

Influence of size dependant ^{14}C uptake rates by phytoplankton cells in zooplankton grazing measurements.

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Abstract : C^{14} is currently used to label natural phytoplankton food in zooplankton grazing experiments. Carbon content per unit volume and rate of carbon uptake both decrease with cell size, following different regressions. When working with multi species phytoplankton assemblages, this physiological property results in a disproportional distribution of tracer to phytoplankton carbon biomass. Because most zooplankton organisms select certain sizes of cells, this disproportionate distribution of tracer results in bias of clearance rates determined on the total phytoplankton population with the ^{14}C method.

This paper combines experimentally determined clearance rates of the copepods *A. tonsa* and *T. longicornis* measured on natural particulate matter distributions in combination with theoretical calculations of the ^{14}C clearance rates. It is shown that in these examples, clearance rates measured by the ^{14}C method would underestimate the grazing pressure on the phytoplankton carbon stock by 7 to 24 %.

Résumé : Le C^{14} est couramment utilisé pour marquer le phytoplancton dans les expériences de broutage par le zooplancton. Le contenu en carbone, ainsi que le taux d'assimilation décroissent en fonction de la taille de la cellule suivant différentes régressions. Lorsque le phytoplancton est constitué de plusieurs espèces, cette propriété physiologique résulte en une distribution disproportionnée du marqueur dans la biomasse phytoplanctonique. Comme la plupart des organismes zooplanctoniques sélectionnent certaines tailles de cellules, cette distribution disproportionnée résulte en une estimation biaisée des taux de filtration déterminés sur le phytoplancton total par la méthode au ^{14}C .

Cet article combine des résultats expérimentaux de taux de filtration sur de la matière particulaire totale par les copépodes *Acartia tonsa* et *Temora longicornis* et des calculs théoriques de ces mêmes taux obtenus par la méthode au ^{14}C . Il a pu être montré, dans ces exemples, que les taux de filtration estimés par la méthode au ^{14}C sous-estiment de 7 à 24 % la pression de broutage exercée sur le phytoplancton.

INTRODUCTION

In general, zooplankton grazing studies aim at quantifying the grazing pressure on a phytoplankton population. This flux is usually quantified in carbon, in order to be intergratable in carbon-flow models of the ecosystems studied.

Daro (1978) introduced a version of the ^{14}C method (Nauwerck, 1959) which allows measurement of zooplankton grazing activity on the totality of a natural phytoplankton population. By introducing a high concentration of ^{14}C to the natural water, ^{14}C uptake by the natural phytoplankton population is sufficiently high to allow its quantification in the phytoplankton and the zooplankton feeding on it after one hour already. Since its introduction, the method has been widely applied. Alternations mainly concerned introduction of a prelabelling period for the natural phytoplankton population prior to the introduction of the zooplankton (Roman & Rublee, 1981 ; Baars & Fransz, 1984).

In order for the ^{14}C method to result in a correct measurement of the grazing pressure on the phytoplankton standing stock, it is necessary that the ^{14}C taken up and the phytoplankton biomass (in terms of carbon) are proportionally distributed over the entire size range in which the phytoplankton population occurs. In other words, if one uses the method to determine the grazing pressure of a zooplankton species on a natural phytoplankton population, one assumes that the ^{14}C taken up by the various phytoplankton species is proportional to the contribution of each species to the total phytoplankton biomass. This condition is essential, because most zooplankton organisms are known to feed size - selectively. Especially adult calanoid copepods are known to graze selectively on phytoplankton volume peaks. These volume peaks are generally caused by large - sized phytoplankton species (Poulet, 1973, 1974, 1978 ; Richman *et al.*, 1977 ; Gamble, 1978 ; Cowles, 1979, Tackx *et al.*, 1989).

If the growth rate ($= ^{14}\text{C}$ uptake) and carbon content of the cells follow disproportional regressions with cell size, the above conditions are not fulfilled, and a discrepancy will arise between the grazing pressure measured by ^{14}C incorporation and the actual grazing pressure on the stock of phytoplankton carbon.

Banse (1976) has established the relationship between growth and respiration rate of various species of phytoplankton from literature data on measurements performed with cultures grown under identical conditions. His results show that growth rate, expressed per carbon weight of the cell, declines with cell size. An overview of the regressions reported by Banse, 1976 is given in table Ia.

TABLE I

Overview of regressions between a) growth rate and cell carbon and b) cell carbon and cell volume for planktonic phytoplankton species as found in literature
with g : growth rate ($\text{pgC cell}^{-1} \text{ hr}^{-1}$) ; C : cell carbon content (pgC cell^{-1}) ; V : cell volume ($\mu\text{m}^3 \text{ cell}^{-1}$).

a) growth rate : $\log g = b \log C + a$

regr.	b	log a	specification	source
1.	0.94	- 1.25		Banse, 1976
2.	0.87	- 1.08		Banse, 1976
3.	0.94	- 1.19		Banse, 1976

b) cell carbon : $\log C = b \log V + a$

4.	0.760	- 0.290		Mullin <i>et al.</i> 1966
5.	0.758	- 0.422	diatoms	Strathmann, 1967
6.	0.806	- 0.460	non diatoms	Strathmann, 1967
7.	0.760	- 0.352	diatoms	Eppeley, 1974
8.	0.940	- 0.600	non diatoms	Eppeley, 1974

The carbon content of unicellular algae has been quantified as a function of cell volume by several authors : Mullin *et al.* 1966 ; Strathmann, 1967 ; Eppeley, 1974. An overview of the regressions found is given in table Ib. This paper evaluates to what extent possible disproportional labelling of various cell sizes can result in bias in ^{14}C measurements of zooplankton grazing pressure on natural phytoplankton populations.

MATERIAL AND METHODS

Experimental grazing data obtained from Tackx *et al.* (1989) were used as a basis for the evaluation of potential bias in ^{14}C experiments. These authors performed grazing experiments using the Coulter counter to quantify the grazing activity of adult calanoid copepods *Acartia tonsa* and *Temora longicornis* on natural particulate matter from the Oosterschelde, a tidal inlet in the South - West Netherlands. Besides the Coulter analysis of the total particulate matter in 20 size classes ranging from 3 to 100 μm Spheric Equivalent Diameter (S.E.D.), the phytoplankton in the samples on which grazing was measured, was counted and sized microscopically, allowing to situate the phytoplankton volume distribution within the particulate matter distribution measured by Coulter. This also allowed to quantify the number of phytoplankton cells eaten in each size class (assuming no discrimination between phytoplankton and non-phytoplankton particles within each size class). For details on experimental procedures and calculations, the reader is referred to Tackx *et al.*, 1989.

Because the degree of bias in ^{14}C results will depend on the combination of the size distribution of available phytoplankton and the pattern of selectivity performed by the zooplankton on this phytoplankton distribution, three different situations of natural phytoplankton distributions were chosen : a) a situation where the phytoplankton is concentrated in a narrow peak around 40 μm S.E.D., b) a situation where the phytoplankton occurs in a major peak around 60 μm and a smaller peak around 20 μm S.E.D. and c) a situation with a low concentration of phytoplankton, in which the phytoplankton is distributed more homogeneously among the 5- 100 μm size range, with a peak around 8 μm and one around 30 μm S.E.D. (fig. 1, a, b, c ; after Tackx *et al.*, 1989).

It was shown by Tackx *et al.*, 1989 that on phytoplankton distributions of the type shown in a and b, both *A. tonsa* and *T. longicornis* select the volume peak of the distribution. On phytoplankton distributions of the type shown in c, both copepod species select the peak of larger particles, but at the same time they also feed on the smaller size range. For *A. tonsa*, this feeding on smaller particles is more pronounced than for *T. longicornis*.

The results of a hypothetical ^{14}C experiment carried out on these three samples were simulated and compared with the results obtained in terms of carbon (calculated from the Coulter results) by the following calculations.

$$\text{Ci} = 0.760 \log V - 0.290$$

$$\text{ICT} = \sum_{i=1}^{20} \text{ICI}$$

$$\text{CT} = \sum_{i=1}^{20} \text{Ci} \cdot \text{ni}$$

$$\text{I}^{14}\text{Ci} = \text{Ii} \cdot {}^{14}\text{Ci}$$

$${}^{14}\text{Ci} = 0.87 \log \text{Vi} - 1.08$$

$$\text{I}^{14}\text{CT} = \sum_{i=1}^{20} \text{I}^{14}\text{Ci}$$

$${}^{14}\text{CT} = \sum_{i=1}^{20} {}^{14}\text{Ci} \cdot \text{ni}$$

$$\text{Fc} = \text{ITC}/\text{CT}$$

$$\text{ICI} = \text{Ii} \cdot \text{Ci}$$

$$\text{F}^{14}\text{C} = \text{I}^{14}\text{CT}/{}^{14}\text{CT}$$

with :

Ci : carbon content per cell in size class i (pg ; calculated following regression 5. in table Ib).

CT : total phytoplankton carbon concentration in the sample (pg C ml⁻¹).

¹⁴Ci : ¹⁴C concentration per cell in size class i after one hour of labelling (pg ¹⁴C cell⁻¹ ; calculated following regression 2. in table Ia).

¹⁴CT : total ¹⁴C concentration in the sample after one hour of labelling (pg ¹⁴C ml⁻¹).

Ii : ingestion rate measured in size class i calculated from the Coulter data following Frost, 1972 (nr ind⁻¹ hr⁻¹). The assumption was made that all particles consumed are phytoplankton cells. Negative ingestion rates were counted as zero.

ICi : ingestion rate in carbon obtained from size class i (pg C ind⁻¹ hr⁻¹).

ICT : total ingestion rate in carbon (pg ind⁻¹ hr⁻¹).

¹⁴ICi : ingestion rate of ¹⁴C obtained from size class i (pg ¹⁴C ind⁻¹ hr⁻¹).

FC : clearance rate on the total phytoplankton biomass on a carbon basis (ml ind⁻¹ hr⁻¹).

F¹⁴C : clearance rate on the total phytoplankton biomass calculated on a ¹⁴C basis (ml ind⁻¹ hr⁻¹).

It was assumed that all cells eaten are entirely consumed, i.e. no sloppy feeding or cell breakage take place, so that all the radioactivity present in consumed cells is measured in the zooplankton.

RESULTS

Fig. 1 d, e, f show the calculated carbon distribution, and the distribution of ¹⁴C after 1 hour in experiment a, b and c respectively. These graphs demonstrate the discrepancy between the distribution of the tracer food and the actual stock of phytoplankton.

Table II shows the FC and the F¹⁴C values calculated for *A. tonsa* and *T. longicornis* in each experiment.

The F¹⁴C values are lower than the FC values in all cases. Expressed as percent of FC values, F¹⁴C values vary from maximally 93 to minimally 76 %. In experiments a and b, the percentages calculated for *A. tonsa* and *T. longicornis* are quite comparable, in experiment c, F¹⁴C for *T. longicornis* is a considerably greater underestimation of FC (76 %) than F¹⁴C for *A. tonsa* (87 %).

TABLE II

FC (ml ind⁻¹ hr⁻¹) and F¹⁴C (ml ind⁻¹ hr⁻¹) calculated for *A. tonsa* and *T. longicornis*.

experiment/species	FC	F ¹⁴ C	F ¹⁴ C as % of FC
a) <i>A. tonsa</i>	1.18	1.09	92
<i>T. longicornis</i>	1.25	1.16	93
b) <i>A. tonsa</i>	0.73	0.62	85
<i>T. longicornis</i>	1.12	0.93	83
c) <i>A. tonsa</i>	0.75	0.65	87
<i>T. longicornis</i>	0.76	0.58	76

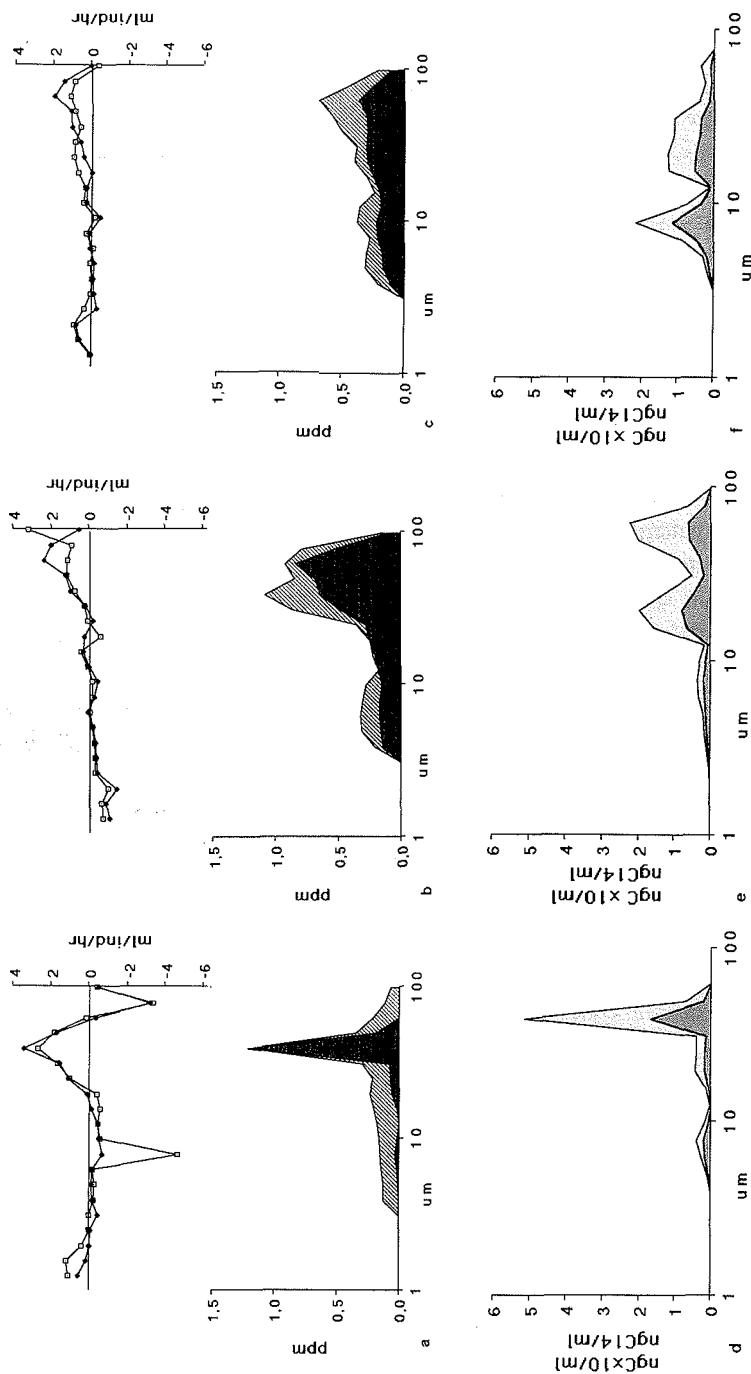


Fig. 1 a, b, c: Size distribution of 3 natural water samples from the Oosterschelde expressed in volume. Total particulate matter (striped, left axis); phytoplankton distribution (shaded, left axis). Clearance rates measured for *A. tonsa* (open squares, right axis) and *T. longicornis* (black diamonds, right axis) on these samples (after Tackx *et al.*, 1989). X axis is in logarithmic scale.

d, e, f: Size distribution of phytoplankton carbon (lightly shaded) and ^{14}C taken up after one hour (darkly shaded) in the samples shown in a, b and c respectively. Note that the ^{14}C concentrations are one order of magnitude smaller than the C concentrations.

DISCUSSION

The use of Coulter obtained results in this paper is not meant to suggest that these results are in any aspect a more reliable measurement of copepod grazing activity than those obtained from other methods. The Coulter and microscopical results were merely used as basic data sets on which to evaluate the performance of the ^{14}C method in quantifying grazing pressure on phytoplankton carbon stocks under natural conditions. Theoretical limits to the degree of variation in phytoplankton biomass distribution and selectivity of zooplankton feeding behavior are hard to define within realistic circumstances. Therefore, we have preferred to use experimentally obtained data as a basis to evaluate the possible degree of bias occurring in ^{14}C measurements. The types of distributions used correspond to other (mainly total particulate matter distributions) reported in zooplankton grazing experiments with natural samples (Allan *et al.*, 1977 ; Gamble, 1978 ; Poulet, 1973, 1974, 1976, 1978). The assumption that all particles eaten are phytoplankton has been argued extensively in Tackx *et al.*, 1989. Even if this assumption is not completely fulfilled, the agreement between clearance rate pattern, and phytoplankton distribution strongly suggests that the amount of phytoplankton cells eaten in each size class is proportional to the concentration of phytoplankton in this size class (Fig. 1 a, b, c). Consequently, the factors of importance to this evaluation would remain within the same proportion and the same type of bias (see below) would occur in ^{14}C measurements.

Regression (2) was chosen from Banses (1976) table 1 because it covers the broadest range of planktonic cell sizes. The formula of Mullin *et al.* (1966) was chosen in accordance to the use of this formula in the paper of Banse (1976).

The results shown in table II demonstrate the dependance of the bias in F^{14}C values on the phytoplankton distribution and the selectivity pattern exerted by the animals feeding on it. The lowest underestimation in comparison with FC occurs in experiment a (7 and 8 % for *A. tonsa* and *T. longicornis* respectively). In this situation, the narrow peak of phytoplankton around 40 μm size, and low concentration of phytoplankton in other size classes necessitates feeding on this one peak. Thus, the disproportionality between the ^{14}C and the carbon distribution is of little consequence, as both *A. tonsa* and *T. longicornis* are feeding in the limited size range where also the bulk of the phytoplankton biomass is situated.

In situation b, the F^{14}C values underestimate FC by 15 and 17 % for *A. tonsa* and *T. longicornis* respectively. In this case both species feed selectively on the peak around 60 μm . Clearance rates (as measured by Coulter) on the second peak of smaller phytoplankton species (around 25 μm S.E.D.) are considerably lower. This second peak has a higher ratio of ^{14}C per unit cell carbon, so that the total amount of ^{14}C taken up by the animals, which is mainly contributed to by the low - labelled big cells, underestimates the proportion of the phytoplankton carbon eaten by 15 and 17 % for *A. tonsa* and *T. longicornis* respectively. In situation c, both *A. tonsa* and *T. longicornis* select cells with a S.E.D. > 40 μm , although the bulk of the phytoplankton volume is situated between 15-40 μm S.E.D. At the same time, both species also feed to a considerable extent on particles in the 10-40 μm size range. In

this case the tendency of *A. tonsa* to feed more on smaller particles than *T. longicornis* results in a considerably less important underestimation of the F value (13 as compared to 24 %).

The data presented here show an underestimation of F^{14}C values in relation to FC values in all cases. Theoretically, the inverse could also be found, in case small particles would be preferably eaten (e.g. by young copepod development stages). Allan *et al.*, 1977 have investigated the selectivity of feeding of juvenile *Eurytemora affinis* and *Acartia tonsa* on natural Chesapeake Bay water. They report that selectivity (i.e. the tracking of biomass peaks measured by Coulter counter) starts with the metamorphosis from Nauplius VI to copepodite I. Poulet (1977) also reports peak tracking by copepodite stages CI-CIII of *Pseudocalanus minutus*, although they obtain the bulk of their food from small sized particles ($< 20 \mu\text{m}$ S.E.D.). Feeding of young juvenile stages being little studied, it seems probable that most ^{14}C measurements with copepods tend towards underestimation rather than overestimation of the real grazing pressure exerted on the phytoplankton stock under study. Even in monospecies cultures, a decline of the $^{14}\text{C}/\text{C}$ ratio will occur with cell size. As most copepods, including copepodite stages, tend to select the right side of the volume peak (Gamble, 1978 ; Richman *et al.*, 1977 ; Poulet, 1973; 1977; 1978) ; this will result in an (limited) underestimation F^{14}C values.

It should be stressed that this underestimation is inherent to the method, because it results from basic physiological properties of algal communities. It occurs in addition to any other, experimentally induced underestimations which can occur in the ^{14}C method, such as loss of radio activity through cell breakage and egestion (Zhang *et al.*, in press).

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