

Utilization of microbial organic aggregates by bay scallops, *Argopecten irradians* (Lamarck)

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Abstract

The use of organic aggregates as a food source by bay scallops, *Argopecten irradians* (Lamarck), was quantified and compared with the use of phytoplankton, a known good food. In two experiments, the first designed to characterize nitrogen incorporation and the second nitrogen release, ¹⁵N was used to trace N into and out of scallops under defined, laboratory conditions. Scallops were fed experimental diets of labelled organic aggregates, produced from the dissolved material released by either a brown (*Fucus vesiculosus*) or red (*Gracilaria tikvahiae*) alga, or phytoplankton, *Thalassiosira weissflogii* (Grunow), for periods of 5–15 h in a flow-through system. More N was incorporated by scallops fed phytoplankton ($523 \mu\text{g} \cdot \text{gDW}^{-1} \cdot \text{h}^{-1}$) than by those fed aggregates ($70 \mu\text{g} \cdot \text{gDW}^{-1} \cdot \text{h}^{-1}$ for aggregates derived from *F. vesiculosus* and $306 \mu\text{g} \cdot \text{gDW}^{-1} \cdot \text{h}^{-1}$ for those derived from *G. tikvahiae*). On both diets, fecal material and ¹⁵N was released rapidly during the first 6 h of depuration. It was estimated that scallops incorporated aggregate N with an absorption efficiency of 77 to 79% as compared with an efficiency of 90% for phytoplankton N. Estimated assimilation efficiency ranged from 14 to 43% for scallops fed aggregates, and 75% for those fed phytoplankton. Despite these differences, aggregates represent a potentially important source of nutrition for suspension-feeding bivalves living in near-shore regions rich in natural seston.

Keywords: Bivalve; Detritus; Nitrogen; Organic aggregates; Scallop

1. Introduction

In the aggregate detritus pathway, dissolved organic material (DOM) released by primary producers is transformed into organic aggregates, which are in turn

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ingested by consumers. In previous work, we described the aggregate pathway and demonstrated that organic aggregates produced from the DOM released by various species of macrophytes can be incorporated by suspension-feeding bivalves (Alber & Valiela, 1994a–c, 1995). We have also shown that more nitrogen can be incorporated by bivalves fed aggregates than those fed either DOM or particulate detritus, and suggested that aggregates may be an important link between producers and metazoan consumers in detrital food webs (Alber & Valiela, 1995).

The next step in determining the potential use of aggregates as a food source for suspension-feeding bivalves in the field is to understand the dynamics of aggregate incorporation and release, and to estimate the efficiencies with which they are used. It is also important to compare the relative use of aggregates with that of a known good food, such as phytoplankton. The use of phytoplankton by filter feeding bivalves is well-described: they absorb phytoplankton with an efficiency greater than 70% (Winter, 1978; Bayne & Newell, 1983; Cranford & Grant, 1990). The use of organic aggregates, however, has never been measured.

The purpose of the present work was to quantify nitrogen incorporation and depuration rates in bay scallops, *Argopecten irradians* (Lamarck), fed aggregates and to compare them with those of scallops fed phytoplankton (*Thalassiosira weissflogii*). Aggregates were prepared from the dissolved material leached from both a brown (*Fucus vesiculosus*) and a red (*Gracilaria tikvahiae*) macroalga. Our results suggest that scallops incorporate aggregates at slower rates than they do phytoplankton, and use them with an estimated absorption efficiency of 78%, as compared with 90% when fed phytoplankton. Nonetheless, when we used our estimate of the absorption efficiency of scallops fed aggregates to calculate the concentration of aggregates that would be necessary in an estuary to meet their requirements, we found that aggregates could meet a substantial portion of the nutritional requirements of scallops in the field.

2. Materials and methods

Two experiments were performed. The first experiment was designed to determine the rate of nitrogen incorporation, and the second the rate of nitrogen release by bay scallops (*A. irradians*) fed aggregates and phytoplankton (*T. weissflogii*, clone Actin). Both experiments used ^{15}N -labelled diets to trace nitrogen into and out of scallops and were performed in a flow-through system with the same overall protocol.

2.1. Labelled diets

Labelled *T. weissflogii* was grown in f/2 medium (Guillard, 1973), which was prepared with silica added but without a nitrogen source. After the medium was autoclaved, 99.9% $^{15}\text{NH}_4\text{Cl}$ was added so the cultures would grow up labelled with ^{15}N . Cells were grown in batch culture and harvested after 3 days, by which

time cell number had increased approximately 20-fold. Samples of the labelled diatoms were filtered onto GF/F (Whatman) filters, dried, and stored desiccated for subsequent ^{15}N analysis.

Labelled aggregate diets were prepared as described previously (Alber & Valiela, 1994c). Briefly, labelled aggregates were produced by leaching ^{15}N -labelled macrophytes (*F. vesiculosus* and *G. tikvahiae*) into 0.22 μm -filtered seawater in the dark (for 5 or 4 days, respectively), removing the macrophytes, filtering the DOM-rich leachate through a GF/F filter, and bubbling the filtered leachate (for 5 or 6 days, respectively). Bubblers were fitted with in-line 0.22 μm filters. Aggregates were operationally defined as any organic material caught on GF/F filters (0.7 μm pore size) after bubbling. Aggregate samples were filtered, dried, and stored desiccated for subsequent analysis of atom % ^{15}N , and, in the nitrogen incorporation experiment, biochemical composition.

2.2. Experimental apparatus

Experiments were run in a flow-through system that delivered 0.22 μm filtered seawater at a rate of 70 $\text{ml} \cdot \text{min}^{-1}$ into each of 12 funnels that were used as tanks to hold experimental animals (Alber & Valiela, 1995). Scallops were placed onto mesh platforms inside the funnels, so that uneaten food and fecal material fell to the bottom, and funnels were stoppered at the bottom. Each funnel was drained through an outlet port positioned at a level that maintained the water volume at 1 l.

All diets were delivered to make a final concentration of approximately 1 μg dry weight (DW) $\cdot \text{ml}^{-1}$ in the funnels. This concentration was chosen because it is roughly equivalent to the dry weight of 3500 *T. weissflogii* cells $\cdot \text{ml}^{-1}$, the concentration of phytoplankton used in both experiments [based on an estimated 164 pg carbon per cell of *T. weissflogii* (Davis, 1982), and a carbon:dry weight ratio of 0.50 for *T. pseudonana* (Epifanio, 1979)]. This concentration of diatoms (3500 cells $\cdot \text{ml}^{-1}$ or 1 $\mu\text{gDW} \cdot \text{ml}^{-1}$) provides a good ration of *T. weissflogii* for juvenile scallops (M. Bricelj, pers. comm.), and is below concentrations where bivalves are reported to start producing pseudofeces. *A. irradians* fed *T. pseudonana* did not produce pseudofeces at concentrations of $\approx 0.4 \mu\text{gDW} \cdot \text{ml}^{-1}$, but did produce them at concentrations of 3 $\mu\text{gDW} \cdot \text{ml}^{-1}$ (Palmer & Williams, 1980): *Crassostrea virginica* fed *T. pseudonana* did not produce pseudofeces until the concentration reached $\approx 10 \mu\text{gDW} \cdot \text{ml}^{-1}$ (Epifanio & Ewart, 1977). Phytoplankton cell counts were done with a haemocytometer (a minimum of 100 cells were counted on at least two replicate slides).

Aggregates were sampled on the day prior to each experiment so that the concentration of aggregates delivered to the funnels could be normalized on the basis of dry weight. Pumping rates were adjusted so aggregate concentration in the funnels would be about 1 $\mu\text{gDW} \cdot \text{ml}^{-1}$. Actual concentrations were measured during the experiments. During the N incorporation experiment, actual concentration was 1.8 $\mu\text{g} \cdot \text{ml}^{-1}$ (SD = 0.3, $n = 3$) for aggregates derived from *F.*

vesiculosus, and $1.1 \mu\text{g} \cdot \text{ml}^{-1}$ ($\text{SD} = 0.4$, $n = 3$) for those derived from *G. tikvahiae*. During the N depuration experiment, actual concentration of aggregates derived from *F. vesiculosus* was $1.2 \mu\text{g} \cdot \text{ml}^{-1}$ ($\text{SD} = 0.2$, $n = 6$).

2.3. Experimental protocol

Juvenile *A. irradians*, spawned in August 1989, were obtained from the Martha's Vineyard Shellfish Hatchery and maintained at ambient temperature in flowing seawater. Experiments were performed in June 1990. At the beginning of an experiment, initial length and wet weight were recorded for each individual. Scallops were placed into funnels (4 per funnel) in the flow-through system, and conditioned on unlabelled *T. weissflogii* for 48 h (delivered at a concentration of $3500 \text{ cells} \cdot \text{ml}^{-1}$). Unlabelled phytoplankton were grown in 12-l batches in carboys, using *f/2* media (Guillard, 1973) with NH_4Cl as the nitrogen source and silica added. Cell counts were done using a Model TAI Coulter Counter.

After the 48 h conditioning period, labelled treatment diets were dripped into funnels for the length of the experimental trial (up to 15 h). There were 3 or 4 funnels for each treatment diet. When they had been fed for the requisite amount of time, scallops were removed randomly from the feeding funnels, moved to separate funnels that received only filtered seawater (FSW), and allowed to depurate for the length of the experimental trial (up to 72 h). Funnels were emptied out and gently rinsed every 24 h during the depuration period. Since scallops within each funnel fed or depurated for different lengths of time, the experimental unit was the individual scallop and not the funnel. In previous work in the same system, where funnels were considered the experimental unit, variability was high among individual scallops and we did not observe a significant funnel effect (Alber, 1992).

At the end of depuration, scallops were removed from the system and their final length and wet weight were recorded. Scallops were then dissected, and adductor muscle tissue was separated from the rest of the body tissue. This was done because ^{15}N incorporated into muscle tissue is an unambiguous measure of assimilated nitrogen: if the depuration period were inadequate it is possible that some of the ^{15}N measured in the whole body might represent material that had been ingested and was still present in the digestive gland as unassimilated ^{15}N . Wet and dry tissue weight (dried in a 60°C oven), and dry shell weight were recorded, and dried body and muscle tissue were stored desiccated and subsequently analyzed for atom % ^{15}N .

2.4. Experimental trials

To obtain rates of labelled nitrogen incorporation into scallop tissue, scallops were fed either labelled aggregates produced from *F. vesiculosus* or *G. tikvahiae* leachate, or labelled phytoplankton, *T. weissflogii*. Two scallops received each of the three treatment diets for 5, 8, 10, or 15 h. All treated scallops were depurated

in FSW for 48 h after receiving the labelled diet, and then dissected and dried (60°C oven), and their tissue analyzed for atom % ^{15}N . An additional six unfed control scallops received the FSW treatment for 5 h and were then removed from the system.

To obtain rates of labelled nitrogen release from scallop tissue, 16 scallops were fed labelled aggregates produced from *F. vesiculosus* leachate and 12 scallops were fed labelled phytoplankton for 15 h each. Scallops were depurated in FSW for 0, 6, 12, 24, 48, or 72 h, according to the schedule in Table 1. After depuration, scallops were removed from the system, dissected and analyzed for atom % ^{15}N . Four unfed control scallops were removed initially and an additional four received FSW for the 15 h feeding period and were depurated according to the schedule in Table 1.

To measure nitrogen released into feces, a subset of the above scallops, four that had been fed aggregates and four that had been fed phytoplankton, were removed from the system after the 15 h feeding period and placed individually into finger bowls containing 250 ml FSW. Scallops were placed on nitex mesh platforms so they would not re-ingest fecal material. After 6, 12, 24, and 48 h the water in the bowls was filtered onto pre-weighed GF/F filters for dry weight and % ^{15}N analysis, and scallops were transferred to new bowls with fresh FSW. At the end of the depuration period (Table 1), scallops were dissected and analyzed for atom % ^{15}N .

2.5. Analytical methods

Atom % ^{15}N was measured in labelled diets and experimental scallops. All samples were prepared with a dry combustion technique (Dumas procedure) and then measured on an atomic emission spectrometer. Specific nitrogen uptake was calculated as follows:

Table 1
Schedule for N release experiment. The number of scallops depurated for each time interval is listed below

Time depurated (h)	Treatment diet		
	Phytoplankton (n)	Aggregates (n)	Unfed control (n)
0	2	2	1
6	2	1	
12	2	2	
24	2 + 1 ^a	1 + 1 ^a	1
48	2 + 2 ^a	1 + 2 ^a	1
72	2 + 1 ^a	1 + 1 ^a	1

^a Scallops depurated in finger bowls in order to measure fecal production.

Specific N uptake

$$\begin{aligned}
 &= \frac{\text{atom } \% \text{ }^{15}\text{N (treatment scallop)} - \text{atom } \% \text{ }^{15}\text{N (control scallop)}}{\text{atom } \% \text{ }^{15}\text{N (treatment diet)} - \text{atom } \% \text{ }^{15}\text{N (treatment scallop)}} \\
 &= \frac{^{15}\text{N incorporated}}{\text{total scallop nitrogen}}
 \end{aligned}
 \quad (1)$$

This calculation is valid as long as the increase in biomass during the experiment is below 10%, and there is no other source of nitrogen in the system (Collos, 1987). Both of these assumptions were valid: the increase in scallop length during the course of the experiment (including the 48 h conditioning period when all scallops were fed unlabelled phytoplankton) was always less than 5%, and there was no other known source of nitrogen.

The amount of nitrogen incorporated by scallops was then calculated as:

$$\frac{\mu\text{g N incorp.}}{\text{gDW}} = \text{Specific N uptake} \times \% \text{N (scallop tissue)} \times \frac{10^6 \mu\text{g}}{\text{g}} \quad (2)$$

Scallop body tissue averaged 9.0% N (SD = 0.6, $n = 15$); muscle tissue averaged 13.9% N (SD = 0.6, $n = 15$) (measured on a Perkin Elmer Model 240C elemental analyzer). These numbers were used in all calculations of nitrogen incorporation. Both specific nitrogen uptake and $\mu\text{g N incorporated gDW}^{-1}$ (Eqs. 1 and 2) could be expressed in terms of time by dividing by the number of hours the scallop was fed labelled diet.

Aggregates produced during the nitrogen incorporation experiment were analyzed for carbon and nitrogen content (Perkin Elmer Model 240C elemental analyzer), percent carbohydrate (measured with the phenol-sulfuric acid assay, Strickland & Parsons, 1972), protein (measured with the Bradford Assay, Biorad, Richmond, VA), and lipid (measured gravimetrically, Bligh & Dyer, 1959).

2.6. Efficiencies

Absorption efficiency (the percentage of consumed food not released as feces) was calculated as follows:

$$\text{absorption efficiency (\%)} = \frac{(C - F)}{C} \times 100, \quad (3)$$

where C represents the amount of material ingested and F the amount defecated during a given period of time. $C - F$ is then a measure of the amount of absorbed ration.

Ingestion (C) was estimated as the difference between the concentration of material entering and that leaving the experimental funnels during the N incorporation experiment. These concentrations were determined by filtering known volumes of water sampled from both the inlet and outlet ports of a funnel onto pre-weighed GF/F filters, drying them in a 60°C oven, and then weighing the dried filters. This method of calculating ingestion may have over- or under-

estimated C , if either uneaten food settled in the funnels and was taken to be ingested material, or feces were resuspended and measured in the outflow as uningested material. There is no way to determine if either of these occurred in the present experiment. Defecation (F) was estimated on a per gram dry weight (gDW) basis using the quantity of material produced during the first 6 h of the fecal production part of the N release experiment.

Assimilation efficiency (that percentage of consumption not rejected as feces or excreted) was estimated with data from the N incorporation experiment. The amount of N incorporated (from Eq. 2) was used as a measure of assimilated N, and the amount of N ingested was estimated as the product of the material consumed (C) times the % N measured in a given treatment diet. Assimilation efficiency was then calculated as follows:

$$\text{assimilation eff. (\%)} = \frac{\mu\text{g N ingested gDW}^{-1} \cdot \text{h}^{-1}}{C(\mu\text{g} \cdot \text{gDW}^{-1} \cdot \text{h}^{-1}) \times \% \text{N (food)}} \times 100. \quad (4)$$

3. Results

3.1. Aggregate biochemistry

Aggregate composition was similar for aggregates produced from the DOM released by either *F. vesiculosus* or *G. tikvahiae* (Table 2). The results obtained in the present study were largely in keeping with the range of values reported previously for the proximate composition of aggregates produced from these two species as well as three other species of macrophyte (*Ulva lactuca*, *Zostera marina*, and *Spartina alterniflora*, Alber & Valiela, 1994b) (Table 2). The one exception was that the aggregates produced here from *G. tikvahiae* leachate had a protein content of 35%, which was higher than any previously reported values.

Table 2
Biochemical composition of aggregates measured during the N incorporation experiment

	Aggregate source		Range
	<i>Fucus vesiculosus</i>	<i>Gracilaria tikvahiae</i>	
% Nitrogen	5.95 ± 1.7 (2)	7.83 (1)	2.7–10.4
% Carbon	26.28 ± 5.9 (2)	34.35 (1)	20.4–65.3
Carbon:Nitrogen	4.46 ± 0.28(2)	4.38 (1)	3.9–6.3
% Protein	24.41 ± 7.84(6)	35.45 ± 5.98(3)	11.9–26.9
% Carbohydrate	4.67 ± 2.64(6)	11.57 ± 2.94(3)	5.7–18.0
% Lipid	5.56 ± 5.36(6)	5.63 ± 0.82(3)	3.6–10.0
% Organic	74.83 ± 7.0 (6)	92.05 ± 7.7 (3)	56.9–100.0

All data are shown with standard deviations; the number of replicates analyzed is in parenthesis. The range of previously reported values (Alber & Valiela, 1994b) is provided for comparison.

3.2. ^{15}N labelling

^{15}N was incorporated into all treatment diets. Labelled aggregates produced from *F. vesiculosus* were 30.3% ^{15}N ($\text{SD} = 0.3$, $n = 3$) in the N incorporation experiment and 28.3% ^{15}N ($\text{SD} = 1.4$, $n = 8$) in the N release experiment, whereas those produced from *G. tikvahiae* were 25.0% ^{15}N ($\text{SD} = 0.8$, $n = 3$) in the N incorporation experiment. Phytoplankton were better labelled than aggregates and had 75% ^{15}N ($n = 1$) in the N incorporation and 74.4% ^{15}N ($\text{SD} = 0.9$, $n = 2$) in the N release experiment. The ^{15}N was subsequently incorporated into all scallops (*A. irradians*) fed labelled diets. The body tissue of aggregate-fed scallops in both experiments ranged from 0.45 to 0.81% ^{15}N , and that of phytoplankton-fed scallops ranged from 2.2 to 20.2%. Unfed controls (fed FSW) averaged 0.40% ^{15}N ($\text{SD} = 0.03$, $n = 6$) in the N incorporation and 0.38% ^{15}N ($\text{SD} = 0.02$, $n = 8$) in the N release experiment, with no differences between those removed right away and those kept in the system for the feeding period and then depurated.

There was a strong correlation between the specific nitrogen uptake measured in adductor muscle tissue and in the rest of the body in both experiments ($r = 0.96$, $n = 64$, $p < 0.001$, Fig. 1). However, the specific nitrogen uptake measured in body tissue was always greater than that measured in muscle tissue. This is to be expected, because muscle tissue is part of the structural pool of nitrogen, which turns over relatively slowly as compared to the more dynamic metabolic pool of nitrogen included in body tissue measurements. Hawkins (1985) estimated that less than 3% of the nitrogen in the blue mussel, *Mytilus edulis*, is in the metabolic pool, with a turnover time of 3-7 days, whereas the remaining material in the structural pool has a turnover time on the order of 1-3 yr.

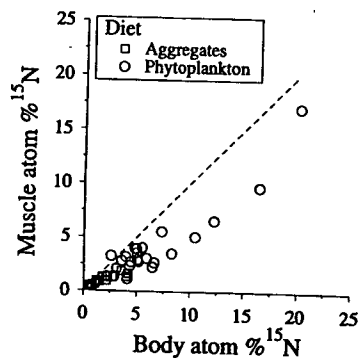


Fig. 1. Atom % ^{15}N measured in both adductor muscle and the remaining body tissue in scallops from both N incorporation and N release experiments fed labelled treatment diets and then depurated for 48 h. Squares represent scallops fed labelled aggregates, and circles scallops fed labelled phytoplankton. 1:1 line (dotted line) is drawn for comparison.

3.3. Nitrogen incorporation

Scallops in the N incorporation experiment averaged 22.8 ± 3.1 (SD , $n = 30$) mm in length and 0.114 ± 0.005 gDW. There were no differences between the lengths or weights of scallops fed the different diets, nor in the ratio of either the dry tissue to shell weight (0.15 ± 0.02) or of adductor muscle to the rest of the body weight (0.40 ± 0.07), both of which could be used as indicators of condition.

Nitrogen uptake by scallops fed either aggregates or phytoplankton was linear during the experiment (Fig. 2). Regression equations for specific nitrogen uptake by scallops versus the number of hours they were fed labelled diets are presented in Table 3. The slopes of each of these relationships were significantly different from 0 for both body and muscle data for all three diets. A test for homogeneity of slopes yielded significant treatment differences between nitrogen incorporation rates in both the muscle and the body tissue of scallops fed the different diets (Table 3). Two planned comparisons were performed on these data. The first comparison supported the notion that the nitrogen incorporation rate of phyto-

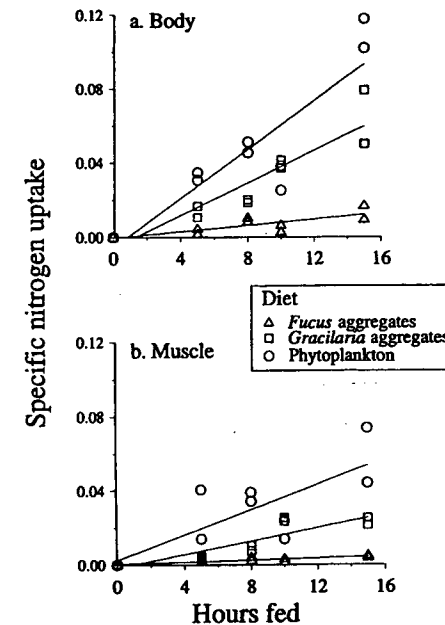


Fig. 2. Specific nitrogen uptake (nitrogen incorporated per unit scallop nitrogen per experimental interval) measured in scallop body and muscle tissue after scallops were fed labelled diets for varying amounts of time in the aggregate uptake experiment. Triangles represent scallops fed *F. vesiculosus* aggregates, squares scallops fed *G. tikvahiae* aggregates, and circles scallops fed phytoplankton. Regression lines are drawn (see Table 3).

Table 3

Regression statistics (a) and analysis of variance results (b) for relationships between the number of hours scallops were fed labelled diets and the specific nitrogen uptake of both body and muscle tissue in the N incorporation experiment. Data in (b) are for comparisons between slopes of all three diets, between phytoplankton and aggregate diets, and between two aggregate diets

(a) Regression statistics						
Diet	Body tissue			Muscle tissue		
	Phytoplankton	Aggregates		Phytoplankton	Aggregates	
		<i>Gracilaria</i>	<i>Fucus</i>		<i>Gracilaria</i>	<i>Fucus</i>
r^2	0.810	0.860	0.665	0.621	0.790	0.678
Slope	0.007	0.004	0.001	0.003	0.002	<0.001
Intercept	-0.006	-0.006	<0.001	0.002	-0.002	<0.001
t	5.839	7.016	3.986	3.620	5.485	4.100
p	<0.001	<0.001	0.004	0.007	0.001	0.003

(b) Analysis of variance results							
Source	df	Body tissue			Muscle tissue		
		SS	F-ratio	p	SS	F-ratio	p
Among regressions	2	0.004	14.852	<0.001	0.001	7.455	0.003
Phytoplankton vs. aggregate diets	1	0.003	18.724	<0.001	0.001	11.396	0.003
<i>Fucus</i> vs. <i>Gracilaria</i> aggregates	1	0.002	10.979	0.003	<0.001	3.514	0.073
Error	24	0.003			0.002		

plankton-fed scallops was significantly greater than that of aggregate-fed scallops (one-tailed test) for both body and muscle tissue (Table 3). The second comparison, made to test whether nitrogen uptake was the same for scallops fed the two aggregate diets, yielded a significant difference in the body tissue but not in the muscle tissue. Since nitrogen incorporation was lower and spanned a smaller range in muscle tissue than in body tissue, treatment differences were more difficult to discern.

3.4. Nitrogen release

Scallops used in the N release experiment were slightly larger than those used in the incorporation experiment, averaging 25.3 ± 2.5 mm (SD, $n = 34$) in length and 0.166 ± 0.005 gDW. As was true in the N incorporation experiment, there were no differences in lengths or weights of scallops fed the different treatment diets, nor in the ratio of either the dry tissue to shell weight (0.16 ± 0.02) or of adductor muscle to the rest of the body weight (0.40 ± 0.14).

The atom % ^{15}N of the body tissue of phytoplankton-fed scallops decreased during the first 12 h of depuration, and then remained relatively constant during the remainder of the 72 h depuration period (Fig. 3a). The final measurements were significantly lower than the initial ones (one-tailed t -test, $t = 20.3$, $p < 0.01$). There was a concomitant increase in atom % ^{15}N of the adductor muscle tissue

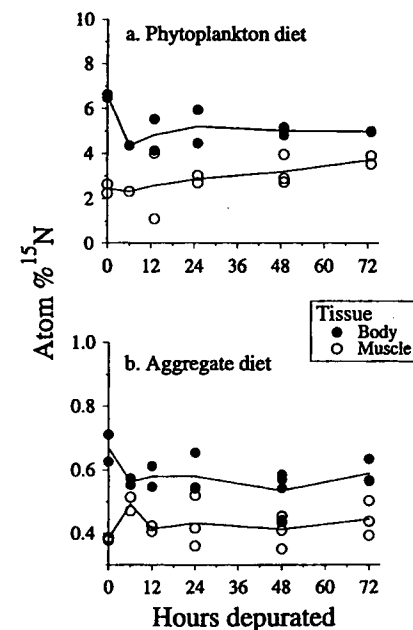


Fig. 3. Atom % ^{15}N measured in the body (closed circles) and muscle (open circles) tissue of scallops fed labelled phytoplankton (a) or aggregate (b) diets for 15 h and then depurated for varying amounts of time.

during the depuration period, ($t = 4.71$, $p < 0.05$), which suggests that some of the absorbed ^{15}N was being assimilated into muscle tissue. The one scallop that had very low atom % ^{15}N in its muscle tissue after 12 h had a cracked shell.

The atom % ^{15}N of aggregate-fed scallops was much lower than that of phytoplankton-fed scallops. Atom % ^{15}N of the body tissue of aggregate-fed scallops decreased quickly during the first 6 h of depuration and then levelled out (Fig. 3b), but there was neither a significant decrease in atom % ^{15}N of the body tissue nor a significant increase in atom % ^{15}N of the muscle tissue over the course of the experiment (t -tests).

Scallops receiving both treatment diets defecated similar amounts of feces over time (Fig. 4a). The average weight of fecal material released by the eight experimental scallops was $320 \mu\text{g} \cdot \text{h}^{-1}$ (SD = 42) during the first 6 h of depuration, and then dropped to $79 \mu\text{g} \cdot \text{h}^{-1}$ (SD = 48) after that. The atom % ^{15}N in the feces was also greatest during the first 6 h of depuration for both treatments (Fig. 4b). After 6 h, the atom % ^{15}N in the feces released by aggregate-fed scallops decreased rapidly. In contrast, the atom % ^{15}N of the material released by phytoplankton-fed scallops remained high after 72 h.

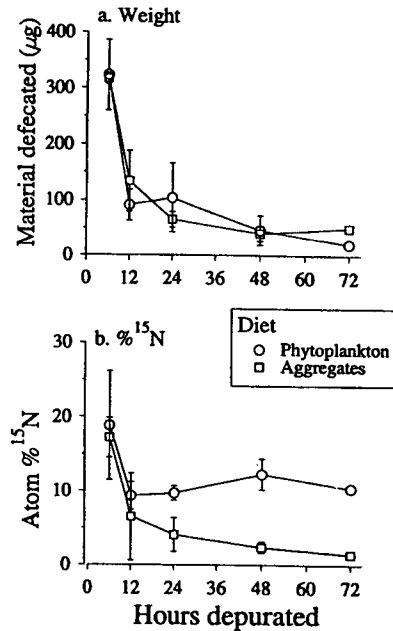


Fig. 4. Weight (a) and atom % ¹⁵N (b) of fecal material defecated by scallops during the fecal production part of the N release experiment. ○ represent phytoplankton-fed scallops and □ aggregate-fed scallops. Error bars represent standard deviations.

3.5. Efficiencies

Estimated ingestion rates (C) of scallops were determined as the differences in particle concentration between incurrent and excurrent flow (Table 4). Scallops consumed phytoplankton at a rate of $36 \mu\text{g} \cdot \text{min}^{-1}$, which is double the rate they consumed aggregates derived from either *G. tikvahiae* or *F. vesiculosus* leachate (17 and $16 \mu\text{g} \cdot \text{min}^{-1}$, respectively). Since all diets were delivered at the same rate, this suggests that scallops were distinguishing between aggregates and phytoplankton, but not between aggregates derived from the different macrophyte sources. Selectivity in bivalve feeding is known to occur for a number of reasons, including preferences for size, shape, or even chemical cue (Hawkins & Bayne 1992). The differences observed here may be due to chemical differences between diets, or perhaps to size differences. Unfortunately, size frequency measurements proved unworkable because particles tended to aggregate in the sample jar.

Defecation (F), was calculated based on the average weight of fecal material released during the initial 6 h of deputation during the N release experiment ($320 \mu\text{g} \cdot \text{h}^{-1}$), normalized to scallop dry weight (0.166 g). It is possible that scallops

Table 4
Absorption and assimilation efficiency calculations (see text for details)

	Diet		
	Phytoplankton	Aggregates	
		<i>Gracilaria</i>	<i>Fucus</i>
(1) Food entering funnel ($\mu\text{g} \cdot \text{ml}^{-1}$)	2.11	1.13	1.67
(2) Food leaving funnel ($\mu\text{g} \cdot \text{ml}^{-1}$)	0.08	0.16	0.78
(3) Consumption in funnel ($\mu\text{g} \cdot \text{ml}^{-1}$) (calculated as [(1)–(2)])	2.03	0.97	0.89
(4) C ($\mu\text{g} \cdot \text{gDW}^{-1} \cdot \text{h}^{-1}$) (based on (3) $\times 70.6 \text{ ml} \cdot \text{min}^{-1}$ food delivered to funnel $\times 60 \text{ min} \cdot \text{h}^{-1}$ (4 scallops $\times 0.114 \text{ gDW}$)	18853	9009	8226
(5) $C \cdot F$ ($\mu\text{g} \cdot \text{gDW}^{-1} \cdot \text{h}^{-1}$) (based on defecation rate (F) of $11928 \mu\text{g} \cdot \text{gDW} \cdot \text{h}^{-1}$)	16925	7081	6298
(6) Absorption efficiency (%) [(5)/(4) $\times 100$]	90	79	77
(7) $\mu\text{g N}$ incorp. $\cdot \text{gDW}^{-1} \cdot \text{h}^{-1}$	523	306	70
(8) % N in food (from Table 2)	3.8	7.83	5.95
(9) $\mu\text{g N}$ ingested [(4) \times (8)]	716	705	489
(10) Assimilation efficiency (%) [(7)/(9) $\times 100$]	73	43	14

responded to starvation during the deputation period by reducing their defecation rates. If this occurred before 6 h, then our estimated defecation rates may be low. This would result in a high estimate of absorption efficiency.

Once ingested, scallops used phytoplankton with an estimated absorption efficiency of 90% and assimilation efficiency of 73% (Table 4). Although the estimated absorption efficiencies of scallops fed aggregates derived from either *G. tikvahiae* or *F. vesiculosus* were similar (79 and 77%, respectively), their estimated assimilation efficiencies on the two diets were very different (43 and 14%, respectively) (Table 4). It therefore appears that scallops are distinguishing between these diets after they have been absorbed.

4. Discussion

4.1. Nitrogen incorporation

Scallops (*A. irradians*) incorporated phytoplankton (*T. weissflogii*) nitrogen at significantly faster rates than they did aggregate N. When expressed as N incorporation per hour per gram dry weight of scallop tissue (from Eq. 2), scallops fed phytoplankton incorporated 523 ± 165 (SD, $n = 8$) $\mu\text{g N} \cdot \text{gDW}^{-1} \cdot \text{h}^{-1}$. These data are in keeping with our results from previous experiments, where juvenile *A.*

irradians incorporated between 395 and 544 $\mu\text{g N} \cdot \text{gDW}^{-1} \cdot \text{h}^{-1}$ when fed *T. weissflogii* (Alber, 1992). Scallops fed aggregates derived from the DOM released from *F. vesiculosus* incorporated $70 \pm 28 \mu\text{g N} \cdot \text{gDW}^{-1} \cdot \text{h}^{-1}$ ($n = 8$). This rate is also in agreement with our previous experiments, wherein incorporation rates of scallops fed aggregates produced from five species of macrophytes ranged from 49 to 119 $\mu\text{g N} \cdot \text{gDW}^{-1} \cdot \text{h}^{-1}$ (Alber & Valiela, 1995) and averaged 18% ($\text{SD} = 6$, $n = 5$) that of phytoplankton.

In contrast, N incorporation by scallops fed aggregates produced from *G. tikvahiae* was unusually high ($306 \pm 80 \mu\text{g N} \cdot \text{gDW}^{-1} \cdot \text{h}^{-1}$ ($n = 8$)). The difference between N incorporation rates of scallops fed *F. vesiculosus*- and *G. tikvahiae*-derived aggregates is not due to differences in ingestion rate or absorption efficiency of scallops fed the two aggregate diets (Table 4). However, once the aggregates were absorbed, there was a marked difference in assimilation efficiency (43% for those fed aggregates derived from *G. tikvahiae* versus 14% for those fed aggregates derived from *F. vesiculosus*). The observed difference in aggregate assimilation may be due to differences in aggregate quality. Aggregates derived from *G. tikvahiae* were higher in percent organic matter, carbon, nitrogen, protein and carbohydrate than those derived from *F. vesiculosus* (Table 2). In fact, the protein content of aggregates produced from *G. tikvahiae* was higher than had been observed in aggregates from any other trial, and may be the reason these aggregates were such a high quality diet for scallops.

4.2. Nitrogen release

Most fecal material was released during the first 6 h of depuration (Fig. 4a). During this initial depuration period, the atom % ^{15}N of body tissue of both aggregate- and phytoplankton-fed scallops showed a rapid decrease (Fig. 3), and there was also a rapid drop in atom % ^{15}N of the feces (Fig. 4b). When taken together, these data suggest that the bulk of the undigested ^{15}N is released during the initial depuration period.

The atom % ^{15}N of fecal material released from scallops fed aggregates continues to drop during the 72 h time-course, with no real change in atom % ^{15}N of body tissue. This is consistent with the data of Kreeger et al. (1988), who found that *Geukensia demissa* cleared their guts of ^{14}C -labelled lignocellulose within 18 h.

Scallops fed phytoplankton also showed a levelling out of body atom % ^{15}N after the initial decrease (Fig. 3a), with some material possibly being assimilated into muscle tissue, as noted earlier, and some being released as feces (Fig. 4b). The atom % ^{15}N of fecal material did remain high for the duration of the 72 h experiment, indicating that scallops may still be processing labelled diet. If the depuration time is inadequate for phytoplankton-fed scallops, this would result in an overestimate of N incorporation rates. However, there are several reasons why this is probably not the case. First, Widdows et al. (1979) distinguishes between intestinal and glandular feces in bivalves, and Hawkins and Bayne (1984) have shown that intestinal feces move through more quickly and are relatively

undigested, compared with glandular feces that pass through the digestive gland. In fact, up to 20% of the N in the feces of *M. edulis* may be of metabolic origin (Hawkins & Bayne, 1985). It is possible that phytoplankton-fed scallops produce more glandular feces than aggregate-fed ones, and that it therefore takes longer to clear their guts. If this so, then the extra N released by these scallops should be included in the pool of absorbed N. Second, the total quantity of fecal material was so low (Fig. 4a) that the actual amount of ^{15}N released is also likely to be low. Finally, the close correlation between muscle and body nitrogen incorporation after 48 h (Fig. 2) supports the adequacy of the depuration period for both aggregate- and phytoplankton-fed scallops, since one would not expect them to correlate so well if body nitrogen incorporation was unduly biased by labelled material still present in the digestive gland.

4.3. Efficiencies

Our estimates of the absorption and assimilation efficiencies of scallops fed phytoplankton (Table 4) are consistent with literature values. In a directly comparable study, Bricelj & Shumway (1991) estimated that *A. irradians* fed *T. weissflogii* had absorption efficiencies ranging from 65% for an algal density of 200000 cells $\cdot \text{ml}^{-1}$, to 90% at a density of 1200 cells $\cdot \text{ml}^{-1}$. In the present study, we calculated an absorption efficiency of 90% at an algal density of 3500 cells $\cdot \text{ml}^{-1}$.

Our estimated assimilation and absorption efficiencies for scallops fed phytoplankton are greater than our estimates for scallops fed aggregates (Table 4). When taken together with the results of the nitrogen incorporation and release experiments, discussed above, a clear picture emerges that phytoplankton are a better food for scallops than aggregates. However, phytoplankton often comprise only a small percentage of the seston, and are subject to marked seasonal variability (e.g. Langdon & Newell, 1990).

In contrast to phytoplankton, detritus is a year-round source of food. As pointed out by Langdon & Newell (1990), bivalves processing water in coastal environments would benefit if they were able to use the abundant detrital material as a food source. In previous work, we have distinguished between amorphous detritus, such as the organic aggregates derived here from DOM, and morphous detritus, which often retains its cellular structure and is derived from the breakdown of particulate material during decomposition (Alber & Valiela, 1994b,c, 1995, see also Bowen, 1984). One can therefore make comparisons between the use of aggregates and morphous detritus.

Morphous detritus is not used effectively by most bivalves. In our studies, we found that *A. irradians* and two species of mussels (*Mytilus edulis*, *Geukensia demissa*) incorporated more N when fed organic aggregates than when fed morphous detritus derived from five species of macrophyte (Alber & Valiela, 1994c, 1995). In fact, N incorporation by bivalves fed morphous detritus was not distinguishable from controls fed FSW. Kreeger et al. (1988) showed that *G. demissa* used refractory lignocellulosic carbon derived from *Spartina alterniflora*

with an absorption efficiency of 14%, and Langdon & Newell (1990) reported an assimilation efficiency for *Crassostrea virginica* fed morphous *Spartina* detritus of only 2.7%. These values are much lower than those estimated here for scallops fed aggregates. Moreover, morphous detritus does not support the growth of bivalves (Kirby-Smith, 1976; Williams, 1981).

Reported values for absorption efficiency in bivalves fed natural seston range from 36 to 75% (Bayne & Newell, 1983). Natural seston is a heterogeneous mixture of living and nonliving material, and includes phytoplankton, amorphous, and morphous detritus. Although high absorption efficiencies could be the result of a diet high in phytoplankton, our data suggest that the presence of organic aggregates in the seston could lead to higher absorption efficiencies than cases where morphous detritus is the dominant form of detritus.

Finally, we have previously hypothesized that organic aggregates are microbial in nature based on the presence of large numbers of bacteria, their biochemical composition, and the fact that scallops did not distinguish between aggregates produced from different macrophyte sources (Alber & Valiela, 1994a,b, 1995). If this is the case, then it is appropriate to compare efficiencies of bivalves fed aggregates with those of bivalves fed bacteria. Crosby et al. (1990) measured assimilation efficiencies of 52% in *C. virginica* fed bacteria, and cite other studies where bivalves assimilated bacteria with efficiencies between 40 and 70%. Although these numbers are in keeping with the assimilation efficiencies calculated here for scallops fed aggregates derived from *G. tikvahiae*, they are higher than those estimated for aggregates derived from *F. vesiculosus*. This suggests that, with the exception of cases where aggregates are very high in protein, they are probably not used as effectively as pure bacteria.

4.4. Nutritional requirements

We estimated the concentration of aggregates that would be necessary in the field to meet the requirements of scallops. This calculation, outlined in Table 5, was performed using published allometric relationships between oxygen consumption and dry weight, as well as the absorption efficiencies estimated in Table 4. Assuming aggregate C and N are utilized similarly, an approximate concentration of 100–500 μg aggregate carbon $\cdot \text{l}^{-1}$ would be required in the water filtered by scallops to meet their carbon demands, depending on estimated retention efficiency. If aggregates are 30% carbon (Table 2), this means that between 300 and 1500 μgDW of aggregates are required l^{-1} .

Field estimates of non-living POC $\cdot \text{l}^{-1}$ range widely, from 100 to 4000 $\mu\text{g C} \cdot \text{l}^{-1}$ (Valiela, 1984). POC in marshes can be as high as 23000 $\mu\text{g C} \cdot \text{l}^{-1}$ (Kennish, 1986). In Great Sippewissett Marsh, Valiela et al. (1978) estimated that POC ranged from 100 to 2200 $\mu\text{g C} \cdot \text{l}^{-1}$. In near-shore estuaries, the concentration of detritus ranges from 100 to 12500 $\mu\text{gDW} \cdot \text{l}^{-1}$ (Kennish, 1986). It is difficult to determine nature of a detrital particle, but these calculations suggest that, depending on the conditions, from less than 10 to 100% of the POM in an estuary would need to be organic aggregates to support the carbon requirements of

Table 5

Aggregate concentration necessary to sustain juvenile scallops (see text for details)

(1) Oxygen required	193 $\mu\text{l} \cdot \text{scal}^{-1} \cdot \text{h}^{-1}$
Calculated as $\log \text{VO}_2$ ($\mu\text{l O}_2 \cdot \text{h}^{-1}$) = $0.794 + 0.725 \log W$	
For N incorporation experiment, $W = 114$ mg	
(from Bricelj et al., 1987)	
(2) Carbon required	137 $\mu\text{g} \cdot \text{scal}^{-1} \cdot \text{h}^{-1}$
Based on 1 ml O_2 per 533 $\mu\text{g C}$ (Lucas & Newell, 1984) and	
C required for maintenance = $1.33 \times \text{C respired}$ (Bayne & Newell, 1983)	
(3) Aggregate C required	175 $\mu\text{g} \cdot \text{scal}^{-1} \cdot \text{h}^{-1}$
Based on absorption efficiency = 78% (see Table 4)	
(4) Water filtered	0.8–1.9 $\text{l} \cdot \text{scal}^{-1} \cdot \text{h}^{-1}$
Calculated as $\log \text{filt. rate}$ ($\text{l} \cdot \text{h}^{-1}$) = $\log 4.742 + 0.82 \log W$	
and $\log \text{filt. rate}$ ($\text{l} \cdot \text{h}^{-1}$) = $\log 5.827 + 0.524 \log W$	
where $W = 0.114$ g (data compiled by Bricelj & Shumway, 1991)	
(5) Aggregate C required in the water filtered to meet C demand	
Assuming retention efficiency = 100%	219–92 $\mu\text{g} \cdot \text{l}^{-1}$
Assuming retention efficiency = 50%	437–184 $\mu\text{g} \cdot \text{l}^{-1}$
(6) Weight of aggregates required in the water filtered to meet C demand	
Calculated assuming aggregates are 30% C (see Table 2)	
Assuming retention efficiency = 100%	730–307 $\mu\text{g} \cdot \text{l}^{-1}$
Assuming retention efficiency = 50%	1458–614 $\mu\text{g} \cdot \text{l}^{-1}$

scallops. Although direct measurements of aggregate material in the field have not yet been performed, our calculations, taken along with the above evidence, suggest it is not unreasonable to expect that a substantial proportion of the nutritional requirements of a bivalve could be met by amorphous organic aggregates.

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