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Gut fluorescence in herbivorous copepods: an attempt to justify the method

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Abstract

Recently the gut fluorescence technique has been critisized because of the possible degradation of chlorophyll into nonfluorescent derivatives during passage through copepod guts and changes of the gut passage time with food concentration. Here pigment budgets have been calculated in 6 experiments with *Calanus finmarchicus* CIV caught 2 km offshore of the Murmansk Marine Biological Institute (the Barents Sea, Dalnije Zelentsi) in September 1992. Copepods were fed with culture of *Platymonas viridis* at different concentrations. Gut pigment and ingestion rate increased with food concentration in a similar way. On average between 78% and 89% of ingested chlorophyll was recovered in the guts and faecal pellets. No trend for a greater loss of fluorescence at low food concentration than at high was observed. Pigment content of faecal pellets incubated in filtered seawater decreased by 20–30% in the first 7–12 h and by up to 60% in 48 h. The decline of pigment content was accompanied by a rapid bacterial growth (by a factor of 3 in 48 h). Gut passage time increased with decreasing food concentration (from 40 min at 9 μg pigm l^{-1} to 64 min at 0.9 μg pigm l^{-1}). These results together with some data by other authors suggest that the gut fluorescence method can be used to estimate *in situ* grazing rate providing gut passage time is measured properly and there are no losses of faecal material. However, careful consideration should be given to the previous feeding history of copepods.

Introduction

Since the introduction of the gut fluorescence technique as an index of feeding activity in herbivorous zooplankton (Nemoto, 1968; Mackas & Bohrer, 1976) it has been widely used to study feeding rates both in the laboratory and in the sea. This method proved to be especially useful for studies of feeding rates, diel feeding rhythms and comparative feeding activity *in situ*.

The importance of this approach can not be overstated as it does not necessarily require lengthy incubations which often cause many artifacts. However, recently questions have arisen as to the possibility of considerable pigment destruction during the gut passage and variance of the gut passage time with food concentration. Evidence on the both problems is controversial. The main focus of the present paper is on the first of these problems though incidentally the second will also be touched upon.

Estimates of feeding rates obtained with the gut fluorescence technique were in several studies close to those derived by other means (Baars & Franz, 1984; Kiørboe *et al.*, 1982, 1985; Baars & Oosterhuis, 1984; Ishii, 1990; Peterson *et al.*, 1990) suggesting that ingested pigment was not broken down during passage through a copepod's gut. On the other hand, however, some authors have reported large and variable losses of pigment during gut passage (Conover *et al.*, 1986; Lopez *et al.*, 1988; Penry & Frost, 1991; Head & Harris, 1992; Cary *et al.*, 1992; Mayzaud & Razouls, 1992). A third group of authors has reported trivial pigment losses of no more than 10–30% of ingested pigment (Dagg & Walser, 1987; Kiørboe & Tiselius, 1987; Pasternak & Drits, 1988).

There also is conflicting evidence regarding the dependence of gut passage time on food concentration. One group of experiments supports its independence from food concentration (Christoffersen & Jespersen, 1986; Kiørboe & Tiselius, 1987; Tsuda & Nemoto, 1987; Ellis & Small, 1989; Drits *et al.*, 1990) and a

second group suggests it is dependent on food concentration (Baars & Oosterhuis, 1984; Wang & Conover, 1986; Dagg & Walser, 1987; Peterson *et al.*, 1990. Dam & Peterson (1989) summarized the dependence of gut passage time on temperature.

In this study 6 grazing experiments evaluating pigment budgets are presented. Simultaneously pigment degradation from egested fecal pellets was examined. Finally, gut passage time at different food concentrations was estimated.

Methods

Collection and storage. Zooplankton was collected 2 km offshore of the Murmansk Marine Biological Institute (Dalnije Zelentsi, the Barents Sea) in September 1992. Samples were taken from the upper 50-m layer with a 500-μm mesh plankton net. Immediately after returning to the laboratory zooplankton was sorted, *Calanus finmarchicus* CIVs were transferred using wide-bore pipettes into the 5-l glass beakers with sea water filtered through 70 μm mesh. The beakers were stored in a dark cold room in a large aquarium with continuous natural sea water flowing to maintain environmental temperature (8–9°C). Every three days animals were transferred into fresh water.

Grazing experiments.
For each grazing experiment (except Exp. 1, performed without replicates) five controls and three experimental replicates were done. The experimental and control beakers were 50-ml glass vessels with inclined walls, which were convenient to examine visually. For each experiment, 1 liter of food medium (*Platymonas viridis* in filtered seawater) at various concentration (0.9–8.9 μg pigm l^{-1}, *i.e.* 2300–20100 cells ml^{-1}) was prepared. Three 10-ml samples of food medium were taken at this time to determine the initial pigment concentration. Five copepods were added to each of the three experimental vessels; five vessels with food medium were left as controls. The copepods used were pre-adapted for 1 day to the appropriate experimental food concentration; only the feeding copepods, *i.e.* the copepods with visible gut contents were used. Each incubation lasted for 5 h , except Exp. 1, which lasted for 6 h. At the end of each experiment, carbon dioxide was added to the experimental vessels to immobilize copepods and prevent their defecation. Copepods were picked with fine forceps and washed in fresh filtered sea water, and then placed in a test tube with two milliliters of 90% aqueous acetone. Faecal pellets were collected under the dissecting microscope with a fine-bore pipette, washed with filtered sea water, and then were placed on a GF/F glass-fiber filter, which was folded over and put in a test tube. Three replicates of 10-ml aliquots of the remaining food medium were then filtered onto GF/F glass-fiber filters and placed in test tubes. Each sample was homogenized by grinding. Other details of the experimental procedure are in Drits *et al.*, (1993). Fluorescence was measured before and after acidification. Chlorophyll and phaeopigment concentration were calculated using the equations of Strickland & Parsons (1968). All values are expressed as Chl *a* equivalents. Pigment content of copepods which had been starved for two days was measured for subsequent subtraction of this 'background' fluorescence level.

As there were no differences in pigment concentration in control beakers at the beginning and at the end of incubation, the observed difference in experimental beakers (Δ, ng pigm) was assumed to result from copepods' ingestion.

As in Dagg & Walser (1987), gut passage time (T) was calculated by dividing the gut content at the end of the incubations (S_c, ng pigm ind $^{-1}$) by the egestion rate (S_f, ng pigm ind^{-1} h^{-1}). The sole distinction is that Dagg & Walser added the difference between the gut pigment measured at the beginning and at the end of the incubation to the faecal pigment to correct for the gut filling of pre-starved copepods. There was no need for the correction in the present study because copepods were pre-fed for 1 day at corresponding experimental concentrations. A error was introduced, however, in transferring animals without immobilizing them from storage into experimental beakers because some of the copepods egested a part of their gut content during the transfer and delayed the beginning of ingestion for some 5–15 min (visual observation).

Three incubations of freshly egested faecal pellets were conducted. For each of them, 50–100 ind of *C. finmarchicus* were allowed to graze for three hours on *Platymonas viridis* at various concentrations. The copepods were removed and the egested faecal pellets were collected and rinsed as described above. Only the faecal pellets of the same size (0.65±0.035 mm length, 0.05±0.001 mm width) were used. Immediately after collection 10–15 faecal pellets were placed onto GF/F filter and their pigment content (S_f) was analyzed. That number was assumed to represent the

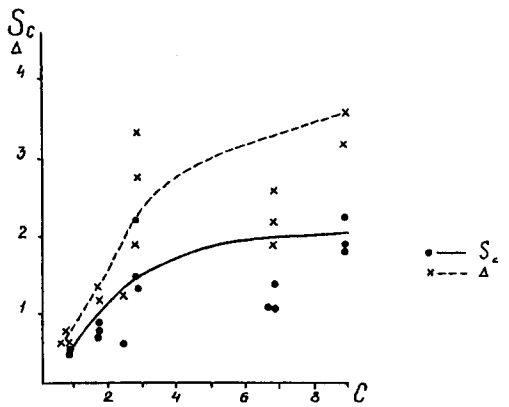

Fig. 1. Changes in gut pigment content (S_c, ng pigm 5 cop^{-1}) and ingestion rate (Δ, ng pigm 5 cop^{-1} h^{-1}) with the increase of food concentration (C, μg pigm l^{-1}).

initial pigment content at the time zero. Subsequent analyses were performed at 7, 12, 18–19, 24 and 48 h of incubation. After the removal of faecal pellets, 10 ml of the incubation medium was filtered onto a black-stained 0.2 μm Nuclepore filter for enumeration of bacteria according to Hobbie *et al.*, (1977).

Results

The ingestion rate of *C. finmarchicus*, calculated from the difference in pigment concentration in experimental vessels, increased with food concentration. The gut pigment content at the end of the incubation was related to food concentration in a similar manner to the ingestion rate (Fig. 1, r=0.95).

The degree of pigment degradation calculated as

$$D = \left(\frac{\Delta - (S_c + S_f)}{\Delta}\right) \%$$

varied from −44 to +30% (Table 1). In 11 of 16 grazing incubations the amount of pigment ingested was greater than the amount recovered in the guts and faecal pellets after 5-h incubation (Table 1). In 7 out of 16 incubations there was 20–30% pigment destruction, in 5 cases there was 0–20% destruction and in 4 cases pigment levels actually increased. The average loss calculated from all the experiments was 11% (the difference between Δ and S_c +S_f was not significant, Student's t-test, P>0.5). No trend for a greater losses of fluorescence at low food concentrations than at high was observed.

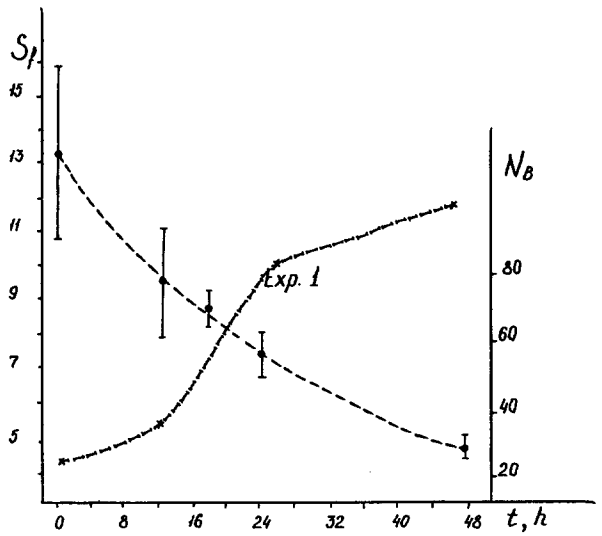

Fig. 2a. Time course of pigment degradation in faecal pellets (S_f, ng pigm in^{-1} 10^{-2}) and simultaneously measured bacterial growth (N, cells 10^4 lm^{-1}).

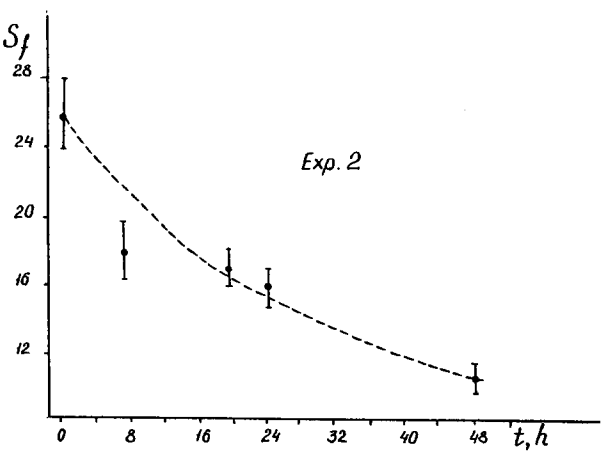

Fig. 2 b.

In all of the faecal pellets incubations, their pigment content decreased at the end of incubation (48 h) by a factor of between 1.8 and 2.8 (Fig. 2 a,b,c). Degradation of faecal pigment was accompanied by bacterial growth (Fig. 2 a,c). These data on bacterial growth are not quantitative, because the data sets are too small and too variable. They can demonstrate only a general trend.

Gut passage time, T, varied from 0.64 h to 1.05 h. There was a distinct decrease of T with the increase of food concentration (Fig. 3).

Table 1. Pigment degradation by *Calanus finmarchicus* feeding on *Platymonas viridis* culture. K - *P. viridis* concentration (cell ml^{-1}); V - experimental volume (ml); C_o and C_t - pigment concentration in the control and grazed beakers ($\mu g\ l^{-1}$), values are means ± SE; Δ - pigment ingested (ng), calculated as the difference between pigment concentration at the beginning and at the end of the incubation; S_c - pigment content of 5 ind of *C. finmarchicus* (ng); S_f - faecal pellets' pigment content (ng); T - gut passage time (h,min); D-degradation efficiency, calculated as $\left(\frac{\Delta-(S_c+S_f)}{\Delta}\right)$%; D* - degradation efficiency, calculated as $\left(\frac{\Delta-S_f}{\Delta}\right)$%.

N exp, K, V	C_0	C_t	$\Delta = C_0 - C_t$	S_c	S_f	S_c+S_f	T	D	D*
1	2.46	2.35	6.5	0.64	4.44	5.08	0.86/51	22	32
6980									
50									
2	2.82±0.030	2.35±0.023	18.8***	2.42	11.60	14.02	1.04/62	25	38
8600		2.43±0.150	15.6*	1.57	9.50	11.07	0.83/50	29	39
40		2.56±0.058	10.4*	1.38	7.77	9.15	0.89/53	12	25
3	1.74±0.087	1.60±0.092	7.0	0.69	4.27	4.96	0.81/48	29	39
5000		1.618±0.099	6.1	0.92	5.24	6.26	0.87/53	−3	14
50		1.62±0.036	6.0	0.75	5.36	6.11	0.70/42	−2	11
4	0.90±0.012	0.846±0.012	2.7*	0.75	3.57	4.32	1.05/63	−44	−32
2280		0.84±0.014	3.0*	0.40	1.89	2.29	1.05/63	24	37
50		0.82±0.003	4.0**	0.52	2.30	2.82	1.13/68	30	43
5	6.83±0.094	6.53±0.156	12.0	1.15	7.76	8.91	0.74/44	26	35
13240		6.48±0.024	14.1*	1.50	14.40	15.90	0.52/31	−14	−3
40		6.57±0.260	10.4	1.15	8.93	10.10	0.64/39	3	14
6	8.92±0.040	8.41±0.018	20.4***	2.10	16.15	18.25	0.65/39	10	21
20100		8.33±0.042	23.6***	2.50	17.00	19.50	0.73/44	17	28
40		8.47±0.074	18.0*	2.08	15.90	17.98	0.65/39	0	12

* Significant $t>t$; $P<0.05$.
** Significant $t>t$; $P<0.01$.
*** Significant $t>t$; $P<0.001$.

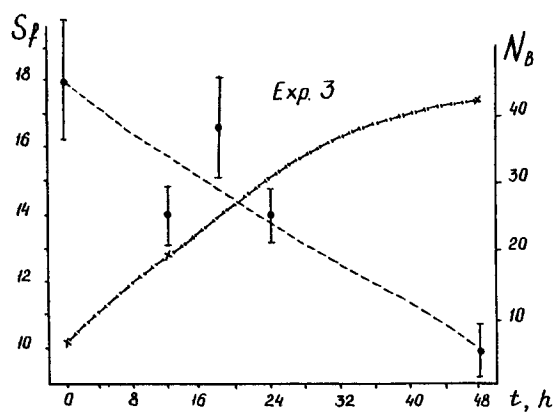

Fig. 2 c.

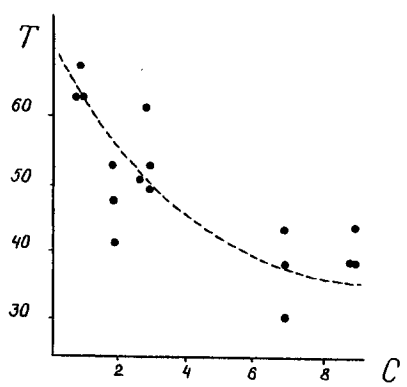

Fig. 3. Gut passage time (T, min) versus initial food concentration (C, μg pigm l^{-1}).

Discussion

It has been suggested that there is not necessarily a functional relationship between gut fullness and ingestion rate (Penry & Frost, 1990) because the latter is

a product of two values: gut content and gut passage time. A large volume of material in the gut and a proportionately long gut passage time or a small volume of material in the gut and a proportionately short gut passage time may both result in the same ingestion rate. Theoretically this is true, but to date there is no experimental evidence for the increase of T with gut fullness. On the contrary, if changes of gut passage time with gut fullness have been observed they were in the opposite sense: a decrease in T with an increase in gut fullness or food concentration (Baars & Oosterhuis, 1984; Wang & Conover, 1986; Dagg & Walser, 1987; Peterson *et al.*, 1990). In this study, ingestion rate of experimental *C. finmarchicus* CIV was closely correlated with gut fullness (measured as amount of pigment in the gut). Perhaps usually high levels of individual variability in gut content and ingestion, as well as intermittent feeding activity patterns, obscure this correlation.

In the present study, an average of 78% of ingested chlorophyll was recovered in the guts and faecal pellets of *C. finmarchicus*. However the amount of pigment recovered at the end of the incubation could have been overestimated. If the part of gut pigment egested during transfer was small compared to the whole gut content, *i.e.* gut pigment content at the beginning of the incubation practically equalled that at the end, then S_c should not have been added to S_f when calculating pigment recovery. So another calculation of the degree of pigment degradation was done:

$$D^* = \frac{\Delta - S_f}{\Delta}\%.$$

D^* varied from −32% to +43%, while D varied from −44% to +30%. The average value of pigment loss increased from 11% to 22%. The true value is likely to be somewhere in between these two estimates. According to the experiments with faecal pellets' degradation, up to 10–20% of initial faecal pigment could have been lost in 5 h incubation. The actual loss of faecal pigment would be lower as pellets were being produced throughout the whole incubation period. The loss of pigment from faecal pellets may partly explain the incomplete pigment recovery.

Irrespective of the chosen way to calculate the degree of pigment degradation, the values obtained are close to those reported by Dagg & Walser, (1987) and are within the range of values reported by the above mentioned 'third group' of authors (who reported not more than 10–30% of pigment destruction). As in those studies, losses of pigment did not covary with food concentration.

It seems that given the high individual variability in ingestion rate and gut pigment content (Mackas & Burns, 1986; Mobley, 1987; Bamstedt *et al.*, 1991; Tseitlin *et al.*, 1991) and inevitable experimental errors, the separation of a 'third group' of data does not make sense. It may well be combined with the first one, reporting the lack of degradation. Now while there need be no doubt in principle of the possibility of chlorophyll *a* degradation into non-fluorescent derivatives during digestive processes, the question is, to what extent and how often it occurs, and whether the gut fluorescence can still be used to investigate *in situ* feeding rates and daily feeding patterns in herbivorous plankton.

Studies where high unrecovered losses of chlorophyll-derived pigments were found reported them to be extremely variable: up to 95% in Conover *et al.*, (1986), from 0 to 92% in Lopez *et al.*, (1988), from 0 to 94% in Penry & Frost, (1991), from 9 to 82% in Head & Harris, (1992), from 0 to 92% in Mayzaud & Razouls, (1992), from 0 to 96% in Cary *et al.*, (1992). The reason for this variability is still unclear. Previous feeding history is considered the main factor affecting the fate of ingested chlorophyll. If this pattern is usually true for herbivorous animals feeding in the wild than the gut fluorescence can hardly be used to evaluate *in situ* feeding activity.

However, in several studies especially designed to compare different methods, gut fluorescence did give results which were close to those obtained using other methods (references in Introduction). Furthermore, in other studies not aimed at such comparisons, reasonable values of daily rations (or clearance rates) were found, which were close to those obtained with other methods (Dagg & Walser, 1987 – Frost *et al.*, 1983; Drits *et al.*, 1990 – Frost, 1972, Arashevich *et al.*, 1980; Peterson *et al.*, 1990 – Kiørboe *et al.*, 1985; Drits *et al.*, 1993 – Schnack, 1985; Schnack-Schiel *et al.*, 1991). If such high levels of pigment degradation often occur then the estimates of daily rations would have to be increased up to 100% body carbon d^{-1} or even more. It seems hardly possible and is inconsistant with other data.

Some of the possible reasons of observed descrepancies in experimental data are summarized here. The weak point of all budget experiments is in measuring the concentration of faecal material. This is the most probable source of pigment loss. Microscopic observation have revealed the presence of a considerable

number of bacteria and a few protozoans inside faeces (present study; Gowing & Silver, 1983; Pasternak & Drits, 1988; Drits *et al.*, 1992) and the organically enriched medium of experimental beakers containing a large number of egested faecal pellets provides bacteria with an additional food resource and may promote rapid growth. Gowing & Silver (1983) observed rapid bacterial reproduction and biomass increase of a factor of 3 within 48 h. In the present study, bacteria increased in number by a factor of 4 within 48 h (Fig. 2 a,c). the most rapid loss of faecal chlorophyll occured in the first 7–12 h of incubation (20–30% of initial faecal pigment, Fig. 2 a,b,c). This period is rather common for incubation experiments, and if there are considerable bacterial and protozoan populations associated with faecal material, they can play an important role in its decomposition and destroy a significant part of faecal chlorophyll (Klein *et al.*, 1986).

In addition, if filtration procedure is involved, there is a risk of breach and escape of broken faecal pieces through the pores as well as leakage from broken pellets. If this problem is to be avoided by extracting pigments directly from experimental animals prior to faecal pellet production, then incubation times should be very short, less than 15–20 min (when copepods have full guts) or 30 min (if animals start to feed with empty guts), as most of the plankton animals studied produce a faecal pellet every 10–15 min at usual food concentration (Arashkevich & Tseitlin, 1978) or even less (6.4 min in Peterson *et al.*, 1990). Moreover, handling of some copepods may cause the premature egestion of their gut content so it seems necessary to narcotize them before handling.

The most convincing evidence that pigment degradation depends on previous feeding history is that of Penry & Frost (1991) in which copepods acclimated for 3 days to high food concentration were transferred then to low food, where they were allowed to feed for 8 hours. The extent of pigment degradation was maximum in that treatment, 89–94%. In this case the excess of digestive enzymes accumulated during incubation at high concentration may have affected the degradation efficiency. Mayzaud *et al.* (cited in Mayzaud & Razouls, 1992) showed that weekly changes in enzyme activity followed variations in ingestion rate. They considered eight hours of feeding at low concentration not sufficient to ensure digestive acclimation to the treatment conditions, so enzyme activity was characteristic for high food concentration and food availability for low concentration. This situation is similar to acute respiration experiments, where animals are transferred from the usual temperature of their habitat to a significantly different temperature. In such a case the result is a large (more than predicted by temperature-related dependence) immediate change of respiration rate, followed by a series of oscillations and, at last, acclimation to the new temperature (Vilenkin & Vilenkina, 1979). This new 'acclimated' rate is close to those predicted by temperature-related dependence.

In the field the situation is applicable where diel vertical migration occurs and there can be a rapid change in food concentration. Head (1992) suggested that copepods which have been previously starved do not destroy pigments very extensively, so that copepods which have not fed during the day might destroy less pigment at night and thus accumulate high gut pigment levels. Enhanced destruction of pigment in copepod guts also may occur during the day when copepods are in the layer of low concentration, although low gut pigment levels are most likely the result of low daytime ingestion rates (Head, personal communication).

Gut passage time obtained in this study decreased from 64 min (at 0.9 μg pigm l^{-1}) to 40 min (at 9 μg pigm l^{-1}), *i.e.* by a factor of 1.7 as the concentration of chlorophyll increased by an order of magnitude. By comparison, Dagg & Walser (1987) reported a 3-fold decrease in T with a 40-fold increase in concentration. In our previous studies (Drits & Pasternak, 1989; Tseitlin *et al.*, 1991) no regular changes of gut passage time were obtained. These experiments were conducted according to the most commonly used scheme: a group of copepods with full guts was placed in filtered seawater with the decrease in gut pigment being monitored every 10–15 min for some period of time. The high variability between replicates characterizing these experiments might have masked the changes over time. An apparent 2-fold difference in T was obtained with a simulation model only by randomly re-grouping individuals with realistic differences in feeding characteristics (initial gut pigment content, time intervals between subsequent defecations) (Tseitlin, in press). Thus, for determination of gut passage time it seems more reliable to follow the procedure of Dagg & Walser (1987) or proposed by Timonin *et al.* (1992).

In conclusion, no important chlorophyll degradation was found in the present study. The obtained results allow the cautious optimism as to the applicability of the gut fluorescence technique for trivial estimation of *in situ* grazing rates. However, it seems reasonable to restrict the use of the method to the situations without abrupt environmental changes. Finally,

attention should be given to the proper estimation of gut passage time and complete account of egested faecal material.

References

Arashkevich, E. G., L. Cahoon, G. G. Nikolaeva, A. G. Pelimski & L. A. Ponomareva, 1980. Qualitative and quantitative characteristics of feeding of dominant zooplankton species (in Russian). In: Pelagic ecosystems of Peruvian region. Moscow, Nauka: 202–215.

Arashkevich, E. G. & V. B. Tseitlin, 1978. Dependence of daily rations on food concentration in herbivorous copepods and their feeding mechanism. Oceanology 18: 37–46.

Baars, M. A. & H. G. Franz, 1984. Grazing pressure of copepods on the phytoplankton stock of the central North Sea. Neth. J. Sea Res. 18: 120–142.

Baars, M. A. & S. S. Oosterhuis, 1984. Diurnal feeding rhythms in North Sea copepods measured by gut fluorescence, digestive enzyme activity and grazing on labelled food. Neth. J. Sea Res. 18: 97–119.

Bamstedt, U., H. C. Eilertsen, K. S. Tande, D. Slagstad & H. R. Skjodal, 1991. Copepod grazing and its potential impact on the phytoplankton development in the Barents Sea. Polar Res. 10: 339–354.

Cary, S. C., J. T. Lovette, P. J. Perl, M. E. Huntley & M. Vernet, 1992. A microencapsulation technique for introducing pure compounds in zooplankton diets. Limnol. Oceanogr. 37: 404–413.

Christoffersen, K. & A. M. Jespersen, 1986. Gut evacuation rates and ingestion rates of *Eudiaptomus graciloides* measured by means of the gut fluorescence method. J. Plankton Res. 8: 973–983.

Conover, R. J., R. Durvasula, S. Roy & R. Wang, 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. & W. E. Walser, 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.

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

Drits, A. V. & A. F. Pasternak, 1989. Effect of food concentration on feeding activity and gut clearance rate of herbivorous copepods in upwelling zones. Oceanology 29: 232–236.

Drits, A. V., A. F. Pasternak, T. N. Semenova & M. V. Flint, 1990. Feeding of herbivorous copepods in upwelling regions. II. Diel rhythms, rations and grazing impact. Oceanology 30: 311–319.

Drits, A. V., E. G. Arashkevich & T. N. Semenova, 1992. Pyrosoma atlanticum (Tunicata, Thaliacea): grazing impact on phytoplankton standing stock and role in organic carbon flux. J. Plankton Res. 14: 799–809.

Drits, A. V., A. F. Pasternak & K. N. Kosobokova, 1993. Feeding, metabolism and body composition of the Antartic copepod *Calanus propinquus* Brady with special reference to its life cycle. Polar Biol. 13: 13–21.

Ellis, S. G. & L. F. Small, 1989. Comparison of gut evacuation rate 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 planktonic copepod *Calanus pacificus*. Limnol. Oceanogr. 31: 449–452.

Frost, B. W., M. R. Landry & R. P. Hasset, 1983. Feeding behavior of large calanoid copepods *Neocalanus cristatus* and *N. plumchrus* from the subarctic Pacific Ocean. Deep-Sea Res. 30: 1–13.

Gowing, M. M. & M. W. Silver, 1983. Origins and microenvironments of bacteria mediating faecal pellets decomposition in the sea. Mar. Biol. 73: 7–16.

Head, E. J. H., 1992. Gut pigment accumulation and destruction by arctic copepods *in vitro* and *in situ*. Mar. Biol. 112: 583–592.

Head, E. J. H. & L. R. Harris, 1992. Chlorophyll and carotenoid transformation and destruction by *Calanus* spp. grazing on diatoms. Mar. Ecol. Progr. Ser. 86: 229–238.

Hobbie, J. E., R. J. Daley & S. Jasper, 1977. Use of Nuclepore filters for counting bacteria by fluorescence microscopy. Appl. envir. Microbiol. 33: 1225–1228.

Ishii, H., 1990. *In situ* feeding rhythms of herbivorous copepods, and the effect of starvation. Mar. Biol. 105: 91–98.

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

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

Kiørboe, T. & P. T. Tiselius, 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.

Klein, B., W. W. C. Gieskes & G. G. Kraay, 1986. Digestion of chlorophylls and carotenoids by the marine protozoan *Oxyrrhis marina* studied by h. p. l. c. analysis of algal pigments. J. Plankton Res. 8: 827–836.

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

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

Mackas, D. L. & K. E. Burns, 1986. Poststarvation feeding and swimming activity in *Calanus pacificus* and *Metridia pacifica*. Limnol. Oceanogr. 31: 383–392.

Mayzaud, P. & S. Razouls, 1992. Degradation of gut pigment during feeding by a subantarctic copepod: Importance of feeding history and digestive acclimation. Limnol. Oceanogr. 37: 393–404.

Mobley, C. T., 1987. Time-series ingestion rate estimates on individual *Calanus pacificus* Brodsky: interactions with environmental and biological factors. J. exp. mar. Biol. Ecol. 11: 199–216.

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

Pasternak, A. F. & A. V. Drits, 1988. On possible degradation of chlorophyll-derived pigments during gut passage of herbivorous copepods. Mar. Ecol. Prog. Ser. 49: 187–190.

Penry, D. L. & B. W. Frost, 1990. Re-evaluation of the gut fullness (gut fluorescence) method for inferring ingestion rates of herbivorous copepods. Limnol. Oceanogr. 35: 1207–1214.

Penry, D. L. & B. W. Frost, 1991. Chlorophyll *a* degradation by *Calanus pacificus*: Dependence on ingestion rate and digestive acclimation to food resources. Limnol. Oceanogr. 36: 147–159.

Peterson, W., S. Painting & R. Barlow, 1990. Feeding rates of *Calanoides carinatus*: a comparison of five methods including evaluation of the gut fluorescence method. Mar. Ecol. Progr. Ser. 63: 85–92.

Schnack, S. B., 1985. Feeding by *Euphausia superba* and copepod species in response to varying concentration of phytoplankton. In: Siegfried, W. B., P. R. Condy & R. M. Laws (eds), Antarctic nutrient cycles and food webs. Springer-Verlag, Berlin, 311–323.

Schnack-Schiel, S. B., W. Hagen & E. Mizdalski, 1991. Seasonal comparison of *Calanoides acutus* and *Calanus propinquus* (Copepoda: Calanoida) in the southeastern Weddell Sea, Antarctica. Mar. Ecol. Progr. Ser. 70: 17–27.

Strickland, J. D. H. & T. R. Parsons, 1968. A practical handbook for sea water analysis. Bull. Fish. Res. Bd Can. 167.

Timonin, A. G., E. G. Arashkevich, A. V. Drits & T. N. Semenova, 1992. Zooplankton dynamics in the northern Benguela ecosystem, with emphasis on the herbivorous copepods. South Afr. J. mar. Sci. 12: 545–560.

Tseitlin, V. B., 1993. Simulation modelling of gut passage time measurement in copepods. Oceanology (in press).

Tseitlin, V. B., A. F. Pasternak & A. V. Drits, 1991. Does gut passage time in copepods depend on food concentration? Oceanology 31: 155–161.

Tsuda, A. & T. Nemoto, 1987. The effect of food concentration on the gut clearance time of *Pseudocalanus minutus* Kroyer (Calanoida: Copepoda). J. exp. mar. Biol. Ecol. 107: 121–130.

Vilenkin, B. J. & M. N. Vilenkina, 1979. Respiration of water-inhabiting invertebrates (in Russian). In: I. E. Zaika (ed.), Advances of science and technique. VINITI, Moscow, 143.

Wang, R. & R. I. Conover, 1986. Dynamics of gut pigment in the copepod *Temora lonicornis* and the determination of *in situ* grazing rates. Limnol. Oceanogr. 31: 867–877.