than 10 % of ingested pigment was unaccounted for. The lack of any pigment digestion can be inferred from 2 other studies. First, in a study similar to ours, Kiørboe et al. (1985) compared several grazing techniques. In their study of 5 copepod species feeding in situ, the gut fluorescence technique gave the same result as did techniques based on chlorophyll, cells or egg production measurements, for *Calanus finmarchicus*, *Acartia* spp., *Centropages hamatus* and *Pseudocalanus* sp., but not for *Temora longicornis*. Second, Kiørboe et al. (1982) found good agreement between ingestion rates for *C. hamatus* estimated using gut fluorescence and disappearance of chlorophyll methods.

Two studies have reported extremely high losses: Conover et al. (1986), 95 % loss, and Lopez et al. (1988), up to 92 % loss. It has been suggested that there may have been methodological problems in the Conover study (Kiørboe & Tiselius 1987). The Lopez study is difficult to evaluate because of extreme variability in their experiments: in 2 of 7 experiments there was no pigment loss but in 3 of 7 the losses were 69, 74, and 92 %. The mean of 7 experiments was 45 % with a coefficient of variation of 0.81. All other gut pigment studies which included some form of pigment budgeting reported an average loss of about 30 % (reviewed in Dam & Peterson 1988 and Lopez et al. 1988). Since the range in pigment loss is 0 to 95 %, we suggest that the degree of pigment digestion should be estimated routinely, by constructing pigment budgets. They can be prepared easily from measurements of ingestion using the method of disappearance of chlorophyll.

We concluded that gut pigment content data underestimated the true ingestion rate because of the man-

ner in which gut passage times were estimated. The method most commonly used is to place a group of copepods with full guts in filtered seawater and monitor the decline in gut pigment content with time. This method provides a measure of the gut evacuation rate, and the inverse is assumed to be an estimate of gut turnover time, similar to gut passage time. The method does provide a measure of gut evacuation rate *in the absence of feeding*, but is it a measure of gut passage time for a copepod that is actively feeding? We suggest, as have others, that it is not. It seems reasonable to expect that the residence time of a packet of food in the gut will be shorter if a copepod is feeding than if it is not feeding. Assuming, on a time scale of minutes, a fairly continuous feeding rate, the rate at which food is consumed must be the same as the rate at which it is egested. Conversely, the rate of defecation of copepods held in filtered seawater is controlled solely by peristalsis. Thus it seems logical to assume that defecation rate will be slower in starved than in feeding animals. Some evidence for this is that Dagg & Walser (1987) showed that gut passage time increased at progressively lower food concentrations. On the other hand, Ellis & Small (1989) found no differences in gut evacuation rates among feeding and non-feeding female *Calanus marshallae*; thus the problem remains open for investigation.

Several other workers have suggested that the method of measuring gut clearance rate in filtered seawater leads to estimates of gut passage time that are too long. Kiørboe & Tiselius (1987) showed that when *Acartia tonsa* were fed the alga *Rhodomonas baltica* and then transferred to feeding on the non-pigmented ciliate *Oxyrhhis marina*, the gut clearance rate, k, was 0.048 $min^{-1}$ as compared to 0.039 $min^{-1}$ for copepods held in filtered seawater. The inverse of k was 20.8 vs 25.6 min respectively, a difference of 19 %.

The value of gut evacuation rate constant obtained from gut clearance measurements is also dependent upon the length of time, t, over which an experiment is conducted. As t approaches zero the value of k increases greatly. There seems to be a growing consensus that k should only be estimated over the initial 30 min after placing the copepods in filtered seawater (see Dam & Peterson 1988 for references). Our k value was derived from measurements made over a 40 min period. We agree with the suggestion of Huntley et al. (1987) that one must take care to collect data at 2 min intervals over the initial 10 to 15 min.

In conclusion, for adult female *Calanoides carinatus*, the gut fluorescence method, as traditionally applied, underestimated ingestion rates. The underestimate resulted from an overestimate of gut passage time (GPT) derived from gut evacuation measurements. When GPT was estimated from fecal pellet production rates and from the ratio, hourly pigment egestion rate/mean gut pigment content, the mean (GPT) was 10.2 min, 37 % faster than the estimate derived from measurements of gut evacuation rate. When the revised estimates of GPT were used to calculate ingestion rate, the result was the same as for the 4 other methods for the experiments conducted at 1500 and 3000 cells $ml^{-1}$. The experiment at 8000 cells $ml^{-1}$ underestimated the true ingestion by about 20 %. Thus we conclude that the gut fluorescence method continues to be a useful technique so long as attention is given to measurement of pigment digestion and to proper estimation of gut passage time.

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## LITERATURE CITED

Attwood, C. G., Peterson, W. T (1989). Reduction in fecundity and lipids of the copepod *Calanus australis* (Brodskii) by strongly pulsed upwelling. J. exp. mar Biol. Ecol. 129: 121–131

Bautista, B., Rodriguez, V Jimenez, F. (1988). Short-term feeding rates of *Acartia grani* in natural conditions: diurnal variations. J. Plankton Res. 10: 907–920

Conover, R. J., Durvasula, R., Roy, S., Wang, R. (1986). Probable loss of chlorophyll-derived pigments during passage through the gut of zooplankton, and some of the consequences. Limnol. Oceanogr. 31: 878–887

Dagg, M. J., Walser, W. E. (1987). Ingestion, gut passage, and egestion by the copepod *Neocalanus plumchrus* in the laboratory and in the subarctic Pacific Ocean. Limnol. Oceanogr. 32: 178–188

Dagg, M. J., Wyman, K. (1983). Natural ingestion rates of the copepods *Neocalanus plumchrus* and *N. cristatus* calculated from gut contents. Mar. Ecol. Prog. Ser. 13: 37–46

Dam, H. G., Peterson, W. T (1988). The effect of temperature on the gut clearance rate constant of planktonic copepods. J. exp. mar. Biol. Ecol. 123: 1–14

Ellis, S. G., Small, L. F. (1989). Comparison of gut-evacuation rates of feeding and non-feeding *Calanus marshallae*. Mar. Biol. 103: 175–181

Frost, B. W. (1972). Effects of size and concentration of food particles on the feeding behavior of the marine planktonic copepod *Calanus pacificus*. Limnol. Oceanogr. 8: 805–815

Harbison, G. R., McAlister, V. L. (1980). Fact and artifact in copepod feeding experiments. Limnol. Oceanogr. 25: 971–981

Huntley, M. E., Marin, V., Escritor, F. (1987). Zooplankton grazers as transformers of ocean optics: a dynamic model. J. mar. Res. 45: 911–945

Jeffrey. S. M., Hallegraeff, G. M. (1987). Chlorophyllase distribution in ten classes of phytoplankton: a problem for chlorophyll analysis. Mar. Ecol. Prog. Ser. 35: 293–304

Kiørboe, T., Møhlenberg, F., Nicolajsen, H. (1982). Ingestion rate and gut clearance in the planktonic copepod *Centropages hamatus* (Lilljeborg) in relation to food concentration and temperature. Ophelia 21: 181–194

Kiørboe, T., Møhlenberg, F., Riisgård, H. U. (1985). In situ feeding rates of planktonic copepods: a comparison of four methods. J. exp. mar. Biol. Ecol. 88: 67–81

Kiørboe, T., Tiselius, P. T. (1987). Gut clearance and pigment destruction in a herbivorous copepod, *Acartia tonsa*, and the determination of *in situ* grazing rates. J. Plankton Res. 9: 525–534

Lopez, M. D. G., Huntley, M. E., Sykes, P. E. (1988). Pigment destruction by *Calanus pacificus*: impact on the estimation of water column fluxes. J. Plankton Res. 10: 715–734

Mackas, D., Bohrer, R. (1976). Fluorescence analysis of zooplankton gut contents and an investigation of diel feeding patterns. J. exp. mar. Biol. Ecol. 25: 77–85

Mantoura, R. F. C., Llewellyn, C. A. (1983). The rapid determination of algal chlorophyll and carotenoid pigments and their breakdown products in natural waters by reverse-phase high performance liquid chromatography. Analytica Chim. Acta 151. 297–314

Nemoto, T (1968). Chlorophyll pigment in the stomach of euphausiids. J. Oceanogr. Soc. Japan 24: 253–260

Omori, M., Ikeda, T (1984). Methods in marine zooplankton ecology. John Wiley and Sons, New York

Pasternak, A. F., Drits, A. V (1988). Possible degredation of chlorophyll-derived pigments during gut passage of herbivorous copepods. Mar. Ecol. Prog. Ser 49: 187–190

Peterson, W. T (1988). Rates of egg production by the copepod *Calanus marshallae* in the laboratory and in the sea off Oregon, USA. Mar. Ecol. Prog. Ser. 47:229–237

Peterson, W. T., Painting, S., Hutchings, L. (1990). Diel variations in gut pigment content, diel vertical migration and estimates of grazing impact for copepods in the southern Benguela upwelling region, in October 1987 J. Plankton Res. 12 (in press)

Roman, M. R., Rublee, P. A. (1980). Containment effects in copepod grazing experiments: a plea to end the black box approach. Limnol. Oceanogr. 25: 982–990

Runge, J. A. (1984). Egg production of the marine planktonic copepod, *Calanus pacificus* Brodsky: laboratory observations. J. exp. mar. Biol. Ecol. 74: 53–66

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