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Utilization of ^{14}C formaldehyde to infer ingestion rates and absorption efficiencies by benthic deposit-feeders

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ABSTRACT: Several experiments were carried out to provide new information on the use of the ^{14}C formaldehyde technique in deposit-feeding studies. Our results show that: (1) uptake and stability of labelling vary with species and types of detritus, (2) there are significant differences between the labelling stabilities of uniformly and ^{14}C formaldehyde labelled diatoms, (3) the use of uniformly and ^{14}C formaldehyde labelled detritus derived from *Pavlova lutheri* results in very similar ingestion rates and absorption efficiencies by *Abra ovata*, (4) ingestion rates and absorption efficiencies of *Abra ovata* fed on 11 macrophytobenthic types of detritus labelled with ^{14}C formaldehyde are consistent with the literature (negative relationship between ingestion and protein content, and between absorption and phenolic content), and (5) absorption efficiencies recorded for ^{14}C formaldehyde labelled sediment trap materials collected at different periods of the year correlate negatively with gross sedimentation rates. These results support the use of the ^{14}C formaldehyde technique with monospecific detritus. The relative values of absorption efficiencies recorded for heterogeneous detritus also seem consistent. However, it is stressed that the exactitude of the absolute values of these absorption efficiencies is yet to be proven.

KEY WORDS: Radiotracer techniques · Sedimentary organic matter · Absorption efficiency · Deposit-feeder · ^{14}C formaldehyde

INTRODUCTION

As mentioned by Lopez et al. (1989), there are 2 specific assumptions for the use of radiotracers in assessing ingestion of organic matter: (1) the radiotracer must be associated to the organic but not to the inorganic fraction, and (2) the tested animal must not select organic matter according to specific activity. When assessing the absorption of sedimentary organic matter, the assumption of labelling uniformity supersedes nonselectivity assumptions.

These assumptions are usually supposed to be met when assessing utilization rates of live microorganisms which quickly incorporate radiolabelled substrates. However, the situation is much more delicate when one considers the utilization rates of complex detritus or naturally available sedimentary organic matter to which these labelling techniques are not transferable. This is why several protocols have been proposed to label detritus or sedimentary organic matter by chemical reaction with specific radioactive compounds such as acetic anhydride (Banks & Wolfinbarger 1981), dimethyl sulfate (Wolfinbarger & Crosby 1983, Crosby 1985), and formaldehyde (Lopez & Crenshaw 1982). Among these methods, the one involving formaldehyde has been the most extensively used so far (Lopez & Cheng 1982, 1983, Bricelj & Malouf 1984, Lopez & Elmgren 1989, Cheng & Lopez 1991, Charles 1993).

The use of ^{14}C formaldehyde to study the utilization of sedimentary organic matter by benthic deposit-feeders was first introduced by Lopez & Crenshaw (1982) in order to provide a radiotracer technique that is specific for organic matter in sediment but nonspecific for different types of organic matter (Lopez et al. 1989). The labelling protocol involves the incubation of organic matter in the presence of ^{14}C formaldehyde in a hypersaline solution (i.e. 30% NaCl) which reversibly inhibits microbial activity (Lopez & Cren-

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shaw 1982). The uptake of label is clearly due to chemical reactions with sedimentary organic matter (Lopez & Crenshaw 1982). Thus the first of the 2 assumptions required for assessing ingestion and absorption rates seems to be met in the case of this particular radiolabelling method. The situation is less clear for the second hypothesis. Lopez et al. (1989) used the dual tracer (i.e. ^{14}C and ^{51}Cr) method initially developed by Calow & Fletcher (1972) to compare the absorption efficiencies of *Hydrobia totteni* and *Mytilus edulis* fed on uniformly and formaldehyde labelled algae and algal detritus. They found very similar absorption efficiencies by using the 2 ^{14}C labelling techniques and, on this basis concluded that the uniformity assumption should not be rejected. However, these authors also reported significant differences in absorption efficiencies of natural sedimentary organic matter measured by using either the ash ratio or the dual tracer method.

To a certain extent, these differences may be due to the existence of selectivity of ingestion relative to organic matter. However, another possible explanation to account for this heterogeneity is that the tests carried out for homogeneous substrates (such as microalgae) are not transferable to highly heterogeneous sedimentary organics. The aim of the present study was thus to provide new information on the interactions between ^{14}C formaldehyde and organic matter.

MATERIALS AND METHODS

Experiment 1: Label uptake. This experiment was designed to test the ability of ^{14}C formaldehyde to label substrates of different origins. Triplicates of 10 mg ash-free dry weight (AFDW) of detritus freshly derived from 11 macrophytes (*Corallina elongata, Rissoella verruculosa, Cystoseira compressa, Cystoseira mediterranea, Colpomenia sinuosa, Dilophus spiralis, Padina pavonica, Stypocolon scoparium, Codium vermilara, Ulva rigida*, and *Posidonia oceanica*), 3 microphytes (*Nitzschia acicularis, Nitzschia sp. and Pavlova lutheri*), and materials collected on 4 different dates (4 August 1992, 29 September 1992, 19 January 1993, and 2 March 1993) in sediment traps located in the bay of Banyuls-sur-mer, France, were labelled with 11.5 µCi of ^{14}C formaldehyde. Fresh materials were frozen and freeze-dried. In addition, macrophytobenthic detritus was ground to a size smaller than 200 µm. All detritus was first washed in filtered (0.2 µm) seawater for 3 h. The labelling protocol was derived from the one proposed by Lopez & Crenshaw (1982). The main differences were: (1) the duration of the incubation (48 vs 36 to 44 h), (2) the number of rinses with seawater (3 vs 1), and (3) preincubation of 3 h in filtered seawater followed by centrifugation to remove any leached label (Charles 1993). All these changes aimed at improving the stability of the label. At the end of this procedure, samples were filtered on a 0.2 µm Nuclepore membrane which was then hydrolyzed in hot NaOH (1 N at 60°C for 24 h). Activities of ^{14}C corresponding to particulate organic matter (POM) were determined by liquid scintillation countings carried out on two 250 µl subsamples of the hydrolyzates. There was no blank to correct for retention of formaldehyde on membranes since the amount of radioactivity adsorbed on membranes, if not negligible, depends on the amount of radioactivity (i.e. ^{14}C formaldehyde) within the dissolved organic matter (DOM) compartment.

Experiment 2: Relationship between uptake and stability of labelling. This experiment was designed to assess the relationship between labelling efficiency and labelling stability. Duplicated samples (10 mg AFDW of the 18 types of detritus used during the first experiment were radiolabelled with 11.5 µCi following the same protocol as in Expt 1. The detritus was then incubated for 48 h within aerated experimental chambers containing 300 ml of filtered (0.2 µm) seawater. At the end of the experiment, the radioactivity corresponding to POM, DOM and CO_2 was measured. The separation of POM and DOM was operational (filtration on a 0.2 µm membrane) (see Charles 1993 for details of the procedure). The relationship between label uptake and labelling stability was assessed using a simple linear regression model.

Experiment 3: Comparison of labelling stability for detritus derived from uniformly and ^{14}C formaldehyde radiolabelled microalgae. This experiment was carried out to compare changes in the partitioning of radioactivity of fresh detritus incubated alone in seawater when either uniformly or ^{14}C formaldehyde labelled. Duplicate samples of 10 mg AFDW of fresh detritus derived from the 3 tested microphytes (*Nitzschia acicularis, Nitzschia* sp. and *Pavlova lutheri*) were either uniformly (48 h incubation in the presence of ^{14}C sodium bicarbonate during the end of the exponential growth phase of the live monospecific cultures) or ^{14}C formaldehyde (same protocol as in Expt 1) labelled. Detritus was then incubated in experimental chambers containing 300 ml of filtered (0.2 µm) seawater for 4, 10, 20 and 48 h. At the end of each experiment, radioactivity was measured in POM, DOM and CO_2 (see Charles 1993 for details of the procedure).

Experiment 4: Comparison of ingestion rates and absorption efficiencies obtained by the 2 ^{14}C labelling techniques. We conducted this set of experiments in order to compare the ingestion and absorption of uni-

formly and formaldehyde labelled detritus freshly derived from *Pavlova lutheri* by the deposit-feeding bivalve *Abra ovata*. Uniformly labelled detritus was prepared by growing an algal culture for 2 d (end of the exponential phase) with 10 µCi ^{14}C sodium bicarbonate l^{-1} Similar, but unlabelled, cultures were grown as well. Cells from both cultures were harvested by centrifugation, frozen and freeze-dried. Detritus derived from the unlabelled cultures was then labelled with ^{14}C formaldehyde (same protocol as in Expt 1). It is important to point out that uniformly labelled detritus was submitted to the same sequence of rinses (i.e. both in seawater and in 30% NaCl) as ^{14}C formaldehyde labelled detritus.

The experimental approach used to assess ingestion and absorption was identical to the one used by Charles (1993). It associated compartmental analysis and analog modelling (Grémare et al. 1991). Compartmental analysis consisted of the measurement of the temporal changes of the distribution of radioactivity between the different compartments (i.e. POM, CO_2, DOM, and bivalves) of a closed system. The analytical procedure used to quantify radioactivity within these 4 compartments is detailed in Charles (1993). Modelling allowed for computation of radioactivity transfers between compartments. The model was almost identical to the one used by Charles (1993). Its structure can be found in Charles (1994) and in Charles et al. (1994). Ingestion rates and absorption efficiencies were computed based on the values of the kinetic coefficients of the fitted models as proposed by Charles (1993). Briefly, the computation of ingestion rates was based on the cumulative amount of radioactivity corresponding to the transfer between detritus and bivalves. There are 3 different ways to estimate absorption. The first is based on the cumulative amounts of radioactivity produced as DOM and CO_2 by the bivalves; it leads to an underestimation of absorption. The second is based on the cumulative amount of radioactivity produced as faeces by the bivalves; it leads to an overestimation of absorption. The third is based on the ratio between kinetic coefficients controlling the production of DOM and CO_2 and the kinetic coefficient controlling the production of faeces. It constitutes a direct estimation of absorption.

Experiment 5: Utilization of detritus derived from macrophytes. This experiment was designed to test the consistency of ingestion rates and absorption efficiencies obtained with the formaldehyde method relative to the existing literature regarding the control of ingestion and absorption of benthic invertebrates. Fresh detritus was prepared from 11 macrophytes and labelled with ^{14}C formaldehyde (same macrophytes and protocol as in Expt 1). This detritus was then used to assess ingestion rates and absorption efficiencies of the deposit-feeding bivalve *Abra ovata*. Compartmental analysis and analog modelling were strictly identical to that used by Charles (1993), and can also be found in Charles (1994) or Charles et al. (in press). The relationships between the main biochemical characteristics (water content, % organics, caloric content, protein, carbohydrate, and total phenolics) of the tested detritus and ingestion and absorption were assessed by using a principal component analysis (Frantzis & Grémare 1992).

Experiment 6: utilization of detritus derived from sediment traps. This experiment was designed to test the use of ^{14}C formaldehyde to assess temporal changes in the utilization rates of sediment trap collected materials by the deposit-feeding bivalve *Abra ovata*. Sediment trap materials were collected in the bay of Banyuls-sur-mer on 4 different dates (4 August 1992, 29 September 1992, 19 January 1993 and 2 March 1993). They were then freeze-dried and labelled with ^{14}C formaldehyde (same station, dates, and protocol as in Expt 1). This detritus was then used to assess ingestion and absorption of the deposit-feeding bivalve *Abra ovata*. Compartmental analysis was identical to those used during Expts 4 & 5. The structure of the model can be found in Charles (1994) and in Charles et al. (1995). Ingestion rates and absorption efficiencies were computed as proposed by Charles (1993).

RESULTS

Experiment 1

The amount of ^{14}C formaldehyde incorporated into the detritus was significantly affected by the origin of the considered detritus (Kruskall-Wallis 1-way ANOVA, $p < 0.001$) (cf. Fig. 1). The amount of incorporated label was between 0.4 µCi (detritus derived from *Ulva rigida*) and 6.1 µCi (detritus derived from *Colpomenia sinuosa*). The amount of incorporated label varied both among and within the 3 broad categories of tested detritus (i.e. macrophytes, microphytes, and sediment trap materials). The amounts of incorporated label were maximal for detritus derived from chromophytes and minimal for that derived from rhodophytes and chlorophytes (with the exception of that derived from the phanerogam *Posidonia oceanica* which exhibited a relatively high value). Detritus derived from microphytes and sediment trap materials showed intermediary incorporations. Regarding sediment trap materials, incorporations of label were maximal during winter and minimal during spring.

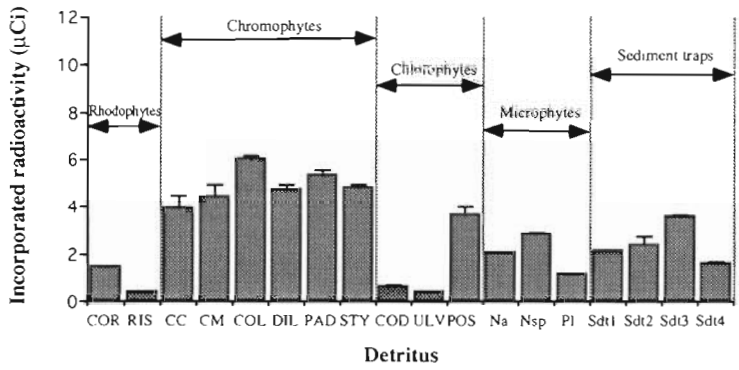

Fig. 1 Expt 1 Amounts of ^{14}C formaldehyde incorporated by the 18 tested types of detritus. The labelling procedure was identical for all tested detritus (i.e. 10 mg OM of detritus incubated in the presence of 11.5 µCi of ^{14}C formaldehyde). Vertical bars are standard deviations, COR: *Corallina elongata*, RIS: *Rissoella verruculosa*, CC: *Cystoseira compressa*, CM: *Cystoseira mediterranea*, COL: *Colpomenia sinuosa*, DIL: *Dilophus spiralis*, PAD: *Padina pavonica*, STY: *Stypocaulon scoparium*, COD: *Codium vermilara*, ULV: *Ulva rigida*, POS: *Posidonia oceanica*, Na: *Nitzschia acicularis*, Nsp: *Nitzschia* sp., Pl: *Pavlova lutheri*, Sdt1: sediment trap material collected on 4 August 1992, Sdt2: sediment trap material collected on 29 September 1992, Sdt3: sediment trap material collected on 19 January 1993, Sedt4: sediment trap material collected on 2 March 1993

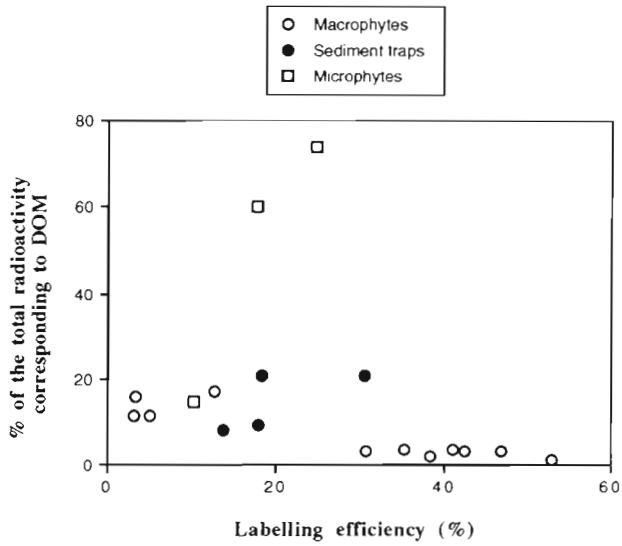

Fig. 2. Expt 2. Relationship between labelling efficiency and the proportion of radioactivity corresponding to DOM after 48 h within the controls. The latter variable is an index of labelling stability

Experiment 2

For all the tested detritus except that derived from *Nitzschia acicularis* (CO_2 corresponding to 8.9% of the total radioactivity after 48 h of incubation), the production of radioactive CO_2 remained negligible (i.e. less than 1.4% of the total radioactivity after 48 h of incubation). Thus, the main exchange of radioactivity occurred between POM and DOM. The relationship between labelling efficiency (defined as the proportion of the radioactivity introduced at the beginning of the labelling procedure which is adsorbed to the detritus) and the % of the total radioactivity corresponding to DOM after 48 h of incubation is presented in Fig. 2. The detritus derived from the 2 diatoms differed clearly from the other 16 types of detritus since it was characterized by a very important loss of radioactivity as DOM (60.0 and 73.7% of the total radioactivity after 48 h for the detritus derived from *Nitzschia acicularis* and *Nitzschia* sp., respectively). If these 2 data points are disregarded, there is a significant negative relationship between labelling efficiency and the amount of radioactivity lost as DOM ($r = -0.680$, $n = 16$, $p < 0.001$). The significance of this relationship is mostly due to the detritus derived from macrophytes.

Experiment 3

For both diatoms, there were significant differences in the temporal changes of the partitioning of radioactivity between detritus labelled with the 2 ^{14}C techniques (cf. Fig. 3). The loss of radioactivity as DOM was much more important when ^{14}C formaldehyde was used. After 48 h of experimentation, the amount of radioactivity corresponding to DOM accounted for 59.9% of the total radioactivity (against only 7.3% in the case of uniform labelling) in experimental chambers containing detritus derived from *Nitzschia acicularis*, and for 73.7% of the total radioactivity (against only 9.7% in the case of uniform labelling) in the experimental chambers containing detritus derived from *Nitzschia* sp. This difference was much less important for the detritus derived from *Pavlova lutheri* (DOM accounting for 14.8% of the total radioactivity after 48 h of incubation for ^{14}C labelled detritus, against 8.9% for uniformly labelled detritus). Diatoms were not examined microscopically, however due to important differences in production of radioactive DOM by (1) uniformly and ^{14}C formaldehyde labelled algae, and (2) the 2 ^{14}C formaldehyde diatoms and ^{14}C formaldehyde *Pavlova lutheri*, it is likely that the bulk of the release of radioactive DOM does not correspond to the physical breakdown of phytoplanktonic cells.

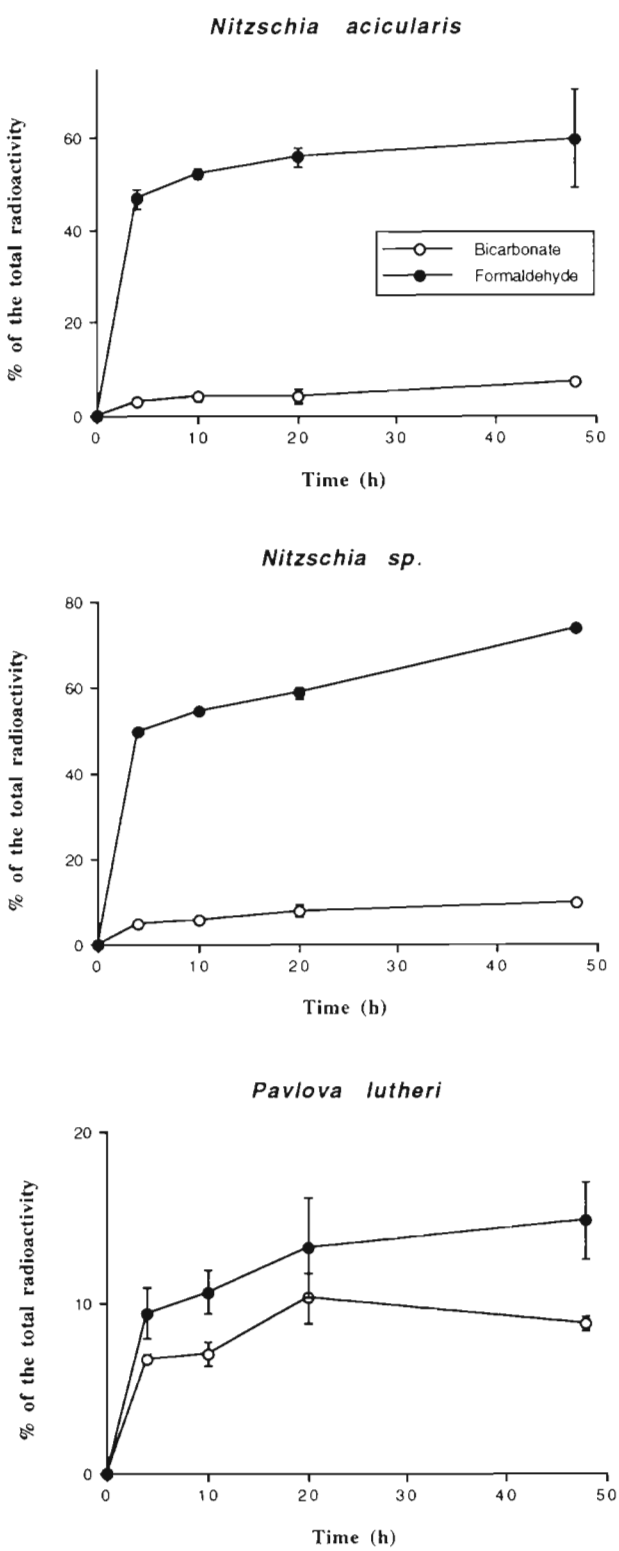

Fig. 3. Temporal changes in the proportion of total radio activity corresponding to DOM within experimental chambers containing detritus derived from uniformly (^{14}C bicarbonate) or ^{14}C formaldehyde labelled microalgae. Vertical bars: SD

Experiment 4

Weight specific ingestion rates of *Abra ovata* fed on detritus freshly derived from *Pavlova lutheri* were estimated at 0.51×10^{-3} mg OM mg DW^{-1} h^{-1} for uniformly labelled detritus and at 0.53×10^{-3} mg OM mg DW^{-1} h^{-1} for formaldehyde labelled detritus (cf. Table 1). Corresponding absorption efficiencies ranged between 13 and 24% (uniformly labelled detritus) versus 16 and 21% (formaldehyde labelled detritus). There was a good agreement between the direct estimations of absorption efficiencies (17% for uniformly labelled detritus vs 18% for formaldehyde labelled detritus; cf. Table 1).

Experiment 5

The main characteristics of the tested detritus are presented in Table 2 together with corresponding ingestion rates and absorption efficiencies by *Abra ovata*. The ingestion rates of *Abra ovata* fed on the 11 tested types of detritus ranged between 0.16 (*Cystoseira mediterranea* and *Dilophus spiralis*) and 8.65×10^{-3} mg OM mg DW^{-1} h^{-1} (*Ulva rigida*). Direct estimations of absorption efficiencies ranged between 0.5% (detritus derived from *Colpomenia sinuosa*) and 12.1% (detritus derived from *Corallina elongata*). The results of the principal component analysis are presented in Fig. 4. The first 3 axes accounted respectively for 5.9, 71.3, and 7.9% of the variance of ingestion

Table 1. Expt 4. Kinetic coefficients, ingestion rates and different estimations of absorption efficiencies corresponding to the fitted models (see Charles 1993) obtained for detritus derived from uniformly (^{14}C bicarbonate) and ^{14}C formaldehyde labelled *Pavlova lutheri*

	Bicarbonate	Formaldehyde
Kinetic coefficients (h^{-1})		
K_1	0.0095	0.0098
K_2	0.3600	0.7000
K_3	0.1300	0.3000
K_4	0.0000	0.0000
K_5	0.0000	0.0080
K_6	0.0750	0.1500
K_7	0.0000	0.0000
K_8	0.0030	0.0010
Ingestion (10^{-3} mg OM mg DW^{-1} h^{-1})	0.51	0.53
Absorption (%)		
Underestimation	13	16
Overestimation	24	21
Direct estimation	17	18

Table 2. Expt 5. Main characteristics of the 11 tested types of detritus and corresponding estimations of ingestion and absorption by *Abra ovata*

Macrophyte	Water (%WW)	Organic matter (%DW)	Energy (J mg DW^{-1})	Proteins (%DW)	Carbo-hydrates (%DW)	Phenolics (mg g OM^{-1})	Ingestion (10^{-3} mg OM^{-1} h^{-1})	Absorption (%)
Cystoseira mediterranea	79.3	70.2	12.5	18.6	20.2	18.2	6.8	2.2
Dilophus spiralis	81.0	70.3	14.3	19.0	16.6	3.4	2.6	1.4
Colpomenia sinuosa	92.0	36.6	6.7	13.3	13.7	9.2	5.9	0.9
Cystoseira compressa	82,9	65.1	10.3	20.8	15.2	53.0	3.8	2.0
Padina pavonica	78.7	48.7	5.6	12.3	11.7	4.9	6.4	0.5
Posidonia oceanica	76.4	78.4	14.8	12.4	33.6	24.0	0.2	10.0
Stypocaulon scoparium	67.0	69.3	11.7	16.3	22.7	3.0	0.2	6.1
Codium vermilara	91.9	50.5	9.0	9.6	27.7	1.1	3.2	6.5
Corallina elongata	27.0	21.5	2.3	4.6	5.1	2.4	8.1	9.1
Rissoella verruculosa	70.9	78.7	13.7	12.4	41.7	9.8	3.2	11.4
Ulva rigida	78.7	68.5	10.2	6.5	37.0	1.0	3.2	6.3

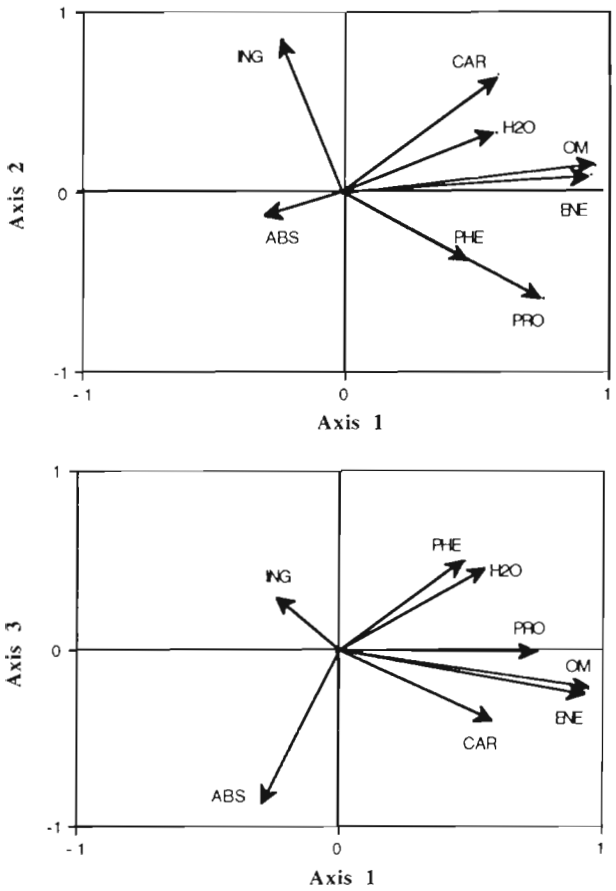

Fig. 4. Expt 5. Principal component analysis based on the values of the following parameters: ingestion (ING), absorption (ABS), water content (H_2O), % organic matter (OM), caloric content (ENE), protein (PRO), carbohydrate (CAR), and total phenolics (PHE). Graphs show the positions of the descriptors on Axes 1 & 2 (top), and on Axes 1 & 3 (bottom)

rates, and for 9.1, 1.7 and 75.6% of the variance of absorption efficiencies. The second axis was characterized by the opposition between ingestion rates and protein and phenolic content. The third axis was characterized by the opposition between absorption efficiencies and phenolic content.

Experiment 6

Gross sedimentation rates, weight specific ingestion rates and absorption efficiencies of *Abra ovata* are presented in Table 3 together with the organic, carbon, nitrogen and protein contents of the sediment trap derived detritus. When expressed in terms of organic matter (OM), ingestion rates were almost constant, between 3.5 (material collected on 29 September 1992) and 4.9×10^{-3} mg OM mg DW^{-1} h^{-1} (material collected on 19 January 1993). Direct estimations of absorption efficiencies were between 6.4 (material collected on 29 September 1992) and 21.0% (material collected on 4 August 1992).

DISCUSSION AND CONCLUSIONS

Preparation of detritus

Degradation of plant detritus shows different stages. The first step corresponds to a rapid loss of soluble and lysable organics, which may be confusing in deposit-feeding studies. It is thus essential to know how artificial detritus is prepared before discussing results of experiments assessing labelling uptake and stability or utilization rates by benthic invertebrates. The detritus used during the present study was prepared from freeze-dried material, and was thoroughly washed

Table 3. Expt 6. Gross sedimentation rates, main characteristics of sediment trap derived detritus and corresponding estimations of ingestion and absorption by *Abra ovata*

Date	Gross sedimentation (g DW m^{-2} d^{-1})	Organic matter (%DW)	Carbon (%DW)	Nitrogen (%DW)	Proteins (%DW)	Ingestion (10^{-3} mg OM µg mg DW^{-1})	Absorption (mg OM g^{-1} h^{-1})
4 Aug 1992	1.6	21.1	8.4	0.74	20.3	4.0	21.0
29 Sep 1992	108.0	8.0	4.9	0.41	7.1	3.5	6.4
19 Jan 1993	3.1	11.1	4.7	0.44	7.3	4.9	16.2
2 Mar 1993	102.6	6.4	2.4	0.12	1.9	4.0	10.1

several times before each experiment (cf. 'Materials and Methods'). In addition, we point out that: (1) we modified the procedure initially proposed by Lopez & Crenshaw (1982) in order to reduce leaching of DOM, and (2) leaching experiments carried out on several types of detritus derived from freeze-dried material have shown that the loss of hydrosoluble molecules occurs during the very first minutes of immersion (Grémare et al. 1989). We are thus confident in stating that the results presented here do not correspond to an artefact due to the leaching of soluble and lysable organics.

Labelling homogeneity (Expt 1)

Although formaldehyde reacts with a wide variety of functional groups (Lopez & Crenshaw 1982), there is no *a priori* evidence that it results in a homogeneous labelling of sedimentary organics (Lopez et al. 1989). Our own results indeed suggest that the efficiency of the labelling procedure is dependent on the type of detritus. Moreover, such differences are related to the origin of the detritus (i.e. maximal for chromophytes, intermediary for microphytes and sediment trap materials, and minimal for chromophytes and chlorophytes). Thus differences in labelling efficiencies probably result from differences in the reactivity of ^{14}C formaldehyde with various chemical compounds. This may cause a serious problem when using ^{14}C formaldehyde to label natural (i.e. heterogeneous) detritus.

Labelling stability (Expts 2 & 3)

Results of the present study clearly show that the stability of the label depends on the origin of detritus. Here again, this may cause a serious problem when using ^{14}C formaldehyde to label natural detritus.

The detritus derived from the 2 tested diatoms was characterized by a very high instability of the label. This result is probably not due to mucus since (1) the reactivity of formaldehyde is much lower for polysaccharids than for proteins (Pottu-Boumendil 1989), and (2) labelling efficiencies are much higher for diatom derived detritus than for detritus derived from *Codium vermilara* (i.e. the macrophyte which exhibits the highest mucus concentration; J. M. Amouroux pers. obs.), and there is no important loss of radioactivity for the detritus derived from *Codium vermilara* (11.3% of total radioactivity after 48 h of incubation). Another hypothesis which may account for this instability is linked with one of the specific actions of formaldehyde on diatoms. Indeed, formaldehyde appears to accelerate the erosion of fine pore occlusions (Round et al. 1990) which may account for the leaching of cytoplasmic labelled material. It would be very interesting to further test this hypothesis by assessing the stability of the label in uniformly labelled diatoms incubated in the presence of nonradioactive formaldehyde (NaCl 30%, 48 h).

If one excepts the case of the 2 tested diatoms, there is a negative relationship between labelling efficiency and the amount of radioactivity lost as DOM. Two different pathways may account for the loss of radioactive DOM: (1) leaching of soluble organics (i.e. bound with ^{14}C formaldehyde), and (2) desorption of ^{14}C formaldehyde in itself. Because of the way detritus was prepared (see the first section of 'Discussion'), we do not believe that the bulk of the production of radioactive DOM is due to leaching Thus, most of the radioactive DOM probably corresponds to desorbed formaldehyde. In any case, control experiments are essential when assessing the effects of the labelling procedure on the measure of ingestion and absorption (such as in Lopez et al. 1989). During the present study, the control experiments led us to limit this comparison to the detritus derived from *Pavlova lutheri* (for which labelling stability was almost equivalent for formaldehyde and uniformly labelled detritus).

Utilization of ^{14}C formaldehyde with homogeneous detritus (Expts 4 & 5)

Two lines of evidence suggest that ^{14}C formaldehyde can be used to label homogeneous detritus: (1) the

comparison of ingestion rates and absorption efficiencies of formaldehyde and uniformly labelled detritus derived from *Pavlova lutheri*, and (2) the nature of the relationships linking the main characteristics of the detritus derived from the 11 tested macrophytes and the levels of utilization of this detritus by *Abra ovata*.

Comparison of ingestion rates and absorption efficiencies obtained with the 2 labelling techniques constitutes a direct test of the adequacy of the ^{14}C formaldehyde labelling technique. This adequacy was already positively assessed by Lopez et al. (1989) for *Mytilus edulis* and *Hydrobia totteni* fed on different types of detritus derived from *Isochrysis* sp. Results from the present study also reveal a good agreement between ingestion rates and absorption efficiencies obtained with the 2 labelling techniques. The main difference between our study and the one carried out by Lopez et al. (1989) is that their measure of absorption was based on the dual tracer method (i.e. on the sole assumption that ^{14}C formaldehyde passes the gut wall at the same rate as non-labelled carbon), whereas our approach (chase experiments) required similar assumptions on the production of DOM and CO_2 by bivalves. The results of the present study constitute thus the first validation of the use of the ^{14}C formaldehyde technique for this particular experimental approach.

The principal component analysis based on the main characteristics of the macrophytobenthic detritus and its utilization rates by *Abra ovata* shows the opposition between: (1) ingestion rates and phenolic and protein contents, and (2) absorption efficiencies and phenolic contents. Both of these results are in good agreement with the literature. The opposition between ingestion rates and protein contents supports the negative correlation between ingestion rates and food contents usually found at relatively high levels of organic contents (Cammen 1980, Phillips 1984, Taghon & Greene 1990). The negative relationship between ingestion rates and phenolic contents was already reported in the case of benthic herbivores (Steinberg 1988). Moreover, the opposition between absorption efficiencies and phenolics is consistent with the reduction of the activity of digestive enzymes by phenolics (Tugwell & Branch 1992). Such a consistency tends to provide a second line of evidence of the adequacy of the use of the ^{14}C formaldehyde technique in the case of homogeneous detritus. However, it should be stressed that this approach is only indirect and that some of the patterns found in the principal component analysis may also result from co-correlation. For example, the opposition between absorption and phenolic content may only reflect the high labelling efficiencies and phenolic contents characterizing detritus derived from brown algae.

Thus our overall conclusion is that this technique is probably appropriate to assess ingestion rates and absorption efficiencies of most monospecific detritus. However, the present study also emphasizes the importance of carrying out preliminary experiments assessing the stability of the label, since it revealed a total lack of stability of the label for the detritus derived from diatoms.

Utilization of ^{14}C formaldehyde with heterogeneous detritus (Expt 6)

It is not presently possible to assess directly the adequacy of the ^{14}C formaldehyde technique for heterogeneous detritus since there is no labelling procedure insuring a homogeneous label of sedimentary organics. In fact, at present, direct labelling techniques constitute the only possible way to assess absorption efficiencies of heterogeneous detritus. The only lines of evidence that can be collected are thus indirect. This situation is why we decided to compare ingestion rates and absorption efficiencies of sediment trap materials collected at different periods of the year. There were significant differences (i.e. between 6.4 and 21.1% of DW) in the organic contents of sediment trap materials collected on the 4 test dates. Thus, the relative constancy of ingestion rates (when expressed in terms of organic matter) supported the existence of a mechanism of compensatory intake in *Abra ovata*, since ingestion of organic matter tends to stay constant when food organic content changes. Moreover, at the studied station there is a negative correlation between gross sedimentation rates and organic contents of sediment trap materials [see Table 3 or Charles (1994) and Charles et al. (1995) for more complete data]. Such a pattern is mainly due to resuspension of sediment cued by the wind (Charles 1994, Charles et al. 1995). The comparison of the absorption efficiencies recorded during the present study shows that: (1) when gross sedimentation is minimal, absorption efficiency is higher for material collected during summer than during winter (21.0 vs 16.2%), and (2) during fall and winter, high absorption efficiencies are related to low gross sedimentation (16.2 vs 10.1 and 6.4%). These results are consistent with the dilution of the material directly sedimenting from the water column with resuspended (and supposedly more refractory) sediment. These results thus contribute to providing indirect evidence supporting the use of ^{14}C formaldehyde with heterogeneous detritus. However, it should be stressed that the present section of the discussion is solely based on the relative values of absorption, and thus does not constitute a proof of the exactitude of the absolute values of these absorption efficiencies, which is yet to be demonstrated.

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