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Absorption and gut passage time of microalgae in a suspension feeder: an evaluation of the $^{51}Cr:^{14}C$ twin tracer technique* **

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ABSTRACT: The bivalve *Mercenaria mercenaria* was pulse-fed labelled microalgae in experiments designed to test the applicability of the $^{51}Cr:^{14}C$ twin tracer technique to the study of food absorption in a suspension feeder. Absorption efficiencies of the chlorophyte *Pseudoisochrysis paradoxa* using Conover's ash-ratio method and the tracer technique were in good agreement. Clams absorbed approximately 14 % of the ^{51}Cr ingested. An assumption of the radiotracer method is that ^{51}Cr and ^{14}C move along the gut at similar rates. The gut passage times of the 2 isotopes differed significantly, however, when clams were fed *P. paradoxa*, a 'good' food source: the gut residence time of ^{14}C was greater than that of ^{51}Cr. Therefore, analysis of a single faecal subsample can cause significant error in the calculated absorption efficiency. The value will be overestimated or underestimated depending on whether the faeces are subsampled early or late, respectively, after transfer of the animals to unlabelled food. Therefore, pulse-chasing, or recovery of faeces over a fairly extended period of time (to be determined for any given experimental conditions) is strongly recommended. Examination of the time-course of ^{14}C egestion revealed that the gut passage time of *P. paradoxa*, which was absorbed with high (82 %) efficiency, was significantly greater than that of 2 chlorophytes (*Nannochloris atomus* and *Stichococcus* sp.) and 2 cyanobacteria of the genus *Synechococcus*, which are inefficiently utilized by *M. mercenaria*. The study provides evidence that clams are able to sort different algal species in their passage through the gut. Control of gut clearance rates, through more rapid elimination of those algal species which are also poorly utilized, may contribute to the species' adaptive strategy.

INTRODUCTION

The main goal of this paper is to describe an application of the $^{51}Cr:^{14}C$ tracer technique (Calow and Fletcher, 1972) to the study of food absorption of a suspension-feeding bivalve *Mercenaria mercenaria*, to test the validity of the original method's assumptions, and to compare the calculated absorption efficiency with that obtained using Conover's ash-ratio method (Conover, 1966). The twin radiotracer indicator technique, developed to estimate absorption efficiencies of aquatic primary consumers, is based on measuring the ratio of ^{51}Cr (an inert tracer) to ^{14}C (the assimilated tracer) in samples of the food and faeces of the test organism. As indicated by the authors, 2 of the conditions which must be met in order to use this method are: (1) that ^{14}C and ^{51}Cr move along the gut at similar rates; (2) that ^{51}Cr is not absorbed to any significant extent.

This method has been successfully applied to the study of a variety of deposit-feeding organisms, e.g. the freshwater gastropods *Planorbis contortus* and *Ancylus fluviatilis* (Calow and Fletcher, 1972), the polychaete *Nereis succinea* (Cammen, 1980), the marine gastropod *Hydrobia totteni* and the bivalve *Nucula annulata* (Lopez and Cheng, 1983). However, the technique has not been extensively used for studies of suspension-feeding bivalves. Stuart et al. (1982) found, in working with the ribbed mussel *Aulacomya ater*, that ^{51}Cr absorption from kelp detritus was significant and highly variable, ranging from 17 to 45 %.

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Therefore, they modified the technique and extended it to a filter-feeding organism by using ^{51}Cr encapsulated in microspheres. As recognized by the authors, since bivalves can exhibit high ingestion selectivity, this modification may be inadequate to estimate absorption efficiencies at high food concentrations, when pseudofaeces are produced. Stuart et al. (1982) also attempted to compare results obtained using Conover's method with those using their modified tracer technique; however, because they lacked a measure of error for Conover's ratio, they were unable to test for the significance of their results.

Conover's method calculates the absorption efficiency from the percentage of organic matter in the food and faeces, and therefore does not require quantitative determination of the food intake or faeces output. One of the limitations of this method is that it does not distinguish between organic matter derived from the food, and from metabolic secretions such as mucus. It could therefore potentially underestimate the true absorption efficiency. Additionally, although the method was originally intended for use with food items with a relatively high ash content, such as diatoms and natural particulates, it has not been verified whether it is readily applicable to naked algal species which are generally used to culture bivalves, and are characterized by a very low ash content. It is therefore important to compare results obtained with the 2 methods under similar conditions, and to validate Conover's method, since it is still the simplest and often, particularly in obtaining field estimates, the only method available to determine absorption efficiencies.

Calow and Fletcher (1972) claimed that the $^{51}Cr:^{14}C$ technique did not require quantitative collection of faeces, provided that the 2 labels had a similar gut passage time. Thus, absorption efficiencies could be estimated from a small sample of the food and faeces. This represented a clear advantage over the gravimetric and other radiotracer methods which require quantitative recovery of feces. Subsequently, Wightman (1975) showed that the absorption efficiency of larvae of a scarabaeid, *Pachnoda ephippiata,* varied significantly over the 6 d following exposure to labelled food. Due to these variations in absorption over time, he recommended that faecal ^{14}C and ^{51}Cr counts be integrated over time to yield an ecologically more meaningful estimate of the absorption efficiency. This paper will attempt to demonstrate that the gut passage times of ^{51}Cr and ^{14}C can be significantly different, so that collection of all or most of the labelled faeces, rather than analysis of a single subsample, is in this case a prerequisite to obtaining accurate values of absorption efficiency with this method.

A second goal of this study is to investigate the differential gut passage time of algal species utilized with very different efficiencies by clams. Five algal species were tested: the chrysophyte *Pseudoisochrysis paradoxa,* VIMS isolate VA-12, (4.0 µm in mean equivalent spherical diameter); 2 small chlorophytes (< 4 µm in diameter), isolated from Great South Bay (Long Island, N. Y.), *Nannochloris atomus* clone GSB, and *Stichococcus* sp. clone Say II (now *Nannochloropsis* sp.), and 2 cyanobacteria (≤ 2 µm) of the genus *Synechococcus. P. paradoxa* is known to be a good food source for clams and is routinely used for culturing of bivalves. The 2 chlorophytes, generically called 'small forms', are abundant in certain areas of Great South Bay contaminated by duck farm wastes (Ryther, 1954, Carpenter, pers. comm.). Bass (1983) has recently demonstrated that they support no significant growth of juvenile *Mercenaria mercenaria.* Using the twin tracer method, she additionally found that *Nannochloris* and *Stichococcus* were absorbed by clams with only 20 and 13 % efficiency, respectively, while the 2 *Synechococcus* strains (Syn a and ASN C-3) were absorbed with about 22 and 28 % efficiency, respectively. The present paper provides evidence that *M. mercenaria* is capable of sorting an indigestible alga in the gut and eliminating it more rapidly than a more digestible algal species.

MATERIALS AND METHODS

Labelling of algae

The labelling procedure was similar to that described by Calow and Fletcher (1972). $^{14}C:^{51}Cr$ labelled algae were prepared by growing the culture in the presence of ^{14}C as $NaH^{14}CO_3$ (1 µCi/10 ml culture) for 5 d, at 17 °C and under constant illumination. ^{51}Cr was added on the fourth day of incubation, as $^{51}CrCl_3$ (100 µCi/100 ml culture), so that the algae were exposed to ^{51}Cr for 24 h before use. Because the ^{51}Cr stock solution was prepared with 0.1 N HCl, it was neutralized upon addition to the culture with 0.1 N NaOH. Under these conditions, at the normal pH of seawater, Cr^{3+} adsorbs to particle surfaces. Prior to use in the experiments, unincorporated label was removed from the algae by repeated centrifugation (8,000 rpm for 15 min) and resuspension in filtered seawater.

Experimental procedure

Clams (28 to 35 mm in shell length) were acclimated to experimental conditions by feeding them *Pseudoisochrysis paradoxa* for 1 wk in the laboratory. For the feeding experiments, they were placed singly in bowls (300 ml capacity) containing 0.22 µm filtered

seawater and labelled algae at an initial concentration of 50×10^6 to 100×10^6 cells l^{-1}. Aliquots (5 ml) of the labelled algal suspension were sampled after thorough mixing and before introducing the animals to the bowls.

Preliminary experiments indicated that orange fluorescent particles ingested by clams took 60 to 90 min to appear in the faeces. Therefore, the animals were allowed to feed on ^{51}Cr:^{14}C labelled algae for only 30 to 45 min, to ensure recovery of all labelled faeces. At the end of this time, the clams were transferred individually to bowls containing filtered seawater and unlabelled algae at a concentration of 30,000 to 40,000 cells ml^{-1}. Algae were added to the bowls (every 1 to 2 h during the first 12 h and every 3 to 6 h thereafter), so that the clams were constantly feeding. The experiments were conducted at an ambient temperature of about 25 °C. The clams that were pulse-fed a labelled chlorophyte or cyanobacteria were fed an equal parts' mixture of *Pseudoisochrysis paradoxa* and the appropriate 'small form' after transfer to unlabelled food. This was done because the clams' feeding activity, or time spent pumping over an extended period of time, was found to be reduced in the absence of *P. paradoxa*.

Faeces were collected periodically over a 48 h period: every 4 to 5 h during the first 12 h after transfer and somewhat less frequently thereafter. (A 48 h period for faeces collection was selected on the basis of a separate, long-term feeding trial, in which clams were fed ^{14}C labelled *Pseudoisochrysis paradoxa*, and faeces were collected over 69 h (see Fig. 1).) Samples of the food and faeces were filtered onto Nuclepore membrane filters (0.6 µm pore size, 25 mm diameter), and placed in glass scintillation vials for subsequent measurement.

Sample preparation and counting

The procedure adopted generally followed that of Lopez and Cheng (1983). The samples were first counted for ^{51}Cr on a gamma counter (Beckman 8000), and the counts corrected for background and decay. The samples were then dampened with 0.1 ml of water and digested overnight by adding 1 ml of tissue solubilizer (NCS, Amersham). Then, 12 ml of organic scintillant (OCS, Amersham) were added, and the samples allowed to equilibrate for 24 h before counting on a liquid scintillation counter (Packard, Tri-Carb^{R}300C). Since there is an overlap in the energy spectra of ^{51}Cr and ^{14}C, a truncated ^{14}C channel (12 to 156 KeV) was used to reduce interference from ^{51}Cr.

Cammen (1977) pointed out the need to correct the counts per minute (CPM) for quenching and convert them to disintegrations per minute (DPM) prior to calculation of the absorption efficiency. Therefore, a set of quenched standards was prepared to establish the quench correction curve for each isotope, by adding filtered algae as quenching agent to ^{14}C-hexadecane and ^{51}Cr standards. ^{14}C counts were thus sequentially corrected for background, ^{51}Cr interference, and quenching. ^{51}Cr and ^{14}C DPM from all faecal samples obtained from each individual were added to calculate the absorption efficiency.

^{51}Cr absorption

Experiments to determine ^{51}Cr absorption were conducted with clams approximately 10 mm in shell length so that the whole animal would fit in a scintillation vial. Fifteen clams were allowed to feed on ^{51}Cr-labelled *Pseudoisochrysis paradoxa* for 30 min, and then transferred individually to scintillation vials for gamma counting. Five of these clams were frozen and subsequently analysed for the initial shell versus meat ^{51}Cr activity. The remaining 10 clams were placed in bowls containing unlabelled algae, and their faeces were collected for 48 h at 4 to 8 h intervals. At the end of the experiment the clams were again counted whole, and after dissection, the tissues and shell were counted separately for ^{51}Cr. The percent ^{51}Cr absorption was then calculated using the following expression:

$$\%\,^{51}\text{Cr absorbed} = \left(1 - \frac{\text{Final } ^{51}\text{Cr DPM in clam tissues}}{\text{Initial } ^{51}\text{Cr DPM in clam tissues}}\right) \times 100$$

The ^{51}Cr egested was used as a means of independently checking the estimate of ^{51}Cr absorption calculated, since:

$$^{51}\text{Cr absorbed} = \text{Initial } ^{51}\text{Cr in whole clam} - \text{Initial } ^{51}\text{Cr in shell} - ^{51}\text{Cr in faeces}$$

RESULTS

The results of a preliminary long-term experiment in which clams were pulse-fed ^{14}C-labelled *Pseudoisochrysis paradoxa* are plotted in Fig. 1. There appears to be a change in the slope of the curve at about 40 h. The fraction (ca. 2 %) of ^{14}C recovered beyond this point is negligible and can be interpreted as loss of absorbed ^{14}C in secretions. Therefore, all subsequent experiments were terminated after 48 h. Since the clams were fed at more frequent intervals during the first 12 h after transfer to unlabelled food, it is not clear whether any significance can be attached to the initial change in slope of the curve.

There was no significant difference ($P > 0.5$)

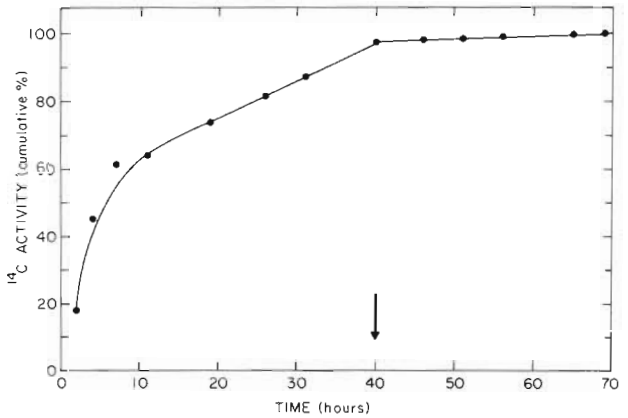

Fig. 1. Long-term record of cumulative ^{14}C activity recovered in faeces of *Mercenaria mercenaria* pulse-fed ^{14}C-labelled *Pseudoisochrysis paradoxa* (averaged for 6 individuals). Arrow: suggested terminating point for absorption efficiency experiments, under the conditions described in this study

between mean absorption efficiencies of *Pseudoisochrysis paradoxa* obtained using Conover's method and the twin tracer method (Table 1), which were compared using analysis of variance of arcsine transformed data. These values (82 and 80 %) represent maximum efficiencies for *P. paradoxa*, since they were obtained at a relatively low algal concentration, equal or less than 5×10^6 cells l^{-1}. Absorption efficiencies of this algal species are no longer concentration dependent at densities below 5×10^6 cells l^{-1} (Bricelj, unpubl.).

The absorption efficiency estimated using the tracer method was corrected for ^{51}Cr absorption by the clams. This was found to be relatively low, equal to a mean value of 13.9 % ± 1.14 SD of the ^{51}Cr ingested.

Table 1. Absorption efficiency of *Pseudoisochrysis paradoxa* by *Mercenaria mercenaria*: comparison between results obtained using the twin tracer method and Conover's ratio

Method	No. of individuals	Mean absorption efficiency % (± SE)
Conover	12	82.2 (± 1.04)
Radiotracer	14	80.7 (± 2.35)
		82.5* (± 1.64)

Mean (n = 13) obtained by eliminating 1 extremely low value of absorption efficiency

Gut passage time of ^{51}Cr and ^{14}C

Measurable activity of ^{51}Cr and ^{14}C appeared in clam faeces even after 48 h of exposure to labelled *Pseudoisochrysis paradoxa*. Therefore, in order to standardize results and compare gut passage times of the 2 isotopes, the time for recovery of 90 % of the label, or t_{90}, was estimated for each clam (Table 2). One hundred % recovery was assumed to occur by the end of 48 h. The t_{90} provides a simple and convenient parameter for comparison of egestion rates of the 2 isotopes. A similar approach was used by Calow (1975b), who defined an egestion half life, or time taken for 50 % of the indigestible material from one feeding to pass out of the gut.

Table 2. Time (h) for cumulative recovery of 90 % of total ^{14}C and ^{51}Cr activity (DPM) in faeces of clams fed labelled *Pseudoisochrysis paradoxa*

Individual No.	t_{90} ^{14}C	t_{90} ^{51}Cr
1	37.9	< 2.0
2	25.4	< 2.5
3	28.8	7.3
4	20.3	< 2.5
5	24.0	4.8
6	29.1	9.4
7	31.9	< 2.5
8	22.6	5.6
9	31.3	14.3
10	18.3	< 2.5
mean	27.0	≤ 5.3
± SD	± 6.0	± 4.0

In this study the t_{90}s were calculated from regression equations relating the cumulative percentage of activity recovered in faeces, as a function of time. The t_{90}s for ^{51}Cr were calculated from polynomial regressions, those for ^{14}C from regressions of the form:

$$1n\ t = a + bC \qquad (2)$$

where t = time in hours; C = ^{14}C activity in faeces (cumulative percentage).

It is clear from examining the values listed in Table 2, that ^{51}Cr, the inert tracer, was consistently egested more rapidly than ^{14}C. That the pattern of recovery differed for the two isotopes is again illustrated in Fig. 2A. This shows a representative example of a time series of gut evacuation of the 2 isotopes for a clam pulse-fed labelled *Pseudoisochrysis paradoxa*. The curves in this figure are fitted by eye. Similar curves were obtained for all other clams fed the same diet.

Due to the differences in gut passage time between ^{14}C and ^{51}Cr, a significant error can be introduced when estimates of absorption efficiency are based on analysis of a fecal subsample collected at a single point in time. In this study, ^{51}Cr and ^{14}C counts of faeces collected over the first 8 h after transfer to unlabelled food, yielded overestimates of as much as 20 %.

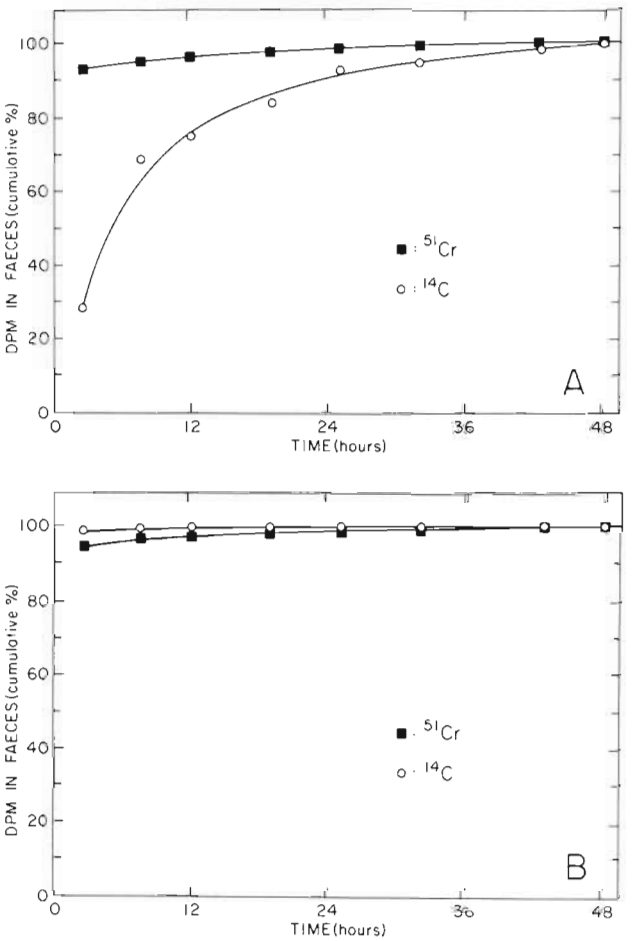

Fig. 2. Cumulative percentage recovery of ^{14}C and ^{51}Cr (DPM) in faeces of *Mercenaria mercenaria* over time. (Recovery at the end of 48 h taken as 100 %.) (A) Clam pulse-fed labelled *Pseudoisochrysis isochrysis;* (B) Clam pulse-fed labelled *Stichococcus (Nannochloropsis* sp.)

Absorption efficiency values based on a 12 h versus 48 h collection period, however, differed by an average of only 6 %.

Gut passage time of different algal species

Fig. 2B shows a typical time series of egestion of the 2 isotopes for 1 out of 7 clams fed the chlorophyte *Stichococcus (Nannochloropsis).* In contrast to the pattern observed in Fig. 2A, in this case the 2 tracers were evacuated more or less synchronously. A similar synchronous pattern was observed for clams fed *Nannochloris,* or either of the 2 *Synechococcus* strains. The t_{90} for ^{14}C of clams fed labelled *Stichococcus* was consistently less than 2.5 h, as compared to a t_{90} of 27.6 h for clams fed labelled *Pseudoisochrysis paradoxa* (Table 3). Therefore, examination of the egestion rate of ^{14}C activity reveals that the chlorophytes and

Table 3. Mean time (h) for cumulative recovery of 90 % of ^{14}C and ^{51}Cr in faeces of *Mercenaria mercenaria* pulse-fed labelled algae (standard deviation indicated in brackets)

Algal food	Mean t_{90} (h)		No. of individuals
	^{14}C	^{51}Cr	
[a]*Stichococcus sp.* (Say II)	< 2.5 (0.1)	< 3.6 (1.6)	7
[b]*Nannochloris atomus* (GSB)	< 6.6 (7.2)	< 7.3 (8.1)	7
[c]*Synechococcus* sp. (Syn a)	< 2.0 (0)	< 2.0 (0)	7
[d]*Synechococcus* sp. (ASN C–3)	< 5.1 (2.3)	< 3.8 (2.3)	7
Pseudoisochrysis paradoxa	27.0 (6.0)	< 5.2 (4.2)	10

[a] Isolated from Great South Bay, N.Y. by Guillard in 1965
[b] Isolated from Great South Bay by Ryther in 1952
[c] Isolated from Long Island Sound, N.Y. by Guillard and Ryther
[d] Isolated from Great South Bay by Sarokin

cyanobacteria, which are inefficiently absorbed by clams, are also egested more rapidly than *P. paradoxa,* which is absorbed with high efficiency (82 %).

DISCUSSION

Good agreement was found in this study between absorption efficiency values for *Pseudoisochrysis paradoxa* obtained with the 2 indicator techniques, although Conover's method measures the absorption of organic matter, and the tracer method measures carbon absorption. This validates, for *Mercenaria mercenaria,* the assumption that mucus did not contribute significantly to faecal organic matter in these experiments.

^{51}Cr absorption by clams was relatively small (13.9 %), and comparable in magnitude to values reported for *Ancylus fluviatilis* and *Planorbis contortus* (9.8 and 10 %, respectively) (Calow and Fletcher, 1972), *Hydrobia totteni* (4 %) and *Nucula annulata* (10 %) (Lopez and Cheng, 1983).

^{51}Cr and ^{14}C were found to have a significantly different gut passage time when clams were fed labelled *Pseudoisochrysis paradoxa,* a highly digestible food source. This finding is somewhat unexpected given the fact that the 2 isotopes are associated with the same food particle. A feasible explanation to account for the observed results is that, following initial breakdown of cells in the stomach, ^{51}Cr, which is adsorbed onto the fragmented and possibly more indigestible cell membranes or cell walls, passes directly

to the intestine. On the other hand, the fraction of the ^{14}C which was incorporated intracellularly by the algae, would be diverted into the digestive gland before egestion or absorption. This theory postulates a separate pathway for the ruptured cell wall and the cell contents of the algae in their passage through the gut of *Mercenaria mercenaria*. The explanation offered is in no apparent conflict with our current understanding of the processes of digestion in bivalves (Purchon, 1977).

The difference in gut passage time of the 2 isotopes results in the need for full or nearly full recovery of labelled faeces. The appropriate time for faeces collection must be established from a preliminary experiment, since it depends on the test organism, food and feeding protocol used. It must be long enough to ensure recovery of most of the labelled food, but not too long, in order to avoid recovery of ^{14}C derived from mucus secretions or excreta. About 40 h was found to be amply sufficient for the feeding regime used in the present study.

An organism that is feeding on a relatively indigestible diet might exhibit different opposing strategies, for example: (1) to increase the food item's residence time within the gut in order to give the digestive processes longer to act upon it, and thus maximize its energy or nutrient gain; (2) to reduce the gut passage time of the food item. This constitutes an adaptive strategy if the food component is almost completely indigestible, in which case there would be little benefit in withholding it longer in the gut for more efficient digestion.

Calow (1975b) suggested that the herbivorous gastropod *Ancylus fluviatilis* might conform to the first type of strategy, whereas the detrivore *Planorbis contortus* illustrates the second type of response. Although his data are insufficient to detect more than a general trend, he described the following pattern for *A. fluviatilis:* diatoms *(Navicula)* were egested more rapidly than unicellular green algae *(Scenedesmus),* and these in turn were egested more rapidly than filamentous blue-green algae *(Rivularia).* This sequence was equivalent to the order of efficiency with which the snail was able to digest the algae (Calow, 1975a).

In contrast, in the present study the passage time through the gut of *Mercenaria mercenaria* of 4 algal species which were poorly utilized was significantly less than that of *Pseudoisochrysis paradoxa,* a species which is absorbed with a high degree of efficiency. The slow egestion of ^{14}C when clams were fed *P. paradoxa* might be explained by diversion of this alga into the digestive gland, while the indigestible algal species are shunted directly to the hind gut. Thus, the ^{14}C of an indigestible food item mimics the behavior of ^{51}Cr, the inert tracer, in its passage through the gut. The indigestible nature of the chlorophytes used as food may be related to the presence of refractory cell walls in these species. A trilaminar cell wall component, indicative of sporopollenin, has been described in this *Nannochloris* strain (Sarokin, 1981). Sporopollenin, a highly refractory material resistant to enzymatic attack and strong acid degradation, is typically found in spores and pollen of higher plants, as well as in the cell walls of other chlorophytes, e.g. strains of *Chlorella* and *Scenedesmus.* There would thus appear to be little advantage in a more prolonged retention of such cells in the gut.

It is now well known that many suspension-feeding bivalves are able to sort particles prior to ingestion, and selectively reject them in pseudofaeces (Kiørboe and Møhlenberg, 1981; Newell and Jordan, 1983). The complex alimentary tract present in all lamellibranchs has also been attributed a role in the sorting of fine and coarse particles after ingestion (Purchon, 1977). Evidence of sorting in the stomach, however, is based largely on the results of feeding experiments using artificial substances, and on the observation of excised tissues and histological sections. More meaningful observations can be obtained from studies of undisturbed animals fed natural labelled particles.

Discrimination between different algae of similar size in the gut might occur through a biochemical sensing mechanism. Ciliary sorting of digestible and indigestible particles has been reported to take place in the gizzard-stomach complex of *Planorbis contortus* (Carriker, 1946). Calow (1975b) invoked this mechanism to explain the fact that an increasing amount of indigestible diluent such as lignin, does not interfere with digestion of bacteria present in the diet of this species.

Taghon et al. (1978) have stressed the importance of determining both the time-specific absorption efficiency, and the gut passage time of different food items. They hypothesized that these 2 parameters might vary between particle types, particularly for a filter-feeder that ingests a mixed diet of both phytoplankton and detritus. The results reported in this paper lend support to his suggestion of a food item-specific gut passage time.

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NOTE ADDED IN PROOF

A 3-phase ^{14}C defaecation pattern has been observed in this study (Fig. 1), in which both the initial rapid phase, and second slower phase were attributed to egestion of unabsorbed label. A similar 3-phase time-course of isotope release has been described in 2 gastropod species starved after exposure to labelled food (Calow, 1975b). The first and second phases (completed within 96 h) were attributed to the production of gizzard and liver string respectively, two faecal components equivalent to intestinal and glandular faeces in bivalves. The feeding regime used in our study was such that clams were essentially starved following exposure to labelled algae, since we estimate that an individual could deplete the experimental vessel of algae in less than 10 min. This probably accounts for the prolonged gut retention of unabsorbed ^{14}C observed when clams were fed *Pseudoisochrysis paradoxa*.

An alternative interpretation of the data (A. J. Hawkins, pers. comm.) might suggest that gut passage of unabsorbed ^{14}C was accomplished within the initial rapid phase of ^{14}C loss (about 12 h), and that subsequent loss was due to the excretion of absorbed ^{14}C. Significant differential gut passage of ^{14}C and ^{51}Cr occurs even under the assumption that 100 % of the unabsorbed ^{14}C was egested in 12 h. For example, in Fig. 2A, this assumption results in egestion of 96.7 % of the ^{51}Cr, but only 38.0 % of unabsorbed ^{14}C after 2 h. Thus, alternative interpretation of the endpoint of defaecation of unabsorbed material does not alter the conclusions of this study. It does, however, point out the need for further study designed to unequivocally identify the various labelled compartments, and model gut evacuation in bivalves at different, constant feeding levels.

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