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CALCULATIONS OF ZOOPLANKTON GRAZING RATES ACCORDING TO A CLOSED, STEADY-STATE, THREE-COMPARTMENT MODEL APPLIED TO DIFFERENT ^{14}C METHODS

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KEYWORDS : zooplankton; grazing; radio isotopes; specific activity; filtering rate; comparison of methods.

ABSTRACT

A re-examination of the numerical example of the three-compartment model by CONOVER & FRANCIS (1973) showed that the warning by these authors for the misuse of radio isotopes in transfer studies within food chains is incorrect and based on a misinterpretation of their results. There is no difference in the estimate of transfer rate by use of specific activities or by use of total radioactivities observed in each compartment.

After adapting the formulae developed by CONOVER & FRANCIS, their model was used to illustrate deviations of the programmed grazing rate in 3 types of grazing experiments; a) with ^{14}C present only in the phytoplankton at the start of the experiment, b) with ^{14}C only in the water, and c) with ^{14}C in both phytoplankton and water. Up to a duration of the grazing experiment of 2 hours, and at various light conditions and grazing pressures, deviations were small and did not exceed 4 %. These results are not directly applicable to practical work because the quantitatively important loss by egestion of radioactive material was not accounted for, only losses by respiration were incorporated in the closed, steady-state model.

Best calculations of the community filtering rate (fraction of the volume of the grazing vessel swept clear per day) were generally obtained with the formula (with t in hours) '((DPM zoo at time t)/(DPM phyto at 0 + DPM phyto at t)/2) x 24/t', applicable to all three types of grazing experiments considered.

INTRODUCTION

The use of radioactive isotopes to measure the transfer of materials in aquatic food chains is widely known. Especially radioactive carbon is being employed extensively and that method gives the outsider the impression that the uptake of carbon by animals is directly measured. In reality, however, one measures only fouraging activity, expressed as that part of the volume of the experimental vessel from which the food is removed, in grazing studies known as the 'filtering rate'. The feeding rate (amount of food ingested per unit time) follows from 'filtering rate x food concentration' so additional measurements of the food stock have to be made.

In grazing studies with the ^{14}C method different types of experiments can be recognized and here 3 of them will be treated. The first originates from NAUWERCK (1959) and was followed by, among others, LAMPERT (1974, 1975) and GULATI *et al.* (1982). Natural water, or a phytoplankton culture, is labelled during a relatively long period of 1 to 10 days,

so that the phytoplankton reaches a high specific activity. Then, the animals are introduced into the medium, or, which is more common nowadays, the incubated phytoplankton is concentrated, washed and introduced in the grazing vessel. After a short period, ranging from some till 15 minutes, during which the zooplankton feeds on the labelled phytoplankton, the experiment is ended by pouring the medium over gauze to retain the zooplankton, followed by collecting a phytoplankton sample by filtering a known volume of the medium through millipore filters. After preparation and counting of these samples (with a liquid scintillation counter, for instance) the counts per minute (CPM) obtained are converted to desintegrations per minute (DPM) to account for count losses due to self absorption and quenching (*cf.* GULATI, 1985). The filtering rate of the zooplankton (ml per animal per unit time) is then calculated according to 'DPM per animal/DPM per ml phytoplankton'. Long grazing periods in this type of experiment are avoided in order to reduce the risk of egestion of radioactive material (*cf.* PETERS, 1984).

In a second method, developed by DARO (1978), ^{14}C bicarbonate is introduced at the start of the experiment, and during incubation in the light, labelling of phytoplankton occurs simultaneously to the grazing by zooplankton. As a result, radioactivity of the phytoplankton increases linearly and that of zooplankton parabolically with time. After one hour the phytoplankton and the zooplankton are collected separately and filtering rates are calculated as '(DPM per animal)/0.5(DPM per ml phytoplankton)'. Note that, compared with the other formula above, a factor 0.5 is introduced. Put simplifiedly, this is because the realized radioactivity of the animals relates to the average radioactivity of the phytoplankton, *i.e.* half of the radioactivity at the end of the incubation. For a detailed derivation of this formula the reader is referred to DARO (1978).

BAARS & OOSTERHUIS (1984, 1985) used a modified version of DARO's method. They preincubated natural water without zooplankton some hours in the light before conducting the grazing experiment. In this way a similar procedure could be followed for experiments in the dark and in the light. In their set-up most of the radioactivity is still present as ^{14}C bicarbonate, as in DARO's method, but the radioactivity of the phytoplankton is high enough to measure grazing rates over relatively short experimental periods.

In this paper the above three methods are treated on basis of the three-compartment model by CONOVER & FRANCIS (1973). These authors classified the use of isotopes by ecologists as naive and showed that ingestion rates calculated with the first formula mentioned above could easily differ from the real ingestion rate by a factor of 2 or more. They urged the use of frequent measurements of the specific activities (the radioactivity related to the carbon biomass of phytoplankton or zooplankton) in the course of a grazing experiment. This recommendation requires much more effort because it implies not only the normal measurement of total radioactivities in both phytoplankton and zooplankton but also the determination of carbon contents in both of these compartments. Here we shall investigate whether their conclusion was valid, and subsequently we shall use their model to study the measuring errors in various experimental conditions of all three ^{14}C methods.

THE THREE-COMPARTMENT MODEL OF CONOVER & FRANCIS

CONOVER & FRANCIS (1973) presented a closed three-compartment model to illustrate possible calculation errors in grazing experiments with labelled food (Fig. 1). The model comprises 3 carbon pools; S_1 water, S_2 phytoplankton and S_3 zooplankton. Transfer rates ρ between these compartments are dependent on constants k and the amount of carbon in the receiving compartment. So, grazing on phytoplankton S_2 by zooplankton S_3 is expressed by $\rho_{23} = k_{23} \times S_3$. The water S_1 looses carbon to the algae by primary production in the light or by assimilation of organic carbon in the dark; $\rho_{12} = k_{12} \times S_2$. Similarly, the dissolved carbon components assimilated by the zooplankton are represented by $\rho_{13} = k_{13} \times S_3$. Carbon

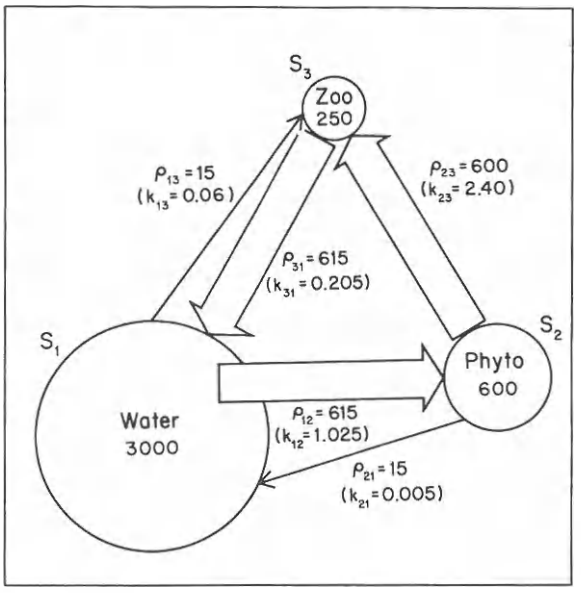


Fig. 1. The three-compartment, closed, steady-state model of CONOVER & FRANCIS (1973) involving water, phytoplankton and zooplankton. Numerical values for stocks (s), rate constants (k) and flows (ρ) as used in an example by CONOVER & FRANCIS. We assumed that these stocks and flows are expressed in μg C/l and μg c/l/day respectively.

respired by phytoplankton and zooplankton returns to the compartment water; $\rho_{21} = k_{21} \times S_1$ and $\rho_{31} = k_{31} \times S_1$ respectively. Because the constants depend on the amount of carbon in the receiving compartment, the meaning of these constants is generally different from variables measured in normal practice. For example, in the model the constants concerning the flows of phytoplankton and zooplankton carbon to the water (respiration) do not depend on the biomass of phytoplankton or zooplankton and, thus, have no relation to respiration values of plankton listed in the literature. Also, the grazing constant, k_{23}, represents the amount of carbon consumed per unit zooplankton carbon, while in grazing experiments with labelled food the filtering rate is measured (see Introduction). These remarks are crucial to understand the numerical example of CONOVER & FRANCIS (1973) as will become clear below.

CONOVER & FRANCIS (1973) assumed the carbon pools to be in a steady state, and then the model works as, what they call, a hydraulic model. If the carbon pools do not change, the production of phytoplankton, for example, is only possible until some of the phytoplankton is eaten and recycling begins. Thus the transfer rates are in balance, in the numerical example given by CONOVER & FRANCIS the carbon assimilated by the phytoplankton stock of 600 μg C/l is 615 μg C/l per day but losses are exactly of the same size; 600 μg is eaten by the zooplankton and 15 μg is excreted (Fig. 1). The carbon consumed daily by zooplankton - equalling 240 % of the zooplankton stock of 250 μg, meaning that CONOVER & FRANCIS used a grazing constant of 2.4 - is supplemented with 15 μg dissolved carbon taken up by the animals, and this total of 615 μg is respired everyday by them (Fig. 1).

For biologists the above couplings seem illogical; in practice, primary production by algae is not limited by the CO_2 concentration in the water, and grazing in incubation bottles often does lead to decrease in algal concentrations. But the advantages of these feedbacks are that algebraic solutions are possible and that a set of formulae can be developed to relate specific activities to processes one wants to measure. In an actual grazing experiment, the plotting of the specific activities for phytoplankton and zooplankton against time will give estimates of the coefficients needed for the calculation of the grazing constant (CONOVER & FRANCIS, 1973). For theoretical purposes, however, one can also select a set of values for carbon pools and flows, and then derive the specific activities at any time

	Compartment		
	Water	Phytopl.	Zoopl.
Specific activity			
Start (t = 0)	0	100	0
End (t = 1/24)	0.061	95.82	9.301
Total activity			
Start	0	60000	0
End	183	57492	2325

Table 1. Numerical values of the specific activity and the total activity in the example of a grazing experiment with labelled phytoplankton lasting one hour, as used by CONOVER & FRANCIS (1973) in a three-compartment model (*cf.* Fig. 1). Units arbitrary, but comparable with DPM/μg and DPM/l for specific and total activity respectively.

in the course of such a programmed grazing experiment. These calculations will give the grazing constant one would have observed in practice, which can be compared with the actual constant put in the model. All the formulae needed are treated in CONOVER & FRANCIS (1973) and the reader is referred to their paper for full details about the derivations. Part of this matter is recapitulated in the Appendix of this paper.

The theoretical example given by CONOVER & FRANCIS is based on pools and flows given in Fig. 1 and concerns the change in specific activities after a grazing experiment lasting one hour and starting with a specific activity of the phytoplankton of 100 units (Table 1). CONOVER & FRANCIS calculated the grazing constant k_{23} as the ratio between the specific activities of zooplankton (after one hour) and of phytoplankton (at the start); $x_3/x_2 = 9.301/100$, *i.e.* 0.093 per hour. This makes 2.23 per day, whereas the real grazing constant put in the model was 2.4 per day, a deviation of 7 %. They also calculated 'ingestion rate' as is normally done in experiments with tracer food, *i.e.* by use of total radioactivities measured in zooplankton and phytoplankton (Table 1, lower line). Total radioactivity in the animals divided by the total radioactivity in the phytoplankton (at the start of the experiment) gives (2325/60000) x 24 hr = 0.93 per day. CONOVER & FRANCIS claim that this is a serious miscalculation compared with the k_{23} of 2.4 put in the model and therefore warn that workers with radio isotopes have to use specific activities instead of total activities. However, they misinterpreted the 'ingestion' calculated with the normal method. The value of 0.93 represents the filtering rate, and in this case it means that 93 % of the water will be swept clear by the zooplankton in the course of one day. To get the real ingestion, we have to multiply with the concentration of phytoplankton, so feeding will amount to 0.93 x 600 is 558 μg carbon consumed per day. Because the feeding put in the model was 600 μg (namely 2.4 x 250), the underestimate is 7 % also with this type of calculation.

A similar mistake is made by CONOVER & FRANCIS when they estimate the zooplankton respiration constant k_{31}. In their method with specific activities (after correcting the activity of the water for excretion by phytoplankton), k_{31} follows from $x_1/x_3 = 0.040/9.301$, *i.e.* 0.0043 per hr. This makes 0.103 per day whereas 0.205 was actually put in the model (Fig. 1). By using total radioactivities they claim that the constant k_{31} will be much more miscalculated : (120.5/2325) x 24 = 1.24 per day. But, 1.24 means that 124 % of the zooplankton biomass is excreted per day so a total of 1.24 x 250 = 310 μg carbon will be transferred from zooplankton to the water while this flow actually involves 0.205 x 3000 = 615. Thus, both calculations show a 50 % deviation of the programmed respiration and this deviation is explicable. The radioactivity of zooplankton starts at zero and increases linearly during the experiment of 1 hour. Consequently, the counts in the water due to excretion by zooplankton are related to the average radioactivity of the animals in that hour, *i.e.* half of the radioactivity at the end of the experiment.

Concluding, the serious warning of CONOVER & FRANCIS (1973) regarding the use

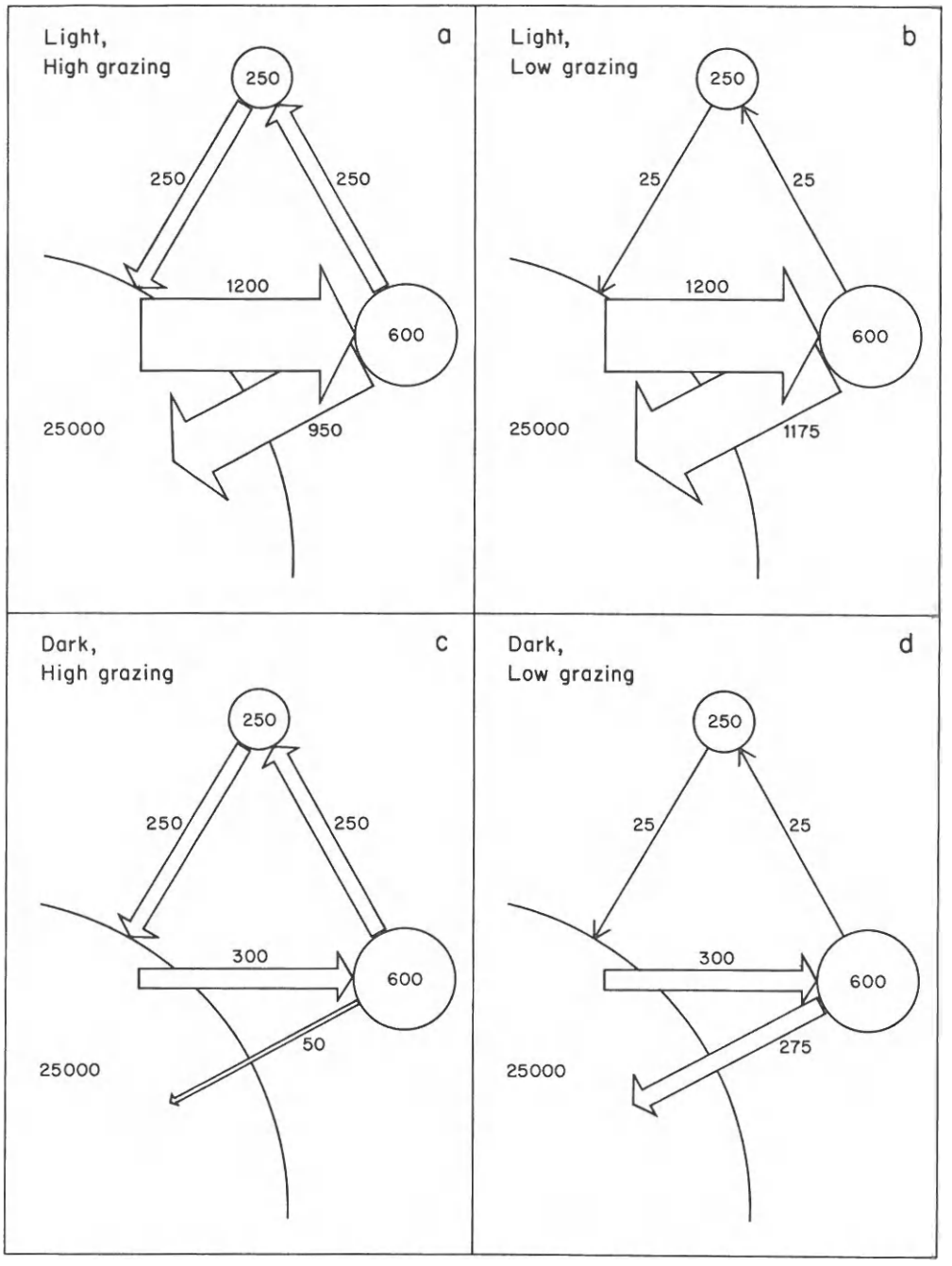


Fig. 2. Three-compartment models according to the model of CONOVER & FRANCIS (1973) but with numerical values varying with light conditions and grazing pressure. Flows (μg C/l/day) between the compartments are in balance, so the stocks (μg C/l) do not change in accordance to the conditions of CONOVER & FRANCIS' model.

of total activities instead of specific activities is wrong. Both calculation methods, if applied correctly, give essentially the same results !

In the next sections we will use the model of CONOVER & FRANCIS to illustrate various situations which can be encountered doing ^{14}C grazing experiments. Special attention will be paid to problems as the one which became apparent above in the calculation of zooplankton excretion, so whether start, end or average values of radioactivity have to be used for a most accurate calculation.

ESTIMATES OF GRAZING RATES IN DIFFERENT EXPERIMENTAL CONDITIONS

In case a large amount of radio isotope is present in the water, as in the method of DARO (1978) or in the modified version as used by BAARS & OOSTERHUIS (1984, 1985), the formulae listed in CONOVER & FRANCIS (1973) are not directly applicable. From the original set of matrices presented in that paper a new set of formulae was derived that includes the possibility of a specific activity in the medium higher than zero right from the start of the experiment. These new formulae, listed in the Appendix, enabled us to calculate the specific activities during a grazing experiment at any of three conditions : a) starting with only the phytoplankton labelled (the classical set-up), or b) with radio isotope only in the water (DARO's method), or c) with most of the radio isotope in the water but with phytoplankton being prelabelled during some hours before the onset of grazing (BAARS & OOSTERHUIS). Calculations for a) and c) were made at high and low grazing pressure in the light, and in the dark. Calculations for DARO's method involved only high and low grazing pressure in the light, because her method is bound to the active incorporation of radioactive bicarbonate by photosynthesis.

We used the same (constant) carbon stocks as CONOVER & FRANCIS for phytoplankton (600 μg/l) and for zooplankton (250 μg/l) but raised the carbon stock in the water to a more natural value for seawater of 25000 μg/l as bicarbonate concentrations in seawater are ca 24500 μg/l. For high grazing pressure we choose a daily ration of 100 % of body carbon (250 μg C/l/day) and for low grazing pressure 10 % (25 μg C/l/day). Limited by the conditions of the model of CONOVER & FRANCIS that carbon stocks do not change, respiration by zooplankton was set at similar amounts (Fig. 2). For both grazing pressures the uptake of dissolved carbon by zooplankton was neglected and set at zero.

For experiments by daylight a high primary production was assumed. In the field daily primary production often equals the phytoplankton stock and this was modelled by applying a carbon uptake of 600 μg/l per light period of 12 hours, giving a rate constant k_{12} of 2 and a primary production over 24 hours in the light of 1200 μg/l (Fig. 2a, b). The uptake of carbon by phytoplankton in the dark should minimally equal the grazing pressure to fulfill the conditions of the model. A four times lower carbon uptake by night than by day was chosen, permitting some respiration by phytoplankton also in the case of high grazing pressure (Fig. 2c).

Specific activities at the start of the experiments were set at 4000 for phytoplankton in the classical experiment (24 x 10^5 DPM/l for the total phytoplankton stock in the grazing vessel), and at 4000 for the water in DARO's experiment (10^8 DPM/l, comparable with 45 μCi per litre). In the experiment with a short prelabelling of the phytoplankton it was assumed that starting with 10^8 DPM/l in the water the specific activity of the phytoplankton reached 1000 after some hours, thus setting the specific activity of the water at 3976 ((10^8 – 600 x 1000)/25000). With the formulae listed in the Appendix specific activities of phytoplankton and zooplankton were calculated at 15, 30, 60 and 120 minutes after the start of the grazing experiments. These specific activities were transformed in total activities, the variable which is normally measured by the worker doing such experiments. The results, listed in Table 2, show trends in radioactivity that could be expected. In the classical

Table 2. Total activities in phytoplankton and zooplankton during grazing experiments in 4 situations with different light conditions and grazing pressure (Fig. 2). Three types of experimental set-ups : a) starting with ^{14}C labelled phytoplankton, b) starting with ^{14}C bicarbonate in the water, and c) with a high amount of ^{14}C bicarbonate in the water but phytoplankton prelabelled for some hours. Dotted lines in zooplankton columns roughly and arbitrarily (**> 1000** DPM/l) indicate the shortest grazing period needed to obtain zooplankton radioactivities most suitable for easy and reliable processing of subsamples of the zooplankton stock.

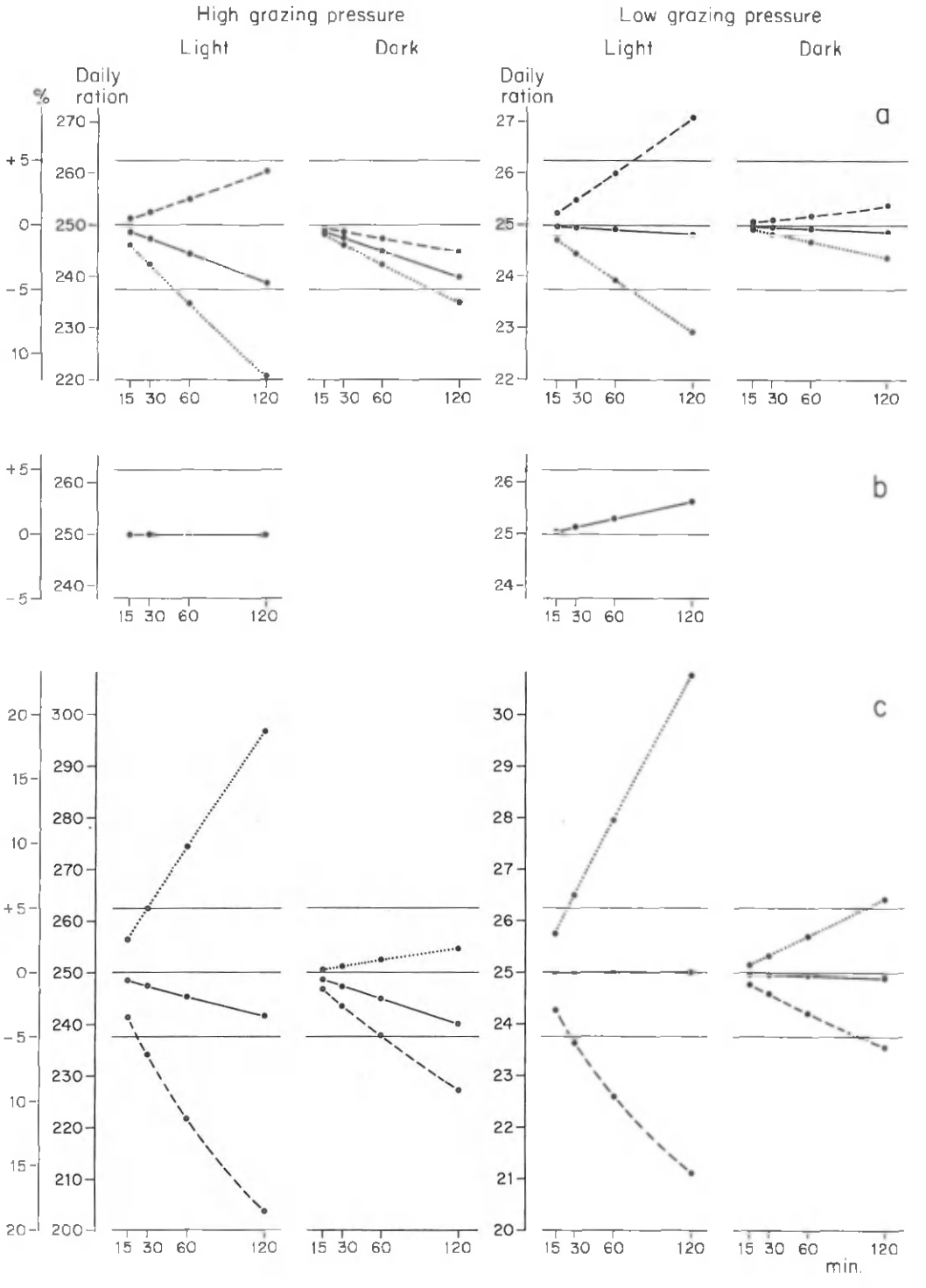
Type of experiment and time (min.)	Total activities (DPM/l) in light conditions				Total activities (DPM/l) in dark conditions			
	High grazing pressure		Low grazing pressure		High grazing pressure		Low grazing pressure	
	Phytopl.	Zoopl.	Phytopl.	Zoopl.	Phytopl.	Zoopl.	Phytopl.	Zoopl.
^{14}C only in phytoplankton								
0	2400000	0	2400000	0	2400000	0	2400000	0
15	2350527	10255	2350529	1030	2387533	10336	2387533	1039
30	2302093	20193	2302102	2038	2375130	20510	2375133	2070
60	2208257	39146	2208292	3989	2350519	40385	2350528	4115
120	2032127	73569	2032257	7644	2302064	78290	2302099	8128
^{14}C only in the water								
0	0	0	0	0				
15	49470	107	49470	11				
30	97897	425	97897	43				
60	191703	1664	191704	169				
120	367720	6384	367728	655				
^{14}C in water & phytoplankton								
0	600000	0	600000	0	600000	0	600000	0
15	636805	2670	636806	268	609275	2611	609275	262
30	672833	5471	672835	552	618501	5234	618501	528
60	742617	11441	742627	1165	636803	10519	636806	1071
120	873545	24738	873585	2562	672825	21227	672834	2202

experiment the highly radioactive phytoplankton is losing more radioactivity with time in the light than in the dark because the dilution with ^{12}C in the light is much higher due to a high primary production coupled with a high respiration/excretion (Fig. 2a, b). In DARO's experiment the radioactivity of the phytoplankton increases linearly with time whereas zooplankton radioactivity quadruples with time periods twice as long. In the experiment with short prelabelling the phytoplankton radioactivity increases, due to the higher specific activity of the water, both in the light and in the dark. The latter phenomenon is absent in actual experiments, of course, but here due to the relatively high uptake of carbon programmed to occur in the dark in order to fulfill the conditions of the model (Fig. 2c, d).

Table 2 was used to reach the amount of carbon grazed away per day by the formula (DPM zoo/DPM phyto) x 24/time x [phytoplankton carbon]. We used three values for the phytoplankton radioactivity : a) that at time zero, b) that at the end of the grazing experiment, and c) the average value between the start and the end of the experiment. Only the latter was used for DARO's experiment because using the value at the start was senseless (with a phytoplankton radioactivity of zero) and because the use of the end value would give estimates of grazing two times too low. The results of the calculations (Fig. 3) show that in short experiments lasting 15 minutes, the deviation from the grazing rate put in the model (250 and 25 μg C per day, respectively) is less than 3 or 4 %, no matter which value of phytoplankton radioactivity is used. With periods of grazing longer than 15 min the deviations generally increase. Now, the use of average phytoplankton radioactivities gave generally the best results. With high grazing pressure the effect of zooplankton respiration becomes apparent in both the classical experiment and in the experiment with short prelabelling, all four curves (bold lines in Fig. 3a and c, left side) decline with time. But although zooplankton respiration rate was programmed as high as the grazing rate, losses by respiration of ^{14}C ingested bring about an underestimate of only ca 4 % over a 2 hour period if average phytoplankton radioactivities are used. This is because the body carbon respired by the zooplankton still consists predominantly of ^{12}C during the grazing experiment. The amount of new material ingested over a 2 hour period is 2/24 x 250 = 20.8 μg. But, on average, in the zooplankton stock of 250 μg only ca 10 μg new carbon will be present during that period. So, the fraction of new material in the amount of carbon respired in that 2 hours is 10/250 = 0.04.

In two cases the use of the phytoplankton radioactivity at the end of the grazing experiment revealed an equally good or even better estimate of the grazing rate than the use of the average phytoplankton radioactivity (Fig. 3a, high grazing pressure). This is because the loss of ^{14}C from the labelled phytoplankton compensates for the loss of ^{14}C from the zooplankton by respiration. In one case the use of the phytoplankton radioactivity at the start of the experiment gave a better result (Fig. 3c, high grazing pressure in the dark). This is due to a comparable counteraction in the calculation; the lower phytoplankton radioactivity at the start rather than during the grazing experiment compensates for the loss of ^{14}C from the zooplankton by respiration.

Fig. 3. Estimates of ingestion rate (μg C per 250 μg of zooplankton carbon) for 4 situations with different light conditions and grazing pressure (Fig. 2, Table 2). Three types of experimental set-ups : a) starting with labelled phytoplankton, b) starting with ^{14}C bicarbonate in the water, and c) starting with a high amount of ^{14}C bicarbonate in the water but with phytoplankton prelabelled for some hours. Different values of phytoplankton radioactivity used in the calculation of ingestion rate : DPM phyto at the start of the experiment (dotted lines), DPM phyto at the end of the grazing period (dashed lines) and mean DPM phyto during the grazing period (bold lines). Ordinates outermost left : percentual deviation of programmed ingestion. Thin horizontal lines : + 5, 0 and − 5 % deviation.


High grazing pressure
Low grazing pressure
Light
Dark
Light
Dark
Daily ration
%
Daily ration
a
b
c
15 30 60 120
min.

GENERAL CONCLUSIONS AND DISCUSSION

This, theoretical, paper has led to the following conclusions.

1) CONOVER & FRANCIS (1973) misinterpreted the results of their model in case of the processing of total radioactivities to reach estimates of the ingestion or feeding rate. There is no principal difference between the outcome of calculations on feeding rate with specific activities and that with total radioactivities. In the latter calculation they overlooked the fact that the first result is an estimate of the filtering rate, not the feeding rate.

2) Within the limitations of CONOVER & FRANCIS' three-compartment model the errors in the estimate of grazing rate are small in different types of ^{14}C experiments. If average phytoplankton radioactivities are used in the calculations, the deviations at the longest experimental duration used (2 hours) did not surpass 4 %.

3) In all the three ^{14}C techniques studied, the best method to calculate the community filtering rate was generally the use of the average phytoplankton radioactivity, according to the formula 'filtering rate = ((DPM zoo at time t)/(DPM phyto at time 0 + DPM phyto at t)/2) x 24/t', with time t in hours (end of the grazing experiment) and the filtering rate expressed as fraction of the total volume of the grazing vessel swept clear per day. If sorting of separate species and stages is done the filtering rate is normally expressed in ml per day per animal according to '(DPM per animal)/(mean DPM phyto per ml) x 24/t'.

One of the remarkable points from Fig. 3 is that experiments in daylight do not necessarily give results worser than those in the dark. Although grazing experiments are generally done in the dark or in dim light to avoid primary production in control vessels (*cf.* PETERS, 1984), in the techniques with radio isotopes such controls are not necessary and the effects of primary production - causing a high loss of label in the classical experiment or an increase in the radioactivity of the phytoplankton in experiments with high concentrations of ^{14}C bicarbonate - do not interfere with the estimate of filtering rate, providing one calculates with the average phytoplankton radioactivity and providing irradiance levels are offered which the animals normally encounter by day. Also in case that the conditions of CONOVER & FRANCIS' model are not fulfilled, *i.e.* that the phytoplankton stock does change due to primary production or grazing, it can be expected that in most cases the use of the average phytoplankton radioactivity will minimize the inaccuracy of the estimate of filtering rate.

More serious objections to CONOVER & FRANCIS' model can be raised regarding the way of programming of the zooplankton compartment as one single carbon pool with complete and instanteneous mixing. They already indicate themselves that this condition is not justifiable. Because of the evidence for a small metabolic pool and a larger structural pool of carbon within the animal, losses of ^{14}C by respiration may be higher than follows from a simple extrapolation of the $^{14}C/^{12}C$ ratio in the entire animal (CONOVER & FRANCIS, 1973; LAMPERT, 1975; LAMPERT & GABRIEL, 1984). Even more serious is the lack of egestion in the model. The carbon content of the faecal pellets egested, will not get recycled immediately; at 15 ^{o}C mineralization will take one or several days. Even if assimilation efficiency is high, for instance 90 %, the carbon not assimilated has about the same specific activity as the phytoplankton cells ingested. If egestion of remains of these cells occurs during the grazing experiment, this will lower the (relative) radioactivity of the animal by 10 % compared with animals in a grazing experiment limited to the time span before the first radioactive pellets appear (*cf.* BAARS & OOSTERHUIS, 1985). In Table 2 we indicated which grazing periods in the different ^{14}C methods are needed to obtain relatively reliable countings for the zooplankton samples, but here we may add that these arbitrary borders also can be used as limiting the duration of the grazing experiment. For instance, at high grazing pressure a period of 15 minutes or less should not be exceeded, whereas at low grazing pressure the duration can be extended to about one hour. DARO's method forms an exception because the food cells (and thus the first pellets produced)

have a relatively low radioactivity in the beginning of the experiment. With her method, the grazing period might be extended to 1 hour at high and to 2 hours at low grazing pressure for the example given in Table 2.

CONOVER & FRANCIS (1973) were well aware of the above mentioned shortcomings in their model but also indicated that more sophisticated models can not be solved anymore in an analytical way. The merit of their simple model is that it inspired others to think about the ^{14}C techniques used and to construct more appropriate models, for example to simulate assimilation and respiration (LAMPERT, 1975; LAMPERT & GABRIEL, 1984). At present more and more data on the carbon budget and dynamics of copepods become available, and it would be worthwhile to simulate the processes influencing the animals radioactivity in ^{14}C grazing experiments according to 4 compartments : ingestion and assimilation in a compartment representing the anterior gut, formation of faecal pellets in a posterior gut compartment, respiration via a small metabolic compartment and growth or egg production in a large structural compartment.

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APPENDIX

A) The specific activities of the compartments presented in Fig. 1 can be calculated for time t according to the equations (see CONOVER & FRANCIS, 1973, p. 274 and p. 281):

$$x_1 = C_{11}\, e^{-\lambda_1 t} + C_{12}\, e^{-\lambda_2 t} + C_E \qquad (1)$$

$$x_2 = C_{21}\, e^{-\lambda_1 t} + C_{22}\, e^{-\lambda_2 t} + C_E \qquad (2)$$

$$x_3 = C_{31}\, e^{-\lambda_1 t} + C_{32}\, e^{-\lambda_2 t} + C_E \qquad (3)$$

in which,

1. time t is part of 24 hr if flows (ρ in Fig. 1) are expressed per day;

2. λ_1 and λ_2 form the roots of the quadratic equation,

$$\lambda^2 - (K_1 + K_2 + K_3)\lambda + K_1K_2 + K_2K_3 + K_1K_3 - k_{12}k_{21} - k_{13}k_{31} = 0$$

(with $K_1 = k_{21} + k_{31}$, $K_2 = k_{12}$, $K_3 = k_{13} + k_{23}$; *cf.* CONOVER & FRANCIS, p. 274);

3. C_E is the final specific activity, to be calculated according to $(x_{10} + x_{20} + x_{30})/(S_1 + S_2 + S_3)$

4. C_{11}, C_{12}, C_{21}, C_{22}, C_{31} and C_{32} have to be calculated according to B).

B) To obtain the solution for C_{ij} two sets of relations are required.
The rates of changes of specific activity in the three pools are :

$$\dot{x}_1 = -K_1x_1 + k_{21}x_2 + k_{31}x_3 \qquad (4)$$

$$\dot{x}_2 = k_{12}x_1 - K_2x_2 \qquad (5)$$

$$\dot{x}_3 = k_{13}x_1 + k_{23}x_2 - K_3x_3 \qquad (6)$$

First we set the derivatives of the equations (1) - (3) equal to equations (4) - (6) at t = 0, so that

$$-\lambda_1C_{11} - \lambda_2C_{12} = -K_1x_{10} + k_{21}x_{20} + k_{31}x_{30} \qquad (7)$$

$$-\lambda_1C_{21} - \lambda_2C_{22} = k_{12}x_{10} - K_2x_{20} \qquad (8)$$

$$-\lambda_1C_{31} - \lambda_2C_{32} = k_{13}x_{10} + k_{23}x_{20} - K_3x_{30} \qquad (9)$$

Then we set the equations (1) - (3) at t = 0, so that

$$x_{10} = C_{11} + C_{12} + C_E \qquad (10)$$

$$x_{20} = C_{21} + C_{22} + C_E \qquad (11)$$

$$x_{30} = C_{31} + C_{32} + C_E \qquad (12)$$

By rearranging equations (10) - (12) and substituting the appropriate variables in equations (7) - (9) the equations for C_{ij} can be obtained. For example, $C_{12} = x_{10} - C_{11} - C_E$ substituted in (7) gives

$$C_{11} = (-\lambda_2x_{10} + \lambda_2C_E + K_1x_{10} - k_{21}x_{20} - k_{31}x_{30})/(\lambda_1 - \lambda_2)$$

In a similar way,

$$C_{12} = (-\lambda_1x_{10} + \lambda_1C_E + K_1x_{10} - k_{21}x_{20} - k_{31}x_{20})/(\lambda_2 - \lambda_1)$$

$$C_{21} = (-\lambda_2x_{20} + \lambda_2C_E - k_{12}x_{10} + K_2x_{20})/(\lambda_1 - \lambda_2)$$

$$C_{22} = (-\lambda_1x_{20} + \lambda_1C_E - k_{12}x_{10} + K_2x_{20})/(\lambda_2 - \lambda_1)$$

$$C_{31} = (-\lambda_2x_{30} + \lambda_2C_E - k_{13}x_{10} - k_{23}x_{20} + K_3x_{30})/(\lambda_1 - \lambda_2)$$

$$C_{32} = (-\lambda_1x_{30} + \lambda_1C_E - k_{13}x_{10} - k_{23}x_{20} + K_3x_{30})/(\lambda_2 - \lambda_1)$$

In case radio isotope is only present in the phytoplankton right at the start of the experiment (so if $x_{10} = 0$ and $x_{30} = 0$), many terms in these equations become zero and the equations then resemble the ones listed by CONOVER & FRANCIS (1973; their Table 1).