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Tracer kinetics in *Daphnia*: an improved two-compartment model and experimental test

By Winfried Lampert and Wilfried Gabriel

With 7 figures and 4 tables in the text

Abstract

An improved two-compartment model for the kinetics of uptake and release of a radioactive tracer in small aquatic animals is presented. Parameters of the model are estimated from experimental results for *D. magna* fed high and low concentrations of ^{14}C-labelled algae. The relative size of the "metabolic" pool appears to be independent of the food concentration (1.6 % of body carbon). The model predicts the "apparent" metabolic rate measured when homogeneously labelled animals are fed algae differing in specific activity from the animal itself. A linear relationship is found for the dependence of the apparent metabolic rate on the specific activity of the food. The slope of this characteristic increases with the concentration of food. Model predictions are tested experimentally. As predicted and experimental results agree very well, we assume that the model is a reasonable description of the kinetics of the tracer. Approximations are given for the model equations. The model can be used to calculate correct metabolic rates of feeding animals from radiotracer experiments.

Introduction

Ten years ago Conover & Francis (1973) critically reviewed the use of radioisotopes by ecologists. They urgently warned that ignoring the theory of tracer kinetics (Atkins 1969) might cause considerable error in the results from studies of uptake or excretion by aquatic animals (Smith & Horner 1981). But despite extensive use of radiotracers in experiments of this kind, the consequences of these authors' warning are still not realized by many researchers.

Conover & Francis (1973) pointed out particularly that, with respect to uptake and release of a tracer, it is highly unrealistic to consider an animal to be a single compartment with instantaneous mixing. There is increasing evidence that at least 2 pools are necessary to adequately describe the behaviour of the tracer, a small intermediate "metabolic" pool and a larger "structural" pool (Conover 1961; Peters & Rigler 1973; Brandl & Fernando 1975; Lampert 1975; Schiller et al., 1977; Taylor & Lean 1981).

It is easy to demonstrate qualitatively that tracer kinetics must be considered very carefully when an experiment with radioisotopes is designed. However, there is a lack of quantitative information about the possible range of

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errors. In this paper we develop an example to show how the interpretation of the results of tracer experiments can be improved by using a more realistic mathematical model. Our example is the measurement of absolute values of respiration by the radiocarbon method as proposed by SOROKIN (1968).

Parameters of an energy budget of an aquatic animal are often estimated by very different methods, e. g. energy intake is measured by using ^{14}C-labelled material, growth by increase in dry weight, and metabolic losses by oxygen consumption. To establish a balance all the measurements must be converted to the same units. Different conversion factors must be applied and thus much uncertainty is introduced. Measuring all the parameters of the budget in the same units, e. g. in terms of carbon, could eliminate this source of uncertainty. Then, assimilation could be measured by carbon-14, growth as increase in carbon, and metabolic losses as production of carbon dioxide (LAMPERT 1983). Unfortunately, monitoring small changes of CO_2 in a buffered aquaeous system is difficult. On the other hand, the measurement of the $^{14}CO_2$ output of labelled animals (SOROKIN 1968) provides a very sensitive and convenient method of estimating metabolic losses. To calculate CO_2 from the excreted radioactivity, the specific activity of the expired CO_2 must be determined. This requires transferring the animals into carbon dioxide free water and handling them repeatedly. Extensive handling can be avoided and the procedure simplified if the animals are labelled homogeneously. In this case one can assume that the specific activity (dpm/mgC) of the expired CO_2 equals the specific activity of the animal. According to SOROKIN (1968), one advantage of the radioisotope method is that the animals can be fed unlabelled food during the experiment. But when a labelled animal assimilates unlabelled carbon, its metabolic pool is diluted and the specific activity of the pool is decreased: hence, the specific activity of the expired CO_2 will be reduced, causing an underestimate of the metabolic losses. SOROKIN (1968) tries to compensate for that reduction in specific activity by using the arithmetic mean of the specific activity of CO_2 before and after the experiment. This is, of course, a laborious procedure. The problem can be solved when homogeneously labelled animals are provided with labelled food of exactly the same specific activity as the animal. In this case the measured output of $^{14}CO_2$ is a true indication of the metabolic losses. Any variation of the specific activity of the food, however, will change the output of $^{14}CO_2$, and thus produce an error. The metabolic rate will be underestimated if the specific activity of the food is too low and will be overestimated if it is too high.

In a recent review KRYLOV (1980) suggests that better knowledge of the tracer kinetics is needed to solve these experimental problems. This study has been initiated to quantify the possible errors introduced by the inadequate use of the tracer and to give advice on how these errors can be corrected. In the beginning we formulate a mathematical model describing the time course of

the specific activity in the different pools. Then we estimate the parameters of the model from feeding experiments with *Daphnia magna*. The model with known pool sizes and transfer rates allows predictions of the outcome of independent experiments. We predict the "apparent metabolic rate" for different conditions when the specific activity of the food does not equal that of the animal. The predictions are supported by experimental determination of these apparent metabolic rates. Finally, we use the model to estimate the range of possible errors for conditions not tested experimentally.

The model

Our model, Fig. 1, is based on the simple two-compartment model (LAMPERT 1975) which described reasonably well the ^{14}C turnover in *Daphnia pulicaria*. It is a simplification of the 4-compartment model by CONOVER & FRANCIS (1973) derived by omitting the "water" and "food" pools under the assumption that the water pool (here the surroundings of the animal) is large enough to avoid any feedback to the food pool. The food is assumed to be replenished, so that the pool size is not changed.

S_1 and S_2 are relative pool sizes of the "metabolic" (pool 1) and the "structural" (pool 2) pools, respectively. Transfer rates are labelled ϱ. To estimate the parameters of the earlier model (LAMPERT 1975), some limiting assumptions were necessary:

The time after injection of the tracer to pool 1 was short, so that the specific activity of pool 2 remained low and the feedback of tracer via ϱ_{21}

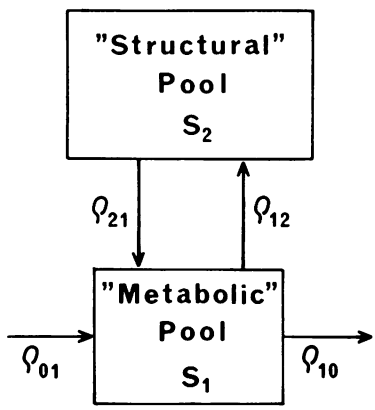

Fig. 1. Graphical representation of the two-compartment model. Pool sizes are labelled (S) and transfer rates (ϱ). The "structural" pool is much larger than the "metabolic" pool. The model represents a daphnid swimming in a very large environment from which it extracts food and into which it releases CO_2. Then ϱ_{01} is the rate of carbon assimilation, ϱ_{10} is the metabolic rate (rate of CO_2-production), and ϱ_{12} and ϱ_{21} are rates of carbon exchange between the pools (see also appendix).

could be neglected. S_1 and S_2 remained constant, i. e. growth of the animal was negligible. Finally, the rate of respiration ϱ_{10} was not measured, but was taken from the literature. Because of considerable variability of the literature data, examples of transfer rates were calculated for different respiratory rates.

Using the data presented by LAMPERT (1975), UMNOV (1980) formulated a new model based on differential equations. But even this model has some shortcomings (GABRIEL, in prep.). The model presented here is improved, so that the system is allowed to grow and the feedback from pool 2 to pool 1 is considered. The transfer rates ϱ_{12} and ϱ_{21} are estimated by the model; ϱ_{01} and ϱ_{10} have both been determined experimentally.

To estimate the respiratory rate from $^{14}CO_2$ output, we start with a homogeneously labelled system. We relate all specific activities to the initial ratio of ^{14}C to ^{12}C (relative specific activity 1). Then the relative specific activities of the pools are

$$a_1 (t = 0) = 1$$
$$a_2 (t = 0) = 1$$

Because the model only allows for CO_2 excretion from the metabolic pool, the relative specific activity of CO_2 will always equal a_1. In order to get detectable levels of excreted $^{14}CO_2$, this must have accumulated in the water for some time. If ingestion of food of the different specific activities starts at $t = 0$, then the relative "apparent" rate of metabolism (RARM) approximates the mean of a_1 during the period of accumulation from the equation

$$\text{RARM} = \frac{1}{\Delta t} \int_0^{\Delta t} a_1 (t) dt \tag{1}$$

The time course of specific activities can be described by the following system of differential equations (for derivation see appendix):

$$\frac{da_1}{dt} = \frac{1}{S_1} [(a_0 - a_1) \varrho_{01} + (a_2 - a_1) \varrho_{21}] \tag{2}$$

$$\frac{da_2}{dt} = \frac{1}{S_2} (a_1 - a_2) \varrho_{12} \tag{3}$$

The change of the relative specific activity of a pool with time is inversely proportional to the pool size but proportional to the input rates and the actual gradient of specific activities.

Though pool sizes may change when the animal grows, we feel it is reasonable to assume that the ratio $S_1 : S_2$ remains constant over the short time intervals used in these experiments. As the amount of carbon retained in the animal is defined as the difference of assimilation and respiration, the transfer rates ϱ_{01}, ϱ_{12}, ϱ_{21}, and ϱ_{10} are not independent. They are connected as follows (see appendix):

$$\varrho_{12} = \frac{S_2}{S_2 + S_1}(\varrho_{01} - \varrho_{10}) + \varrho_{21} \quad (4)$$

Experimental material and methods

All experiments for the estimation of parameters of the model and for the test of the predictions were carried out with *Daphnia magna* of 0.25 to 0.35 mg individual dry weight (0.12—0.15 mg C). The food was *Scenedesmus acutus* grown in a chemostat as described by LAMPERT (1976). Food suspensions of appropriate densities (low: 0.1 mg $C \cdot liter^{-1}$, 2.8×10^3 cells ml^{-1}; high: 1.5 mg $C \cdot liter^{-1}$, 4.2×10^4 cells ml^{-1}) were prepared by diluting stock suspensions with membrane filtered lake water. Particular carbon concentration of the stock was estimated by measuring the light extinction (800 nm) in a photometer, using a previously established calibration curve.

Algae were labelled with ^{14}C-sodium-bicarbonate in a closed Erlenmeyer flask under fluorescent light with gentle shaking overnight (LAMPERT 1977). Daphnids were labelled with ^{14}C-labelled *Scenedesmus*. 10—20 new-born animals were placed into stoppered glass bottles completely filled with about 1.2 liter of food suspension (2 mg $C \cdot liter^{-1}$). The specific activity of the food was adjusted by diluting with unlabelled algae to 3.2×10^6 dpm per mg C. To prevent sedimentation of the algae the bottles were placed on a tissue culture apparatus which rotated them along their long axis once a minute. The daphnids were transferred to fresh food suspension every second day. To ensure that they became homogeneously labelled, the food was always carefully adjusted to the same specific activity (i.e. 3.2×10^6 dpm per mg C). Animals were assumed to be uniformly labelled after about 8—10 days, at which time they had their second clutch of young and were used in the release experiments. That they were well fed under these conditions was demonstrated by the large numbers of eggs they carried.

Rates of carbon assimilation and respiration were measured simultaneously. 7—8 unlabelled *D. magna* were placed into glass stoppered bottles of 300 ml with ^{14}C-labelled algal suspension. After 4 hours the label retained in the animals and the $^{14}CO_2$ excreted were determined. The sum of both yielded the assimilation rate. Oxygen consumption was measured in the same bottles by a membrane electrode and converted to CO_2-excretion to estimate the respiratory rate. A wide range of food concentrations was tested in this way (BOHRER & LAMPERT, in prep.) but only the rates for 0.1 mg $C \cdot liter^{-1}$ and 1.5 mg $C \cdot liter^{-1}$ were used for this study.

The increase of $^{14}CO_2$ output by previously unlabelled *Daphnia* fed labelled food was measured for the estimation of pool sizes. 8 unlabelled daphnids of 2.7 mm average size were placed into glass stoppered bottles with labelled food suspension for different periods of time. 100 ml bottles were used for the 20 and 40 min. runs, 300 ml bottles for the longer periods (1, 1.5, 2, 3, and 4 hours). Controls without animals were run simultaneously. The bottles were incubated for every time interval in a temperature controlled waterbath (20 °C) under weak illumination. At the end of the experimental time, each bottle was opened and two 15 ml subsamples were withdrawn by a syringe. The subsamples were transferred to vials containing 0.1 ml N NaOH. 10 ml of each sample were filtered through a 0.45 μm membrane filter. The filter was dissolved in a special cocktail (Filtercount, Packard) and counted in an LSC. 3 ml of the filtrate was pipetted into a scintillation vial and mixed with a water absorbing fluor (Picofluor, Packard) which had been enriched with 5 % of a CO_2-absorbant (Carbosorb, Packard) to avoid losses of $^{14}CO_2$. Samples treated in this way were stable for many days. The

remaining 7 ml of filtrate were acidified by 0.2 ml of N HCl and bubbled with air for 10 minutes to remove the CO_2. Then another 3 ml was mixed with the fluor. The difference of radioactivity before and after acidification was considered to be $^{14}CO_2$. To measure the initial value of $^{14}CO_2$, the food suspension was treated in the same way when the bottles were filled at the beginning.

The animals were poured through a sieve, rinsed, narcotized in carbonated water, flushed into a petri dish containing a few drops of formalin and transferred immediately into weighed aluminum containers. They were dried at 102 °C for two hours, cooled in a desiccator, and weighed to the nearest μg on an electro balance. After weighing, the dry animals were transferred to a scintillation vial, wetted with a drop of distilled water, and incubated at 60 °C overnight with 1 ml of tissue solubilizer (Soluene 350, Packard). The cool samples were counted with a toluene cocktail.

Controls without animals were used to measure the $^{14}CO_2$ excretion of the algae. The controls were corrected for the number of algae consumed by the animals in the experimental bottles. The number of algae at the end of the experiment in the bottles with animals and in the controls was estimated from the radioactivity of the membrane filters. It was assumed that the rate of ^{14}C-excretion per alga was constant and was the same in the experimental bottles and in the controls. Also the per capita loss rate of algae in the animal bottles was assumed to be constant resulting in an exponential decrease of the algal number. Therefore, the following correction was applied:

$$^{14}CO_2 \text{ control (corr.)} = \frac{1}{K \cdot t}[1-\exp(-Kt)](C_c - C_i) + C_i$$

$$K = \frac{1}{t} \ln \frac{F_c}{F_a}$$

where: C_c and C_i are the concentrations of $^{14}CO_2$ in the controls and in the initial suspension; F_c and F_a are the radioactivity of the filters from the controls and the bottles with animals. $^{14}CO_2$ excretion in the controls never exceeded 30 % of the animal bottles. Reduction of food in the animal bottles was usually in the order of 20 %.

The ability of the algae to re-incorporate $^{14}CO_2$ under the dim light conditions was tested. Daphnids were fed labelled algae overnight. The suspension was then filtered, and the filtrate containing $^{14}CO_2$ and labelled DOC was used to make up a new suspension with unlabelled algae. After 4 hours under the experimental conditions, the algae were filtered on a membrane filter and their radioactivity was measured. The uptake did not exceed 2 %, thus this possible error was neglected.

These experiments yielded a time series of measurements of ^{14}C incorporated into the animal and $^{14}CO_2$ excreted. The sum of the two measures estimated the amount of carbon assimilated. The respired ^{14}C was then expressed as percentage of total assimilated ^{14}C. However, the radioactivity retained in the animal may be partly due to undigested food contained in the gut which is not yet assimilated. This error is assumed to be small because of the high assimilation efficiency our daphnid exhibits on a diet of *Scenedesmus*. A regression on the uptake of ^{14}C in time (see LAMPERT 1977) at high food concentration shows only a small intercept representing the material in the gut. If the animals were allowed to clear their guts at the end of the experiments to remove the undigested material, they would lose additional ^{14}C from the metabolic pool by the mechanisms discussed in the introduction. This loss from the metabolic pool would probably introduce a much greater error than the one caused by assuming that all the radioactivity contained in the animal represents assimilated carbon. The error introduced by assuming that all the radioactive material was assimilated is more important during the shorter experimental periods.

Homogeneously labelled animals were used to test the predictions of the model. Food suspensions of varying specific activity were prepared by mixing labelled and unlabelled algae. Specific activities ranged from 0 to more than twice the value of the animals. Experiments lasted 4 hours. Samples of food suspension and animals were treated as described above. Rates of $^{14}CO_2$-output were calculated as % of body-^{14}C. When an animals and its diet are of exactly the same specific activity, this must reflect the true respiratory rate. Therefore, all the "apparent" metabolic rates obtained with food of a defined specific activity are given relative to the "ideal conditions".

Parameter estimation

Because the rates of transfer are related to each other as in (4) and ($S_1 + S_2 = 1$) and because ϱ_{01} and ϱ_{10} can be measured directly, the system of differential equations (2) and (3) contains only 2 unknown parameters, e. g. S_1 and ϱ_{21}. These parameters can be estimated from experimental data. We measured the average ratio of respired to assimilated ^{14}C (E) for different periods of time (Δt). Results of these measurements are presented in Fig. 2.

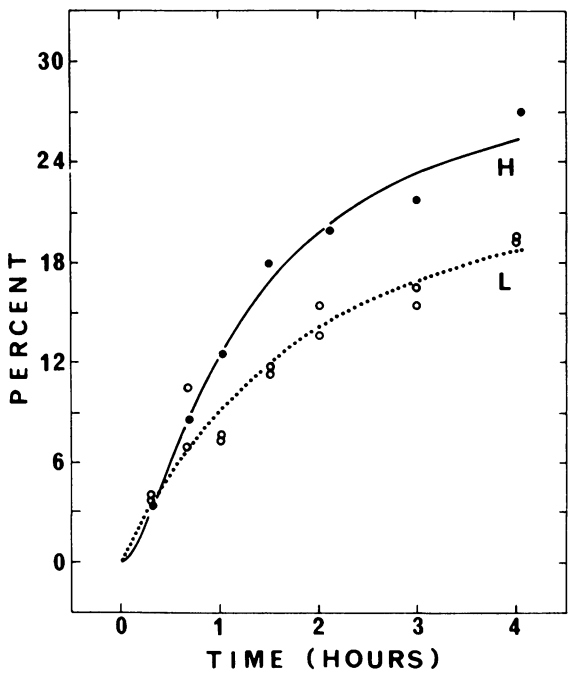

Fig. 2. Experimentally determined fraction of the assimilated ^{14}C which was excreted as $^{14}CO_2$ after different periods of time. Unlabelled *D. magna* were fed labelled *Scenedesmus* at high (H) and low (L) concentration. Lines are the results of a least-squares fit (see text).

The mean ratio for a given period of time is:

$$E_i = \frac{1}{\Delta t_i} \int_0^{\Delta t_i} \frac{a_1(t)\, \varrho_{10}}{\varrho_{01}} dt \tag{5}$$

As the daphnids in our experiments were unlabelled at the beginning, but the food was labelled, we had the following initial conditions:

$$a_0 = 1$$
$$a_1(t = 0) = 0$$
$$a_2(t = 0) = 0$$

By numerical integration of (2) and (3) the corresponding values of the model (T_i) can be calculated as functions of the parameters S_1 and ϱ_{21}. Then these parameters can be estimated by a least squares fit, i.e. by minimization of

$$\chi^2 = \sum_{i=1}^{n} \frac{[E_i - T_i(\varrho_{21}, S_1)]^2}{\sigma_i^2} \tag{6}$$

(σ_i^2: variance of E_i)

with respect to ϱ_{21} and S_1. The minimization is independent of σ_i if, as in the experiments presented, the absolute errors are approximately identical at all measuring points. Fig. 2 shows how well the fitted lines approximate the experimental results. Having estimated S_1 and ϱ_{21} we know all the parameters of the model. They are listed in Table 1.

Two complications must be considered:
First, 0.1 mg C · liter^{-1} is below the incipient limiting level concentration. Thus the ingestion rate depends on the concentration of food. A closed system was required to estimate the parameters experimentally. Consequently, the concentration of algae decreased during the experiments. Therefore, ϱ_{01} and ϱ_{10} and also ϱ_{21} vary with time. The numerical values given in Table 1 were calculated with the estimated mean food concentration during

Table 1. Parameters of the two-compartment model (cf. Fig. 1) of *D. magna*, as determined for low and high concentration of food. Pool sizes (S) in % of total body carbon; transfer rates (ϱ) in % of body carbon per hour.

Model parameter	Algal concentration (mg C · liter^{-1})		Mode of determination*
	0.1	1.5	
S_1	1.6	1.6	pf
S_2	98.4	98.4	c
ϱ_{01}	0.665	2.25	m
ϱ_{10}	0.4	0.8	m
ϱ_{12}	1.22	1.76	c
ϱ_{21}	0.96	0.33	pf

* pf = least-squares-fit; c = calculated; m = measured

the experimental period. We also estimated the parameters by allowing the transfer rates to vary with time. The differences between the results of this calculation and the calculation with the means were negligible. Thus we present only the latter values.

Second, at the high food concentration the gut of the animals is densely packed with unlabelled food at the beginning of the experiment, when ingestion of labelled food starts. Hence, the gut acts like an additional small pool, preceding the "metabolic" one. If this complication is not ignored, a_0 becomes time dependent. We can approximate this situation by adding a third differential equation (7) to the system (2) (3):

$$\frac{da_0}{dt} = \frac{1}{S_0}(a_F - a_0)\,\varrho_{01} \tag{7}$$

where a_F is the specific activity of the food and S_0 the pool size representing the gut content. By fitting simultaneously for S_0, S_1, and ϱ_{21} a pool size S_0 = 0.34 % of total carbon is obtained. This approximation assumes that food entering the gut mixes instantaneously with the gut content. However, when labelled food enters the fore gut, only part of it is mixed by peristalsis, whereas unlabelled food is defecated at the same time. The problem appears only at the high food concentration. If the same procedure is carried out for 0.1 mg $C \cdot liter^{-1}$, S_0 is not significantly different from zero.

The time course of the specific activities of pools 1 and 2 (a_1 and a_2) is presented in Figs. 3 and 4. During the first 4 hours a_2 changes only very little. If, as an approximation, we consider $a_2 = 0$, we are able to integrate the differential equation (1). For the initial conditions $a_0 = 1$ and $a_1\,(t=0) = 0$ we obtain the following equation:

$$a_1(t) = \frac{\varrho_{01}}{\varrho_{01} + \varrho_{21}}\left[1 - \exp\left\{-\frac{1}{S_1}(\varrho_{01} + \varrho_{21})t\right\}\right] \tag{8}$$

The results of this approximation are included in the figures. At the low food concentration (Fig. 3) the approximation comes very close to the exact solution. At the high food level (Fig. 4), the deviation is due to the effect of the initially filled gut [Equ. (7)]. By this complication a delay in the time course of a_1 is produced.

Prediction of the "apparent" rate of metabolism and experimental test

Knowing all the parameters of the equations (2) and (3) we can try to predict the results of independent experiments. Coming back to the initial problem, we ask: Which "apparent" rate of CO_2 excretion will we measure if the specific activity of the food and of the labelled animals are not identical?

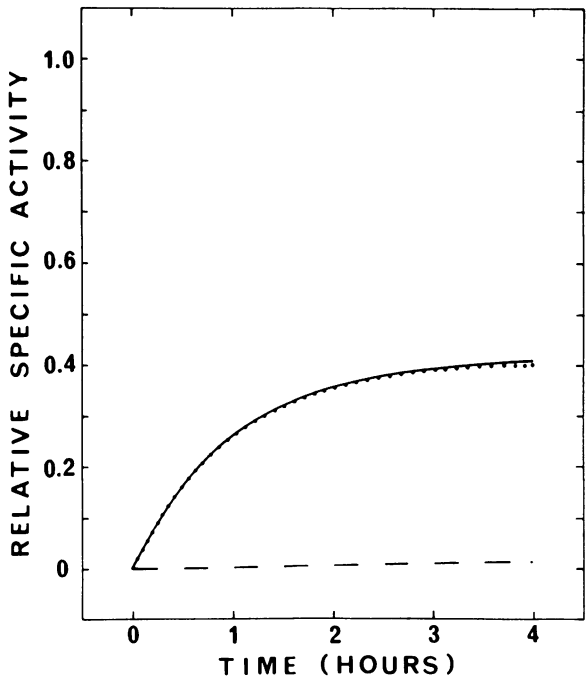

Fig. 3. Model estimation of the time course of the specific activities in the "metabolic" (solid line) and the "structural" (broken line) pool for low food concentration, using the parameters listed in Table 1. Specific activities are given relative to the specific activity of the food. The dotted line indicates the results of the calculations by the approximative equation (8).

We integrate the system of differential equations (2) and (3) with the initial conditions $a_1\,(t=0) = a_2\,(t=0) = 1$ and calculate the relative apparent rate of metabolism (RARM) from equation (1). The period of accumulated $^{14}CO_2$ is set at 4 hours. The specific activity of the food (a_0) varies from 0 (unlabelled food) to 2.5 (2.5 times specific activity of the animals).

The model predicts a linear relationship between the relative specific activity of the food and the RARM (Fig. 5). When the specific activity of the food is 0, the RARM is 69 % at the low and 25 % at the high food concentration. Thus, the measurements yield a serious underestimation.

After predicting the apparent rate, we tested the model by measuring the $^{14}CO_2$ output with food of different specific activity. The measured points have been added to Fig. 5. They agree very well with the predicted ones. Slopes and intercepts of the predicted line and linear regressions for the measured results are compared in Table 2. From the excellent agreement we conclude that our model describes the behaviour of the tracer in *Daphnia magna* reasonably well.

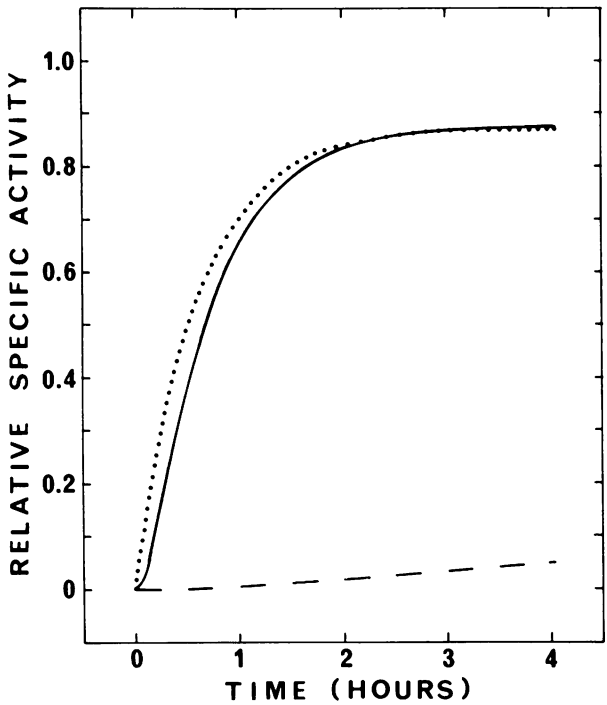

Fig. 4. The same as Fig. 3, but for high algal concentration.

Further calculations

For practical purposes it might be useful to know the slope of the line relating the relative specific activity of the food and the RARM for concentrations of food other than the two tested here. Therefore, we will have to interpolate between the food concentrations. As the relative pool sizes do not change with the food concentration (cf. Table 1) and ϱ_{01} and ϱ_{10} are known, only ϱ_{21} has to be determined for the calculation of the RARM. According to Equ. (5) only one measured point (E) is needed because the system of differential equations has only one degree of freedom left. The incipient limiting level concentration for *Daphnia magna* ingesting *Scenedesmus* at 20 °C is about 0.3 mg C · liter^{-1}. Assimilation rate and metabolic rate do not change in the range from 0.3 to 1.5 mg C · liter^{-1} (Bohrer & Lampert, in prep.). Hence, E must be constant in this range. Below the incipient limiting level we assume a linear increase of E (Δt = 4 hours) from 0.1 mg C/l (E = 0.191) to 0.3 mg C/l (E = 0.25) (cf. Fig. 2). Using E and the respective measured values of ϱ_{01} and ϱ_{10}, ϱ_{21} was fitted, so that the RARM could be calculated [Equ. (1)]. The numerical values of the RARM which reflect the degree of underestimation

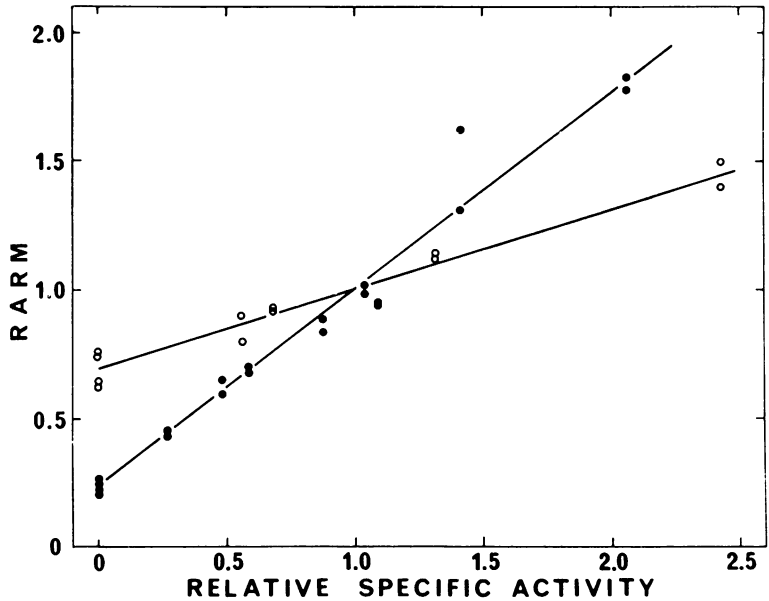

Fig. 5. Effect of the specific activity of the food on the estimated rate of CO_2 production from homogeneously labelled daphnids. Specific activities of the food are given relative to the animals (1.0). Relative apparent rates of metabolism (RARM) are related to the correct rate measured when the food has exactly the same specific activity as the animals (1.0). Deviations of RARM away from one, therefore, reflect the degree of underestimation or overestimation. Lines represent model predictions (not regressions!). Points are results of the experimental test. Parameters of the regression equations on the experimental values are given in Table 2. (dots 1.5 mg C · liter^{-1}; open circles 0.1 mg C · liter^{-1}).

Table 2. Parameters of the linear equation $y = a + b \cdot x$ relating the relative apparent rate of metabolism (y) and the specific activity of the food relative to the animals (x) (cf. Fig. 5). Comparison of the lines predicted by the model and linear regressions on the experimental results. 95 %-confidence limits are given in parentheses.

Algal concentration (mg C · liter^{-1})		Model prediction	Experiment
0.1	a	0.693	0.687 (0.050)
	b	0.307	0.311 (0.043)
1.5	a	0.239	0.241 (0.020)
	b	0.761	0.753 (0.021)

or overestimation of the correct rate of metabolism when food of different specific activity is offered are plotted in Fig. 6.

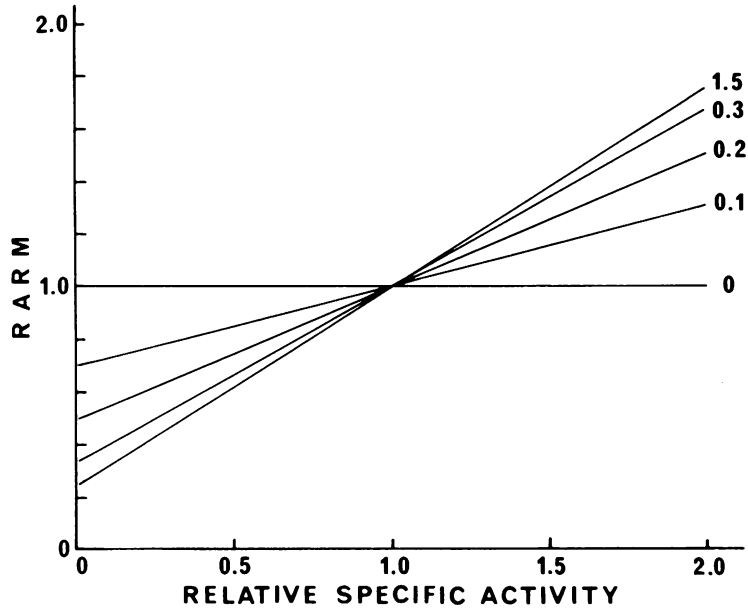

Fig. 6. Interpolation of the characteristics relating the relative specific activity of the food to the RARM for algal concentrations not tested experimentally. Lines are predicted by the model for the concentrations (mg C · liter^{-1}) indicated.

Instead of using a fit to E, ϱ_{21} can be approximated according to Equ. (8) from the following equation:

$$E = \frac{\varrho_{10}}{\varrho_{01} + \varrho_{21}} \left(1 - \frac{S_1}{\Delta t\,(\varrho_{01} + \varrho_{21})} \left[1 - \exp\left\{-\frac{1}{S_1}(\varrho_{01} + \varrho_{21})\,\Delta t\right\}\right]\right) \qquad (9)$$

ϱ_{21} is obtained by numerical solution of Equ. (9). Numerical values of ϱ_{21} generated by this approximation come very close to the results of the fit.

The time period of accumulation of $^{14}CO_2$ in the experiment has a striking influence on the result. To quantify the error to be expected we calculated the RARM for time periods of 1 to 24 hours, using the model parameters listed in Table 1 (Fig. 7). When unlabelled food is offered to a labelled animal for 24 hours at high concentration, only 13 % of the correct metabolic rate is observed. But even after only one hour the error is already 45 %.

Finally, we calculated the time needed to label an animal homogeneously, again using the parameters of Table 1. At high concentrations of food our *D. magna* would need 149 hours to reach 90 % of the specific activity of the food and 297 hours to reach 99 %. At the low concentration these time periods are much longer (457 hours for 90 % and 914 hours for 99 %). However, the transfer rates of Table 1 refer to adult *D. magna*. Labelling will proceed faster

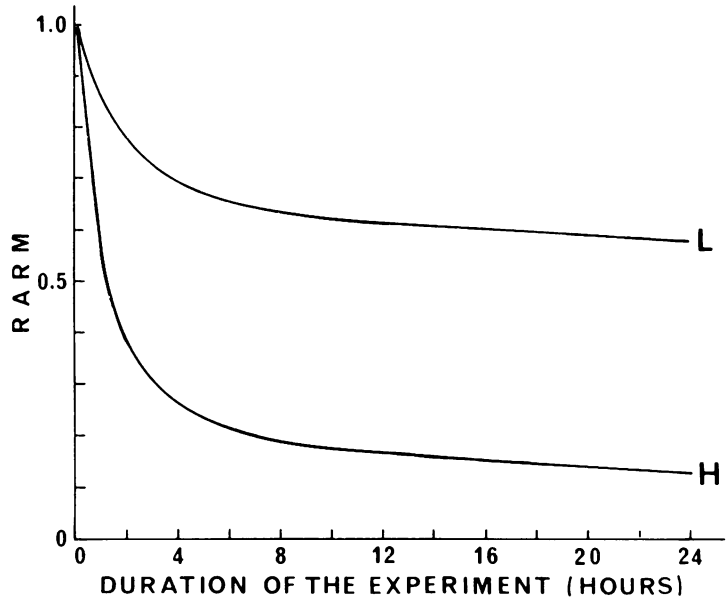

Fig. 7. Model predictions of the effect of prolonged duration of an experiment on the RARM when labelled animals (spec. activity = 1.0) are fed unlabelled food (spec. activity = 0). Underestimation of the correct metabolic rate increases over time and is more pronounced at high (H) than at low (L) concentration of food.

when it starts with young animals because these have relatively higher metabolic rates.

We tested this by raising homogeneously labelled neonates (offspring of labelled animals; 7 μg dry weight) in unlabelled food suspension (1 mg C · liter^{-1}). Every day some daphnids were removed and their dry weight and radioactivity were determined. The specific activity of the animals decreased rapidly. 10% of the initial value was reached after 78 hours when the daphnids had a dry weight of 47 μg. A 99% reduction was recorded after 250 hours at 320 μg dry weight per animal.

Discussion

The model presented here should describe the kinetics of the tracer better than the previous one (Lampert 1975) because growth of the animal and feedback from pool 2 to pool 1 were not neglected when the parameters were estimated. The predictions based on the two-compartment model are in a very good agreement with the experimental test. Thus we assume that for given experimental accuracy no further compartments are necessary to describe tracer

Table 3. Reported numerical values for the relative size of the "high-turnover" pool of different species of zooplankton (% of total body phosphorus or carbon respectively).

Species	Tracer	Relative size of "metabolic" pool (%)	Reference
Calanus finmarchicus	^{32}P	1.3–6.1	CONOVER 1961
Daphnia rosea	^{32}P	2.6	PETERS & RIGLER 1973
Mesocyclops edax	^{14}C	3.0	BRANDL & FERNANDO 1975
Daphnia pulicaria	^{14}C	2.6–3.1	LAMPERT 1975
Chydorus sphaericus	^{14}C	3.0	SCHILLER et al. 1977
Daphnia magna	^{14}C	1.6	This study

kinetics in a *Daphnia*, unlike in animals which contain an intermediate "storage" pool (STREIT 1975).

Transfer rates reported here are, of course, only valid for the special conditions, but the principle seems to be rather general in small aquatic animals. Pool size estimates have been obtained in very different ways, from decrease of label in the animal's body (CONOVER 1961; BRANDL & FERNANDO 1975; SCHILLER et al. 1977), appearance of tracer in the medium from labelled animals (PETERS & RIGLER 1973), or increase of tracer output when previously unlabelled animals receive labelled food (LAMPERT 1975; this study). Nevertheless, the results are very similar (cf. Table 3). Moreover, experiments of GARDNER & SCAVIA (1981) demonstrated a very fast response of *Daphnia* to deprivation of food with respect to ammonium excretion. This may be an indication that a similar model can also be applied to nitrogen metabolism.

We found the relative size of the metabolic pool to be nearly identical below and above the incipient limiting level concentration of food. The relative size of pool 1, however, in *D. magna* is a little smaller than the values estimated for other species (Table 3). This might be because of the large size of the animals, compared to the other species, or because the parameters were estimated in different ways. The large size has probably influenced other parameters of the model, too. We observed that the fraction of assimilated ^{14}C respired after some period of time was lower at the low food concentration (cf. Fig. 2). This effect of the food concentration was not found with smaller species of *Daphnia*. Actually, the fraction of ^{14}C respired after 3 hours was a little higher at very low food concentrations for *D. pulex* and *D. pulicaria* (Table 4). We cannot explain this contradiction from our data. In other experiments with *D. magna* (BOHRER & LAMPERT, in prep.), this reduction was not always as pronounced as in this study. Besides the physiological differences between small and large species, there might be an artifact of the experimental design involved. The reduction of food in the bottles is measured from the decrease of radioactivity on the filters. This does not provide in-

Table 4. Fraction of assimilated ^{14}C which is excreted as $^{14}CO_2$ in experiments of 3 hours duration, when unlabelled daphnids are fed labelled food. Values are given for algal densities below (0.1 mg C · liter^{-1}) and above (1.5 mg C · liter^{-1}) the incipient limiting level concentration. All data from experiments of the authors (partly unpublished).

Daphnia species	Individual dry weight (μg)	Algal species	Fraction respired	
			Low concn	High concn
D. pulicaria	60	*Scenedesmus acutus*	0.34	0.30
D. pulicaria	60	*Asterionella formosa*	0.31	0.27
D. pulex	35	*Scenedesmus* sp.	0.29	0.24
D. pulex	35	*Chlamydomonas reinhardi*	0.31	0.26
D. magna	320	*Scenedesmus acutus*	0.18	0.24

formation on the quality of the food. As part of the food is reingested after defecation, the qualitative effect of the grazing may be more pronounced than the quantitative one. This artifact would be more important for large animals at low concentrations of food and for longer experimental periods.

Table 4 demonstrates that the results of experiments with different daphnids and diets give similar results. The evidence suggests that correct tracer kinetics must be considered in uptake and release experiments. For examples there is a considerable loss of tracer in assimilation experiments. For reasons discussed in LAMPERT (1975), it is not sufficient to refer to SCHINDLER (1968) who did not find $^{14}CO_2$ losses during the first 14 hours of an experiment. Fig. 2 shows that a measurable loss of tracer may occur already after 20 minutes in *D. magna*, and this process may even be faster in animals with a higher metabolic turnover.

The results of this study may also help to revive the measurement of the metabolic rate of aquatic animals by the method of SOROKIN (1968). The effect of the specific activity of the food on the results of such experiments is already known qualitatively.

FEDOROV & SOROKIN (1967, cited in CONOVER & FRANCIS 1973) reported that feeding a labelled animal with a non-labelled diet reduces the production of $^{14}CO_2$. Labelled bream larvae given higher ratios of non-labelled food exhibited a lower rate of ^{14}C-excretion (SOROKIN & PANOV 1966). Now we have some quantitative information which enables us to calculate the correct metabolic rate. Because it is difficult to feed animals with food of exactly the same specific activity, it is important to know that the relationship between the relative specific activity of the food and the apparent metabolic rate is linear (Fig. 5). If this relationship is known, the measured rate of $^{14}CO_2$ excretion can be corrected to the relative specific activity 1.0. The specific activities of the animal and the food can be measured easily. Only two points are nec-

essary for the experimental determination of the correction line. The first value can be obtained by feeding unlabelled food to the animal (specific activity = 0); the second point, from the experiment with labelled food. Using this method would help to overcome problems presented by factors such as an unknown respiratory quotient. It would, therefore, allow the parameters of the carbon budget for aquatic animals to be measured much more accurately.

Acknowledgements

We are grateful to Dr. V. HRBÁČEK, Prague, for identifying our stocks of *Daphnia pulicaria*, which in the first version of this model (LAMPERT 1975) were assumed to be *Daphnia pulex*. We also thank Dr. BARBARA TAYLOR and Dr. RICHARD E. CONDREY for improving the manuscript.

Appendix

Definitions

ϱ_{ik}	transfer rate from i to k (fraction of total carbon per hour)
ϱ_{01}	rate of carbon assimilation
ϱ_{10}	rate of CO_2-production (metabolic rate)
C_1	total carbon in pool 1
C_2	total carbon in pool 2
S_1	$= \frac{C_1}{C_1 + C_2}$ relative size of pool 1
S_2	$= \frac{C_2}{C_1 + C_2}$ relative size of pool 2
a_i	relative specific activity (normalized to some fixed ratio of $^{14}C/^{12}C$)
a_F	rel. spec. activity of the food
a_0	rel. spec. activity of assimilated carbon
a_1	rel. spec. activity of pool 1
a_2	rel. spec. activity of pool 2
	Example: for homogeneously labelled animals $a_1 = a_2 = 1$
$M_1 = a_1 \cdot C_1$ $M_2 = a_2 \cdot C_2$	product of the total carbon of a pool and its relative specific activity

Derivation of equations (2), (3), and (4)

From these definitions the following equations result for C_1, C_2, M_1, and M_2:

$$(A1)\quad \frac{dC_1}{dt} = (C_1 + C_2) \cdot (\varrho_{01} + \varrho_{21} - \varrho_{12} - \varrho_{10})$$

$$(A2)\quad \frac{dC_2}{dt} = (C_1 + C_2) \cdot (\varrho_{12} - \varrho_{21})$$

$$(A3)\quad \frac{dM_1}{dt} = (C_1 + C_2) \cdot (a_0 \cdot \varrho_{01} + a_2 \cdot \varrho_{21} - a_1 \cdot \varrho_{12} - a_1 \cdot \varrho_{10})$$

$$(A4)\quad \frac{dM_2}{dt} = (C_1 + C_2) \cdot (a_1 \cdot \varrho_{12} - a_2 \cdot \varrho_{21})$$

From the definitions $a_1 = \frac{M_1}{C_1}$ and $a_2 = \frac{M_2}{C_2}$ and using the quotient rule follows:

(A5) $$\frac{da_1}{dt} = \frac{1}{C_1} \cdot \frac{dM_1}{dt} - \frac{a_1}{C_1} \cdot \frac{dC_1}{dt}$$

(A6) $$\frac{da_2}{dt} = \frac{1}{C_2} \cdot \frac{dM_2}{dt} - \frac{a_2}{C_2} \cdot \frac{dC_2}{dt}$$

By substitution of (A1), (A2), (A3), and (A4) into (A5) and (A6) and by the definitions for S_1 and S_2, equations (2) and (3) are obtained.

The assumption $\frac{C_1}{C_2} = \text{const.}$ is equivalent to $\frac{d}{dt}\left(\frac{C_1}{C_2}\right) = 0$

therefore, $$C_1 \cdot \frac{dC_2}{dt} = C_2 \cdot \frac{dC_1}{dt}$$

By substituting (A1) and (A2) and by solution for ϱ_{12} and the definitions for S_1 and S_2 we obtain equation (4).

Zusammenfassung

Das Zwei-Kompartiment-Modell für das Verhalten eines Radiotracers (^{14}C) in *Daphnia* (Lampert 1975) wurde auf der Basis eines Differentialgleichungssystems verbessert und neu beschrieben (Fig. 1). Das neue Modell berücksichtigt den Rückfluß des Tracers vom "Strukturpool" zum "Stoffwechselpool" und das Wachstum des Tieres. Die Modellgleichungen werden mathematisch abgeleitet und Näherungsformeln gegeben.

Die Raten der Kohlenstoffaufnahme und CO_2-Abgabe wurden bei *Daphnia magna* experimentell mit der Radiokohlenstoffmethode gemessen (Fig. 2). Die Größe des kleinen Pools mit dem hohen Umsatz (Stoffwechselpool) liegt bei niedriger und hoher Futterkonzentration bei 1,6 % des gesamten Kohlenstoffs. Entsprechend sind 98,4 % im "Strukturpool" festgelegt (Tabelle 1).

Das Modell wird eingesetzt, um mögliche Fehler bei der Methode der Messung der CO_2-Abgaberate fressender Daphnien mit Hilfe homogen radioaktiv markierter Tiere zu quantifizieren. Es sagt einen linearen Zusammenhang zwischen der scheinbaren Rate der CO_2-Exkretion (RARM) und der spezifischen Aktivität des Futters im Verhältnis zum Tier voraus (Tabelle 2, Fig. 5). Erhalten zum Beispiel markierte Daphnien für vier Stunden unmarkiertes Futter, so liegt der gemessene Wert der CO_2-Abgabe bei nur 25 % des tatsächlichen Wertes.

Die Voraussagen des Modells wurden experimentell überprüft. Die experimentellen Daten bestätigen die Modellvoraussagen sehr gut (Fig. 5). Daraus wird geschlossen, daß das Zwei-Kompartiment-Modell das Verhalten des Tracers im Tier hinreichend genau beschreibt, so daß auch Voraussagen über den Einfluß der Futterkonzentration und der Fütterungszeit auf die scheinbare CO_2-Abgabe möglich sind. Wenn markierte Tiere Futter anderer spezifischer Aktivität fressen, so wird der auftretende Fehler immer größer, je höher die Futterkonzentration (Fig. 6) und je länger die Fütterungszeit ist (Fig. 7).

Der lineare Zusammenhang zwischen der Unter- und Überschätzung der CO_2-Abgaberate und der relativen spezifischen Aktivität des Futters ermöglicht eine einfache Korrektur der Ergebnisse von Stoffwechselmessungen mit radioaktiv markierten Tieren, wie sie Sorokin (1968) vorgeschlagen hat. Damit sind präzise Messungen der CO_2-Abgabe von Wassertieren auch in gepufferten Systemen möglich.

Am Beispiel der Messung von CO_2-Abgaberaten wird deutlich gemacht, daß die Kenntnis der Kinetik unbedingte Voraussetzung für die Verwendung radioaktiver Tracer bei physiologischen Experimenten mit aquatischen Organismen ist. Keinesfalls kann ein Organismus, wie oft geschehen, als ein einzelnes Kompartiment aufgefaßt werden, in dem der Tracer gleichmäßig verteilt wird.

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