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Importance of refractory plant material to the carbon budget of the oyster *Crassostrea virginica*

M. P. Crosby [1] *, C. J. Langdon [2] and R. I. E. Newell [1]

[1] Horn Point Environmental Laboratories, Center for Environmental and Estuarine Studies, University of Maryland, P.O. Box 775, Cambridge, Maryland 21613, USA
[2] Hatfield Marine Science Center, Oregon State University, Newport, Oregon 97365, USA

Abstract

The ability of the oyster *Crassostrea virginica* (Gmelin) to filter, ingest and assimilate ^{14}C-labeled *Spartina alterniflora* as a carbon source was investigated under laboratory conditions. The oyster assimilated crude-fiber carbon extracted from *S. alterniflora* with an efficiency of approximately 3%. Enteric bacteria did not enhance this process. The annual average (April 1984 to November 1985) of crude fiber in the Choptank River sub-estuary of the Chesapeake Bay, Maryland, USA, from which the oysters were collected, was 15.7 $\mu g\,l^{-1}$ (range 4.3 to 34.3 $\mu g\,l^{-1}$). The potential food value of crude fiber to oysters in this system was estimated to be less than 1% of their carbon demand. However, the potential contribution of crude fiber to the carbon requirements of other oyster populations, such as those in southeastern USA, may be as great as 20%, due to higher crude-fiber concentrations in the seston.

Introduction

The relative importance of the non-living refractory cellulosic matrix detritus and associated living microheterotrophs to the nutrition of marine invertebrates is unknown. However, the potential value of the detrital complex as a food source for some estuarine organisms is well recognized (Darnell 1967, Frankenberg and Smith 1967). Newell (1965) concluded that the bivalve *Macoma balthica* ingested detrital complexes but digested only the associated microbes. Other studies lent credence to the view that consumers of detrital complexes assimilate the microbial communities associated with the complexes rather than the non-living particulate organic material (NL-POM) [e.g. the polychaetes *Cirriformia tentaculata* (George 1964) and *Capitella capitata* (Tenore 1977); crusteans (Johannes and Satomi 1966); amphipods (Fenchel 1970)]. Birkbeck and McHenery (1982) showed that the mussel *Mytilus edulis* is capable of selectively degrading lysozyme-sensitive bacteria and absorbing certain bacterial compounds (i.e., diaminopimelic acid and glucose). The gastropod *Nucula annulata* digests bacteria attached to the surface of particles with an efficiency of 72% (Lopez and Cheng 1983). Due to the high efficiency of conversion of nitrogen by bacteria, Newell and Field (1983) proposed that bacteria may contribute as much as 73% of the nitrogen requirements, but only 9% of the carbon requirements of the filter-feeding community of a kelp-bed system.

Newell and Field (1983) proposed that filter-feeding communities of kelp-bed ecosystems in South Africa may utilize large amounts of carbon directly from NL-POM of fragmented kelp. Similarly, Findley and Tenore (1982) reported that *Capitella capitata* derived a major portion of its nitrogen from NL-POM of the red seaweed *Gracilaria foliifera*. Wetzel (1977) reported that the structural carbohydrate fraction of the marsh grass *Spartina alterniflora* was not directly assimilable by the mud snail *Nassarius obsoletus*, while Kirby-Smith (1976) reported that the bivalve *Argopecten irradians* could not derive any nutrition from detritus derived from *S. alterniflora*. Kelp is a submerged non-vascular plant, which lacks the types of structural compounds predominant in vascular plants such as *S. alterniflora*. It may be that vascular plants contain a high percentage of unavailable energy (Tenore 1983) that can only be efficiently utilized after microbiological decomposition and enrichment. However, equivocal results have been obtained from studies that have utilized *S. alterniflora* as the detrital matrix. Crosby (1985) found that the grass shrimp *Palaemonetes pugio* utilized NL-POM derived from *S. alterniflora*.

A dominant suspension-feeding bivalve in estuaries of the east coast of the USA is the oyster *Crassostrea virginica*. The algal portion of seston is important in the diet and

* Present address: Belle W. Baruch Institute for Marine Biology and Coastal Research, Baruch Marine Field Laboratory, University of South Carolina, P.O. Box 1630, Georgetown, South Carolina 29442, USA

energetics of this oyster (Savage 1925, Galtsoff 1964, Jørgensen 1966, Owen 1966, Walne 1970, Mathers 1972, Webb and Chu 1982). The relationship between the detrital component of seston and oyster nutrition is poorly understood, although detritus may be the dominant constituent of the particulate organic matter (POM) of seston. Non-phytoplankton POM contributes 69% (Stuart 1982) to 80% (Kirby-Smith 1970) of total POM, and "detritus" in the Chesapeake Bay constitutes an average of 77% of total seston particle numbers (Van Valkenburg et al. 1978). The crystalline styles of *C. virginica* and the mussel *Geukensia demissa* contain amylases and cellulases capable of releasing reducing sugars from aged marsh grass, *Juncus roemerianus* and *Spartina alterniflora* (Lucas and Newell 1984). Based on enzymatic rates of release of carbohydrates, Lucas and Newell suggested that up to 40% of the carbon-absorption requirements may be met by utilization of detritus from *J. roemerianus* and *S. alterniflora*. Although such an enzymatic approach to the study of cellulose digestion can demonstrate the presence or absence of a particular enzyme, it is difficult to assess the quantitative importance of the activity of such enzymes to the oysters' digestive process and nutrition. In addition, it is often technically difficult to determine if the complete suite of cellulases is present (Newell and Langdon 1986). More realistic determinations of the ability of bivalves to digest refractory cellulosic material and the quantitative importance of this source of carbon to the oyster's carbon requirements can only come from feeding studies utilizing tracer techniques.

This study was designed to compare the ability of *Crassostrea virginica*, filter-feeding in a normal manner, to utilize ^{14}C-labeled *Spartina alterniflora* under non-antibiotic (sterile water) vs antibiotic (sterile water and antibiotically treated oysters) conditions. The seasonal variability in estuarine seston concentrations was examined to ascertain the ecological importance of the refractory component of seston to oyster nutritional requirements. Biochemical assays of the seston also yield more precise data on the concentration of refractory NL-POM than assays based on microscopic techniques and the use of a Coulter Counter (Van Valkenburg et al. 1978).

Materials and methods

Feeding experiments

Juvenile *Crassostrea virginica* (Gmelin) (shell length 1.5 to 2.5 cm) were collected in summer 1985 from the Choptank River sub-estuary of the Chesapeake Bay (76°8′W; 38°44′N). The oysters were maintained in a flow-through system receiving ambient river water (13 to 15‰S, 16° to 19 °C). Just prior to the experiments (fall, 1985), the oysters were briefly soaked in a 1% commercial bleach (Clorox) solution in distilled water and the shells thoroughly brushed to remove all epiphytic organisms. The oysters were then maintained at 22 °C for 2 wk in a 10-liter flow-through aquarium, with the water recirculated through a large aquarium (1 100 liters) and a subsurface gravel-bed filter (15‰S). The alga *Isochrysis* aff. *galbana* (Clone T-ISO) and refractory cellulosic material were continuously pumped into the 10-liter aquarium (final concentrations, $\sim 5 \times 10^7$ cells and ~ 15 mg l^{-1}, respectively) as a food source. Respiration rates (ml O_2 h^{-1}) were determined for each individual, using a radiometer oxygen electrode system (Bayne et al. 1977). The rates of oxygen consumption were converted to weight-specific values (ml O_2 mg^{-1} dry tissue wt h^{-1}) using the allometric relationship (Bayne and Newell 1983) with a weight exponent of 0.72 (Newell, unpublished data). These experimental oysters were then placed in the feeding chambers (Fig. 1) for 24 h to acclimatize prior to the addition of ^{14}C-labeled refractory cellulosic material. This material (< 20 μm) was prepared by chemically extracting the labile components from *Spartina alterniflora* that had been grown in a $^{14}CO_2$ atmosphere [see Newell and Langdon (1986) for further details]. During the acclimation period the oysters were fed *I. galbana*. Two separate experimental treatments were employed. The first will be referred to as "non-antibiotic" (15‰S filtered at 0.2 μm then autoclaved at 121 °C and 15 psi for 15 min) and the second as "antibiotic" [rifampicin (5 ppm) and chloramphenicol (5 ppm) added to similarly prepared water]. These antibiotics have no adverse affect on oyster feeding or growth at these concentrations (Langdon and Bolton 1984, Langdon and Siegfried 1984). In both treatments, the prepared ^{14}C-*S. alterniflora* (3 mg l^{-1}) with kaolin (3 mg l^{-1}) as an inorganic supplement was fed to the oysters for 36 h. The suspensions were introduced to the feeding chambers via peristaltic pumps (Fig. 1).

Carbon budgets for *Crassostrea virginica* fed on ^{14}C-labeled *Spartina alterniflora* detritus were calculated using a specific activity (determined in this study) of 5.126×10^5 disintegrations per minute (dis/min) mg^{-1} extracted *S. alterniflora*. Since this extracted *S. alterniflora* is predominantly (> 85%) cellulose (Newell and Langdon 1986), 1 mg *S. alterniflora* equals 0.444 mg C and 8.66×10^{-7} mg C dis/min. Total ^{14}C ingested was calculated by Eq (1):

$$I = F + WB + R \quad , \tag{1}$$

where I = total ingested ^{14}C oyster^{-1}, F = total fecal ^{14}C oyster^{-1}, WB = total ^{14}C in whole-oyster tissue, and R = total $^{14}CO_2$ produced oyster^{-1}.

Feces (F) were collected from each individual over the course of the experiment. The crude fiber was extracted from each sample. To the final pellet, 1 ml of Soluene-350 (New England Nuclear) tissue-solubilizer was added, the slurry was incubated for 18 h at 55 °C, added to 15 ml AQUASOL II (New England Nuclear), and counted on a Packard (Model 460C) scintillation counter. For all liquid scintillation counting, an external standard mode with 10 min counts was used as well as internal standards to establish quench curves. To obtain corrected dis/min, whole-body assays were conducted using individual oyster tissue homogenized (Brinkman tissue homogenizer) in sterile 15‰S water. Two 500 μl aliquots were taken for weight measurements (mg dry wt at 70 °C) and a 1 ml aliquot for whole-body radioactive burden. A correction for undigested

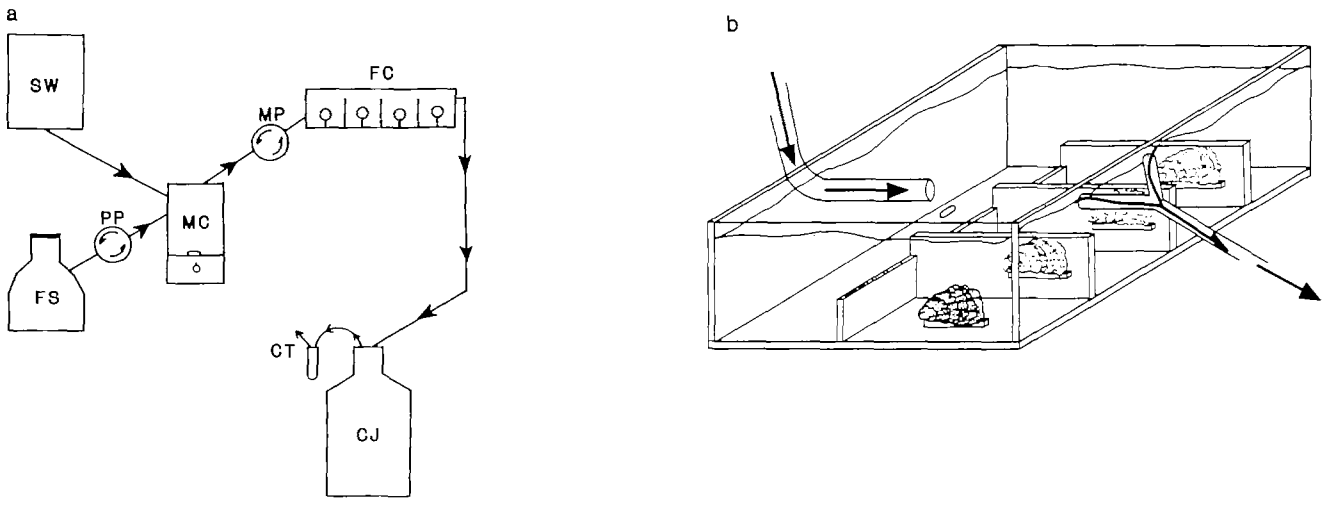

Fig. 1. Feeding apparatus (a) consisting of four feeding chambers (FC) each of 1-liter capacity; three of these each contained four oysters affixed atop fecal separators which facilitated separate collection of pseudofeces and feces; flow rate through each chamber was adjusted to 1 liter h^{-1} via a Brinkman MCP 2500 microprocessor pump (MP); inflow line to each chamber originated from a single premixing chamber (MC), the purpose of which was to mix inflowing seawater (SW) with food stock (FS) prior to its presentation to the oysters (*Crassostrea virginica*); food stock was fed into the microprocessor pump via a peristaltic pump (PP) while seawater flowed via gravity; all outflow water from each feeding chamber was collected in individual carboys (CJ) with outgoing air passing through CO_2 traps (CT). (b) Inflow lines (arrows) were located at bottom of each feeding chamber adjacent to a microstirbar to mix the chamber water; outflow lines were located at top of water line on opposite side of the feeding chamber from the inflow

^{14}C-*Spartina alterniflora* still within the oyster was made by determining the crude fiber (Newell and Langdon 1986) in 1 ml tissue aliquots of the remaining homogenate. The activity due to crude fiber was subtracted from the non-extracted tissue activity to yield corrected body burdens.

Analyses for $^{14}CO_2$ production involved the collection of all outflow water from each chamber into individual 20-liter carboys, and was based on techniques described by Lampert and Gabriel (1984). The CO_2 was maintained in solution in the carboys by raising the pH to ≥ 9 with the addition of 2.4 g NaOH. Bacterial respiration was inhibited by the addition of 200 ml formalin that gave a final concentration of 1% in full carboys. As the carboys were filling, the outgoing air passed through a CO_2-stripper filled with 25% NaOH. In addition, a vial with 5 ml 25% NaOH and Whatman No. 1 filter-paper wicks was hung inside each carboy. At the end of the 36 h feeding period, triplicate samples (3 ml each) were taken from each carboy, filtered (0.2 μm) and the filter (+10 ml Filtercount) and filtrate (+750 μl Carbosorb +15 ml Aquasol II) counted as previously described. The carboys were then acidified (pH < 1.5) by the addition of 60 ml 11.6 *N* HCl and bubbled with N_2 for 24 h. Preliminary experiments demonstrated that this acidification and stripping procedure was 100% effective in removing $^{14}CO_2$. The total radioactivity for each carboy was calculated by subtracting the post-acidification dis/min from the pre-acidification dis/min, and multipling by the volume of the carboy. In addition to measuring the carboy $^{14}CO_2$, in the initial experiments the feeding chambers were sealed and any $^{14}CO_2$ lost from the water to the atmosphere was collected by CO_2 traps inside the individual chambers. Less than 0.3% of the total $^{14}CO_2$ was released to the atmosphere. Thus, the chamber CO_2 traps were not used in subsequent experiments.

Bacterial assay

To ascertain the effect of the antibiotic treatment in reducing bacterial populations and activity, [^{3}H]TdR (^{3}H-thymidine)-uptake (Fuhrmann and Azam 1980, 1982) measurements were conducted for both non-antibiotic and antibiotic-treated whole-oyster tissue homogenates. Bacterial cell numbers in the homogenates were determined using acridine orange direct-counts (Hobbie et al. 1977). Incorporation of [^{3}H]TdR into cold TCA-insoluble material was determined with the specific techniques described by Crosby and Peele (1987).

Seston analysis

The seston was sampled at two primary sites (one in Tred Avon River, the other in Broad Creek) in the Choptank sub-estuary of the Chesapeake Bay, Maryland, on two consecutive days (one day at each site) at approximately monthly intervals (April 1984 to November 1985). A submersible pump was used to draw water from 1 m above the oyster bar, three times daily. On each sampling date, water was also collected from 1 m above the bottom at the upper reaches and mouths of each system, Approximately two weeks following the primary monthly sampling, secondary water samples were collected from 1 m above the two primary oyster-bar sites and the upper reaches and mouth of each system. All water samples were pre-filtered through 130 μm Nitrex screening. Filters for each time period and site from a given sample were individually assayed and a mean value determined. Seston quantity was determined by vacuum-filtering (< 200 mm Hg) known volumes (approximately 300 to 750 ml) of each water sample through duplicate heat-treated (450 °C, 20 min) and weighed 55 mm Whatman GF/

Table 1. *Crassostrea virginica.* Assimilation efficiencies (mg C mg^{-1} tissue dry wt) of oysters fed ^{14}C-*Spartina alterniflora* cellulosic material. Antibiotic-treated oysters were exposed for 24 h prior to feeding, and during the experiment, to 5 mg l^{-1} of the antibiotics rifampicin and chloramphenicol. All oysters were held for 24 h after feeding to allow depuration of ingested ^{14}C material. WB: whole body. —: no ^{14}C above background level recorded

Oyster No.	Tissue wt (mg)	Carbon voided in biodeposits (F)	Carbon present in oyster tissue (WB)	^{14}C respired (R)	Assimilation efficiency $=(WB+R)/(F+WB+R)$ (%)
Oysters not treated with antibiotics					
1	33.3	8.54×10^{-2}	1.02×10^{-3}	–	1.2
2	60	5.79×10^{-2}	1.44×10^{-3}	–	2.4
3	50	1.54×10^{-2}	5.59×10^{-4}	–	3.5
4	60	3.87×10^{-2}	1.50×10^{-3}	–	3.7
5	50	4.25×10^{-2}	1.86×10^{-3}	–	4.2
6	40	1.47×10^{-1}	4.66×10^{-3}	–	3.1
7	63.3	6.29×10^{-2}	1.41×10^{-4}	–	0.2
8	36.7	6.42×10^{-2}	2.96×10^{-3}	–	4.4
9	30	5.62×10^{-2}	4.17×10^{-3}	–	6.9
10	40	1.51×10^{-2}	8.37×10^{-5}	–	0.6
x		5.85×10^{-2}	1.84×10^{-3}		3.02
(SE)		(1.20×10^{-2})	(5.06×10^{-4})		
Oyster treated with antibiotics					
1	64	1.62×10^{-3}	7.98×10^{-5}	–	4.4
2	36	5.51×10^{-3}	9.60×10^{-5}	–	1.7
3	42	2.62×10^{-3}	7.97×10^{-5}	–	3.0
4	20	5.44×10^{-3}	4.74×10^{-5}	–	0.9
5	22.5	1.80×10^{-2}	6.74×10^{-4}	–	3.6
6	37	3.00×10^{-3}	6.57×10^{-5}	–	2.2
7	50.5	6.39×10^{-3}	1.60×10^{-4}	–	2.4
8	33.5	1.03×10^{-2}	5.27×10^{-5}	–	0.5
x		6.61×10^{-3}	1.57×10^{-4}		2.34
(SE)		(1.90×10^{-3})	(7.50×10^{-5})		
Grand mean					2.72
(SE)					(0.41)

C filters. Each filter was then rinsed with isotonic ammonium formate to remove salts, wrapped in aluminium foil, and frozen at −25°C until analyzed. Seston analyses consisted of weighing the dried (80°C, 24 h) filters to obtain total mg seston l^{-1} followed by ashing (450°C, 3 h) and weighing the filters to obtain mg organic seston l^{-1} by subtraction.

Biochemical analyses

Biochemical analyses for carbohydrate were determined by vacuum-filtering known volumes of all bottom-water samples through heat-treated 55 mm Whatman GF/C filters, as previously described. Filters were homogenized and analyzed for total carbohydrate using the phenol-sulfuric acid method outlined by Dubois et al. (1956). Crude-fiber concentrations were ascertained using the above total carbohydrate methods on samples that had been pre-treated with acid (0.9% v:v H_2SO_4) and alkali (1.25% w:v NaOH) (Strickland and Parsons 1972).

Results

Feeding experiments

Because production of $^{14}CO_2$ from the feeding chambers was not significantly different from that for the empty control chambers, Eq. (1) was reduced to:

$$I = F + WB \quad . \tag{2}$$

Data for fecal (F) and whole-body (WB) ^{14}C, as well as assimilation efficiencies (AE) for individual *Crassostrea virginica* are presented in Table 1. The mean arcsine-transformed AE between the feeding chambers of the treatments and between the treatments were not significantly different (ANOVA). The mean AE of the two treatments was 2.7%. Correlations between individual oyster weights and AE were not significantly different from zero. Although no significant difference in AE was found between the two treatments, the ^{14}C fecal production and incorporation into tissue in the oysters treated with antibiotics was an order of magnitude less than in non-treated oysters (Table 1). The rates of oxygen consumption at 25°C for the oysters treated with antibiotics (mean $= 17.9 \pm 1.83\times10^{-3}$ ml O_2 mg^{-1} tissue dry wt h^{-1}) were significantly higher (ANOVA; $p<0.001$) than the rates (mean $= 7.06 \pm 0.82\times10^{-3}$ ml O_2 mg^{-1} h^{-1}) for the non-treated oysters.

Bacterial assay

Results of acridine orange direct-counts of tissue homogenate are presented in Table 2. The mean number of bacte-

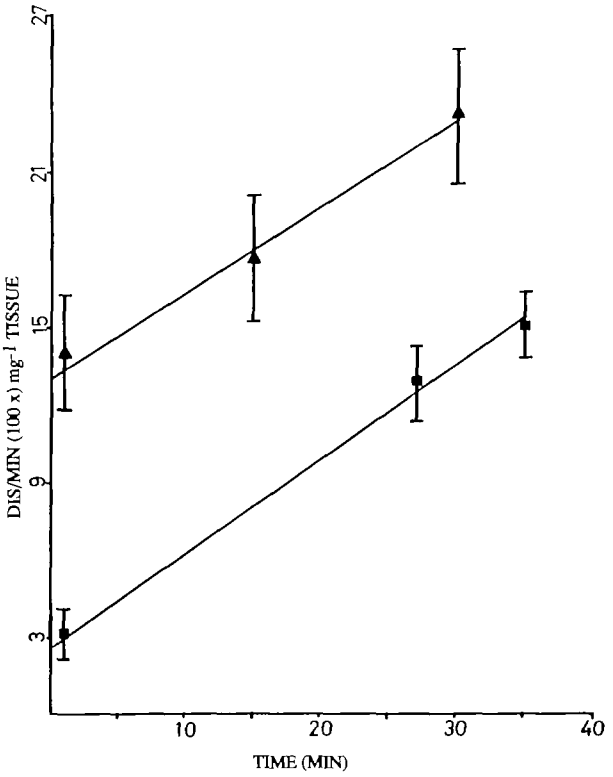

Fig. 2. *Crassostrea virginica.* Regression lines for [^{3}H]TdR incorporation mg^{-1} dry wt of oyster tissue during short-term incubation assays of non-treated tissue, where dis/min = 1340 + 31.4 (min), $r = 0.6$, $p = 0.014$ (▲), and antibiotically treated tissue, where dis/min = 286 + 35.5 (min), $r = 0.9$, $p < 0.001$ (■)

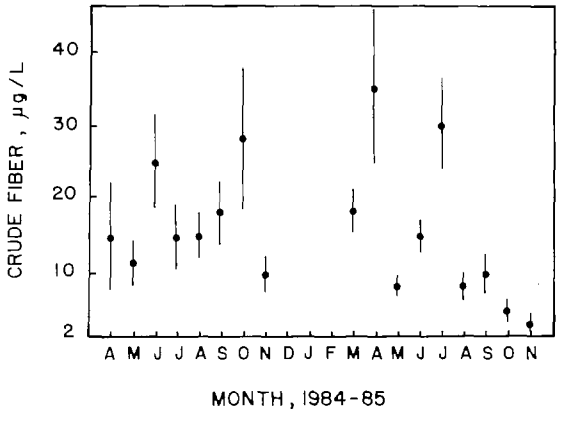

Fig. 3. Mean (±SE) crude-fiber concentration ($\mu g\, l^{-1}$) in seston samples plotted against time for consecutive months from April 1984–November 1985

ria cells mg^{-1} dry wt oyster tissue was significantly (Student's t-test, $p < 0.001$) lower (over 80% reduction) in homogenates treated with antibiotics than in controls. Incorporation of [^{3}H]TdR was linear (Fig. 2) during the short-term (< 35 min) experiments for untreated samples ($r = 0.582$; $p = 0.014$) and samples treated ($r = 0.9$; $p < 0.001$) with antibiotics. Slopes of the two regression lines were tested for significance and for parallelism, with the rates of [^{3}H]TdR incorporation not found to be significantly different (Crosby and Peele 1987). The intercepts were significantly ($p < 0.001$) hetergeneous, indicating that the antibiotic treatment reduced both bacterial numbers and overall radioactivity in the tissue homogenates.

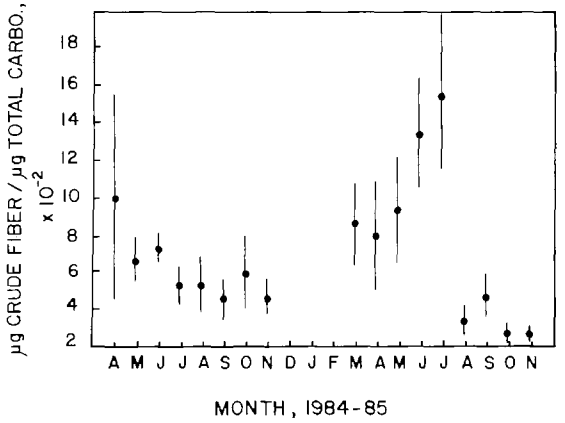

Fig. 4. Crude fiber as a proportion of total carbohydrate (μg crude fiber μg^{-1} total carbohydrate) in seston samples plotted against time for consecutive months from April 1984–November 1985. Error bars represent one standard error about means

Table 2. *Crassostrea virginica.* Results of acridine orange direct-counts of bacterial numbers in tissue homogenates (mean no. bacteria cells mg^{-1} dry wt). Control oyster tissue was pooled sample of three tissue homogenates from oysters not treated with antibiotics. Individuals Nos. 1–3 were three oysters that had been treated with antibiotics. n: number of fields counted in two replicates

Oyster No.	Means	(SE)	n
Control	1.26×10^7	(7.07×10^5)	20
1	2.34×10^6	(1.71×10^5)	20
2	2.26×10^6	(1.68×10^5)	20
3	2.66×10^6	(2.26×10^5)	20
x (1+2+3)	2.42×10^6	(1.22×10^5)	3

Seston assay

Crude-fiber concentrations in the seston were significantly correlated with particular organic matter ($p = 0.012$), total carbohydrate ($p < 0.001$), total carbon ($p < 0.001$), and total nitrogen ($p < 0.001$). However, correlations of crude fiber with month, and weight of seston were not significant. A large pulse of crude fiber was evident during the fall of 1984 and spring of 1985 (Fig. 3). Values ranged from 4.3 to 34.3 μg crude fiber l^{-1}, with a mean of 15.7 μg for the two years. The proportion of the total carbohydrate composed of crude fiber ($\mu g\, \mu g^{-1}$) is plotted against time in Fig. 4. The highest level occurred in the spring for 1984, and in the summer for 1985. The percentages ranged from ca. 3 to 16%, with an average for the two years of 7.5%. Crude fiber never exceeded 1% of POM during the two years, with a mean value of 0.4% (Fig. 5).

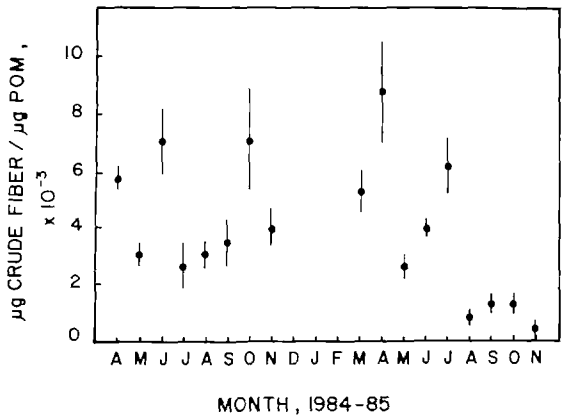

Fig. 5. Crude fiber as a proportion of particulate organic matter (μg crude fiber μg^{-1}POM) in seston samples plotted against time for consecutive months from April 1984–November 1985. Error bars represent one standard error about means

Discussion

If an oyster's ability to assimilate ^{14}C-cellulosic detritus is enhanced by bacteria in the digestive system, a significant reduction of these bacteria would be expected to be followed by a significant reduction in the oysters' assimilation efficiency. However, our study demonstrated no statistically significant reduction in the efficiency of utilization of ^{14}C-*Spartina alterniflora* by *Crassostrea virginica* when bacterial populations were reduced (Table 1). Lucas and Newell (1984) and Brock et al. (1986) reported the presence of cellulase in style preparations of *C. virginica*, although no indication was given as to whether the enzyme was endogenously produced. Crosby and Reid (1971) reported that the gut microflora of bivalves played a significant role in the extracellular digestion of cellulose, in combination with cellulases secreted by the bivalve digestive diverticula. The relative contributions of exogenous versus endogenous sources of cellulolytic enzymes were not determined. Payne et al. (1972) found cellulase in the clam *Scorbicularia plana*, but no evidence of the cellulolytic bacteria in the clam's gut. Conversely, Fong and Mann (1980) demonstrated that the gut bacteria of the sea urchin *Strongylocentrotus droebrachiensis* aided in the digestion of cellulose. Mayasich and Smucker (1986) reported cellobiase, maltase, and chitobiase activities in crystalline style and digestive diverticula extracts from *C. virginica* that had been rendered aseptic by treatment with toluene. Newell and Langdon (1986) observed no difference in cellulolytic activities of gut fluids from *C. virginica* treated with antibiotics versus those of untreated oysters. In addition, the cellulolytic activity of crystalline styles was not altered when *in vitro* incubations were made with bacterial activity controlled by the addition of thimersol and toluene. The feeding studies we employed confirm the potentials described by the above enzymatic reports. However, although our results do indicate that oysters can produce endogenous cellulolytic enzymes, the enzymatic activity may be relatively low. Our pretreatment of feeding the oysters a combination of algae and refractory cellulosic material ensured that they had an adequate source of nutrition. In addition, the cellulose would stimulate the natural levels of any inducible cellulase enzymes. Thus, the ability of oysters to utilize the cellulosic material in our experiments would not have been compromised by them being in a starved and stressed physiological condition nor being presented with a food item to which they had not been exposed.

Much of the work dealing with cellulase activities in invertebrates has involved the breakdown of dissolved substrates [for review see Brock et al. (1986), Newell and Langdon (1986)] and particulate substrates such as cellulose filterpaper (Payne et al. 1972) and cotton (Koopmans 1970). Carbohydrases necessary to digest detrital material (as determined by enzymatic studies) have been reported for *Choromytilus meridionalis* and *Perna perna* (Seiderer et al. 1982), *Crassostrea virginica* and *Geukensia demissa* (Lucas and Newell 1984), and other bivalves (Brock et al. 1986). Although an enzymatic approach can demonstrate the presence or absence of a particular enzyme, it is difficult to assess the quantitative importance of such enzyme to the bivalve's nutrition. In order for refractory plant cellulose to be directly and completely utilized as a source of carbon by *C. virginica*, it must possess a suite of three cellulose enzymes: C_1 cellulase, β1,4 glucanase; and β-glucosidase (Newell and Langdon 1986). Using crystalline style extracts of *C. virginica*, Newell and Langdon were unable to detect C_1 cellulase capable of degrading crystalline cellulose, nor β-glucosidase activities (using cotton fiber and cellobiose as substrates, respectively). They suggested that the principal function of style cellulase activity is the partial depolymerization of refractory amorphous cellulose, perhaps aiding in the digestion of algal cells and detritus. This does not rule out the possibility of endogenous cellulolytic enzyme production elsewhere within the oyster.

Stuart et al. (1982) reported an assimilation efficiency of 50% for *Aulacomya ater* fed on freshly ground kelp particles. Seiderer and Newell (1985) calculated, on the basis of ecological energetics, that the bivalve *Choromytilus meridionalis* actively digested kelp detritus. In our experiments, an assimilation efficiency of only 2.7% was found for refractory particles of *Spartina alterniflora*. This would help explain the results of Kirby-Smith (1976) and Williams (1981) who attempted, unsuccessfully, to grow the scallop *Argopectin irradians* and *Mytilus edulis*, respectively, on detritus derived from marsh grasses. The difference between reports may be due to biochemical differences between the two types of substrate. Factors such as particle size and "age" may vary in importance depending on the source of the NL-POM (Tenore et al. 1982, Tenore 1983). Stuart (1982) reported that the scope for growth in *Aulacomya ater* decreased with age of kelp detritus, which was a function of declining energy content of food with age. Stuart concluded that filter-feeders in the natural environment obtain maximum benefit from particles in the earlier stages of decomposition, before most of the nutrient and energy-rich compounds (such as low molecular weight carbohydrates and alginates) are utilized by microheterotrophs. Kelp are submerged, nonvascular plants, which lack the cellulosic types of structural com-

Table 3. *Crassostrea virginica.* Estimation of importance of non-living particulate organic material (NL-POM) in Choptank River to carbon demand of 100 mg wt oyster. AE: assimilation efficiency

Oxygen consumption (R) at 25 °C	$=3.711 \times 10^{-1}$ ml O_2 h^{-1} $=1.978 \times 10^{-1}$ mg C h^{-1}	(measured in this study) (Lucas and Newell 1984 for conversion)
Total carbon required at 25 °C	$=1.333$ (R) $=1.333$ (1.978×10^{-1} mg C h^{-1}) $=2.636 \times 10^{-1}$ mg C h^{-1}	(Bayne and Newell 1983)
Assume: total carbon required	$=263.6$ μg C h^{-1}	
AE for NL-POM	$=3\%$	
total NL-POM	$=34.3$ μg l^{-1}	
filtration rate	$=2$ liters h^{-1}	
crude fiber	$=44.4\%$ C	
Oyster is 100% efficient at filtering NL-POM		
Then: carbon absorbed from NL-POM	$=(34.3$ μg C $l^{-1})$ (0.444) (0.03) (2 liters h^{-1}) $=0.91$ μg C h^{-1}	
This represents $<1\%$ of the oysters' carbon demand		

pounds predominant in vascular plants. It may be that because vascular plants contain a high percentage of unavailable energy in the form of refractory structural carbohydrates, they can only be utilized after microbial decomposition and energy enrichment. A greater amount of refractory, highly complex, structural material occurs in aerial (e.g. *Spartina* sp.) versus aquatic (e.g. *Thalassia* sp.) plant species due to the buoyancy support given to the aquatic plant species by the surrounding water. Tenore (1983) has proposed that the nutritional quality of NL-POM is a function not only of nitrogen enrichment, but also available energy content (which he defined as that portion of total energy content hydrolyzed by 1 *N* HCl for 6 h at 20 °C). The growth of *Capitella capitata* fed low-nitrogen diet rations of seaweed detrital complexes is best correlated with nitrogen content, while growth of worms fed vascular plant detrital complexes is best correlated with available calories (Tenore 1981). Tenore (1981) showed that most of the detritus derived from seaweeds has a high energy availability compared to that from marshgrasses and seagrasses.

The assimilation efficiencies for the oysters untreated and treated with antibiotics did not differ statistically (Table 1). No $^{14}CO_2$ was respired by oysters under either treatment. It is likely that had the oysters been fed for a longer period of time ^{14}C from the labeled substrate might have become a larger component of the oysters' metabolic pool and would have been respired at a measurable rate. The decrease in total ^{14}C ingested by oysters treated with antibiotics compared with the controls was statistically significant (ANOVA; $p<0.01$). This suggests that the ability of oysters treated with antibiotics to ingest the cellulosic particles was reduced, even though both groups of oysters were acclimated for 14 d in the laboratory to uniform salinity, temperature and food regimes. This difference in ingested ration is unlikely to be due to an inhibitory effect of the antibiotics on the oyster's metabolism, because the metabolic rate of the antibiotic-treated oysters, as measured by rate of oxygen consumption, was significantly higher than that of the non-antibiotic treated oysters. Other studies (e.g. Langdon and Bolton 1984, Langdon and Siegfried 1984) have found no significant deleterious effects of the antibiotics rifampicin and chloramphenicol on the filtration and feeding processes of *Crassostrea virginica*. Our data did demonstrate that although both bacterial cell numbers and overall bacterial metabolic activity decreased significantly in *C. virginica* treated with antibiotics, their assimilation efficiencies for cellulosic carbon did not differ significantly from controls. The interpretation of our observations is based on the efficiency with which the oyster can assimilate carbon from the ingested ration and is independent of the absolute amounts of cellulosic carbon ingested. This approach to ascertaining the importance of enteric bacteria to the ability of the host organism gaining nutrition from organic matter has been used successfully by Fong and Mann (1980) with the sea urchin *Strongylocentrotus droebachiensis*, and Wainwright and Mann (1982) with the mysid *Mysis stenolepis*.

We calculated that oyster populations in the Choptank River satisfy $<1\%$ of their carbon demand through assimilation of NL-POM (Table 3). If one assumes the composition of extracted *Spartina alterniflora* used in our study to be essentially the same as cellulose (44.4% C by weight), then the minimum required ration of refractory NL-POM needed to meet the carbon demand of a 100 mg dry wt oyster would be (263.6 μg C $h^{-1}/0.03)/0.44$ μg C μg^{-1} NL-POM or 19.79 mg NL-POM h^{-1}. These oysters, having a filtration rate of ~ 2 liters h^{-1}, would be required to be in an environment having ~ 10 mg NL-POM l^{-1} in order to satisfy their minimum carbon demand. The highest monthly mean crude-fiber concentration in the seston was 34.3 $\mu g\, l^{-1}$, while the greatest value for a single sample was 131.1 $\mu g\, l^{-1}$. It is apparent, then, that in the Choptank River, oysters can obtain less than 1% of their carbon demand from the direct utilization of crude fiber in seston (Fig. 3). Further evidence of the lack of importance of crude fiber in the Choptank River to oyster nutrition is evident in its low percent occurrence in both total carbohydrate and POM in the seston (Figs. 4 and 5). This does not imply that NL-POM may not significantly contribute to oysters' nutritional re-

quirements in other ecosystems. In the salt-marsh systems of the southeastern USA, inputs of refractory carbohydrate to the water column would be much greater than for the Choptank sub-estuary and, therefore, form a larger portion of the oysters' ingested ration. Using data from Dame et al. (1986) for the North Inlet salt marsh, South Carolina, we calculated an estimate for NL-POM C in these salt-marsh tidal creeks (Table 4). Our calculated value of 899 μg NL-POM C l^{-1} cannot be directly compared with refractory crude fiber. Due to the manner in which it was calculated, the North Inlet value will include less refractory material (i.e., fecal pellets, etc.). However, the calculated value does illustrate a great increase in NL-POM compared to that found in estuaries not dominated by *Spartina* sp. marshes.

To date, few of the numerous studies of salt-marsh and estuarine systems have analyzed the seston for crude fiber. Heinle and Flemer (1976) presented some information on detrital fluxes in a small, low-salinity marsh in the Patuxent River of the Chesapeake Bay. Crude-fiber values from their work, values for one year from a salt marsh in Delaware (Kreeger et al. 1988), and values obtained in the Choptank River during this study, are presented in Table 5. Much greater concentrations of crude fiber than those for the Choptank River are evident in the marsh systems. These marshes are orders of magnitude smaller in size and production than more expansive *Spartina* sp. marshes found in the southeast USA (Heinle and Flemer 1976). In addition, many of the marshes south of the Chesapeake Bay region have greater tidal amplitude, resulting in the populations of oysters being largely intertidal. At low tide, the exposed oysters close their shells and stop feeding, resulting in food material remaining in their guts until reimmersion at the rising tide when feeding begins again. The intertidal oyster has an increased time for enzymatic digestive action to occur on ingested crude fiber, which may result in increased efficiency of utilization of that substrate. This would be dependent on the oyster's ability to carry out aerial digestion and absorption. It is possible that in large intertidal salt marshes, where crude-fiber concentrations are high and gut residence-times in oysters longer, contributions of NL-POM to oyster energetics may be quite significant. Based on the NL-POM levels calculated (Table 4) to exist in North Inlet tidal creeks, we can estimate (Table 6) that North Inlet oyster populations may be able to meet up 20% of their total carbon requirements through the absorption of NL-POM C. Peterson et al. (1985, 1986), using multiple stable isotopes, reported that *Geukensia demissa* had carbon, nitrogen, and sulfur compositions which indicated that 80% of their diet originated from *S. alterniflora* in the interior of the Great Sippewissett marsh. It could not be determined if the detritus was utilized directly or via a microbial conduit. The importance of bacteria and the total detrital complex to oyster energetics

Table 4. Computations for determining estimates of detrital concentrations in North Inlet salt-marsh tidal creeks, South Carolina. Values utilized in computations are from Dame et al. (1986) and are based on efflux from North Inlet per year. POC: particulate organic carbon; ATP-C: estimate of bacterial carbon

Total water discharge $= 27.0 \times 10^8$ m^{-3}
Total POC efflux $= 3.0 \times 10^6$ kg C
Total ATP-C efflux $= 5.3 \times 10^5$ kg C
Total phytoplankton $= 4.2 \times 10^4$ kg C

Thus:

$$\text{NL-POM C} = \frac{[3.0 \times 10^6 \text{ kg C} - (5.3 \times 10^5 \text{ kg C} + 4.2 \times 10^4 \text{ kg C})]}{27.0 \times 10^8 \text{ m}^3 \text{ H}_2\text{O}}$$

$$= \frac{[2.428 \times 10^6 \text{ kg C}]}{2.7 \times 10^{12} \text{ liters}}$$

$$= 899 \ \mu\text{m C l}^{-1}$$

Table 6. *Crassostrea virginica.* Estimation of importance of NL-POM in North Inlet, South Carolina, to carbon demand of 100 mg dry wt oyster. AE: assimilation efficiency

Assume:	total carbon requirement	$= 263.6$ μg C h^{-1}
	AE for NL-POM	$= 3\%$
	total NL-POM	$= 899$ μg C l^{-1}
	filtration rate	$= 2$ liters h^{-1}
Then:	carbon absorbed from Nl-POM	$= (899 \ \mu\text{g C l}^{-1})(0.03)(2 \text{ liters h}^{-1})$ $= 53.94 \ \mu\text{g C h}^{-1}$

Therefore: 20% of total carbon requirement may be met through absorption of NL-POM C

Table 5. Reported values (μg l^{-1}) for crude fiber in the seston

Area and year	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sep.	Oct.	Nov.	Dec.	Source
Low-salinity marsh													
1973	–	–	–	–	–	–	31.5	38.5	36.5	47.0	27.0	28.0	Heinle and Flemer (1976)
1974	52.5	51.5	43.0	72.5	37.0	66.0	–	61.5	–	45.0	–	97.0	
1975	–	58.5	–	34.5	–	55.5	–	–	–	–	–	–	
Choptank river													
1984	–	–	–	14.3	11.4	25.0	14.6	14.1	17.5	27.3	10.4	–	This study
1985	–	–	18.2	34.3	8.6	14.7	29.5	7.8	9.7	5.4	4.3	–	
Salt marsh													
1986	–	–	92.3	77.4	29.8	29.1	58.7	49.6	–	38.4	17.7	15.8	Kreeger et al. (1988)

has also been examined in our laboratory, and the results are currently being prepared for publication.

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