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A simplified ^{14}C method for grazing measurements on natural planktonic populations

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ABSTRACT: The method used consists of adding highly radioactive material (40 μCi/l $NaH^{14}CO_3$) to sea water with its natural concentrations of zoo- and phytoplankton, incubating this water in the light, separating zoo- from phytoplankton after 1 h or at the most 2 h and measuring the radioactivity of both. Under such conditions, the concentration of the tracer in phytoplankton can be simplified as a linear function of time, and that of the zooplankton as a parabolic function of time. This simplification leads to an overestimation of grazing of at most 2–3 %. Comparisons with the Coulter Counter method are given and discussed.

INTRODUCTION

The aim of this study is to find a method to determine the grazing of herbivorous zooplankton under natural conditions with natural populations of phyto- and zooplankton. Because primary production is determined as a total value for mixed populations of different parameters such as light, depth, nutrients, the assumption was made that the same kind of approach could possibly be applied to the feeding of herbivorous zooplankton, in order to study "grazing" as a whole and as function of different concentrations of phytoplankton taken as another whole. For this purpose, the ^{14}C technique was chosen, as it is the most sensitive one. Another goal was to find as simple a method as possible for routine use on board a ship.

THE MODEL

General

Conover & Francis (1973) demonstrated that the ^{14}C method used in feeding experiments (prelabelling the culture for a long time) can lead to underestimations, if not all exchanges between the different compartments of the system are measured, or taken into account, for example: respiration, excretion of phyto- and zooplankton.

Such a system is complicated and we attempted to simplify it. First of all, we

tried to eliminate all feed-back systems, i.e. the reinjection of ^{14}C in the water. The simplest way to obtain this result, is to use short-time experiments where ^{14}C is not yet excreted or respired in phyto- and zooplankton. For zooplankton, Schindler (1970) reported on the 1961 results of Malavitskaya & Sorokin indicating that there are different phases in uptake of the ^{14}C labelled food: a first phase of true ingestion ($^1/_2$–1 h), followed by a second phase where ^{14}C egestion occurs during the 15–16 h, after which respiration of ^{14}C occurs. Thus, for zooplankton, 1-h experiments avoid excretion of ^{14}C. If for phytoplankton the experiment is made immediately after adding ^{14}C to the water, respiration of ^{14}C is negligible after a 1-h experiment.

Haney (1971, 1972) used 5-minutes experiments with prelabelled cultures added to natural water in his in-situ feeding experiments. Sorokin (1966) used 3- to 6-h time experiments.

The model chosen is a stationary 3-compartment system (indeed the masses of the compartments do not change in a short time) where we follow the tracer immediately after adding to the water.

	λ_1		λ_2		λ_3
water		phytoplankton		zooplankton	
q_1		q_2		q_3	

q_1, q_2, q_3 are the concentrations of ^{14}C in water, in phytoplankton and in zooplankton respectively; λ_1 is the rate of ^{14}C uptake by phytoplankton, λ_2 the rate of ^{14}C-labelled phytoplankton uptake by zooplankton and λ_3 is the rate of excretion of zooplankton.

The differential equations of the evolution of ^{14}C in the 3 compartments are as follows:

$$\frac{dq_1}{dt} = -\lambda_1 q_1$$

$$\frac{dq_2}{dt} = \lambda_1 q_1 - \lambda_2 q_2$$

$$\frac{dq_3}{dt} = \lambda_2 q_2 - \lambda_3 q_3 \qquad \lambda_3 = 0 \text{ from experimental conditions}$$

$$\frac{dq_3}{dt} = \lambda_2 q_2$$

Integrating we obtain:

$$q_1 = q_{10}\, e^{-\lambda_1 t}$$

$$q_2 = q_{20}\, e^{-\lambda_2 t} + \frac{\lambda_1 q_{10}}{\lambda_2 - \lambda_1} (e^{-\lambda_1 t} - e^{-\lambda_2 t})$$

$$q_3 = q_{10} + q_{20}\,(1 - e^{-\lambda_2 t}) + q_{30} + \frac{q_{10}}{\lambda_2 - \lambda_1} (\lambda_1 e^{-\lambda_2 t} - \lambda_2 e^{-\lambda_1 t})$$

where $q_{10} = q_1$ at time 0
$q_{20} = q_2$ at time 0
$q_{30} = q_3$ at time 0

From the experimental conditions $q_{20} = 0$ and $q_{30} = 0$

$$q_1 = q_{10}\, e^{-\lambda_1 t} \tag{1}$$

$$q_2 = \frac{\lambda_1 q_{10}}{\lambda_2 - \lambda_1}(e^{-\lambda_1 t} - e^{-\lambda_2 t}) \tag{2}$$

$$q_3 = q_{10} + \frac{q_{10}}{\lambda_2 - \lambda_1}(\lambda_1 e^{-\lambda_2 t} - \lambda_2 e^{-\lambda_1 t}) \tag{3}$$

If q_{10} is very high, and the time is very short, we can simplify $q_1 = q_{10}$ and $\lambda_2 q_2 <<< \lambda_1 q_1$, so that we have

$$\frac{dq_2}{dt} = \lambda_1 q_{10}$$

$$\frac{dq_3}{dt} = \lambda_2 q_2$$

Integrating

$$q_2 = \lambda_1 q_{10} t \tag{4}$$

$$q_3 = \frac{1}{2}\lambda_1 q_{10} \lambda_2 t^2 \text{ or } q_3 = \frac{1}{2}\lambda_2 q_2 t \tag{5}$$

the grazing rate being $\lambda_2 = 2\frac{q_3}{q_2 t}$ (6)

METHODS

We came to the following experimental scheme: One-l bottles of natural water (without alterations in the concentrations of phyto- and zooplankton) were used.

40 μCi NaH $^{14}CO_3$/l were added, and the bottles incubated at the most 2 h in light (10 TL lamps = 10 000 lux) and at sea water temperature. After 1 or 2 h, zooplankton was separated from phytoplankton on using a 50 or 100 μm silk mesh.

Phytoplankton was filtered on a millipore filter of 0.45 μm. Zooplankton was formolized, very gently, using a solution which does not exceed 2–3 ‰ (to avoid excretion caused by death due to stress).

Afterwards, the animals were sorted under the binocular microscope with a bent dissection needle into different species or developmental stages.

Following sorting the zooplankton, the remaining filtrate (phytoplankton greater than 50 or 100 μm) which was isolated together with the zooplankton on the 50- or 100-μm silk was also filtered on through a 0.45 μm filter, in order to get the total phytoplankton radioactivity. It is important to use many duplicates of 1 l. When working with natural populations, we commonly used 5 duplicates for one experiment.

APPLICATION AND RESULTS

Let us calculate λ_2 (the grazing rate) with the two models following equations (2) or (3) for the general model and following equations (6) for the simplified model,

with the results for natural phyto- and zooplankton from the North Sea. Three cases will be considered: high, low and mean phytoplankton biomasses.

High phytoplankton biomasses

If we take 40 μCi/, i.e., $q_{10} = 10^8$ cpm/l, we obtain the highest values of q_2 (cpm in one l natural phytoplankton incubated for 1 h under 10 000 lux on the order of magnitude of 10^5 cpm/l. With the different values of q_3 (radioactivity in cpm in zooplankton from 1 l which grazed during 1 h) we calculated λ_2 as shown in Table 1.

Table 1

Grazing rate calculated following the simplication (λ_2 from [6]) or following the complete model (λ_2 from 2 or 3) in the case where the radioactivity of phytoplankton (q_2) is much higher as the radioactivity get into the zooplankton (q_3). The difference between the two calculators is negligible

q_3 (cpm)	$\frac{q_2}{q_3}$	λ_2 from (6)	λ_2 from (2) or (3)	Error (%)
25	4000	$5\ 10^{-4}$	$4.999\ 10^{-4}$	0.02
50	2000	$1\ 10^{-3}$	$0.998\ 10^{-3}$	0.2
100	1000	$2\ 10^{-3}$	$1.999\ 10^{-3}$	0.06
200	500	$4\ 10^{-3}$	$3.997\ 10^{-3}$	0.09
400	250	$8\ 10^{-3}$	$7.988\ 10^{-3}$	0.15
800	125	$1{,}6\ 10^{-2}$	$1.595\ 10^{-2}$	0.27

Low phytoplankton biomasses

The lowest possible results obtained in feeding experiments, giving 10^8 cpm/l as q_{10}, are of the order of magnitude of $q_2 = 10^3$ cpm/l (for natural phytoplankton incubated 1 h). We calculated λ_2 for different conditions (see Table 2).

Table 2

Grazing rate calculated following the simplification (λ_2 from [6]) or following the complete model (λ_2 from 2 or 3) in the case where the phytoplankton radioactivity (q_2) is low. When the relation radioactivity of phytoplankton on the radioactivity of zooplankton $\frac{q_2}{q_3}$ becomes too low, 10 or 5, is the difference between the two calculation methods not negligible

q_3 (cpm)	$\frac{q_2}{q_3}$	λ_2 from (6)	λ_2 from (2) or (3)	Error (%)
25	40	0.05	0.050	0.89
50	20	0.1	0.098	1.66
100	10	0.2	0.194	3.24
200	5	0.4	0.376	6.27

Mean phytoplankton biomasses

A mean of 300 results obtained at the end of a phytoplankton bloom in May–June 1976 in the Fladenground (North Sea) is about $q_2 = 5\ 10^4$ cpm/l. Table 3 shows the calculations of λ_2 in different feeding experiments.

Table 3

Grazing rate calculated following the simplification (λ_2 from [6]) or following the complete model (λ_2 from 2 or 3) in the case of mean phytoplankton radioactivity (q_2). In all cases of zooplankton radioactivities q_3 is the relation $\frac{q_2}{q_3}$ higher than 50 and is the difference between the two calculations negligible

q_3	$\frac{q_2}{q_3}$	λ_2 from (6)	λ_2 from (2) or (3)	Error (%)
25	2000	$1 \cdot 10^{-3}$	$0.999 \cdot 10^{-3}$	0.07
50	1000	$2 \cdot 10^{-3}$	$1.999 \cdot 10^{-3}$	0.07
200	250	$8 \cdot 10^{-3}$	$7.99 \cdot 10^{-3}$	0.13
250	200	0.01	0.01	0.18
500	100	0.02	0.02	0.34
1000	50	0.04	0.04	0.67

DISCUSSION OF THE CALCULATION

The first case (V, a) concerns the maximum of a phytoplankton bloom in the North Sea when the zooplankton has not yet reached its maximum. This results in q_3 values of at most 100–200; in this case we overestimate the grazing by 0.09 %.

The second case (V, b) is exemplified by the phytoplankton biomass of the months February–March, when zooplankton is very scarce and we obtain values of q_3 of 20–30 cpm at most; in this case we overestimate the grazing by 0.887 %.

This can also be related to the summer phytoplankton at certain times of a bloom by a certain herbivorous species, when grazing values can be high: therefore we must look carefully at the values of q_3.

The third case (V, c) concerns the decline of phytoplankton bloom during the maximum of a zooplankton bloom. The highest q_3 values obtained are 500 cpm and in this case we overestimate the grazing by 0.34 %.

All these overestimations due to the simplification of the calculation model are in any case negligible, since the counting error at the scintillation counter is always 1 to 2 % (for every 50 min of counting).

DISCUSSION OF THE EXPERIMENTAL METHOD

The most important advantage of this method is its simplicity. Because of the short experiment time, we can repeat the experiment many times a day using different

phytoplankton and zooplankton concentrations to investigate the diel feeding rhythm of different species. It therefore provides more precise results for in situ 24-h feeding rates.

The disadvantage is the uncertainly that all phytoplankton cells are labelled, especially in such a short time; in this case we underestimate the grazing rate. Nevertheless, after having performed comparisons with another method, we think that this underestimation is of no great importance.

COMPARISON WITH THE COULTER COUNTER METHOD

Culture experiments

We set up feeding experiments in the laboratory, using adults *Artemia salina* feeding on a culture of *Duraliella primolecta*. For both methods the same concentrations were used: 10 *Artemia*/l and 21.309 cells/ml of *Dunaliella primolecta*. The experiments were carried out in 1-l bottles. The feeding time was 24 h for the Coulter Counter experiment and 2 h for the ^{14}C experiment.

Two experiments were carried out at 18° C: one in the light (artificial light from the culture room) and one in the dark (the bottles remained in silver paper and in a black box).

The filtering rate in the Coulter Counter experiment is calculated employing Gauld's formula. In the ^{14}C experiment the results of 2 h were multiplied by 12.

For the dark experiment using the ^{14}C method, the *Dunaliella* culture was first prelabelled (without animals); thereafter the animals were added and put into the dark for 2 h.

The grazing rate is $\lambda_2 = \frac{q_3}{q_2 t}$ because q_2 is constant when labelled phytoplankton is put into the dark.

Table 4

Comparison of the mean values obtained by applying the two methods: ^{14}C method and particles counting method with the Coulter Counter using adult *Artemia salina* grazing on a culture of *Dunaliella primolecta* (laboratory experiments)

^{14}C method		Coulter Counter method	
Filtering rate (ml/animal/24 h)	Ingestion rate (cells/animal/24 h)	Filtering rate (ml/animal/24 h)	Ingestion rate (cells/animal/24 h)
	Light experiment		
108.6 ± 18.0 n = 5	2 314 583 ± 383 974 n = 5	102.3 n = 2	2 136 434 n = 2
	Dark experiment		
96.22 ± 3.15 n = 5	2 050 437 ± 67 247 n = 5	103.11 n = 1	1 955 043 n = 1

Experiments with natural phyto- and zooplankton

Furthermore, we made comparisons between the two methods used at the Sluice-Dock of Ostend. The phytoplankton in this area is mostly composed of nannoplankton, but there is also detritic material, which was counted by the Coulter Counter and not by the ^{14}C method.

In the Coulter Counter experiment, 1-l bottles were used with natural phytoplankton and 100 copepods of the species *Acartia bifilosa* (most of them were adults, with a few old copepodites stages). The bottles were incubated in Sluice-Dock water at 0.5-m depth for 24 h.

In the ^{14}C experiment natural concentrations of zooplankton were used (10–90 copepods/l); the length of the experiment was 1 h. The different developmental stages were sorted after the experiment, which was carried out as described in the chapter "Methods".

The results obtained by using the ^{14}C method are on the order of magnitude of those obtained with the Coulter Counter; however, the values measured are a little lower, probably due to the ingestion of detritic material (Table 5).

Table 5

Comparison experiments between the ^{14}C method and the particles counting method with the Coulter Counter with natural phytoplankton and adult *Acartia bifilosa*

Concentration of phytoplankton in the water (μm^3/ml)	^{14}C method Ingestion rate (μm^3/animal/24 h) (adults only)	Coulter Counter method Ingestion rate (μm^3/animal/24 h) (adults + few copepodites)
$0.8–1.1 \cdot 10^6$	$0.43–0.48 \cdot 10^6$	$0.85–3.1 \cdot 10^6$
$1.7–2 \cdot 10^6$	$0.96–1.44 \cdot 10^6$	$0.5 –2.42 \cdot 10^6$
$3 –3.5 \cdot 10^6$	$3.5 –5.5 \cdot 10^6$	$2.7 –9.2 \cdot 10^6$

THE METHODICAL PROBLEM OF ESTIMATING GRAZING RATES OF NATURAL PHYTOPLANKTON

(a) The method described does not provide any information on the selectivity of zooplankton for some size classes of phytoplankton. It gives only the grazing as a whole. (b) Concerning large phytoplankton biomasses it was demonstrated by the light experiments, that it is possible to get an idea of the amount of the phytoplankton retained on the silk with zooplankton (see "Methods"). (c) In the dark experiment, when phytoplankton is prelabelled in the light without zooplankton present, it is impossible to know the grazing rate on large phytoplankton concentrations retained with the zooplankton. To solve this problem we had to do the experiment in two steps: a series of bottles were incubated with ^{14}C in the light with phyto- and zooplankton. After 1 h ($t_{(1)}$) a first series was manipulated as described in the chapter "Methods", and the grazing rate determined was

$$\lambda_{2(1)} = 2 \frac{q_2}{q_{3(1)} t_{(1)}}$$

The remaining series of bottles was then put into the dark, and after another hour $t_{(2)}$ zooplankton was separated from phytoplankton.

$$q_{3(3)} = q_{3(1)} + q_{3(2)} = \frac{1}{2} q_2 \lambda_{2(1)} t_{(1)} + q_2 \lambda_{2(2)} (t_{(2)} - t_{(1)})$$

$q_{3(1)}$: concentration of the tracer in zooplankton after the time $t_{(1)}$ in the light
$\lambda_{2(1)}$: grazing rate in the light
$t_{(1)}$: grazing time in the light
q_2 : concentration of the tracer in phytoplankton at the time $t_{(1)}$
$q_{3(1)}$: concentration of the tracer in zooplankton between time $t_{(1)}$ and $t_{(2)}$
$\lambda_{2(2)}$: grazing rate in the dark
$(t_{(2)} - t_{(1)})$: grazing time in the dark
$q_{3(3)}$: total concentration of the tracer after the time $t_{(2)}$

In this formulation only $\lambda_{2(2)}$: grazing rate in the dark, is unknown.

CONCLUSION

The method described allows to measure the "grazing" of zooplankton on living phytoplankton only, the latter taken as a whole. Because of the very short experimental time it was possible to determine more precisely the instantaneous grazing rate of zooplankton, and this, done by experiments repeated during a 24-h period, leads to a better estimate of the 24-h grazing. It does not give any information about size-class selectivity just as detritic material. This is reason why two methods: Radiocarbon and Coulter Counter method were used together in order to obtain a complete information about ingestion of zooplankton.

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