Assimilation of bacteria by the dwarf surf clam *Mulinia lateralis* (Bivalvia: Mactridae)

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**ABSTRACT:** Estimation of assimilation efficiency of bivalves, fed with radiolabeled bacteria, differs substantially depending on choice of tracer. We have investigated assimilation of bacteria by the dwarf surf clam *Mulinia lateralis* (Say) using bacteria labeled with either L-[^35]S-methionine or [methyl-^3H]-thymidine. Labeled bacteria were fed to *M. lateralis* 6.18 ± 0.45 mm (mean ± SD) in shell length in static pulse-chase experiments. After feeding and 4 consecutive 1 h chases in 0.2 μm filtered seawater, *M. lateralis* retained 93.3 ± 6.45 % (n = 20) of incorporated ^35S-methionine radioactivity. Alternatively, when ^3H-thymidine was used, only 51.4 ± 16.54 % (n = 13) was retained. During our chase procedure, excretion patterns also differed conspicuously. For excreted ^35S activity, 49.05 ± 13.30 % was recovered in the particulate phase (> 0.2 μm) and 50.94 ± 13.30 % was dissolved. The corresponding values for excreted ^3H activity were 11.43 ± 4.70 % and 88.57 ± 4.70 %. Assimilation efficiency, estimated using ^35S-methionine, is considerably higher than previously reported values using ^14C, ^3H and ^15N tracers. We can explain assimilation and excretion differences between tracers in terms of metabolic pathways associated with the labeled moieties. The assimilation of methionine and high retention of ^35S tracer demonstrates the role of bacteria as a source of proteins and mineral nutrients for *M. lateralis*.

**INTRODUCTION**

The dwarf surf clam *Mulinia lateralis* (Say) is a common bivalve that inhabits soft substrata and exhibits high growth and reproduction rates. Opportunistic species such as *M. lateralis* increase dramatically in numbers under favorable conditions and can become dominant in benthic communities (Levinton 1970, Santos & Simon 1980). Over short time periods, *M. lateralis* may attain local average densities of up to 21 000 ind. m⁻² (Santos & Simon 1980). Elevated filtration and metabolic rates suggest that *M. lateralis* is adapted to exploit high food concentrations (Shumway 1983, Shumway & Newell 1984). The possibility that food resources may seasonally fall below the energy requirements of *M. lateralis*, and the inability to catabolize protein reserves during starvation, may account for the mass mortalities that characterize this species (Shumway & Newell 1984).

It is conceivable that suspended bacteria may constitute a food source for *Mulinia lateralis* in its natural habitat. The ability of bivalves to utilize bacteria has been verified by many authors (reviewed by Langdon & Newell 1990). Effective clearance of free-living and natural-sized bacteria has been demonstrated by Wright et al. (1982), Lucas et al. (1987), and Langdon & Newell (1990). Kemp et al. (1990) have shown that small- (shell height = 16 mm) to large- (40 mm) sized individuals of the ribbed mussel *Gekkensia demissa* (Dillwyn) can filter bacteria-sized particles with differing efficiency. The assimilation of both free-living and particle-bound bacteria (not necessarily natural bacterioplankton) has been demonstrated by Birkbeck & McHenery (1982), Harvey & Luoma (1984), Amouroux (1986), and Crosby et al. (1990). In addition to clearance and assimilation studies, the presence of bacteriolytic enzymes has been confirmed in bivalves by McHenery et al. (1979), Seiderer et al. (1984), and Jamieson & Wardlaw (1989).

Many researchers studying feeding and assimilation of bacteria by suspension-feeding bivalves have utilized isotopic tracers to label bacteria. These studies have included the use of ^3H-thymidine (Hollibaugh et al. 1980, Birkbeck & McHenery 1982), ^14C-glutamic acid (Amouroux 1986), ^14C-[u]-D glucose (Harvey & Luoma 1984, Crosby et al. 1990) and ^15N]-ammonium sulfate (Crosby et al. 1990). In this study, we investigated the ability of *Mulinia lateralis* to assimilate a mixed assemblage of bacterioplankton using ^35S-methionine in comparison with ^3H-thymidine as tracers.

The goal of our study was 2-fold: (1) to investigate
the ability of *M. lateralis* to assimilate bacterioplankton, and (2) to compare assimilation efficiency estimates obtained by using 2 isotopic tracers to label different macromolecules. The amino acid 35S-methionine labels bacterial protein and has been used in trophic studies by Wikner et al. (1986). The sulfur in methionine plays an important structural role in intracellular proteins and functions as part of important coenzymes in enzyme reactions (Linder 1985). The tracer 3H-thymidine labels bacterial DNA and was originally proposed for trophic studies by Hollibaugh et al. (1980). Under certain circumstances, however, 3H-thymidine may label other bacterial cellular components (see Hollibaugh 1988). Few tracer studies have been addressed towards differential utilization of bacterial macromolecules. We hypothesized that assimilation efficiency estimates would vary depending on the metabolic pathways associated with the different tracers.

**MATERIALS AND METHODS**

The specimens of *Mulinia lateralis* used in our experimental procedures were raised at the Rutgers Shellfish Research Laboratory, New Jersey, USA. Specimens were maintained in flow-through containers and fed daily with algae batch-cultured in Kalwall™ tubes. Experimental organisms averaged 6.18 ± 0.45 mm in shell length (mean ± SD, n = 33). Experiments were conducted at room temperature (24.0 ± 0.5°C) in sterile tissue culture wells (Falcon, 3.6 ml capacity). Bivalves were maintained in 0.45 µm filtered seawater for 6 to 8 h before being used in experiments. During that period, if bivalves defecated, the water was changed. A Tracer Analytic Mark III, Model 6881 LSC, was used for scintillation counting. Radioactivity measurements in disintegrations per minute (dpm) were corrected for quenching and counting efficiency using external standards to establish a quench curve.

Our experimental protocol consisted of 3 steps: (1) isolation of bacteria and radiisotope labeling; (2) pulse-feeding; and (3) chase, which includes purging of residual radioactive activity and excretion.

**Bacteria isolation and labeling.** Bacterial assemblages used in this study were obtained from Tahitian *Isochrysis* aff. *galbana* Green (strain T-JSO) cultures raised in Kalwall™ tubes. Bacteria were separated from algae by filtration through 47 mm diameter, 1 µm pore size polycarbonate filters (vacuum < 20 kPa). After separation, bacteria were counted by the acridine orange direct count method (AODC) developed by Hobbie et al. (1977). Subsamples of 100 µl were taken from the stock and 10 random fields per filter were enumerated under oil immersion. Bacterioplankton present in 50 to 60 ml of the bacterial suspension were caught on 47 mm diameter, 0.2 µm pore size polycarbonate filters. They were then washed (10 ml, 0.45 µm filtered seawater) and resuspended by shaking (1 min) in 50 ml polypropylene centrifuge tubes with 5 ml of 0.45 µm filtered seawater (salinity 22 ppt). Additional seawater was added to the initial volume. Bacteria were counted after resuspension by AODC to determine percentage recovery. Resuspended bacteria were subsequently incubated in 50 ml sterile plastic bags (Whirl-Pak) in subdued light with either L-[35S]-methionine (specific activity = 1134 Ci mmol⁻¹, final conc. = 0.002 nmol ml⁻¹; NEN Research Products) or [methyl-3H]-thymidine (specific activity = 2.0 Ci mmol⁻¹, final conc. = 11.25 nmol ml⁻¹). Bacteria were incubated in 35S-methionine for 60 min; incubation time for 3H-thymidine was either 30, 60 or 120 min depending on rate of 3H-thymidine uptake. We used 35S-methionine to label bacterial protein (Wikner et al. 1986) and 3H-thymidine to label bacterial DNA (Hollibaugh et al. 1980; see also Hollibaugh 1988).

**PULSE-FEEDING**

1. Labeled bacteria caught on filter were resuspended. Bacteria were enumerated by AODC. Volume brought back to 60 ml.

2. Labeled bacterial suspension divided into two equal aliquots.

3. 30 ml labeled bacterial suspension with associated DOM.

4. 30 ml bacterial suspension.

5. 2 ml aliquots added to each feeding-chamber.

![Fig. 1 Protocol for pulse-feeding *Mulinia lateralis* with labeled bacteria in tissue culture wells. DOM: dissolved organic matter; PC: polycarbonate](image-url)
Pulse-feeding. After incubation, labeled bacteria were collected on 47 mm diameter, 0.2 μm polycarbonate filters and rinsed twice with 5 ml each of 0.45 μm filtered seawater to remove uncultivated label. Bacteria were then resuspended as described above. Bacteria were counted again (AODC) to estimate percentage, recovery and degree of clumping.

The resulting resuspended bacteria were used for feeding and assimilation studies. Bacterial concentrations added to feeding chambers ranged from 4.0 to 8.5 × 10^6 cells ml^-1. Radioactivity in the bacterial suspension was determined for particles caught on 0.2 μm pore size filters.

Preliminary experiments indicated that some label from bacteria was released during our resuspension procedure. Therefore, prior to feeding experiments, the labeled bacterial suspension was divided into 2 equal portions (Fig. 1). One portion was filtered through a 25 mm diameter, 0.2 μm pore size polycarbonate filter to remove bacteria. The filtrate was collected in acid-washed and combusted glass scintillation vials. This filtrate was used for dissolved-phase uptake controls. Each experimental run consisted of 2 replicate sets of feeding chambers. One set was used for bacteria plus associated dissolved organic matter (DOM), the other for DOM only. Each set consisted of at least 12 chambers: 6 wells with bivalves (usually 3 live and 3 formalin-killed) and 6 wells without. For each chamber, 2 ml of suspension (bacteria + DOM or DOM only) was added. Suspensions were added to empty wells as controls for label disappearance resulting from feeding-chamber wall adsorption. Radioactivity in 3 wells without bivalves were sampled at the beginning of the experiment. The 3 remaining no-bivalve wells were sampled after 1 h. The difference in activity was used to correct uptake values.

Feeding was carried out in a laminar flow hood under continuous lighting. Bivalves were allowed to feed for 1 h, after which they were removed and placed in new sterile wells. Remaining radioactivity was assayed by taking 1 ml samples from each well. Samples were assayed in 7 ml plastic MiniVials with 5 ml ScintiVerse LC cocktail.

Chase. In the chase procedure, live bivalves incubated with both bacteria and DOM, and with DOM only, were blotted dry and placed in new culture wells with 2 ml of 0.2 μm filtered seawater. After each 1 h incubation (total 4 h), bivalves were removed from the wells, blotted dry, and transferred to new wells. Radioactivity excreted in wells was assayed by filtering each well’s total volume through 25 mm diameter, 0.2 μm polycarbonate filters. Particles caught on each filter were washed with a total of 3 ml of 0.45 μm filtered seawater and assayed (particulate excretion). Filtrate from each well (1 ml) was assayed separately for dissolved excretion.

After termination of the chase procedure, bivalves were blotted dry, placed in scintillation vials, and digested in Scintiget tissue solubilizer for 24 h at 60°C. After digestion, the shells were removed with fine forceps and 35 μl glacial acetic acid was added to the digestion mixture to decrease chemiluminescence. Tissue dpm values were corrected for variable quench and for self-absorption. The pulse-chase experiments were repeated 6 times for 35S-methionine and 3 times for 3H-thymidine, using different batches of bacteria.

Added radioactivity. Radioactivity added in particulate phase (R_p) was calculated by subtracting dissolved (filtrate) radioactivity (R_d) from total radioactivity in stock bacterial suspension (R_{p+d})

R_p = R_{p+d} - R_d (1)

Incorporated radioactivity. Incorporated particulate radioactivity (IR_p) was calculated by subtracting radioactivity remaining in dissolved set (R_d) from total remaining radioactivity in wells (R_{p+d}) and subtracting this remaining dpm value from added particulate radioactivity:

IR_p = R_p - (R_{p+d} - R_d) (2)

Isotope excretion. For each experimental set, excreted radioactivity was obtained by subtracting excreted radioactivity from treatment controls (dissolved) from corresponding total excretion values.

Particulate excretion: For calculation of particulate phase excretion we used the following formula:

EPR_p = EPR_{p+d} - EPR_d (3)

where EPR_p = excreted particulate radioactivity from particulate uptake; EPR_{p+d} = excreted particulate radioactivity from total uptake; and EPR_d = excreted particulate radioactivity from dissolved uptake.

Dissolved excretion: For calculation of dissolved phase excretion resulting from particulate uptake we used the formula:

EDR_p = EDR_{p+d} - EDR_d (4)

where EDR_p = excreted dissolved radioactivity from particulate uptake; EDR_{p+d} = excreted dissolved radioactivity from total uptake; and EDR_d = excreted dissolved radioactivity from dissolved uptake.

Total excretion: Cumulative excretion (particulate phase and dissolved phase) after 4 h was calculated as:

Σ ER_p = Σ EPR_p + Σ EDR_p (5)

where Σ ER_p = total excreted radioactivity from particulate uptake after 4 chases, Σ EPR_p = particulate excretion from particulate uptake after 4 chases, and Σ EDR_p = dissolved excretion from particulate uptake after 4 chases.
**Assimilation efficiency.** Assimilation efficiency (AE) was calculated as:

\[
AE = \frac{IR_p - \sum ER_p}{IR_p} \times 100
\]  

(6)

**RESULTS**

Preliminary clearance studies showed that within 1 h *Mulinia lateralis* could clear 33.48 ± 5.50% of bacterial cells from suspension. Incubation of bacteria with isotopes did not noticeably alter size or appearance of cells. We observed no clumping of bacteria after resuspension. The recovery of bacteria from filters ranged from 50 to 100% (mean 77%).

Uptake of particulate radioactivity by clams was variable for both isotopes and was apparently related to activity of the organisms during feeding. An average of 74.92 ± 18.54% of methionine was taken up (range = 19.05 to 94.82), whereas the corresponding value for thymidine was 49.51 ± 15.28% (range = 19.88 to 69.60).

Average tracer radioactivity present in bivalve tissue (assimilation) after digestion and scintillation counting was within 5% of values obtained by subtraction (Table 1). Assimilation estimates reported in this study were obtained by subtraction.

The discrepancy in tracer retention is shown graphically in Fig. 2. Assimilated radioactivity values, expressed as percentage of incorporated radioactivity, after 4 chases were 93.34 ± 6.45% (n = 20) for 35S-methionine and 51.40 ± 16.54% (n = 13) for 3H-thymidine. Retention of incorporated 35S was higher than that of 3H from the beginning of the chase period.

The difference between particulate and dissolved fractions of excreted radioactivity, in relation to time,

Table 1. *Mulinia lateralis*. Label activity remaining in tissue from digestion and scintillation counting, compared to values obtained by subtraction of excreted radioactivity. Experiments with 35S-methionine were run 6 times. Experiments with 3H-thymidine were run 3 times. Average *M. lateralis* 35S background dpm = 49 ± 7 (n = 18); 3H background dpm = 176 ± 42 (n = 9); Subtr.: subtraction; Scint.: scintillation.

<table>
<thead>
<tr>
<th></th>
<th>35S-methionine</th>
<th>3H-thymidine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean dpm n</td>
<td>Mean dpm n</td>
</tr>
<tr>
<td>Subtr. Scint.</td>
<td>89 150 3</td>
<td>205 659 5</td>
</tr>
<tr>
<td></td>
<td>407 586 3</td>
<td>224 767 3</td>
</tr>
<tr>
<td></td>
<td>132 512 3</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>256 570 3</td>
<td>246 689 3</td>
</tr>
<tr>
<td>Diff. (%)</td>
<td>3.9</td>
<td>1.6</td>
</tr>
</tbody>
</table>

Cumulative excretion in both fractions for 35S-methionine and 3H-thymidine after 4 chases is shown in Fig. 3A,B. Particulate activity decreased rapidly, with a proportional increase in dissolved excretion. A comparison between isotopes indicates that 3H activity was excreted primarily in dissolved form from the beginning of the chase.

**DISCUSSION**

Assimilation in this study is defined as incorporated radioactivity minus radioactivity lost as excreta (particulate and dissolved; see also Bayne 1983 p. 312). The use of radiolabeled food to measure absorption and assimilation poses considerable problems of interpretation (Conover & Francis 1973). The focus of our study was the assimilation of bacterial (particulate) radioactivity. However, we have utilized a 2-compartment
From our experiments, we have shown that *Mulinia lateralis* can clear and assimilate bacteria smaller than 1 μm. Assimilation efficiency estimated by using $^{35}$S-methionine in this study (93.34%) is higher than most previously reported values. Birkbeck & McHenery (1982) utilized several cultured bacteria as food and $^3$H and $^{14}$C as tracers in their studies with *Mytilus edulis* L. Assimilation estimations based on $^3$H tracers used in their study differed depending on the labeled bacterial macromolecule. When $^3$H-thymidine was used to label DNA in bacteria, AE ranged from 7% to 36%. When $^3$H-
diaminopimelic acid was used to label bacterial cell wall, an AE of 81% was reported. An efficiency of 84% was reported for 14C-labeled bacterial macromolecules. Birkbeck & McHenery (1982) were the first authors to demonstrate differential utilization of bacterial macromolecules in bivalves.

The tracer 14C-glucose was used by Harvey & Luoma (1984) in their studies on Macoma balthica (L.). Assimilation efficiency reported as 14C activity remaining in the bivalve’s foot after 14 d was ca 20%. Amouroux (1986) studied Venus verrucosa L. and reported 14C assimilation values between 39.9 and 51% during a 40 h period. Studying Crassostrea virginica (Gmelin) and using 14C and 15N as tracers, Crosby et al. (1990) reported AE values of 52.5 and 57.2% for bacterial carbon and nitrogen respectively.

Hollibaugh et al. (1980) were the first researchers to propose the use of 3H-thymidine as a tracer in bacteria-feeding studies. Using Donax gouldii Dall and Mytilus edulis as study organisms, they reported extremely high uptake values for both species after 1 h feeding. Average clearance rates for the 2 species used in their study were 4.6 ml h⁻¹ for D. gouldii and 6.5 ml h⁻¹ for M. edulis. Based on these clearance values D. gouldii incorporated 99.69% of cleared particulate radioactivity after 1 h. During the same time period, M. edulis incorporated ca 100% of cleared particulate radioactivity. These results suggest 100% retention of natural-sized bacteria. Later studies have, however, shown that M. edulis and related Donax spp. are not efficient at clearing natural-sized bacteria (Wright et al. 1982, Matthews et al. 1989). We attribute the high ‘assimilation’ values of Hollibaugh et al. (1980) to a possible underestimation of clearance rate. Methods for estimation of clam and mussel clearance rates were not given in this pioneering study.

Direct comparison of assimilation efficiencies reported in different studies is difficult. Generally, information is not given concerning the specific bacterial components labeled. Apart from the discrepancy in choice of tracers, assimilation values are reported after variable time periods ranging from 1 h to 14 d. An appropriate time period of several hours to allow for purging of unabsorbed food is necessary. Extremely long ‘chase’ periods, however, increase the chances of isotope recycling within the system (Conover & Francis 1973), especially in static systems.

The discrepancy in assimilation and excretion of radiolabel between isolopes in this study can be explained by the difference in labeled moieties. Tritiated thymidine used in this study is labeled at its methyl group at the C5 position of thymine. The tracers 35S-methionine and 3H-thymidine were chosen to selectively label bacterial proteins and DNA. Tritiated thymidine, however, has recently been shown to be less specific than originally proposed. Hollibaugh (1988) has demonstrated that some microbial communities incorporate up to 53% of [methyl-3H]-thymidine into protein. [Methyl-3H]-thymidine can be degraded rapidly within bacterial cells. The 3H on the methyl group may subsequently be transferred to other compounds and incorporated by de novo pathways into RNA, DNA and proteins (Moriarty 1986). In our studies, as much as 47.75 ± 5.32% of radioactivity associated with 3H was found in hot TCA precipitated macromolecules (non-DNA) after 1 h incubation. Because 3H-methyl can enter a general CH3 pool, interpretation of its production and excretion pathways is difficult.

CH3 has many functions in cellular metabolism (Zubay 1988), making 3H a non-specific label. The large portion of dissolved 3H may have included a variety of substances, in particular 3H2O, a product of cellular respiration. That this dissolved 3H radioactivity was not taken up by bacteria in earlier pulse-chase experiments suggests that it is not in a utilisable form for bacteria. Release of dissolved 3H from digestion of labeled bacteria was first noted by Birkbeck & McHenery (1982). These authors suggested that Mytilus edulis selectively utilized different bacterial polymers, rejecting DNA.

Methionine is classified as an essential amino acid in mammalian nutrition studies (Zubay 1988). The mineral nutrient sulfur can be absorbed either as part of amino acids or as inorganic sulfate with equal efficiency (Linder 1985). Sulfur is the site for attachment of CH3 has many functions in cellular metabolism (Zubay 1988), making 3H a non-specific label. The large portion of dissolved 3H may have included a variety of substances, in particular 3H2O, a product of cellular respiration. That this dissolved 3H radioactivity was not taken up by bacteria in earlier pulse-chase experiments suggests that it is not in a utilisable form for bacteria. Release of dissolved 3H from digestion of labeled bacteria was first noted by Birkbeck & McHenery (1982). These authors suggested that Mytilus edulis selectively utilized different bacterial polymers, rejecting DNA.

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Table 2. Studies investigating clearance and/or assimilation of attached or unattached planktonic bacteria and cultured bacteria by suspension-feeding bivalves. NR: not reported; NS: not studied; (+) positive result for clearance or assimilation; (-) negative result for clearance

<table>
<thead>
<tr>
<th>Species</th>
<th>Clearance</th>
<th>Assimilation</th>
<th>Bacterial source</th>
<th>Bacterial dimensions (µm)</th>
<th>Cells ml⁻¹</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Mytilus californianus</em></td>
<td>+</td>
<td>+ Growth</td>
<td>Cultured</td>
<td>0.8 x 1.0</td>
<td>2.0 x 10⁶</td>
<td>ZoBell &amp; Landon (1937)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.8 to 1.8-7.0 x 9.2</td>
<td></td>
<td>ZoBell &amp; Feltham (1938)</td>
</tr>
<tr>
<td><em>Donax gouldii</em></td>
<td>+</td>
<td>+</td>
<td>Natural</td>
<td>0.3 (coccis)</td>
<td>0.5-5.0 x 10⁶</td>
<td>Hollibaugh et al. (1980)</td>
</tr>
<tr>
<td><em>Mytilus edulis</em></td>
<td>+</td>
<td>+ [Methyl-³H] thymidine</td>
<td>Natural</td>
<td>0.5 x 2.0 (rods)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>+ Lysozyme-sensitive sp. [Methyl-³H] thymidine</td>
<td>Cultured</td>
<td>NR</td>
<td>0.5-1.0 x 10⁷</td>
<td>Birkbeck &amp; McHenery (1982)</td>
</tr>
<tr>
<td><em>Geukensia demissa</em></td>
<td>+</td>
<td>NS</td>
<td>Natural</td>
<td>0.2-1.0</td>
<td>4.2-5.2 x 10⁶</td>
<td>Wright et al. (1982)</td>
</tr>
<tr>
<td><em>Mytilus edulis</em></td>
<td>-</td>
<td></td>
<td>Natural</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Mya arenaria</em></td>
<td>-</td>
<td></td>
<td>Natural</td>
<td></td>
<td></td>
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</tr>
<tr>
<td><em>Venus verrucosa</em></td>
<td>+</td>
<td>+ ¹⁴C-L glutamic acid</td>
<td>Cultured</td>
<td>0.5 x 5.0</td>
<td>NR</td>
<td>Amouroux (1986)</td>
</tr>
<tr>
<td><em>Mytilus edulis</em></td>
<td>+</td>
<td>NS</td>
<td>Natural</td>
<td>0.513-0.524 mean spherical diameter: 1.38-3.43 x 10⁶</td>
<td>Lucas et al. (1987)</td>
<td></td>
</tr>
<tr>
<td><em>Donax serra</em></td>
<td>-</td>
<td>NS</td>
<td>Natural</td>
<td>0.25-1.15 mean spherical diameter: 1.17-6.14 x 10⁶</td>
<td>Matthews et al. (1989)</td>
<td></td>
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<tr>
<td><em>Mactra lilacea</em></td>
<td>-</td>
<td></td>
<td>Natural</td>
<td></td>
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<tr>
<td><em>Crassostrea virginica</em></td>
<td>+</td>
<td>+ [¹⁴C] glucose</td>
<td>Cultured</td>
<td>1-2</td>
<td>1.12-3.14 x 10⁶</td>
<td>Crosby et al. (1990)</td>
</tr>
<tr>
<td><em>Crassostrea virginica</em></td>
<td>+</td>
<td>+ [¹⁵N] ammonium sulfate</td>
<td>Natural</td>
<td>NR</td>
<td>5.58 x 10⁶</td>
<td>Langdon &amp; Newell (1990)</td>
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<tr>
<td><em>Geukensia demissa</em></td>
<td>+</td>
<td>+</td>
<td>Natural</td>
<td>NR</td>
<td>5.58 x 10⁶</td>
<td>Langdon &amp; Newell (1990)</td>
</tr>
<tr>
<td><em>Mulinia lateralis</em></td>
<td>+</td>
<td>+ [Methyl-³H] thymidine</td>
<td>Algal culture</td>
<td>&lt;1</td>
<td>4.0-8.5 x 10⁶</td>
<td>Present study</td>
</tr>
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*Macoma baltica* is a facultative deposit feeder (J. Lin pers. comm.)
Results from our study show that approximately half of the excreted 35S label was recovered in dissolved form. Dissolved 35S-labeled compounds were also found by Allen & Awapara (1960) and Allen & Garrett (1972) in their studies using 35S-methionine. Using 14C-glutamic acid as a tracer, Amouroux (1986) detected radioactivity associated with unidentified DOM in his studies on Venus verrucosa. Based on previous studies, the dissolved 35S could have resulted from bacterial degradation of fecal material, as suggested by Amouroux & Amouroux (1988), or from excretion of dissolved SO4\(^{2-}\) (Linder 1985, Allen & Garrett 1972). Activity from 35S caught on filters ("particulate") during later parts of the chase may have been incorporated into sulfonated mucopolysaccharides, a major component of molluscan mucus (Allen & Garrett 1972).

The ability of bivalves to effectively clear and assimilate bacteria-sized particles seems to be species-specific (Table 2). Comparative particle-retention studies in bivalves indicate that particles smaller than 2 to 4 μm are not cleared efficiently (Mohnenberg & Riisgård 1978, Riisgård 1988). These studies are, however, based on particle counts using electronic particle counters which cannot routinely detect particles <2 μm. Although clearance of larger particles may be high, clearance of nano- and picoplankton-sized particles may have been underestimated.

In this study, we have utilized 'natural'-sized bacteria to estimate \( AE \) in Mulinia lateralis. Many previous investigators assessing assimilation of marine bacteria by bivalves have relied on radiolabeled bacteria grown on enriched organic media (Birkbeck & McHenery 1982, Harvey & Luoma 1984, Amouroux 1986). Cultured marine bacteria are known to be much larger than natural forms (Lee & Fuhrman 1987). Therefore, use of large bacteria may overestimate the contribution of bacteria to bivalve nutrition.

To estimate bacterial contribution to bivalve nutrition, investigators should also use bacterial numbers commensurate with what their species of interest might encounter in natural systems. In early studies, ZoBell & Landon (1937) and ZoBell & Feltham (1938) demonstrated growth in Mytilus californianus Conrad using high concentrations of cultured bacteria (2.0 \( \times \) 10^8 to 5.0 \( \times \) 10^8 cells ml\(^{-1}\)). There have been no recent studies documenting growth in adult bivalves fed natural bacteria.

Clearance and assimilation of free-living bacteria by bivalves demonstrates the potential of these particles as sources of nutrition (Seiderer et al. 1984, Crosby et al. 1990, Langdon & Newell 1990). Bacterioplankton also play an important role in the mediation of nutrients from detritus. The role of bacteria in mediating the utilization of refractory carbon and nitrogen from detritus complexes has been documented by many authors (Stuart et al. 1982, Newell & Field 1983, Seiderer et al. 1984, Benner et al. 1988, Crosby et al. 1990). Compared with other planktonic components, natural planktonic bacteria have a lower C:N ratio (Nagata 1986, Lee & Fuhrman 1987). Nitrogen and crude protein content in bacteria can be as high as 12.5 and 78% respectively (Young & Scrimshaw 1975).

Although the contribution of bacterial carbon and nitrogen to bivalve nutrition has been well documented, little information exists on the contribution of specific bacterial macromolecules. The degradation of methionine and high retention of 35S found in this study suggest a potentially important role of bacteria as a source of mineral nutrients, as well as proteins, for Mulinia lateralis. Caution in isotope selection for use in bacteria-assimilation studies is recommended. The importance of bacteria as a food source is dependent upon both bacterial quantity and quality.

**Acknowledgements.** We thank Drs S. K. Allen and J. Scarpa for supplying specimens of Mulinia lateralis. We also thank Drs S. R. Fegley, R. G. Gustafson, J. N. Kraeuter, B. A. MacDonald and S. C. Wainright, and Mr W. J. Ganzonier, for critically reading the manuscript. Drs S. E. Ford and Ms H. L. Motto assisted in figure preparation. Comments from 2 anonymous reviewers helped improve the manuscript. K. Chalermwat is under scholarship from the Royal Thai Government. This is New Jersey Agricultural Experiment Station Publication no. D-32406-2-90 and Contribution no. 90-41 of the Institute of Marine and Coastal Sciences, Rutgers University, supported by New Jersey State funds and the Fisheries and Aquaculture Technology Extension Center.

**LITERATURE CITED**


Bishop, S. H., Ellis, L. L., Burcham, J. M. (1983). Amino acid...


This article was presented by S. Y. Newell, Sapelo Island, Georgia, USA

Manuscript first received: November 5, 1990
Revised version accepted: January 16, 1991