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A Method for Estimating the Biomass of Bacteria in Aquatic Sediments and Its Application to Trophic Studies

D. J. W. Moriarty
Departments of Microbiology and Zoology, University of Queensland, St. Lucia, Queensland

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Summary. A method is described for estimating the biomass of bacteria in aquatic sediments by an enzymic measurement of D-lactic acid derived from hydrolysis of muramic acid. A correlation is shown between muramic acid and biomass. The Gram-negative rod bacteria contain about 20 μg muramic acid/mg carbon whereas the Gram-negative or Gram-variable pleomorphic and Gram-positive bacteria contain about 100 μg muramic acid/mg carbon. Thus to measure biomass, the relative proportions of these bacteria in the population must be measured. The method is limited at present to sediments in which the biomass of blue-green algae is insignificant compared to bacteria. It is particularly suited to measuring the biomass of bacteria in sediments ingested by animals. This is illustrated by analysis of the gut contents of two deposit-feeders, a mullet (*Mugil cephalus*) and a prawn (*Metapenaeus bennettae*), in which it is shown that bacteria are an important component of their diet.

Introduction

Bacteria have an important trophic role in benthic ecosystems, especially in inshore areas. Much of the primary production from macrophytes such as mangroves and sea-grasses becomes available to higher trophic levels after it has been utilised by bacteria (Jorgensen, 1966; Newell, 1970; Fenchel, 1971; Mann, 1972). Studies such as these mostly suggest, rather than state explicitly, that bacteria are the major source of food for deposit feeders. Quantitative studies have been hampered by the difficulty of measuring the biomass of bacteria in sediments. The best available technique has been direct counting with fluorescence microscopy, but this suffers from some drawbacks and inaccuracies (Wood, 1953; Zhukova, 1963; Fenchel, 1971; Hobbie *et al.*, 1972; Dale, 1974; Jones. 1974). The ATP[1] method for measuring total microbial biomass (Holm-Hansen, 1973) is not applicable to the analysis of stomach contents because some ATP may be hydrolysed after ingestion. In the sediments, however, estimates of total microbial biomass may provide an independent check of bacterial biomass measured by the method described below.

While investigating feeding in the mullet *Mugil cephalus* (L.) and the prawn *Metapenaeus bennettae* (Racek and Dall) a method was required to measure the amount of bacteria in terms of carbon, in their food (Moriarty, 1975). Millar and Casida (1970) suggested that muramic acid in soils might be used as a measure

1 *Abbreviations.* ATP = adenosine triphosphate; LDH = lactic acid dehydrogenase; NAD = nicotinamide adenine dinucleotide; tris = tris(hydroxymethyl)aminomethane; C = carbon; M = muramic acid.

of the bacterial population. They showed that within each of the two major types of bacteria, the Gram-negative and the Gram-positive, the amount of muramic acid was closely related to bacterial biomass. This would be expected because muramic acid is an important component of peptidoglycan in the cell wall and occurs nowhere else in the cell. Muramic acid is present in the cell wall of all prokaryotes (bacteria and blue-green algae), except the halophilic bacteria (Rogers and Perkins, 1968).

This suggested a method for measuring bacterial biomass, and a technique has been developed which has given useful results in studying the estuarine sediments ingested by the prawns and mullet. The determination of muramic acid is adapted from Tipper (1968) and strictly speaking, it is D-lactic acid rather than muramic acid which is measured. Muramic acid, liberated from the cell wall by acid hydrolysis, is hydrolysed with alkali to release D-lactate. The lactate is oxidised to pyruvate by D-LDH with the concomitant reduction of NAD which is measured in a spectrophotometer. A disadvantage of the method is that it cannot be used in the presence of large numbers of blue-green algae (see "Discussion").

Methods

Bacteria. Bacteria, obtained from the culture collection in the Department of Microbiology, University of Queensland, were grown on a shaker at 28° or 37° (depending on their optimum temperature). The medium contained peptone 10 g, yeast extract 5 g, NaCl 5 g and distilled water 1 l; pH was 7.5. Bacteria, isolated on agar plates from sea-grass flats, were grown in Zobell's medium 2216E (Zobell, 1946: medium 2216 with 1% yeast extract) at 28°. Cultures were harvested in a centrifuge at 20°, washed twice in 0.5% (w/v) NaCl, or filtered sea-water if they were grown in sea-water medium, and then freeze-dried.

Sediments. Stomach contents were removed from 10 mullet soon after capture on a sea-grass (*Zostera capricorni*) flat in Moreton Bay, Queensland. Sediment was collected from the same area by coring to a depth of about 1.5 cm. About 50 prawns, caught in the Brisbane River estuary at night, were brought on dry ice to the laboratory where their proventriculus contents were removed and combined. A sample of the top 1 cm of mud just below the low water level was collected from the same area. All sediments and gut contents were kept on dry ice after collection and then freeze dried before analysis.

Muramic Acid. The procedure was modified from that of Tipper (1968) [see also Hohorst (1963) for further details]. Reagents: HCl: 3 M. NaOH: 5 M. Na_2HPO_4: 0.5 M. Buffer containing glycine 0.5 M and hydrazine 0.4 M, pH 9.0 (solid NaOH was used to adjust pH). NAD: 20 mg/ml in tris-HCl pH 7.5, 0.02 M. D-lithium lactate: 105.5 µg/ml (*i.e.* 100 µg/ml D-lactate). D-LDH: 1 mg/ml (diluted in tris buffer from stock 5 mg/ml) from Boehringer, Mannheim, W. Germany.

A weighed sample was hydrolysed with HCl (about 1 ml per 200 to 500 mg sediment or 10 to 20 mg bacteria) in a sealed tube at 100° for 6 hrs. After adding phosphate (0.2 ml per ml HCl) the sample was neutralised with hydroxide and centrifuged or filtered to remove particulate matter. At this stage portions of samples were put aside for assay of D-lactate from sources other than muramic acid. More hydroxide was added to raise the pH to 12.5 and the solution was incubated at 35° for 2 hrs. The pH was then reduced to about 8 to 8.5 with HCl and if necessary, it was centrifuged again. All samples were stored at −15° until assayed for lactate.

D-Lactate Assay. To small test tubes in crushed ice the following were added consecutively with mixing after each addition; glycine buffer (kept at 4°) 1 ml; sample 100 µl; NAD 100 µl; LDH 40 µl. Sample volumes were adjusted to contain about 1 to 12 µg D-lactate/100 µl. If they were more dilute than this, up to 300 µl was added to the reaction tube and buffer volume was correspondingly reduced. A duplicate set of sample reaction tubes was set up, but no LDH was added. To test for inhibition of D-LDH, a replicate assay should be carried out with a smaller volume of sample (*e.g.* half that used in the first assy tube). Standards

containing 0, 10, 20, 40, 60 and 120 µl of D-lactate solution were prepared, with complementary alterations to the buffer volume. Replicates were made of all reaction mixtures. After incubation, the reaction tubes were transferred to ice. Extinction at 340 nm was measured in a 1 cm × 1.5 ml cuvette, after allowing 1 minute for the solution to warm up.

The standard curve was almost linear over this range (0–12 µg D-lactate). For each sample, the E_{340} value without LDH was subtracted from that with LDH and the difference read off the standard curve to give µg D-lactate per sample. From this, muramic acid was calculated as shown in the following example for mud from mullet stomachs.

$\Delta E_{340} = 0.04 \pm 0.05 \equiv (0.9 \pm 0.1)$ µg D-lactate; 0.454 g sediment was hydrolysed; final volume 3.1 ml; 0.1 ml in each reaction tube. Molecular weight of muramic acid = 251, and of lactate = 89. $0.9 \times (3.1/0.1) \times (1/0.454) \times (251/89) = 170 \pm 20$ µg muramic acid/g sediment.

Carbon. Carbon was determined using Pregl's dry combustion method (Pregl, 1951; Steyermark, 1961). Magnesium perchlorate was used to absorb water and manganese dioxide for nitrogen oxides. Potassium persulphate was found not to be necessary to give maximum release of carbon (Newman and Tomlinson, 1964). A balance weighing to 0.01 mg was used. The furnace was operated at about 900°. Sediment samples were combusted with and without prior treatment with 1 M HCl to remove inorganic carbon.

Chlorophyll. Chlorophyll *a* was extracted with 90% (w/v) acetone at 4° for 6 hrs. It was estimated as described by Wetzel and Westlake (1969), using a value of 84 for the specific absorption coefficient.

ATP. The method for extraction of ATP was based on that of Holm-Hansen (1972) and measurement of ATP on that of Stanley and Williams (1969). Centrifuge tubes containing 9 ml tris buffer (0.02 M, pH 7.5) and 1 ml potassium phosphate buffer (0.01 M, pH 7.5) were heated in a boiling water bath. Freshly collected sediment was mixed in a small beaker and about 500 mg added to each of twenty tubes. To ten of these tubes, 0.5 ml of an overnight culture of bacteria (isolate no. 3, Table 1) was then added. A further ten replicate extractions were made of the bacteria only. After 5 min in the boiling water bath, tubes were cooled in tap water, centrifuged for 1 min and the supernatants frozen until assayed for ATP. The pellet of sediment was freeze-dried and weighed.

Assay solutions contained 1 ml potassium phosphate buffer (0.01 M, pH 7.5) with magnesium sulphate (4 mM) and 0.1 ml sample or standard ATP in tris buffer. A Nuclear Chicago Scintillation Counter Mark II was used out-of-coincidence.

Firefly extract (20 µl) was added as the print-out of the preceeding sample commenced. Counting was carried out for 0.4 min. Inhibition of the luciferase by material in the samples was monitored by assaying replicates of the sediment samples with an additional 20 ng ATP. Firefly extract and ATP were purchased from Sigma Chemical Co. Ltd., St. Louis, USA.

Composition of Bacterial Populations. Sediments were diluted in sterile sea-water, in ten-fold steps, with vortex mixing at each step. Aliquots of 0.1 ml from the 10^4 and 10^5 fold dilutions were plated on 2216E agar and incubated at 24° for 1 week. Smears from plates having about 25–50 colonies, were Gram stained and examined microscopically. The diluted sediment samples were placed in a boiling water bath for 5 min and further aliquots plated out to count the number of spores.

Blue-Green Algae. About 1 mg samples of freeze-dried sediment were weighed to the nearest 10 µg on microscope slides. Distilled water was added and all blue-green algal cells counted.

Results

Relationship between Muramic Acid Content and Biomass

According to the ratio of muramic acid to total cellular carbon, the bacteria occurring, or likely to occur in aquatic sediments, may be divided into three groups (Table 1). The Gram-negative rods analysed here all have about 20 µg M/mg C. The Gram-negative or Gram-variable, pleomorphic bacteria seem to have a more diverse relationship between muramic acid and carbon. On average however, they have about the same ratio as Gram-positive bacteria and so all are considered as one group with 100 µg M/mg C. As the actinomycetes were not

Table 1. Muramic content of various bacteria

Type of bacteria	μg muramic acid/mg carbon		Number of determinations
	Mean	Range	
Gram-negative rods			
Escherichia coli	20	17–22	4
Enterobacter aerogenes	20	17–22	2
Proteus vulgaris	20	19–20	2
Serratia marcescens	22	20–25	2
Pseudomonas aeruginosa	23	23–23	3
Isolate No. 5	17	—	1
Isolate No. 6	19	15–23	2
Average	20		
Gram-negative or variable, pleomorphic			
Arthrobacter globiformis	85	80–90	2
Isolate No. 1	110	approx. ± 20[a]	4
Isolate No. 2	110	approx. ± 20[a]	4
Isolate No. 3	110	approx. ± 20[a]	4
Isolate No. 4	60	approx. ± 20[a]	4
Gram-positive			
Bacillus subtilis	106	104–110	3
Micrococcus aurantiacus	115	107–123	2
Average	100		
Actinomycetes			
Streptomyces venezuelae	52	52–52	2

[a] Determined graphically: see text.

found in the sediments studied here, only one representative is included, which is shown to have a muramic acid to carbon ratio intermediate between the first two groups.

An apparent inhibition of the D-LDH was noticed in a few samples. When successively smaller volumes of sample were assayed, the amount of D-lactate per unit weight of sample increased to a maximum. This was observed with the Gram-negative pleomorphic bacteria isolated from the sea-grass flat (numbers 1–4, Table 1). A graph of μg M/mg C against volume assayed was plotted and extrapolated to zero volume, to give the mean figures quoted in Table 1. The amount of D-lactate present at low levels of dilution was near the lower limit for detection by this method, hence errors were large. An attempt to remove the interference with an ion exchange resin was unsuccessful.

A formula for calculating biomass from muramic acid derived from the data in Table 1 is:

$$C = \frac{M}{20n + 100p}.$$

Where C = carbon (mg), M = muramic acid (μg), n = proportion of Gram-negative rod bacteria and p = proportion of Gram-positive and Gram-negative or variable

pleomorphic bacteria ($n+p=1$). Muramic acid from other microorganisms and spores is neglected in this formula because they were uncommon in the sediments studied here (see "Discussion").

Effect of Hydrolysis on D-Lactate Recovery

Sediment samples from the stomachs of mullet have been hydrolysed with acid for periods of 4, 6 and 8 hrs. The variation in amount of D-lactate thus obtained was no greater than the variation between replicate samples hydrolysed for 6 hrs. The range of variation is about 10% for samples containing *ca.* 100 to 500 μg M/mg dry weight.

Extending the time of alkaline hydrolysis from 2 to 4 hrs did not alter the amount of D-lactate present.

The presence of sediment had no effect on the recovery of D-lactate (muramic acid) from *B. subtilis* cells added to it (Table 2).

Table 2. Recovery of muramic acid from *Bacillus subtilis* cells added to sediment from a sea-grass flat

Sediment and *B. subtilis* were mixed before acid hydrolysis. The range of variation for 3 determinations is shown.

Sample	Weight (mg)	Muramic acid (μg/g)	μg M in sample
Sediment	488	21 (±10)	10 (±5)
B. subtilis	2.29	41000 (±2000)	94 (±4)
Combined (expected recovery)			104 (±9)
Combined (actual recovery)			103 (±10)

Controls for Other Sources of D-Lactate

No D-lactate was found in samples which were hydrolysed in acid only. Sediments from sea-grass flats, mullet stomach and prawn proventriculus were tested.

Application to Trophic Studies

Substantial quantities of muramic acid were found in the sediments ingested by the mullet and prawns (Table 3). Muramic acid was also present in the sediments in the localities from which these animals were caught, but as the amounts were small and near the lower limit for detection, errors were proportionately large. The proportion of Gram-negative rods to Gram-negative pleomorphic and Gram-positive bacteria were determined from 4 plates with a total of 126 colonies, for the sea-grass flats and 2 plates (58 colonies) for the Brisbane River. Spores numbered about 1 per 6000 colonies in both localities.

The bacterial biomass thus derived is compared to algal biomass and total organic carbon in Table 4. In the sediments from both localities, bacterial biomass is greater than algal. It should be noted, however, that the algae (mainly diatoms) occur mostly on the surface, whereas these cores were taken to a depth of about 1.5 cm. Blue-green algae numbered less than 1×10^4 cells/g dry weight of the sediment in the stomachs of these mullet. The mullet have concentrated

Table 3. Muramic acid in sediment from various sources and equivalent bacterial biomass

The range of variation in assay of muramic acid is shown. The proportion of Gram-negative rods in the bacterial population ingested by the animals is assumed to be the same as that in the sediments of the locality of capture.

Sediment source	Muramic acid μg M/g ash	Proportion of gram-negative rods	Bacterial biomass mg C/g ash
Sea-grass flat	20±10	0.70	0.4
mullet stomach	170±20		3.8
Brisbane River	80±20	0.86	2.6
Prawn proventriculus	4415±200		142

Table 4. Examples of the application of the muramic acid method for measuring bacterial biomass

Bacterial biomass was measured as in Table 3. Algal carbon was measured as chlorophyll $a \times 30$.

Sediment source	Total organic	Algal	Bacterial	Bacterial C
	mg C/g ash			% of total C
Sea-grass flat	2.4	0.2	0.4	16
Mullet stomach	16.4	6.3	3.8	23
Brisbane River	19.4	1.0	2.6	14
Prawn proventriculus	456	0	142	31

Table 5. Comparison with ATP method for microbial biomass

Sediment, mainly sand, was from the top 1 cm of two different sea-grass flats. Total microbial C was calculated as ATP × 250; algal C as chlorophyll $a \times 30$; bacterial C as muramic acid/44. The range of variation due to experimental error is shown, except for ATP, where the standard error of 10 replicates is given.

Sample number	Total organic C	Algal C	Bacterial C	Total microbial C
	mg C/g ash			
1	2.7 (±0.1)	0.24 (±0.01)	0.47 (±0.25)	0.5 (±0.05)
2	2.1 (±0.1)	0.10 (±0.01)	1.3 (±0.3)	1.2 (±0.1)

the organic carbon, including bacteria by about 1 order of magnitude from the sediment. The prawns show much greater selectivity. They have selected not only organic from inorganic matter, but organic matter containing a high proportion of bacteria.

Comparison of ATP and Muramic Acid Methods

Two samples of sea-grass sediment, collected at different times from that in Table 4, have been analysed for ATP as well as the other components (Table 5).

The estimate of total microbial biomass thus obtained is only a little less than the combined algal and bacterial biomass. Addition of cultured bacteria containing a known amount of ATP to the sediments showed that ATP recovery was probably complete. Inhibition of the luciferase reaction by material in the extracts did not exceed 5%.

Discussion

Correlation of Muramic Acid with Biomass

The method reported here for determining bacterial biomass is dependent on a reasonably close correlation between muramic acid and total carbon in a cell. Since muramic acid has a key role in the cell wall and occurs nowhere else (Rogers and Perkins, 1968), its amount in the cell will be directly related to cell size and thus carbon. Some variation is expected of course, in the constituents of a cell during growth, but this would not be great. What does matter though, is whether the ratio of muramic acid to carbon varies greatly between different bacteria. From the data presented here, it seems that the bacteria can be divided into two main groups, those with 20 μgM/mgC and those with 100 μgM/mgC. The variability encountered with the Gram-negative or variable, pleomorphic bacteria may well be due to experimental problems rather than an actual variation in proportions of muramic acid. Some of these bacteria are characterised in young colonies, by the presence of rods and in older colonies by rods and cocci of variable size. This suggests they may be *Arthrobacter* spp.

A study of muramic acid levels with cell-cycle in the *Arthrobacter*-like organisms and methods for purifying D-lactate are under investigation. This present problem does not detract from the usefulness of the method for showing whether bacteria are an important component in the diet of deposit feeders. For example in the sediment from the mullet stomachs, the uncertainty in muramic acid content of the pleomorphic bacteria contributes at the most, a variation of ± 0.5 mgC/g ash.

Escherichia coli and the other Gram-negative bacteria were selected partly for comparison with the data of Millar and Casida (1970). In terms of dry weight, they found about half the amounts reported here of muramic acid in the Gram-negative bacteria and about one-third the amounts in Gram-positive bacteria. There are many differences in technique which might contribute to the different results.

Factors Affecting D-Lactate Measurement

Tipper (1968) has shown that losses during assay of D-lactate are less than 7% and racemisation less than 3%. He demonstrated complete recovery of muramic acid after acid hydrolysis for 18 hrs with other cell wall constituents. Although 4 hrs hydrolysis in acid seems to be sufficient, 6 hrs has been chosen here to ensure complete hydrolysis.

Alkaline hydrolysis at 37° for 2 hrs was shown by Tipper (1968) to be sufficient for maximum release of D-lactate from muramic acid.

Sediment does not adsorb or otherwise depress the recovery of muramic acid or D-lactate from bacteria as shown by the results reported here.

The possible inhibition of D-LDH by material in the extracts needs to be checked as described above.

Controls for Other Sources of D-Lactate

Experiments showing that no D-lactate was formed until extracts were hydrolysed in alkali, provide strong presumptive evidence that muramic acid is the only source of D-lactate. Tipper (1968) found that no D-lactate was released from other cell wall constituents. He showed also that the amounts of muramic acid measured in cell walls of various bacteria as D-lactate were similar to the amounts detected directly by amino acid analyser. As D-lactate has been reported in various organisms (*e.g.* Warburg *et al.*, 1957; Camien *et al.*, 1963; Long and Kaplan, 1968) the control described here should be used. In the examination of sediments or gut contents, it is unlikely that D-lactic acid from sources other than muramic acid would be present in concentrations of the same order of magnitude. Where actual amounts of D-lactate per unit weight of sample have been given (*e.g.* Camien *et al.*, 1963), they are several orders of magnitude lower than those derived from muramic acid in cell walls. Although Camien *et al.* (1963) argued to the contrary, it seems from later work (especially Tipper, 1968), that the D-lactate they found in bacterial cell walls was derived from muramic acid.

Composition of Bacterial Populations

The proportions of the main groups of bacteria shown in Table 1 need to be determined for the environment under study. For greatest accuracy in a heterogeneous population of bacteria, the main types should be isolated, characterised and their ratio of muramic acid to carbon measured. The simple counting procedure used here is probably sufficient for most purposes. It is assumed that the ratio of numbers of heterotrophs which form colonies on plates, is in fact equivalent to the ratio of total biomass of the different types of bacteria. The reasonably close correlation between total microbial biomass (ATP) and combined bacterial and algal biomass (Table 5) would suggest that this assumption may be justified. Further work is needed to check the validity of this assumption. Studies of aquatic and sediment bacterial populations in other localities (Wood, 1967; Hodgkiss and Shewan, 1968; Stevenson *et al.*, 1974) indicate that Gram-positive bacteria may be more numerous than found here. At this stage a detailed study of bacterial populations has not been undertaken and it is possible that estimates of Gram-positive bacteria in the present study were low and thus the biomass from muramic acid data has been overestimated. If Gram-positive bacteria made up 20% of the population in the sea-grass mud flat, for example, the bacterial biomass in the mullet stomachs would be 3.0, rather than 3.8 mgC/g ash. In other words, uncertainty of the exact composition of the bacterial population does not greatly influence the usefulness of this method.

Simple methods for characterising bacterial populations using selective media (Pratt and Reynolds, 1974) may be useful here. Crystal violet cannot be used for counting marine Gram-negative bacteria because, unlike soil bacteria, their growth is inhibited by it (Pratt and Reynolds, 1973).

The formula given here is applicable to environments were bacterial spores and blue-green algae are uncommon. If a locality is being studied in which it is found that spores are numerous, an allowance may be made for them in the formula since they contain about 3.5 times more muramic acid than Gram-positive bacteria (Millar and Casida, 1970). Obligate anaerobic bacteria and their spores are probably uncommon in marine muds (Wood, 1953).

Blue-Green Algae

Blue-green algae contain muramic acid in their cell walls (Rogers and Perkins, 1968). The cell size and thickness of their peptidoglycan layer is generally greater than that of the common Gram-negative bacteria in water (Carr and Whitton, 1973). As a rough estimate, blue-green algae may contain 500 times more muramic acid per cell. Calculation shows, therefore, that when blue-green algal cells number less than about 10^7/g sediment, their muramic acid contribution would be negligible (about 2 μg M/g).

Comparison with Other Methods

Direct counting of bacteria is probably the best alternative method with which to compare the muramic acid method. Wood (1953) counted about 10^9 bacteria/ml in estuarine mud. Dale (1974), using direct counts to study bacteria in intertidal sediments of Nova Scotia, has found from 1.17×10^8 to 9.97×10^9 bacteria/g dry sediment. Assuming the dry weight of each bacterial cell to be 2.2×10^{-13} g, he has transformed his data to biomass. The above figures become 2.6×10^{-2} mg to 2.2 mg/g dry sediment or about 1.5×10^{-2} to 1.0 mg C/g dry sediment, which is in good agreement with the data presented here. Dale comments that his estimates may be low because of losses in the technique and because only objects clearly distinguishable as bacteria were counted. In addition, he faced a considerable statistical problem in counting about 500 bacteria in a population of 10^8 to 10^{10}/g.

Bacteria can vary by more than one order of magnitude in size (Luria, 1960), so converting from numbers to biomass is not accurate, unless counting is made even more tedious by estimating size as well. The advantages of the muramic acid method over counting are that bacteria do not have to be removed from surfaces on which they might be adsorbed; they are readily distinguished from inert particles and micro-flagellates, and biomass is estimated directly.

The muramic acid, or more precisely D-lactic acid, is presumed to occur in living cells only (at least near the surface of the sediments). It is assumed that most bacteria either divide or are eaten, rather than die, or if they die, autolysis and decomposition by other bacteria rapidly degrade the cell walls. This assumption is difficult to test, but there are reports that the surface layers of the sediments are turned over constantly by the deposit-feeding animals (Odum, 1970; Whitlatch, 1974). Ciliates are presumed to be active in keeping bacteria in the growth phase (Lackey, 1967).

That the muramic acid method described here provides a reasonable estimate of bacterial biomass is substantiated by the comparison with total microbial and algal carbon (Table 5). If much muramic acid were derived from cell wall fragments, a large discrepancy would be expected between the results of these two methods. Biomass of protozoa is unknown, but is included by the ATP method for total microbial biomass. It is unlikely to be as high as the algal or bacterial biomass. The ATP method probably provides a minimum estimate of bacterial biomass, since losses during processing can occur easily. Cells in a dormant or resting state may contain lower amounts of ATP. The muramic acid method provides a maximum estimate, as some cell wall fragments may well be present. For studying gut contents, the ATP method cannot be used except to provide a minimum figure for microbial biomass. The muramic acid method

was developed for, and is particularly suited to, measuring bacterial biomass in gut contents. Losses are unlikely to occur during the initial stages of digestion and bacteria are present in large numbers in gut contents of deposit-feeders.

Application

The large amount of muramic in the gut contents of the animals investigated here shows that bacteria are important in their diet. There were less than 1×10^4 cells of blue-green algae per g sediment in the mullet gut and thus all the muramic acid is bacterial. In the sediments from the localities where the animals were caught, the concentration of muramic acid was near the lower limit of sensitivity of the assay. Errors, therefore, were large in proportion. Future improvements in the procedure may enable measurements of smaller quantities, thus making the method more widely applicable.

As mentioned above, a detailed study of the bacterial populations in these sediments was not undertaken. The conversion of muramic acid values to biomass is, therefore, an approximation (Table 3). In these prawns, bacteria and detritus were the only food. It has been found in prawns from a similar area, that muramic acid relative to ash in the intestine is about 60% of the amount in the proventriculus, which indicates that bacterial cell walls are digested (Moriarty, 1975). This conclusion is supported by laboratory studies with ^{14}C-labelled bacteria (Moriarty, 1975). Algae (mainly diatoms) as well as bacteria are the main food items in this group of mullet.

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Dr. D. J. W. Moriarty
Division of Fisheries and Oceanography
C.S.I.R.O.
Box 120
Cleveland, Queensland 4163, Australia