dissolution technique. The results reported here are based on the acid-soluble fraction.
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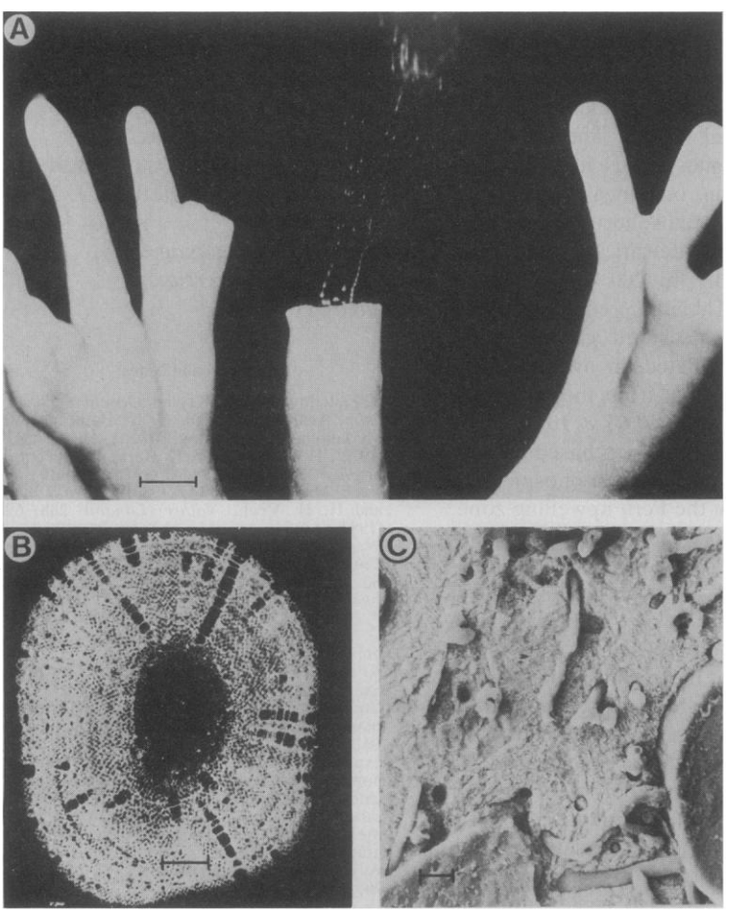
Coral Gas: Oxygen Production in *Millepora* on the Great Barrier Reef

Abstract. *Large volumes of a gas consisting of 69 percent molecular oxygen and 31 percent molecular nitrogen with trace amounts of carbon monoxide, carbon dioxide, and methane have been found trapped inside skeletons of the common hydrozoan* Millepora. *Volumes were low in the morning and reached a maximum by late afternoon. The oxygen was probably produced by the endolithic (boring) algae, with which the* Millepora *skeletons are very heavily infested. Oxygen production by endolithic algae in* Millepora *and in other substrates could influence estimates of reef productivity based on measurements of dissolved gases.*

Concentrations of dissolved gases in reef waters have been used for some time to estimate rates of calcification, respiration, and photosynthesis (*1*, *2*). In some of these studies (*2*), it has been suggested that "skeletal algae" (in fact, endolithic or boring algae) may play a major role in O_2 production, but most recent studies have tended to ignore the endoliths completely (*3*). Recent observations on the Great Barrier Reef of gas trapped inside skeletons of the common hydrozoan *Millepora* (stinging or fire coral) suggest that large amounts of O_2 can be produced by endolithic algae in reef ecosystems.

During scuba diving associated with general research on bioerosion in the central region of the Great Barrier Reef, we incidentally observed gas bubbling from freshly broken branches of several species of *Millepora*. Further observations showed that, although the volumes of trapped gas were very low or undetectable in early morning, by late afternoon a freshly broken branch of *Millepora* could exude a fine stream of bubbles for up to half a minute (Fig. 1A). The gas is under such pressure that it will bubble down and out of a broken-off branch held vertically. One of us (M.J.R.) insists that he heard the hiss of escaping gas on several occasions when a piece of coral was broken. The gas has been found in *Millepora* at all depths between −1 and −20 m.

Samples collected for analysis (*4*) showed the gas to be, by volume, 69.3 percent O_2, standard deviation = 8.0, and 30.7 percent N_2, standard deviation = 3.5 (N = 15), with traces (< 0.1 percent) of CO, CO_2, and CH_4. Although O_2 is produced by symbiotic algae (zooxanthellae) associated with coelenterate hosts (*5*), this process is unlikely to be the source of the O_2 described here. The



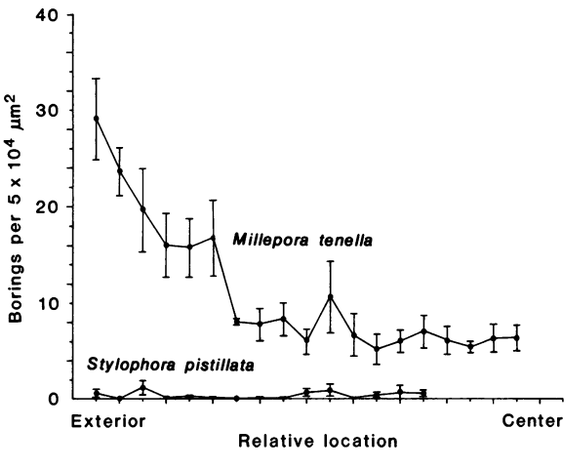


Fig. 1 (left). (A) Gas escaping from a freshly broken branch of *Millepora tenella*: sample from −10 m, Britomart Reef. Scale bar, 5 mm. (B) Thin section of *Millepora tenella* skeleton showing the porous coenosteum, corallites (dactylopores and gastropores) with complete tabulae, and the highly porous central region. Scale bar, 1 mm. (C) Scanning electron micrograph of borings in *Millepora tenella* (coenosteum: Spurr impregnation, weak acid etch). Scale bar, 20 µm. Fig. 2 (right). Relative intensity of algal borings in *Millepora tenella* and *Stylophora pistillata* (a scleractinian coral with a branching habit similar to that of *Millepora*). Samples were from −10 m, Britomart Reef. The bars represent the range of at least five measurements in all cases, from several different coral samples.

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living portion of *Millepora* colonies (the coenosarc) is restricted to the superficial layers of the skeleton (*6*). Individual polyps are sealed off from the inner part of the skeleton by a series of complete tabulae, and, although a system of organic filaments occupies minute canals in the outer layers of the skeleton, these filaments degenerate below the surface, leaving the canals open (Fig. 1B). Oxygen produced by zooxanthellae would have no direct access to the internal skeleton and therefore would not be trapped inside.

On the other hand, the architecture of the *Millepora* skeleton is such that it could easily trap gas produced internally, which may explain why this is the only coral in which this phenomenon has been found to date. The central region is occupied by large, open pores. This is surrounded by the coenosteum, in which the polyps are located. The coenosteum is highly porous but has little communication with the surrounding seawater. In addition, it has a dense peripheral rim. The *Millepora* skeleton therefore has a very high porosity but relatively low permeability. In contrast, scleractinian skeletons have higher permeabilities, and any gases produced inside them can easily diffuse into the surrounding seawater.

Millepora skeletons collected for detailed morphological analysis showed phenomenally high incidences of infestation by boring algae (Fig. 1C). Infestation was highest near the periphery, and in fresh specimens it is evidenced by an intense green rim just below the surface, but algal borings were present throughout the colonies (Fig. 2). Borehole diameters were variable (between about 6 and 20 μm). It is likely that photosynthesis by these algae produced the O_2, causing N_2 to come out of solution in the internal water to equilibrate with the O_2. The origins of the trace gases are uncertain, but the CO_2 is probably respiratory.

How much, if any, of this O_2 escapes to the surrounding seawater is unknown. Much of it could be used by the endoliths in nighttime respiration. By midmorning, however, significant amounts of gas are usually present in the skeletons. Conservative rough estimates of the amount of gas trapped inside *Millepora* colonies by late afternoon (*7*) suggest that the reservoir of O_2 at -10 m is at least 300 ml per square meter of colony vertical projection area.

This gas has been found in *Millepora* heads on the Great Barrier Reef from Lizard Island in the north at least as far south as the fringing reefs of the central region, and it is likely that the phenomenon is even more widespread. There are wider implications to the findings: if endolithic algae in coral heads can produce large quantities of O_2, their contribution in other substrates may be considerable. Boring algae are ubiquitous in shallow marine carbonates and are especially abundant in tropical regions (*8*). It is possible that they could affect the O_2 and CO_2 concentrations of overlying waters and hence influence measurements of reef productivity. Future investigators of reef dynamics who are engaged in analyses of dissolved gases should make every effort to assess the contribution by endolithic algae.

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4. We collected the gas underwater by breaking coral branches under an inverted glass funnel connected to a separatory funnel. The gas was transferred underwater to prerinsed (distilled water and then seawater) 5-ml Teflon septum glass vials. Only minute amounts of seawater were included with the samples. Samples were refrigerated immediately after extraction. Analyses were performed at the Queensland Government Chemical Laboratories in Brisbane; in the analyses a Hewlett-Packard 5840 gas chromatograph fitted with a thermal-conductivity detector was used.
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7. Whole *Millepora* fronds covering a vertical projection area of about 50 cm^2 were broken out of larger colonies and placed inside plastic bags. Working at the same depth as that at which the coral grew, we placed the bagged heads on a plywood board and bashed them with hammers to produce the maximum coral destruction and gas release consistent with a minimum number of holes punctured in the bags. (Gas volumes as produced are therefore underestimates of the total volume present.) Gas was extracted from the bags underwater with a hypodermic syringe. Volumes were read underwater directly from the syringe and were not corrected to sea level.
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Proliferative Capacity of Murine Hematopoietic Stem Cells in vitro

Abstract. *Large numbers of granulocytes can be collected repeatedly from the supernatant medium of long-term cultures of mouse bone marrow cells. A constant relationship was found between the number of adherent hematopoietic stem cells and the lifetime cell production per culture. The data indicate that there is a limit to the proliferative capacity of normal and of irradiated stem cells. A similar limitation was found in the production of marked granulocytes from clonal cultures of "beige" C57 (*bg/bg^J*) stem cells placed in limiting dilutions into stromal culture layers.*

Long-term cultures of mouse bone marrow cells maintain stem cell differentiation and undergo extensive self-renewal (*1*). Hematopoiesis is established on the flask bottom, where a stromal network supports the formation of large, confluent, granulopoietic aggregates (*2*). As they mature, the flat, spread granulocytes round up and become suspended in the culture medium. If the system's longevity is tested by repeated harvesting of the nonadherent cells, one observes three phases which resemble those first seen by Hayflick (*3*) in serially subcultured, diploid human fibroblasts. A typical example, shown in Fig. 1a, displays an initial lag phase, a middle plateau phase, and a terminal senescent phase. The plateau is affected by the frequency of feeding. Twice weekly feedings elicit higher cell production early on when compared to the once weekly feeding mode. Yet, the terminal phase invariably occurs and thus appears unrelated to nutritional factors. Senescence in vitro has been interpreted as expression of a general biological limit to the division capacity of somatic cells (*4*). Despite the obvious relevance of this concept for cellular regeneration, and although a large amount of work has been conducted in the area (*5*), very few reports have