# Marine bacteria — more useful than we think?

Bacteria ... we are all familiar with these organisms that cause a long list of human maladies, ranging from infected cuts and scratches to bubonic plague. Yet, as agents of decay, they perform a vital role in the biological processes on this planet, recycling essential elements like carbon, nitrogen, and phosphorus from the dead to the living.

**Measuring bacterial productivity in marine sediments.**

These tiny primitively organized cells have successfully invaded a wide range of habitats on land and sea. The same process of bacterial fermentation that makes smelly compost heaps and curdled milk also produces cheese, yoghurt, and vinegar. Having, as they do, the ability to encase themselves in protective cysts to withstand harsh environmental conditions, bacteria have a toughness that matches their ubiquity.

Bacteria form significant links in food chains — for example, in the rumens of sheep and cows bacteria digest grass. In the sea, few animals have a fermentative gut flora, so bacteria in the open water and in the sediment digest plant material, providing food for some marine fauna.

On the land, we can put a cow into a paddock, measure its feeding rate and weight gain, and thus estimate the food conversion efficiency via bacterial decomposition. In the sea, scientists can't do this: they are testing new techniques to study the way in which plants (whether they be seagrass or algae) are broken down by bacteria — and how much of the marine animals' diet the bacteria themselves constitute. Marine researchers face many problems in dealing with an 'open' system like the sea, where material can be washed away by currents and tides.

### Improved techniques

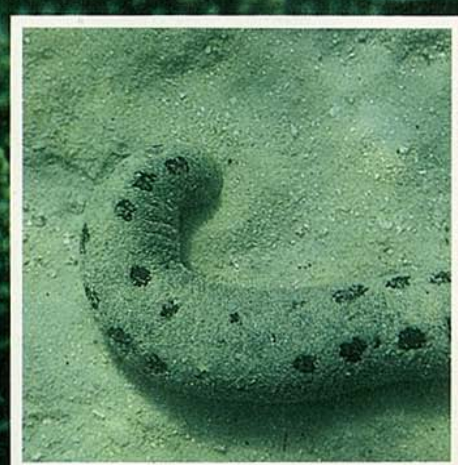
Relatively little is known about marine bacteria compared with other groups of seawater organisms. But scientists, using improved microscopic and biochemical techniques, are finding that bacteria are more abundant and active and occupy a more significant position in the marine food chain than was previously thought.

One of these scientists, Dr David Moriarty of the CSIRO Division of Fisheries Research in Cleveland, near Brisbane, has been studying the role of bacteria in seagrass beds and coral reefs in Australia and in aquaculture ponds in South-East Asia. His aim has been to quantify their role by developing and adapting techniques that accurately measure their productivity and biomass.

**Eel grass, *Zostera capricorni*, in a Moreton Bay seagrass bed.**

Classical microbiological techniques, such as culturing bacteria on agar in petri dishes or trying to count them under an ordinary light microscope, were inadequate for a number of reasons. To begin with, these bacteria are often bound in aggregates of clay and organic matter and can't be dis-

**Measuring bacterial productivity in coral reef sediments.**



**A sea slug on the floor of a Lizard Island reef.**

persed into isolated cells easily. This makes counting them inaccurate and tedious (if not downright impossible). For similar reasons, a more recent technique, in which a special fluorescence microscope reveals bacteria stained specifically with a fluorescent dye, has proved to be inappropriate for studying bacteria in some sediments.

Newer biochemical techniques can not only be used to measure biomass more accurately but can also measure dynamic processes such as growth rates of bacterial populations.

One method, developed by Dr Moriarty, is based on the characteristic biochemical structure of bacterial cell walls. Scientists determine the amount of muramic acid, which is present only in those walls, in a sample and can use it to estimate the bacterial biomass.

The site of much of Dr Moriarty's work is Moreton Bay, Qld. The seagrass flats there provide shelter for mobile animals including prawns and mullet, but very few of these feed directly on the seagrass. Under the seagrass, the sediments harbour the main food source — an invisible mass of mud-dwelling organisms including tube worms, crustacea, shellfish, fungi, protozoa, and bacteria.

Initially, Dr Moriarty analysed the gut contents of mullet and juvenile prawns to see just what parts of the mud and sand the animals digested. Using muramic acid content as an index he found that bacteria provided 7–15% of the organic carbon in the mullet samples, but they made up less than 10% of the juvenile prawns' diet. Mullet seemed to feed by filtering small bacteria-covered particles from the sediment, progressively digesting cells as the particles passed along the gut. Dr Moriarty concluded that, in muddy areas, mullet would probably assimilate sizable amounts of bacteria. Prawns were more selective in their feeding, eating bacteria only when they could not get the small shellfish and crustaceans they usually prefer — these smaller animals feed directly on bacteria in the sediment.

To measure the rate of bacterial cell production in the seagrass sediments on which the mullet and prawns lived, Dr Moriarty adapted a technique based on the process of chromosomal replication. Bacterial cells are structurally simple, having a single circular chromosome that, unlike the genetic material of higher plant and animal cells, is not contained within a nucleus.

The DNA within the chromosome is made up of four sub-units, the so-called base pairs (two purines and two pyrimidines), with each having an associated sugar group. By labelling one of the pyrimidine sub-units — thymidine — with the radioactive hydrogen isotope tritium, providing it to bacterial samples, and measuring how quickly the bacteria converted it to DNA, Dr Moriarty could determine how fast the cells were reproducing. Most bacteria seem to prefer using this external source of thymidine when it is supplied in sufficient amounts, rather than depending on the normal source inside the cell.

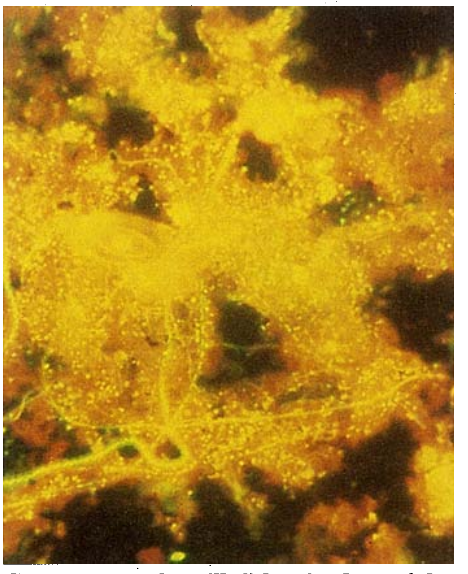
### Fermenting seagrass

Dr Moriarty and a team of eight other researchers — from the CSIRO Division of Fisheries Research, Canberra's Baas Becking Geobiological Laboratories, Brisbane's Griffith University, and Florida's State University in Tallahassee — used the tritiated thymidine technique to estimate bacterial productivity in the Moreton Bay seagrass flats. They found that, for every square metre of sediment, the production of new biomass by oxygen-using microbes and anaerobic (non-oxygen-using) microbes that cause fermentation averaged 60 milligrams of carbon per day; the production in the water above it averaged a further 23 mg. The seagrass itself had a daily productivity of more than 1000 mg of carbon per square metre.

In order to determine the total flow of carbon and nitrogen in seagrass sediments through bacterial activity, the researchers needed to know the major groups of microbes present, what they did, and their rates of growth and activity. The tritiated thymidine method did not show the full extent of bacterial growth in the sediments because groups such as the sulfate-reducing *Desulfovibrio* spp. in anaerobic sediments can't take up thymidine.

A narrow oxidized layer on the surface of the sediments and around the roots of the seagrass overlies the much deeper oxygen-deprived sediments. Undoubtedly, subsurface bacteria like *Desulfovibrio* spp. were responsible for much plant decomposition. In fact, a comparison of carbon flux measured using rates of sulfate reduction and DNA synthesis showed that bacterial productivity of the deeper anaerobic zone equalled that of the aerated top 3 mm of sediment. Dr Moriarty suggests that 90 mg of carbon per square metre per day, which includes an estimate of production by sulfate-reducing bacteria, would be close to the total figure for bacterial production over the top 50 mm of sediment.

By comparing the rate of DNA synthesis with the rate of sulfate reduction in the sediments, the team guessed that, altogether, bacteria were decomposing about 200 mg of organic carbon from every square metre of seagrass per day. As in the rumen of a cow, microbes were breaking down the plant matter into smaller digestible particles. Bacteria convert seagrass detritus to living cells with an efficiency of about 33%; the rest of the carbon disappears as carbon dioxide.



**Gut contents of a milk fish. Tiny bacterial cells show up as bright dots under the fluorescence microscope.**

Microbes may be just bugs to some, but to Dr David White, an organic chemist from Florida State University, U.S.A., they have their own distinctive biochemical 'signatures' written in the ink of their fatty acid content. Dr White's analysis of the phospholipid content of the Moreton Bay sediment samples revealed that anaerobic bacteria made up more of the microbial biomass than the oxygen-dependent forms. The biomass of bacteria was much greater than that of the meiofauna (animals less than 1 mm long, including worms, crustaceans, and nematodes).

Bacterial production was also high in the water above the sediments. Most of the bacteria there cling to particles suspended in the water and stirred up from the surface of seagrass leaves and the sediment layer by turbulence. The suspended cells are easily swept away by the tide, effectively exporting some production from the seagrass flats.

Bacteria help seagrass grow by making nitrogen available in the form of ammonia. Together with a Ph.D. student, Mr Paul Boon, Dr Moriarty estimated that the daily consumption of ammonia in the Moreton Bay seagrass community is about the same as the amount produced per day. Although bacteria and bacterial grazers rapidly recycle a considerable quantity of ammonia, the combined activities of nitrifying and denitrifying bacteria may lead to the loss of

## Cultivated prawns

The Coastal Aquaculture Development Project at Gelang Patah, Malaysia, invited Dr Moriarty to study aquaculture ponds there, with funding provided by the United Nations Food and Agriculture Organization. Manures and compost have been used in aquaculture for centuries in South-East Asia. Bacteria, protozoa, and meiofauna together mineralize organic matter, and provide — either directly or indirectly — food for the farmed fish.

Microbial activity can be a hindrance to intensive fish-farming where feed pellets are supplied directly to the fish, as the microbes tend to decompose the pellets and use up oxygen in the water. One of Dr Moriarty's aims was to determine whether that gardener's gold — chicken manure — could replace expensive pelleted feed, lowering production costs of cultured prawns.

Previous work done by Dr Gerald Schroeder of the Agriculture Research Organization, Israel, had shown that, rather than being directly eaten, manure supplied nutrients like nitrogen and phosphorus to the ponds. This promoted high bacterial productivity when complemented by an organic carbon source such as straw. The food chain of manured ponds appears to follow the route of manure–bacteria–meiofauna–prawns.

Dr Moriarty's work revealed that most of the commercial pelleted feed added to the Gelang Patah ponds was not eaten by prawns but supported bacterial growth. Meiofauna grazing on bacteria appeared to keep bacterial densities down. When Dr Moriarty stimulated bacterial growth in the ponds by adding chicken manure, the meiofauna responded very quickly, increasing tenfold 1–2 weeks after bacteria had increased. Meiofauna almost disappeared, however, when the ponds were stocked with prawns.

From these types of studies, scientists can predict more accurately the effect of supplemental feeding or of substituting manuring for pelleted food. Dr Moriarty suggests that applying techniques such as tritiated thymidine measurement of bacterial productivity in aquaculture — providing a scientific basis for pond management — could boost production of fishes like mullet or tilapia (milk-fish), which are extensively cultivated in South-East Asia.

**These figures for the Gelang Patah ponds show how important bacteria are.**

**Bacteria in fish farms**

| | biomass (mg carbon per sq. m) | production (mg carbon per sq. m per day) |
|---|---|---|
| bacteria | 2500 | 360 |
| nematodes | 67 | 10 |
| copepods | 28 | 4 |

**Pellets or manure**

| bacteria in water | numbers (million per litre) | production ($\mu$g carbon per L per hour) |
|---|---|---|
| pellet-fed ponds | 8800–26 000 | 39–87 |
| manured ponds | 12 000–13 000 | 37 |
| seagrass beds | 2800–6800 | 0·1–0·3 |
| open ocean | 500–2500 | 0·08–0·8 |

| bacteria in sediment | numbers (million million per sq. m) | production (mg carbon per sq. m per hour) |
|---|---|---|
| pellet-fed ponds | 70–2100 | 6–21 |
| manured ponds | 53–83 | 12–17 |
| seagrass beds | 43–170 | 2–7 |

**Bacterial production in manured Gelang Patah ponds proved comparable with that in pellet-fed ponds and much greater than that in seagrass beds or the open ocean.**

a lot of nitrogen from the seagrass bed. Nitrifying bacteria oxidize ammonia to nitrate, which is then changed to nitrogen gas by the denitrifying bacteria.

The researchers found evidence that insufficient nitrogen might have limited the productivity of bacteria that decompose the seagrasses and convert their organic matter into bacterial protein and other nutritionally important compounds. This would mean that seagrasses and bacteria were competing for ammonia.

Bacterial productivity followed seagrass photosynthesis — growth rates showed a daily cycle that paralleled the release of organic matter during seagrass production. Cells proliferate at 5–10 times the average rate during the morning; in an afternoon 'siesta' bacterial production slows down. No seasons, however, exist in the Moreton Bay bacterial populations — they appear to be as active in winter as they are in summer.

### Slime

The slime coats covering many bacterial cells may be as important a food for underwater deposit-feeding animals as the bacteria themselves. These coats have a range of physical structures. Scientists have suggested that the outermost layers protect bacteria by buffering them from harsh environments. In many cells from Moreton Bay bacterial colonies, the slime layers resemble those of soil bacteria.

Dr Moriarty has studied the feeding habits of the filter-feeding sea slugs (or holothurians) inhabiting the reefs of Lizard Island in the northern Great Barrier Reef. Sea slugs feed like lazy vacuum cleaners, sucking detritus from the reef floor. Considered a delicacy in some Asian countries, they form the basis of the beche-de-mer fishery.

During grazing experiments, the sea slugs, enclosed in caged areas on the reef bed, each ate about 20–50 mg of bacterial carbon per day in summer. This represents about 10–40% of the abundant bacteria produced during the warmer months.

Measurements of bacterial growth rates over the Lizard Island reefs indicate that the whole population doubles as often as once every 2–3 hours during peak production on some summer afternoons. Studies during the early seventies by Dr Yuri Sorokin of the Institute of Oceanology, U.S.S.R., indicated that bacterial production in water over coral reef flats was even higher than primary production. However, he had used a relatively inaccurate technique for measuring production, involving dark fixation of the radioactive isotope $^{14}C$, so his results appeared uncertain.

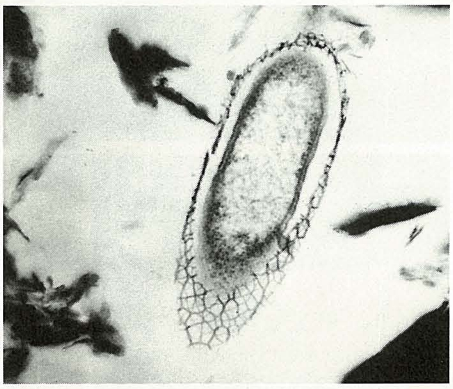
Dr Moriarty's results, obtained using tritiated thymidine to measure growth rate, indicated that the high production of bacteria over the Lizard Island reef flats was due to nutrients derived from seagrasses, algae, or coral mucus in the flats. Past studies have shown that corals do secrete large amounts of carbon-containing mucus, and bacterial populations can develop very rapidly on this.

The 'standing crop' of bacteria on the Lizard Island flats at any one time was low — a clue to the extensive grazing activity by sea slugs and other filter feeders. Earlier work had shown that up to half of the bacteria over the reefs were aggregated in particles, and that bacteria comprised about 20% of particulate organic matter. So sinking of particulate matter may cause additional losses of bacteria. But grazing is probably more important, with zooplankton and many different bottom-dwelling filter feeders eating the bacteria-laden detritus (or mucus or slime).

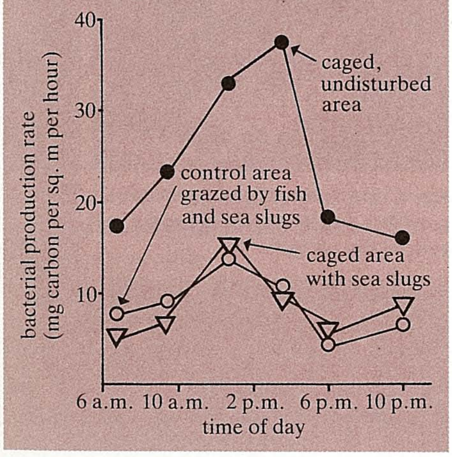
Much of the primary productivity of mangrove belts and kelp beds, as well as that of seagrass beds and coral reefs, is probably channelled through bacteria rather than plant grazers. Dr Moriarty has studied seagrass beds in Florida, U.S.A., and found that up to 50% of the net primary production passes through bacteria in mid summer. He plans to identify the fate of the 70% of the seagrass in Moreton Bay that has not yet been shown to be degraded by microbial activity, answering questions like 'how much is used by fungi, or in bacterial decomposition not yet accounted for; how much is eaten directly by grazers, and how much simply floats away?'

*Mary Lou Considine*

**A slime capsule — made up of a complex network of polysaccharides — surrounds this bacterium.**



**Time of day and bacterial productivity**




**In the absence of predators, notably sea slugs, the day-time rise in the bacterial production rate in surface sediment from a Lizard Island reef was much greater than when predators were present. Production was measured by the tritiated thymidine method.**

## More about the topic

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## Stromatolites at Shark Bay

On the other side of the continent from Moreton Bay — at Hamelin Pool, Shark Bay, W.A., — bacteria form an integral part of a different coastal feature — the sheltered pools containing relic sedimentary formations known as stromatolites. These strange structures are formed when organic material and sediment become trapped in mats of algae, made brittle and rigid by chalky calcium carbonate deposits mixed with the plant material. The resulting knob-like projections are survivors of a life-form that arose in an earlier epoch, thousands of millions of years ago, when stromatolites were abundant.

Now stromatolites are confined to a few scattered locations around the globe. Hamelin Pool contains the largest array of living specimens. They grow freely here because the very salty water deters any potential grazers.

After measuring the microbial biomass and growth rates in four different types of stromatolite mats, Dr Moriarty estimated that bacteria contributed substantially to the community's carbon cycle, using up 20–30% of the organic carbon from blue-green algae in the mats.

He found that many of the stromatolite microbes were embedded in slime layers, made up of polysaccharides produced and excreted by the cells themselves. Cells require extra fuel for synthesis of slime that can amount to many times the cellular biomass of the bacteria — they not only need extra carbon for the slime, but use extra respiratory fuel to synthesize the material. The rate of cell production estimated from tritiated thymidine measurements doesn't account for this additional carbon, so the bacteria at Hamelin Pool would be more productive than indicated by thymidine calculations.

**Stromatolites at Shark Bay, W.A.**

Bacterial biomass and productivity in sediments, stromatolites, and water of Hamelin Pool, Shark Bay, Western Australia. D.J.W. Moriarty. *Geomicrobiology Journal*, 1983, **3**, 121–33.