DECOMPOSITION OF TURTLEGRASS (*THALASSIA TESTUDINUM* Konig) IN FLOWING SEA-WATER TANKS AND LITTERBAGS: COMPOSITIONAL CHANGES AND COMPARISON WITH NATURAL PARTICULATE MATTER

By: Parke A. Rublee and Michael R. Roman

Rublee, P.A. and M.R. Roman. 1982. Decomposition of Turtlegrass (*Thalassia testudinum Konig)* in flowing seawater tanks and litterbags: Compositional changes and comparison with natural particulate matter. *Journal of Experimental Marine Biology and Ecology* 58:47-58.

Abstract:

Thalassia testudinum Konig litter was incubated up to 3 yr in flowing sea-water tanks and litterbags in the field. Weight loss of litter was evident within 1 wk and 50% loss of ash free dry weight took < 35 days. Carbon and nitrogen were lost from litter, but the C: N ratio (by weight) changed only slightly over the first 180 days of incubation. Protein, carbohydrate, and lipid content of litter decreased with time. Number of bacteria on litter increased from ≈ 9 to 37-60 × 10^9 cells • g dry wt^{-1}. Lipid synthesis activity suggested a succession in the litter microbial community. *Thalassia* detritus from long-term tank incubations (2 to 3 yr) exhibited similarities in biochemical composition with particulate matter in natural water.

Article:

INTRODUCTION

Interest in the decomposition of macrophyte material, although evident since early in the 20th century (Boysen-Jensen, 1914), was stimulated by recognition of the potential importance of extensive detrital production and export in coastal ecosystems (Odum & de la Cruz, 1967). The concept of macrophyte production followed by microbial decomposition of detritus and subsequent consumer utilization was incorporated into our understanding of the functioning of coastal ecosystems. Recently, however, this paradigm has been reexamined and questioned in terms of production, export, and energy transfer processes (cf. Peterson et al., 1980).

Our understanding of the macrophyte decomposition process is derived mainly from litterbag or "batch culture" studies (e.g. Olah, 1972). Early in the decomposition process there is a rapid loss of weight due to the leaching of soluble substrates. A later, overlapping process is microbial colonization and utilization of the more labile components of the litter. A still later process is saprophytic breakdown of the more refractory components. Overlapping all of these are chemical breakdown of the litter substrate and more importantly, physical breakup of the particulate matter which may occur due to water movement and organisms which scrape, chew, or ingest the litter.

The use of litterbags, however, has been criticized for various reasons. Within natural systems macrophytes "dieback-, a gradual process which induces trans-location of nutrients to roots and rhizomes and is seldom simulated in decomposition studies. Thus, should live or dead material (e.g. dried to constant weight) be used in litterbags? Additionally, the use of litterbags or artificial enclosures may alter the rates of these decomposition processes and, in particular, may lead to emphasis of the leaching process if fresh material is used as the litter source. What is the appropriate opening for litter containers: too small and the exchange of dissolved and particulate materials (including organisms) may not mimic natural processes - too large and little or no material is retained. Finally. litterbags are indicators of material retained, the material which has presumably undergone the greatest amount of decomposition is lost from the bags.

We examined several facets of these questions as part of a study of utilization of suspended detritus by zooplankton. Our goal was to produce small particles of aged detrital material of known origin that simulated natural particulate matter. Our hypothesis was that we could do this in flow-through sea-water tanks which could retain small detrital particles longer than litterbags. We compared litter aged in litterbags with that aged in the flow-through sea-water tanks on the basis of biochemical and microbial parameters. We also compared the aged detritus to natural particulate matter from coastal areas of Florida, the Bahamas and to literature values. *Thalassia testudinum* Konig was chosen as our litter source since it is the dominant seagrass in the shallow, sub-tropical, near-shore systems we studied.

MATERIALS AND METFIODS

Fresh *Thalassia* was harvested from a grass bed near Key Biscayne, Florida on 23 May, 1979. Our procedure was to excise the leaves near the sediment surface and to use all plant material harvested within a given area. Our decision to start with freshly harvested material was based simply on obtaining natural macrophyte material that had not yet gone through extensive decomposition. Thus, our initial material consisted of ≈ 60% green leaf blades, 4% brown blades, and 27% blades exhibiting a mixture of green, yellow, anti brown portions. Epiphytic cover ranged from 0 to 100% of the leaf blade, averaging 18%. The average blade was 17 cm in length and 0.8 cm wide. Leaf blades were kept submerged in sea water throughout the harvesting and all subsequent processes to prevent desiccation of the cuticle which results in accelerated decomposition (Zieman, 1968: S. Y. Newell pers. comm.).

We placed 40 to 60 g wet wt of *Thalassia* blades in each of 28 litterbags. Litterbags were nylon mesh netting with openings of 3 mm and measured 10 × 20 cm. Closed bags were tied at evenly spaced intervals to a 4-rn line which was secured to a subtidal mud bottom near the grass bed where the material was collected. At various time intervals three or more bags were harvested from the set and returned to the laboratory in a bucket of sea water for subsequent analysis.

The tank treatment consisted of triplicate 40-1 fiberglass tanks with 375 to 400 g wet wt of *Thalassia*. Sea water was introduced from the bottom of each tank and an overflow at the top was circled by a screen with ≈ 1 × 3 mm mesh openings (Roman, 1977). Flow was adjusted to about two to three flushings per day. The source of the sea water was Bear Cut, Florida and it passed through a settling tank and a coarse swimming pool filter prior to tank inflow. Thus, larger animals were excluded, but microorganisms, some meiofauna, and invertebrate larval forms had access (and were found) in the tanks. The water temperature of these tanks was similar to the in situ temperature, and illumination of the indoor tanks consisted simply of constant overhead fluorescent lighting. At various time intervals, all material from each tank was gently placed on screens (1-mm mesh), water allowed to drain, a wet weight taken, known amounts of litter removed for various analyses, and the remaining material replaced. When the litter became delicate, such that handling would significantly accelerate physical breakup of the material, this procedure was changed. Thereafter, a single tank was harvested in total by collection on a 53-µm mesh net. Samples for 180 and 365 days of incubation were collected in this manner. One additional sample of *Thalassia* litter aged 3 yr in a tank was also used for analysis. This material was handled and aged in a similar manner, but had been decomposing since 1977.

Wet weight changes were determined on all samples. The samples from each treatment/time combination were then pooled, and subsamples were taken for various analyses. Five subsamples were dried at 60 °C to constant weight. Ash free dry weight (AFDW) was determined after ashing at 550 °C overnight. Weight loss on a dry or ash free dry weight basis could then be determined for the entire sample.

Chemical analyses included determinations of carbon, nitrogen, lipid, acid-soluble carbohydrate, and protein content. Carbon and nitrogen were assayed on both dried and ashed (to correct for carbonates) subsamples on a Perkin Elmer Elemental Analyzer. For lipid and carbohydrate analyses, replicate dried sub-samples (≈ 10 mg) were homogenized in a tissue grinder with 5 ml distilled water. Two ml of homogenate from each subsample was then extracted for lipids by a modified Bligh & Dyer (1959) method. Test tubes containing the sample, 2 ml additional distilled water, 10 ml absolute methanol, and 5 ml chloroform were shaken vigorously and allowed to

stand for at least 2 h. Addition of 5 ml distilled water and 5 ml chloroform with vigorous shaking broke the single phase. After at least 12 h the lower phase was removed and the solvent evaporated. The residue was then assayed against a cholesterol standard for total lipids by the sulphophosphovanillin method (Barnes & Blackstock, 1973). Carbohydrates were extracted and hydrolyzed from 3 ml of homogenate following the procedure of Holland & Gabbott (1971), except that proportionally larger volumes of solutions were used. Carbohydrates were analyzed against a sucrose standard by the method of Dubois et al. (1956). Duplicate subsamples for protein were homogenized in 5 ml 1 N NaOH and allowed to stand for at least 1 h at 60 °C. Samples were centrifuged and aliquots of the supernatant neutralized with an equal volume of 1 N HC1, followed by protein determination by the micro-assay Bio-Rad method (Bio-Rad Laboratories), which uses Coomassie blue dye and a serum albumin standard.

Subsamples for bacteria counts and microbial activity were taken immediately after weight determinations while the material was still wet, usually within 2 h of sample collection. For determination of bacteria associated with the sample, 4 to 10 subsamples (≈ 0.2 to 0.5 g dry wt) were placed in 20 ml of a prefiltered (0.2-μm Nuclepore filter) 5% formalin solution and stored at 4 °C. Within 1 wk samples were homogenized with 100 ml prefiltered sea water in a blender and aliquots counted by the acridine orange direct count (AODC) method (Hobbie et al., 1977; Rublee, 1978). The remaining homogenate was passed through a glass fiber filter (VVhatman GFC) for sample weight determination. Microbial activity of detrital material was determined by lipid synthesis of ^{14}C-acetate and $H_3{}^{32}PO_4$ precursers as described by White et al. (1977) and Morrison et al. (1977).

Field samples of natural particulate material were collected from the *R.V. Orca* and the *R.V. Calanus* during 1979 and 1980 in coastal waters of Florida and the Bahama Islands. Generally, 250 to 1000 ml of water was passed through a glass fiber filter (Whatman GFC) which was dried at 60 °C for later analysis. Natural subsamples were treated in a manner similar to litter samples for all analyses.

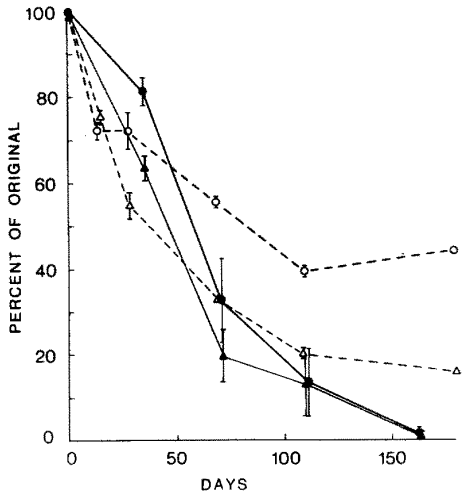

Fig. 1. Weight loss from *Thalassia* litterbags and tanks (mean ± SE): ———, litterbag incubations; – – – –, tank incubations; ●, ○, dry wt values; ▲, △, AFDW.

RESULTS AND DISCUSSION

Loss of weight from *Thalassia* litter in this study exhibited a similar pattern in both litterbags and tanks during the first 50 days of the experiment (Fig. 1). Thereafter, litter in the tank lost weight at a slower rate than that in the bags, and even exhibited an increase in dry weight between 110 and 180 days. This was not unexpected since the tank treatment is considerably more effective at retaining small particulates which would be lost from the litterbags, including carbonate precipitates from the sea water. In fact, by 180 days litterbags no longer contained any *Thalassia* detritus. Additionally, a smaller mesh net (53 μm) was used to harvest the material from the tank at the 180-day sample, and this certainly introduced a significant bias, resulting in the increased weights at that sampling point. The patterns of weight loss are similar to those found in other batch culture and litterbag studies of *Thalassia* decomposition (Zieman, 1968; Knauer & Ayers, 1977; S. Y. Newell, pers. comm.).

Carbon and nitrogen changes in the litter (Fig. 2) were also generally consistent with literature reports. A rapid loss of carbon and nitrogen during the first 2 to 5 wk was probably associated with rapid losses of soluble compounds and labile organics. The carbon to nitrogen ratio appeared to decrease slightly over the 180-day incubation period, but slopes of the regressions of C: N vs. time were not significantly different from zero ($F = 0.047$, $n = 5$ for litterbags: $F = 0.418$, $n = 6$ for tanks). The carbon to nitrogen ratio of a 1-yr-old *Thalassia* litter sample from a flowing sea-water tank, 9.0, was below the range of 10.3 to 19.0 found in samples incubated less than 6 months. *Thalassia* incubated for 3 yr had a C: N of 19.5. Knauer & Ayers (1977) found C: N ratios averaging 17.9 during the first 31 days. and 27.0 for the next 21 days in their batch culture study of *Thalassia* decomposition.

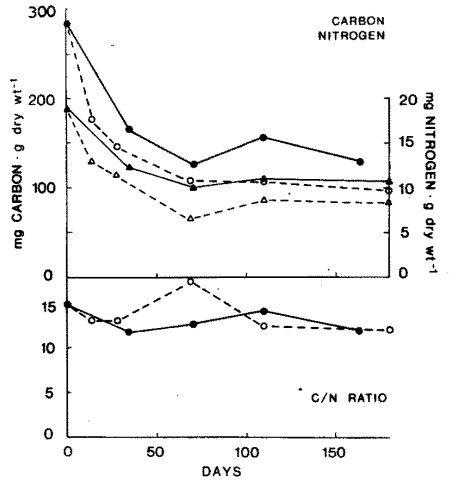

Fig. 2. Carbon and nitrogen changes in *Thalassia* litter: upper figure: ●, ○, carbon values; ▲, △, nitrogen values; both figures: ———, litterbag samples; – – –, tank samples.

Protein content of the litter decreased significantly in both litterbags and tanks during the first 35 days (Fig. 3). Over the entire incubation period protein decreased from 6.6 to 1.3% of dry wt. Our initial value of 6.6% is similar to that reported by Dawes et al. (1979), but contrasts with the value of 13.1"/„ determined by Burkholder et al. (1959). The discrepancy is explained by their use of a conversion factor of 6.25 x the nitrogen content as an estimate of protein. Using the same conversion and our nitrogen content of 19 mg • g dry wt^{-1} would yield an estimate of 12%. Odum et al. (1979) have noted that such a conversion factor is inappropriate for macrophytes due to substantial amounts of non-protein nitrogen in vascular plants. Furthermore, Knauer & Ayers (1977) pointed out that changes in carbon to nitrogen ratios are not tightly coupled to development of microbial communities on detritus, at least in culture systems. Thus, much of the earlier work which estimated protein content of various detrital or food sources for nitrogen content must be interpreted cautiously, as should the importance of carbon to nitrogen ratios in nutritional considerations.

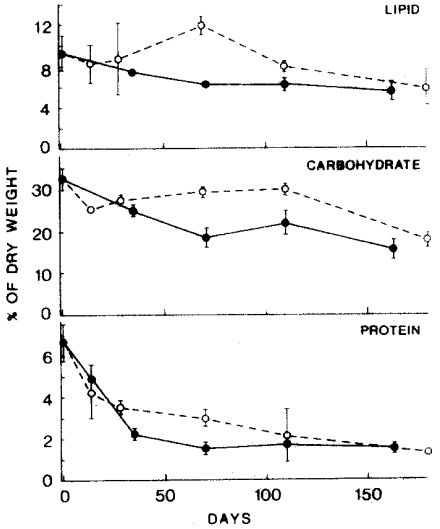

Fig. 3. Lipid, carbohydrate, and protein losses from *Thalassia* litter (mean ± SE of replicate analyses from pooled samples): ———, litterbag samples; – – –, tank samples.

Carbohydrate and lipid content of the litter did not show the dramatic decreases with time as did weight and protein content. Carbohydrate content of fresh litter in this ,study was 32.8% of dry wt and decreased to 15% after 6 months exposure. The initial carbohydrate value is similar to the value of 35.6% found by Burkholder et al. (1959) but higher than the values for soluble carbohydrate found by Dawes et al. (1979); our hydrolytic method includes both soluble carbohydrates and some insoluble cellulose. Lipid content of fresh material was 9.2% by weight and decreased to 5 to 6% in the older samples. The pattern of change was similar in both litterbags and tanks.

Colonization of decomposing litter material by bacteria was significant. Fresh Thalassia exhibited $9.36 \pm 2.60 \times 10^9$ ($\bar{X} \pm$ SE) cell g dry wt^{-1}. On both litterbag and tank samples the number of bacteria increased from 4 to 6 times through the first 5 wk, followed by a more gradual decrease (Fig. 4). Samples from the litterbags showed higher numbers of bacteria than those from the tanks. The pattern of microbial activity, evidenced by lipid synthesis, varied from the pattern of bacterial numbers (Fig. 4). Synthesis of ^{32}P into lipids was highest in the 14-day sample from the tanks and in the 35-day sample from the litterbags. Incorporation of ^{32}P decreased rapidly after the initial peak and remained low thereafter. The maximum incorporation of ^{14}C into lipids occurred after 10 wk in both treatments, followed by a decrease in total lipid synthesis.

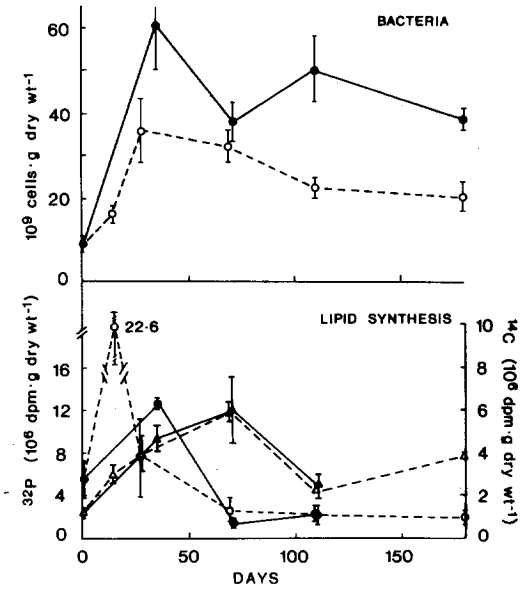

Fig. 4. Bacterial colonization and lipid synthesis in *Thalassia* litter (mean ± SE): both figures: ———, litterbag samples; – – – –, tank samples; lower figure: ●, ○, ^{32}P incorporation; ▲, △, ^{14}C incorporation.

The patterns of bacterial colonization and lipid synthesis activity suggest a microbial colonization and succession pattern compatible with our understanding of litter decomposition. Bacteria are ubiquitous in aquatic systems and some populations have the potential to colonize appropriate substrates rapidly. Addition-ally, the bacterial community is generally effective at utilizing dissolved substrates over a broad range of concentrations. They are not as effective, however, at hydrolyzing structural carbohydrates rapidly, and in this regard may be outcompeted by fungi which have both the appropriate exoenzymes and growth form to attack solid substrates (Harley, 1971). Thus, one would expect rapid colonization by bacteria with a successional pattern toward a more diverse community, possibly emphasizing fungi.

Rapid colonization of litter by bacteria has been demonstrated in this study (Fig. 4) and in others (Knauer & Ayers, 1977; Rublee, 1978). Furthermore, several studies (Morrison et al., 1977; White et al.,1977) examined the development of the microbial community on allochthonous litter in a Florida estuary and found correlation between lipid synthesis activity and successional changes determined by scanning electron microscopy observations. They pointed out that prokaryotic organisms contain proportionally more phospholipids than eukaryotes. Thus, the peak of activity of ^{32}P incorporation in lipids. corresponding with the rapid increase in bacterial numbers, is suggestive of the importance of bacteria during the early stages of decomposition on both a mass and activity basis. The later peak in total lipid synthesis, as evidenced by the ^{14}C incorporation, suggests

a successional population which includes eukaryotes: likely those better able to utilize the more refractory structural carbon of the older litter material. This is not to say that bacteria are absent from older detritus. On the contrary, as material is broken up and the surface area increases. bacteria are effective colonizers of particle surfaces and may still play a role in enhancing the nutritional quality of the particles by immobilizing dissolved nutrients from surrounding water and producing additional bacterial biomass (Paerl, 1975). Thus. the successional pattern during aerobic decomposition would indicate that bacteria play important roles in both the early and later stages of decomposition, while fungi may predominate in the intermediate stages of breakdown. Lee et al. (1980) and J. E. Hobbie & J. Helfrich (pers. comm.) have noted that fungi are present in quantities equal to or greater than bacteria in decomposing *Spartina alterniflora*. Recently, Newell & Fell (1980) noted the presence of fungi in *Thalassia*, but suggested that their importance in *Thalassia* decomposition is confined to intertidal locations.

How does the litter produced by litterbags or flow-through sea-water tanks compare to natural particulate material? Generally, to collect "pure" detritus one must of necessity collect fresh or nearly fresh material. Older particulate matter is usually a mixture of detritus from various sources, inorganic material and phytoplankton (Fenchel, 1970). Nevertheless, some comparisons can be made on the limited data available from the literature and samples of natural particulate matter that we have taken in Florida and Bahamian waters.

Carbon to nitrogen ratios for natural particulate matter collected in Florida and Bahama waters ranged from 6.5 to 9.8 (Table I). These values are within the ranges reported for most marine waters (see Parsons et al., 1977). Particulate matter contained from 7.4-14.1 μg •l^{-1} protein; 31.2-86.5 μg • 1^{-1} carbohydrate, and 28.9-55.4 μg • 1^{-1} lipid. The protein concentrations are similar to those reported by Holm-Hansen (1972) and Handa et al. (1972) for stations in the Pacific Ocean, but lower than those reported for northern Atlantic and North Sea stations (Mayzaud & Taguchi, 1979; Lancelot-Van Beveren, 1980). The carbohydrate and lipid values lie within the ranges reported in three of those studies; Holm-Hansen's (1972) values were lower. It should be noted that in all these data the values include the contribution of phytoplankton. Lancelot-Van Beveren (1980) used a statistical method to separate the contribution of phytoplankton vs. bacteria/detritus and found them to be nearly equal throughout most of the year. Our data also compare reasonably well with the biochemical composition of 6-, 12-, and 36-month aged *Thalassia* detritus in flow-through sea-water tanks (Table I). As the *Thalassia* ages it becomes more similar to the particulate matter.

The microbial community on the *Thalassia* litter also exhibited characteristics similar to that found on natural particulate samples. Fenchel (1970) reported 3 x 10^9 bacteria • g dry wt^{-1} on *Thalassia* detritus of undetermined age from Biscayne Bay. Our values from the tank and litterbag samples are nearly an order of magnitude higher and the discrepancy is probably explained by our use of epifluorescent illumination and Fenchel's use of transmitted light. This allowed us to view smaller cells as well as those on the surface of detrital particles which might not have been visible with transmitted light. We also counted bacteria retained on 3.0-μm pore size Nuclepore filtered water samples from the Bahamas stations. Such filters allow the majority of the free-living bacteria to pass (Azam & Hodson, 1977) and thus the counts represent bacteria associated with particulate matter. These observations yielded values of bacteria attached to particulate matter of 4.2 x 10^9 cells • g dry wt^{-1} for surface and 16-m samples over a coral reef off Grand Bahama Island. Thus, numbers of bacteria are not greatly different among the laboratory produced detritus and natural particulate matter.

Lipid synthesis values for whole natural water samples also suggested a microbial community of similar activity to litter samples. Water incubated with label was collected on Whatman GFC filters, which should allow many of the free-living bacteria to pass through (cf. Sheldon, 1972; Azam & Hodson, 1977). Thus, the resultant uptake of label should, to some degree, reflect particle-associated lipid synthesis activity. Values for the low and high tide samples in Bimini Harbor were 2.23 × 10^6 and 4.98 x 10^6 dpm • g dry wt^{-1} for ^{14}C incorporation and 1.63 × 10^7 and 5.88 × 10^7 •g dry wt^{-1} for ^{32}P. For surface and bottom water samples at Grand Bahama the values, in dpm • g dry wt^{-1}, were 1.01 × 10^6 and 0.24 × 10^6 for ^{14}C incorporation and 1.28 × 10^7 and 1.00 × 10^7 for ^{32}P incorporation. These values lie within the ranges we found for *Thalassia* aged in tanks and litterbags.

TABLE I

Comparison of some biochemical characteristics from tank-decomposed *Thalassia* and samples of natural particulate matter: coefficient of variation in carbon and nitrogen analyses is generally $< 5\%$; for proteins, carbohydrates, and lipids ranges are given where replicates of pooled samples were analyzed.

Source of sample	Dry wt ($\mu g \cdot l^{-1}$)	Carbon (as % of dry weight)	Nitrogen (as % of dry weight)	C : N	Protein (as % of dry weight)	Carbohydrate (as % of dry weight)	Lipid (as % of dry weight)
Fresh *Thalassia* blades	-	28.20	1.90	14.8	6.10–8.10	30.80–34.8	7.14–10.58
Thalassia detritus							
6 months old	–	9.80	0.82	12.0	0.91–1.36	15.40–19.90	4.63–7.06
1 yr old	–	7.30	0.81	9.0	–	–	
3 yr old	–	6.81	0.35	19.5	0.45–0.62	0.31	0.66–0.69
Bimini Harbor							
Low tide	4.5	2.93	0.41	7.2	0.12–0.28	0.74–3.83	0.63–0.90
High tide	2.8	7.77	1.20	6.4	0.25–0.28	1.21	0.97–1.09
Grand Bahama Island							
Neritic surface water	4.28	2.83	0.36	7.9	0.28	–	1.29
Reef, 16 m	4.89	2.54	0.26	9.8	0.22–0.31	0.30–0.32	0.92–1.60
Biscayne Bay, Florida	8.53	5.06	0.83	6.1	0.53–0.55	1.87–2.11	2.47
Gulf Stream	3.87	4.47	0.58	7.7	0.12	1.41–1.64	2.27

In summary, this study has demonstrated that both litterbags and flowing sea-water tanks can produce detritus that is similar in some microbial characteristics and some gross biochemical measures to natural particulate matter. We have not demonstrated an exact chemical similarity, and a more complete analysis would be necessary to do so. Litterbags have been severely criticized as a method for the study of decomposition. Certainly some of this criticism is justified; litterbag loss rates may not be equivalent to field decomposition rates. However, some of the qualitative changes in litterbag or tank incubated detrital material do appear to be similar to those which yield natural particulate matter and therefore these systems are adequate for production of food sources to measure detrital consumption and utilization.

REFERENCES

AZAM, F. & R. E. HODSON, 1977. Size distribution and activity of marine microheterotrophs. Limnol. Oceanogr., Vol. 22, pp. 492-501.

BARNES, H. & J. BLACKSTOCK, 1973. Estimation of lipids in marine animals and tissues: detailed investigation of the sulphophosphovanillin method for 'total' lipids. J. Exp. Mar. Biol. Ecol., Vol. 12, pp. 103-118.

BLIGH, E. G. & W. J. DYER, 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol., Vol. 37, pp. 911-917.

BOYSEN-JENSEN, P., 1914. Studies concerning the organic matter of the sea bottom. Rep. Danish Biol. Stn., Vol. 22, pp. 1-39.

BURKHOLDER, P. R., L. M. BURKHOLDER & J. A. RIVERO, 1959. Some chemical constituents of turtle grass, Thalassia testudinum. Bull. Torrey Bot. Club, Vol. 86, pp. 88-93.

DAWES, C. J., K. BIRD, M. DURAKO, R. GODDARD, W. HOFFMAN & R. MCINTOSH, 1979. Chemical fluctuations due to seasonal and cropping effects on an algal-seagrass community. Aquat, Bot., Vol. 6, pp. 79-86.

DuBois, M., K. A. Gues, J. K. HAMILTON, P.A. REBERS & F. SMITH, 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem., Vol. 28, pp. 350-356.

FENCFIEL, T., 1970. Studies on the decomposition of organic detritus derived from the turtle grass Thalassia testudinum. Limnol. Oceanogr., Vol. 15, pp. 1420.

HANDA, N,, K. YANAGI & K. MATSUNAGA, 1972. Distribution of detrital materials in the western Pacific Ocean and their biochemical nature. In, Detritus and its role in aquatic ecosystems, edited by U. Melchiorri-Santolini & J. W. Hopton, Mem. 1st. Ital. Idrobiol. Dort Marco de Marchi Pallanza Italy, Vol. 29, Suppl., pp. 53-71.

HARLEY , J. L., 1971. Fungi in ecosystems. J. Appl. Ecol., Vol. 8, pp. 627-642.

HOBBLE, J. E., R. J. DALEY & S. JASPER, 1977. Use of nuclepore filters for counting bacteria by

fluorescence microscopy. Appl. Environ. Microbiol., Vol. 33, pp. 1225-1228.
HOLL AND, D. L. & P.A. GARROTT, 1971. A micro-analytical scheme for the determination of protein, carbohydrate, lipid, arid RNA levels in marine invertebrate larvae. J. Mar. Biol. Assoc. U.K., Vol. 51, pp. 659-668.
HOLM-HANSEN, O., 1972. The distribution and chemical composition of particulate material in marine and fresh waters. In, Detritus and its role in aquatic ecosystems, edited by U. Melchiorri-Santolini & J. W. Hopton, Mem. 1st. Ital. Idrobiol. Dott Marco de Marchi Pallanza Italy, Vol. 29, Suppl., pp. 37-51.
KNAUER, G. A. & A. V. AYLRS, 1977. Changes in carbon, nitrogen, adenosine triphosphate, and chlorophyll a in decomposing Thalassia testudinum leaves. Limnol. Oceanogr, Vol. 22, pp. 408-414. LANCELOT-VAN BEVEREN, C., 1980. A statistical method to estimate the biochemical composition of phytoplankton in the Southern Bight of the North Sea. Estuarine Coastal Mar. Sci.. Vol. 10, pp. 467-478.
LEE. C. W., R. W. HOWARTH & B. L. HOWES, 1980. Sterols in decomposing Spartina alterniflora and the use of ergosterol in estimating the contribution of fungi to detrital nitrogen. Limnol. Oceanogr., Vol. 25, pp. 290-303.
MAYZAUD, P. & S. TAGUCHI, 1979. Spectral and biochemical characteristics of the particulate matter in Bedford Basin. J. Fish. Res. Board Can., Vol. 36, pp. 211-218.
MORRISON, S. J., J. D. KING, R. J. BOBBIE, R. E. BE(HTCH D & D. C. W11111. 1977. Evidence for microfloral succession on allochthonous plant litter in Apalachicola Bay. Florida. U.S.A. Mar. Biol., Vol. 41, pp. 229-240.
NEWEL , S. Y. & J.W. FELL, 1980. Mycoflora of turtlegrass (Thalassia testudinum KonigI as recorded after seawater incubation. Bot. Mar., Vol. 22, pp. 265-275.
ODUM, E. P. & A.A. DE LA CRUZ, 1967. Particulate organic detritus in a Georgia salt marsh-estuarine ecosystem. In, Estuaries, edited by G. H. Lauff, AAAS Publication No-. 83, pp. 383-388.
ODUM, W. E., P. W. KIRK & J. C. ZIEMAN, 1979. Non-protein nitrogen compounds associated with particles of vascular plant detritus. Oikos, Vol. 32, pp. 363-367.
OLAH. J., 1972. Leaching, colonization, and stabilization during detritus formation. In, Detritus and its role in aquatic eco.systems, edited by U. Melchiorri-Santolini & J. W. Hopton. Mem. 1st. Ital. Idrobiol. Dott Marco de Marchi Pallanza Italy, Vol. 29, Suppl., pp. 105-127.
P11 RE , H. W., 1975. Microbial attachment to particles in marine and freshwater ecosystems. Microb. Ecol., Vol. 2, pp. 73-83.
PARSONS, T. R., M. TAKAHASHI & B. HARGRAVE, 1977. Biological oceanographic processes. Pergamon, Oxford, pp. 58-64.
PETERSON, B. J., R. W. HOWARTH, F. LIPSCHULTZ & D. ASHENDORF, 1980. Salt marsh detritus: an alternative interpretation of stable carbon isotope ratios and the fate of Spartina alterniflora. Oikos, Vol. 34, pp. 173-177.
ROMAN, M. R.. 1977. Feeding of the copepod Acartin tmisa on the datom Nitzschia closterinm and brown algae (Elkus vesiculosas) detritus. Mar. Biol.. Vol. 42, pp. 149--155.
RUBLEE P.. 1978. Bacteria in a North Carolina salt marsh: standing crop and importance in the decomposition of Spartina alternillora. Ph.D. thesis, North Carolina State University, Raleigh, 80 pp. SIII I
SHELDON. R. W., 1972. Size separation of marine seston by membrane and glass filters. Limnol. Oceanogr., Vol. 17. pp. 494--498.
WHITE D.C.. R. J. BOBBIE, S. J. Mokkison, D. K. Oosii Rtioo. C. W. IAN t oit S.: I). A. MEI 11.12, 1977. Determination of microbial activit). of estuarine detritus by relative rates of lipid biosynthesis. Limnol. Oceanogr. Vol. 22, pp. 1089 1099.
ZIEMAN, J. C.. 1968. A study of the growth and decomposilion of the sea-grass Thalassul testudinum. VI S. thesis. University of Miami, Coral Gables, 50 pp.