IN SITU MONITORING OF THE EFFECTS OF WATER QUALITY ON BENTHIC DETRITAL
DECOMPOSITION.

John J. Lee, Carmine Mastropaolo, Marie McEnery, John H. Tietjen and
Judith Garrison

Department of Biology, City College of NY
Convent Avenue at 139th Street
New York, New York 10031

MASTER

ABSTRACT: Detrital decomposition is an important marine benthic process which contributes to the fertility of seas, particularly in estuarian and coastal waters. The process in the sea involves a complex community of microorganisms and small animals which interact with each other in a manner similar to that which occurs in forest litter and in composts. Plastic chambers for measuring decomposition rates of Spartina alterniflora (a marsh grass which is a major contributor to detritus on U.S. East and Gulf coasts) were placed on the bottom of the sea at four sites in the northeast: Towd Point, Southampton, New York; the effluent quarry of the Millstone power plant on Niantic Bay, Long Island Sound; Winsor Cove, Cataumet, Massachusetts (the site of an oil spill); and Sippewissett marsh, Falmouth, Massachusetts (a control site for Winsor Cove). The stations were visited monthly. By various means we measured the rates of decomposition and growth of sediment microbial and animal populations. This long term method is promising as a useful means to measure the integrated effects of pollutional stresses on important but slow environmental processes. It may be more environmentally meaningful than LD50 or similar short term measures of transient stresses.

DISCLAIMER

This book was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise, does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

DISTRIBUTION OF THIS DOCUMENT IS UNLIMITED

DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency Thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

DISCLAIMER

Portions of this document may be illegible in electronic image products. Images are produced from the best available original document.

INTRODUCTION

Two principal types of food webs lead to the highly productive estuarine marine ecosystems found on the East and Gulf Coasts of the United states. One, the plankton grazing web, is based on phytoplankton growth in the nutrient-rich estuarine waters; the other, the detrital food web, is based on the production and decomposition of sea grasses and cord grasses that grow in shoal waters and in the wetlands that border the sea.

Only a small quantity of the sea and cord grasses enters marine productivity by direct grazing; the bulk of the material is processed by microorganisms and small animals. Marine systems are not unique in this respect because similar processes take place in grasslands and forest soils by litter decomposition. As much as two-thirds of the annual production of forests enters the litter decomposition cycle. Estimates of the amount of sea and cord grasses entering the detrital food web suggest that ~ 90% of the annual production is channeled into this web.

The detrital marine food web, particularly in the Northeast and Middle Atlantic states, is under heavy assault from 2 major directions: 1) rapid destruction and removal of the annual production of many salt marshes from the ecosystem; and 2) fouling, polluting, poisoning, destroying, and otherwise disturbing production associated with the detritus-based benthic habitats. Although many factors have contributed to the sharp decline of the commercial fish landings in the New York marine district over the last 20 years (Jensen, 1977), the sharp reduction in the quantity and decline in the quality of the habitat suitable to support the detrital food web must

have been a major contributing factor.

We have to quibble on this last point. Assessment of impact implies that one has an ability to make objective judgements on the quality of the habitat and its suitability to support detrital decomposition. Unfortunately assay methods are not yet available. We accepted the challenge to try to test the feasibility of one method because the problem seemed technically solvable by extension of the work that we and our co-workers were doing with various laboratory microcosms (Lee et al. 1975; Tenore 1975, 1976; Tenore et al. 1977).

MATERIALS AND METHODS

Flow-through reaction vessels/respirometers were built of plexiglass to test the effects of water quality on detritus decomposition under conditions as close to ambient as practical. The lower part of the reaction vessel is a solid box (Plate I, Figs, A, B) that holds the sediment and the detritus. The upper portions are pierced with 3/8" holes that are covered on the outside with fiberglass window screens and the inside with nylon and fiberglass Millipore filters (NRWP 14250; 1 μ m pore). Plastic shields surround the reaction vessels to deflect horizontal waves and to prevent damage from browsing animals. During the periods when respired $^{14}\text{CO}_2$ is being trapped, the caps of the reaction vessels are removed and replaced with water-tight, glass-enclosed vessels containing a filter paper saturated with 40% KOH (Plate II). Measurements of $^{14}\text{CO}_2$ release were at low tide during the day and lasted from 2-3 hrs.

Since it was reasonable to assume that aufwuchs growth might plug up the pores of the nylon filters during incubation in the field, we examined this point experimentally. During the development of prototypes we found that weekly scrubbing of the surfaces of the plastic

apparatus surrounding the filters with commercial plastic pads designed for cleaning cooking pots, aufwuchs buildup was prevented. At the same time the filter surfaces were gently brushed with a 1.25 cm paint brush. At the termination of each experiment we removed each reaction vessel from its holder under water and placed it in a plastic bucket of sea water. This was done to prevent drying and changing of the characteristics of the small aufwuchs community on the vessels, and to prevent

damage to the membranes by unbalanced water pressure during transportation to the laboratory. Upon return to the laboratory ^{24}Na was thoroughly mixed with the seawater in the reaction vessels so that it could be used as a tracer to measure eflux rates. The sea water in the buckets surrounding the vessels was sampled at 5 min intervals. Radioactivity was measured by γ spectrometry. The rate of flux of the sea water in the water column of the reaction vessels was at least 12 x its own volume per hour in the most fouled cases. It was much higher ($> 20 \times$) in most cases.

The sediment in the reaction vessels was thoroughly washed beach sand, with approximately the same frequency grain size distribution as we noted in Southampton Long Island salt marshes (Matera and Lee, 1972) enriched with 4% (by weight) ground dried Spartina alterniflora (total weight of Spartina in each reaction vessel, 50 g). The Spartina was collected in the winter (December) and, after it was washed and dried, it was ground in a Wiley Mill to particles which were $\sim 120 \mu\text{m}$ in size. The Spartina detritus was enriched with dried and ground radionuclide labeled (^{14}C) Spartina to give specific activity of $2.72 \mu\text{Ci/gram}$ Spartina. The Spartina used as a tracer was grown from seedlings (obtained from Environmental Concern, P. O. Box P, St. Michaels, Maryland 21663) in a transparent plexiglass cabinet in a $^{14}\text{CO}_2$ environment following the method of Ferguson and Williams (1974). After the S. alterniflora reached $\sim 0.8 \text{ m}$ in height the photoperiod in the chamber was shortened and the temperature was lowered to 15°C during the dark cycle to induce senescence before harvesting from the chamber. After harvest, the leaves and stems were placed in gently flowing freshwater for 2 weeks, dried in glass trays in a hood for 2 months, before being ground in.

a Wiley Mill and assayed for activity.

The reaction vessels and their holders were placed in sublittoral zones in: 1) the Millstone Atomic Power Plant effluent quarry; 2) at a control site at the nearby New England Environmental Laboratory at Millstone; 3) Winsor Cove, on Buzzards Bay, Bourne, Massachusetts, the site of a minor #2 fuel oil spill; 4) Greater Sippewissett Marsh on Buzzards Bay, Falmouth, Massachusetts, a control site nearby; and 5) Towd Point on North Sea Harbor, an embayment of Greater Peconic Bay between the forks at the eastern end of Long Island, New York, a control site. At mean low tide the vessels were covered by ~ 1 m of water. Depending upon ambient conditions and lunar cycles they are covered by 2-3 m of water at high tide. In each site the reaction vessels were divided into 2 groups: 1) microflora; and 2) meiofauna, microflora, and microfauna. There were 2 or 3 replicate reaction vessels at each station. With one exception both groups were inoculated with microflora from indigenous detritus rafts. The microflora was dislodged from the detritus by vigorous agitation of the detritus and filtered through 8 μ m pore membrane filters before inoculation into the reaction vessels. Because the benthos of Winsor Cove is still oil saturated and does not have an indigenous meiofauna, the inoculum for this site came from the other side of nearby Scraggy Neck. In addition to the microflora, one group of vessels at each site also was inoculated with a detrital fraction that was gently sieved through a 0.5 mm screen. This fraction contained meiofauna, microflora, and microphytes. At each site this fraction (~ 1.50 g) was diluted with Millipore SC (8 μ m) filtered seawater until newspaper print was easily read through the graduated cylinder used for the dilutions. This is an

optical density approximately equal to a 1×10^7 /ml nutrient broth culture of E. coli. An inoculum of 70 ml was added to all vessels. Samples of the inoculum were fixed in a Rose Bengal-formalin mixture for later analysis of the meiofaunal species structure.

Once a month we visited each site, aseptically took samples from the vessels with the aid of sterile pipettes and measured rates of $^{14}\text{CO}_2$ liberated from the entire reaction vessel containing decomposing Spartina. In the field each sample of sediment and pore water was divided into 3 aliquots and either: 1) frozen on dry ice for carbon/nitrogen analysis; 2) extracted in cold (4°C) 35% perchloric acid containing 17 mM of EDTA for one-half hr before freezing on dry ice for ATP analysis; or 3) fixed in 4% seawater-buffered glutaraldehyde for later examination with the SEM. The ATP in the frozen perchlorate extracts were neutralized with ~ 0.17 ml of 2.6N KOH to pH 7.2 to 7.4 and then measured using a JRB luminescent biometer and a purified, lyophilized, luciferin-luciferase preparation (E. I. DuPont de Nemours Co.) in MOPS buffer (pH 7.4) following the procedure outlined by Falkowski (1977). Precision was facilitated by the automated rapid injection and switching system of Chapman and coworkers (1971).

Material for particular carbon and nitrogen analysis was filtered onto precombusted (2 hr at 200°C) Gelman (cat. #61631) glass fiber filters. The filters were dried, weighed, and analyzed on a Hewlett-Packard C-H-N analyzer (Model 185) following the procedures detailed by Sharp (1974). The elemental analyzer combines a Micro-Dumas controlled pyrolysis step (1025°C) with gas measurement on a thermal conductivity gas chromatograph. Acetanilide was used as a standard.

The filter papers with captured $^{14}\text{CO}_2$ were carefully folded in the field and placed in small polycarbonate capsules and frozen on the dry ice. The capsules were counted for activity after pretreatment with an Inter-technique Sample Oxidizer (Model IN 4101) (Peterson et al., 1969). The radioactivity of the samples was measured in a Beckman β liquid scintillation counter (LS 250). Carbon-14 standards (Amersham-Searle CFR 101) were oxidized in each batch of 40 samples so that recovery losses and alkaline quench in counting could be calculated and compensated.

Due to the small numbers of samples (3) at each location and sample date all figures are plotted as the arithmetic mean. Samples for SEM examination were dehydrated through a graded series of ethyl alcohol, placed in iso-amyl acetate and then placed on lens paper for easier manipulation during critical point drying and mounting on stubs. The stubs were then coated with an Au-Pd mixture in a vacuum evaporator and examined in a Cambridge Stereoscan scanning electron microscope (Model 54-10).

RESULTS AND DISCUSSION

In general there was good reproducibility among the replicate reaction vessels incubated at the various test sites. Carbon/Nitrogen ratios in the detritus and trends at all sites were very similar. After one month, values dropped from ~ 30:1 (range 36:1 to 26:1) to ~ 25:1 (range 30:1 to 15:1) 6 months later. In general the C/N ratios of the reaction vessels with and without meiofauna were very close to each other with the ratio tending to be slightly lower (0-45%) in the vessels with meiofauna (Fig. 1).

There was a gradual build-up of ATP in all of the vessels during the course of incubation. After 1 month incubation, values for ATP were ~ 3 ng ATP/g detritus (range 1-9 ng ATP/g sediment). Highest values were observed in the Millstone Plant effluent quarry in reaction vessels with meiofauna. At the end of 6 months ATP values at different sites were quite variable. The slowest growing communities were at Towd Point, Southampton, where ATP values reached ~ 30 ng ATP/g detritus with the microflora alone (Fig. 2). In contrast, reaction vessels at Sippewissett and Winsor Cove had ~ 300 µg ATP/g detritus (Fig. 3).

Differences among the vessels with and without meiofauna were quite variable. In some sets there was only 3 to 5% difference between pairs in a set, in other sets the reaction vessels with meiofauna had 100% more ATP than those without the meiofauna. At the Millstone control site, 3 reaction vessels (2 with meiofauna and 1 without) had approximately 120 ng ATP/g detritus (Fig. 4). The fourth reaction vessel (no meiofauna) had less than

half (~ 54 ng ATP/g detritus). The effluent quarry vessels with meiofauna had only 22 ng ATP/g detritus, while those with only microorganisms had 55 ng ATP/g sample (Fig. 5), only a fraction of the controls.

As expected, because detrital carbon is mineralized during the process, the rates of $^{14}\text{CO}_2$ release from the detritus declined during incubation. Another factor underlying the decline is the change in ambient temperature. During the course of incubation, temperatures at various sites increased from an average $\sim 14^\circ\text{C}$ in June to $\sim 25^\circ\text{C}$ in August before falling to lows of only $\sim 4^\circ\text{C}$ (range 2.5°C - 6.5°C) in December. Temperatures in the effluent quarry at Millstone were, of course, correspondingly higher ($\sim 21^\circ\text{C}$ in December). Another factor affecting the overall rate of mineralization is the change in the ratio of less refractory (more easily decomposed) fraction of detritus to the more refractory fraction. The less refractory fraction is more or less rapidly decomposed during the initial stages of microbial attack leaving the more refractory constituents behind (Lee et al., 1975).

At 2 of the sites, Winsor Cover and Southampton, there were consistent and significant differences in the amount of $^{14}\text{CO}_2$ released from reaction vessels with and without meiofauna (Fig. 6). After a month, rates of mineralized carbon respired were ~ 4.5 mg C/hr in the sediments in reaction vessels with meiofauna and only 0.75 mg C/hr in the sediments in reaction vessels with microflora alone. At the same sites in September, after 3 months incubation, when the water was still fairly warm (15 - 17°C) at both sites, rates of mineralization had declined but was still between 1-2 mg C/hr. At the Sippewissett and Millstone control

sites the mineralization rates of vessels with and without meiofauna were much closer and generally followed the same trends as found at the other 2 sites (Fig. 7). The rates of mineralization in the reaction vessels within the Millstone effluent quarry were 30% of the rates in the nearby control sites (Fig. 7).

SEM observations of samples from the reaction vessels suggested that there was great variation in the microbial colonization of the Spartina fragments. Some particles were heavily colonized after only 1 month of incubation whereas others in the same reaction vessel were sparsely colonized after 5 months incubation. In general there was greater variety of morphological types of microbes and more clumping of particles into small aggregates in vessels with meiofauna than those without. This avenue of research seems to offer promise of yielding much more information and should be pursued in future studies.

Of the 4 variables observed in the reaction vessels during decomposition (changes in C/N ratio; build-up of detrital based microbial and meiofaunal communities as observed in the SEM, or as observed in ATP build-up; and CO₂ evolution) only two seem reasonably practical as means to monitor the effects of pollutant stress on this major benthic process. There was so little change in C/N ratios because there is so much undecomposed cellulose relative to the fraction ^{of nitrogen} that can be fixed and acquired from the water column during incubation by the biota. Too little is known about the stochastic aspects of microbial attack of cellulose in shallow coastal waters, or the consequences of favoring some flora over other, to be able to use the SEM or selective growth media to identify detrital communities

which are under pollution stress but still functioning at some lower level. On the other hand, total ATP (or energy charge) and release of mineralized carbon seem to offer promise that they could be reasonably simple and routine methods for measuring the effects of various types of stress on the health of detrital decomposing systems in the near shore areas.

Clouding the issue of using ATP (or energy charge) as a practical means for this measurement is the problem of relating total ATP to the decomposition rate or health of the system. Recent studies (Yingst, 1978; Sikora et al., 1977) have shown that much of the ATP measured in sediment samples is in the meiofauna. While many meiofauna eat cellulose decomposers, many others eat other microbes and algae which are less directly or only partially linked to cellulose decomposition. Much more knowledge is needed on the functional interrelationships between the various assemblages of the detrital food web before one could feel comfortable with using total ATP (or energy charge) as an index of benthic community health.

Although the data base was small for this preliminary study (2-3 replicates at each site, 2 variables) we are surprised that the range of results was so close between replicates and between sites so geographically separated. To establish a more realistic idea of the 'normal' ranges of initial rates of colonization and decomposition the data base must be expanded. This is particularly true because of the stochastic aspects of the process. While we used a uniformly assembled substrate in this study it would be important to use the native substrate in monitoring programs. Nowhere is this point made more clearly than at Winsor Cove (Plate III). The mussels have returned to this oil impacted site and are filter feeding

on the available plankton. After 5 years Spartina alterniflora is still receding along the shore line (Hampson and Moul, 1978) and meiofauna are rarely found in the oil soaked sediment which oozes oil as one walks on it. While our study shows that the water quality at the site is quite satisfactory for detrital decomposition to take place, it is probably not occurring there. One approach we have used before in laboratory flow-through chambers with detrital decomposition systems is to withdraw the native substrate, freeze and thaw it, and then "spike" it with ^{14}C labeled ground Spartina detritus as a label (Tenore et al., 1977). There do not seem to be any technical obstacles to doing this, and in fact, it might be advantageous to incubate native tracer "spiked" substrate and artificially constructed substrate side by side as controls of each other. Although of more academic than pragmatic interest, some experiments on relating $^{14}\text{CO}_2$ release during decomposition to the total carbon mineralized and some longer experiments that could relate cropping of the microbial and meiofaunal assemblages to the rates of in situ decomposition seem quite worthwhile.

Overall the system that we describe here shows considerable promise as a practical means to monitor the effects of water and sediment qualities on detrital decomposition in the near shore benthic habitats. Considerable effort is still needed, however, to develop the assay for routine applications.

ACKNOWLEDGEMENTS

Many people aided us in different ways while we were doing this study. Dr. Howard Sanders and Mr. George Hampson of the Woods Hole Oceanographic Institute helped us set up the experiments at Winsor Cove, Massachusetts. Drs. John Teal of WHOI and Ivan Valiella of MBL-BUMP helped us set up in the Greater Sippewissett Marsh in Falmouth, Massachusetts. Dr. William Renfro and Mr. Paul Jacobson of the North East Nuclear Energy Co. provided us with lab space and helped us set up the experiments and controls at the Millstone Atomic Power Plant at Niantic, Connecticut. Mr. Tom Rewinski of the Board of Trustees in Southampton Township granted us permission to place the experiment in the Towd Point Sanctuary in North Sea Harbor, Southampton.

The ATP analysis was done under the tutorledge and in the laboratory of Dr. Paul Falkowski, Department of Oceanography, Brookhaven National Laboratory. The C/N measurements were made in the Biology Department, Lamont-Doherty Geological Observatory of Columbia University. We are grateful to Dr. Thomas Malone and Ms. Elizabeth Cosper for instructing us in the use of the equipment and making it available to us.

Most of the study was supported by DOE contract (11-1) 3395. Support from NSF grant OCE 78-09962 to complete the project is gratefully acknowledged.

REFERENCES

1. Chapman, A. G., L. Fall, and D. Atkinson. 1971. Adenylate energy charge in Escherichia coli during growth and starvation. J. Bact., 108: 1072-1086.
2. Falkowski, P. 1977. The adenylate energy charge in marine phytoplankton: the effect of temperature on the physiological state of Skeletonema costatum (Grev.) Cleve. J. exp. mar. Biol. Ecol., 27: 37-45.
3. Ferguson, R. L. and R. B. Williams. 1974. A growth chamber for the production of ¹⁴C labeled salt marsh plants and its application to smooth cordgrass, Spartina alterniflora Loisel. J. exp. mar. Biol. Ecol., 4: 251-259.
4. Hampson, G. and E. Moul. 1978. No. 2 fuel oil spill in Bourne, Massachusetts: immediate assessment of the effects of marine invertebrates and a 3 year study of growth and recovery of a salt marsh. J. Fish. Res. Bd. Can., 35: 731-744.
5. Jensen, A. C. 1977. New York's marine fisheries: changing needs in a changing environment. N.Y. Fish and Game J., 24: 99-128.
6. Lee, J. J., K. Tenore, J. Tietjen and C. Mastropaolo. 1975. An experimental approach toward understanding the role of meiofauna in a detritus based marine food web. In: Proc. 4th Nat. Symp. Radioecol., "Radioecological Problems Associated with the Development of Energy Sources". Dowden, Hutchinson and Ross, Inc. In press.
7. Peterson, J., F. Wagner, S. Siegel, and W. Nixon. 1969. A system for convenient combustion preparation of titrated biological samples for scintillation analysis. Analytical Biochem., 31: 189-203.

8. Sharp, J. H. 1974. Improved analysis for "particulate" organic carbon and nitrogen from seawater. *Limnol. Oceanogr.*, 19: 984-992.
9. Sikora, J. P., W. B. Sikora, C. Erkenbrecher and B. Coull. 1977. Significance of ATP, carbon and caloric content of meiobenthic nematodes in partitioning benthic biomass. *Mar. Biol.*, 44: 7-14.
10. Tenore, K., J. Tietjen, and J. Lee. 1977. Effect of meiofauna on incorporation of aged eelgrass, Zostera marina, detritus by the polychaete, Nephtys incisa. *J. Fish. Res. Bd. Can.*, 34: 563-567.
11. Yingst, J. Y. 1978. Patterns of micro- and meiofaunal abundance in marine sediments, measured with the adenosine triphosphate assay. *Mar. Biol.*, 47: 41-54.

Reprint Removed

PLATE I

- A. Schematic Diagram of flow-through reaction vessel for detritus decomposition assay.
- B. Cut-away diagram of reaction vessel in plexiglass holder.

PLATE II

- A. Reaction vessels at lowest tide with respirometer caps in place.
- B. Edge of the marsh at Winsor Cove where an oil spill killed most of the Spartina 5 years ago. Erosion and crumbling of the marsh peat obvious in foreground.
- C. Close-up picture of marsh peat showing extensive mussel repopulation of the littoral zone.

PLATE III

SEM photographs of representative microbial populations on the surfaces of Spartina fragments from reaction vessels.

- A. Typical colonization on the surface of Spartina after 1 month incubation. x 2,800.
- B. Showing extensive microbial colonization on 1 fragment (upper third) and no colonization on an adjacent one (lower two-thirds) x 2,000.
- C. Showing extensive colonization by bacillus and cocci on fragments incubated for several months. x 2,800.
- D. Showing extensive mycelial-like growth of filamentous bacteria and flexibacteria. x 2,750.

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

Fig. 6

Fig. 7