

Fig. 2. Microbial food chain. Three types of microbes are required for the decomposition of complex biomass to methane and carbon dioxide. The principle intermediates and the major route of carbon flow is shown in red.

Most of the methane in anaerobic food chains is derived from the methyl group of acetate (Fig. 1) (6). In methanosarcina (7), acetate is activated to acetyl-coenzyme A (CoA), which is cleaved by the nickel-containing CO dehydrogenase/acetyl-CoA synthase, yielding enzyme-bound methyl and carbonyl groups.

The subsequent steps all require unusual coenzymes and cofactors with novel structures only found in the methanoarchaea (8). After cleavage, the methyl group of acetate is transferred to the cofactor tetrahydrosarcinapterin (THSPt) and then from CH_3 -THSPt to coenzyme M (HS-CoM). The CH_3 -S-CoM is reductively demethylated to methane by methyl-CoM reductase, which uses coenzyme B (HS-CoB) as an electron donor. An additional product of the reaction catalyzed by methyl-CoM reductase is the heterodisulfide CoM-S-S-CoB, which is reduced by heterodisulfide reductase to regenerate the reactive sulfhydryl forms of the coenzymes. The reduction of CoM-S-S-CoB is accomplished with an electron pair originating from oxidation of the carbonyl group of acetyl-CoA.

In the other pathway (reactions 2a and 2b), carbon dioxide is reduced to the methyl level with hydrogen or formate by use of still other novel cofactors unique to the methanoarchaea (8). The methyl group is attached to tetrahydromethanopterin (THMPt), a structural and functional analog of THSPt. Conversion of CH_3 -THMPt to methane is the same as for the acetate pathway except the pair of electrons for reduction of CoM-S-S-CoB originate from the oxidation of hydrogen or formate. Thus, methyl-CoM reductase is a key enzyme in both pathways.

The aa'bb'gg' methyl-CoM from the CO₂-reducing *Methanobacterium thermoautotrophicum* contains two molecules of an unusual nickel-porphinoid prosthetic group F₄₃₀. What does the new crystal structure tell

us about the catalytic mechanism and the functions of the novel F₄₃₀, HS-CoM, and HS-CoB structures? Each F₄₃₀ is positioned at the bottom of identical narrow channels formed by residues from the aa'bg or a'ab'g' subunits; however, separation of the F₄₃₀ molecules by about 50 Å clearly marks two independent catalytic sites. The high-resolution crystal structures with either HS-CoM and HS-CoB or CoM-S-S-CoB bound to the enzyme allowed Ermler *et al.* to propose a detailed and highly plausible mechanism largely consistent with previous studies.

The protein environment and arrangement of the coenzymes relative to F₄₃₀ suggests a nucleophilic attack of Ni(I) on CH_3 -S-CoM and formation of an unusual [F₄₃₀]Ni(III)-CH₃ intermediate. The Ni(III) oxidizes HS-CoM, producing a thiyl radical intermediate. Finally, protonolysis releases methane, and the thiyl radical couples with -SCoB to form the heterodisulfide. Evidence for the proposed carbon-nickel bond is eagerly awaited. Such an intermediate has only been proposed for one other enzyme, found in a CO₂-reducing acetogenic anaerobe (9, 10).

The Archaea domain is filled with diverse microbes with uncommon metabolic activi-

ties, portending uncommon structures. Two other crystal structures are reported for enzymes from the methanoarchaea; one of these, a novel class of carbonic anhydrase, displays an unusual protein folding pattern (11). On the basis of this limited sample, one can expect many more novel proteins. These discoveries will surely be expedited by the recently completed sequence of the genomes from the methanoarchaeons *M. thermoautotrophicum* (12) and *Methanococcus jannaschii* (13). Less than half of the predicted protein coding regions can be assigned a role from database sequences. These are truly exciting times for microbial structural biology.

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ECOLOGY

Marine Managers Look Upstream for Connections

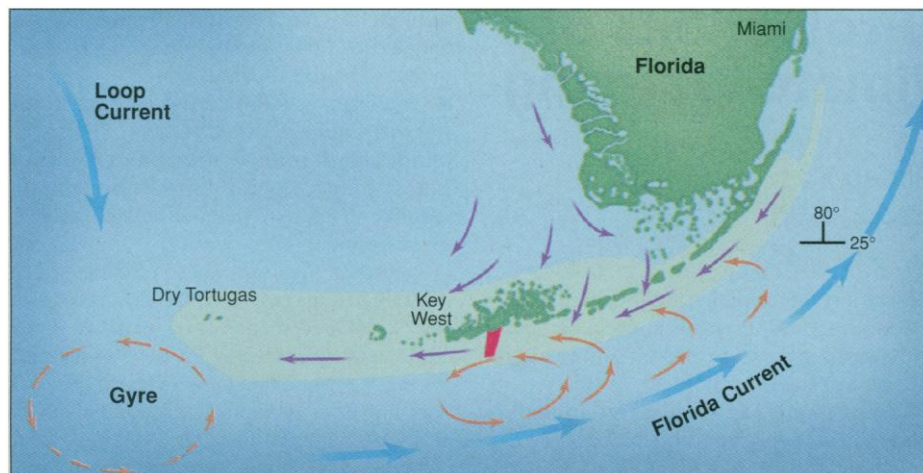
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The Florida Keys are the focus of a major national effort to manage a large land-sea area. In response to drastic declines in coral reefs and fishes and to create a buffer zone from shipping, Congress in 1990 created the 9500-km² Florida Keys National Marine Sanctuary. Although it is now exceeded in size by the Monterey Bay National Marine Sanctuary, its unique management plan includes virtually every conceivable human interaction with the marine environment. A state-federal partnership implementing this pioneering effort at coastal marine resources management was signed on 1 July.

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Over the 6 years of its development, by far the most contentious element of the management plan was the no-take marine reserves, in which all fishing and collecting is banned. These reserves are widely perceived as the best option for conservation of marine biological diversity in shallow waters, particularly in the tropics, where most organisms live in intimate association with specific sites (1).

A portion of a coral reef, even if only a few hectares in size, that is protected from human disturbances will develop larger populations of organisms composed of larger individuals within periods as short as a few years (2). In the Florida Keys, where coral reefs are heavily visited by tourists, this fact alone argues strongly for the establishment of reserves. People like to see big fish. In eco-



The Florida Keys National Marine Sanctuary. The Western Sambos Marine Reserve is shown in red. Nearshore countercurrents and seasonal current gyres may retain locally produced marine larvae within the system.

nomic terms, a reef fish is much more valuable swimming in front of an underwater photographer than it is on a fishing line.

In spite of the efforts of marine scientists, conservation groups, and over a hundred public information meetings organized by federal and state agencies, politically organized fishers mounted a very effective campaign against the marine reserves. They argued that the reserves would deny them traditional access to marine areas for recreational and commercial fishing and that scientific understanding of the functioning and effectiveness of marine reserves was incomplete. Thus, in response, sequential drafts of the Sanctuary management plan proposed marine reserves of a steadily eroding size and number. The initial drafts proposed three sites totaling approximately 10% of the 9500-km² Sanctuary; then in the next draft, the proposal shrank to three reserves at 6% of the area; and finally, the last version settled on the single 30-km² Western Sambos Ecological Reserve (see figure)—less than 0.5% of the total Sanctuary area.

Clearly, a tiny isolated reserve cannot be self-sustaining, and the geographic scale for management and research of such a small area must be expanded to encompass the geographic scale of the physical ocean processes supporting life in the sea (3). Underlying this conviction is a salient feature of most marine organisms—a planktonic larval phase of 1 to 2 months during which ocean currents can transport the organism over long distances. Identification of “sources” of marine larvae and “sinks”—areas dependent on the import of larvae from other locations—is critical for placement of marine reserves.

Scientists and managers have long been aware that the Florida Keys are downstream from the Caribbean, but the management implications of this idea have never been fully realized. Now Roberts (4) on page 1454 of this issue, in a simple but revealing geo-

graphical analysis, combines the speed and direction of surface currents with hypothetical larval lives of 1- and 2-months' duration to define “transport envelopes,” areas from which larvae might be imported to a location and to which they might be transported from a location. These patterns of interregional connectivity allow a measure of the risk of overexploitation and an assessment of the potential for recovery from disturbance. For example, locations such as Barbados are at potential risk from human disturbances as there is little upstream coral reef area providing larvae, whereas the Florida Keys receive larvae originating from over 40,000-km² of upstream ocean, including many coral reefs. For organisms with longer larval lives, the upstream area may be much larger. For example, the spiny lobster has a larval life of from 6 to 9 months. This makes the entire Caribbean region a potential source area for the Florida lobster fishery, worth over \$10 million annually.

The analysis by Roberts *et al.* brings out the political dimensions of connectivity, showing, for example, that a network of marine reserves in Cuba, Mexico, the Cayman Islands, and some of the shallow banks of the western Caribbean would be most effective in sustaining the biological diversity of south Florida. Thus, U.S. support over the last decade of a network of Caribbean marine laboratories and reserves (5) is a case of self-interest.

However, Roberts makes several simplifying assumptions. First, many marine larvae are not carried passively on ocean currents, but are capable of directed swimming in response to currents and environmental gradients. Second, the current patterns used in his large-scale geographic analysis do not show the fine local details of coastal ocean circulation. For example, although it appears that the Florida Current and the Gulf Stream surrounding the Keys will move passively, drifting larvae rap-

idly to the north, both nearshore countercurrents and seasonal current gyres can actually retain marine larvae in the region (see figure). As Roberts argues, retention of larvae by these mechanisms would decrease the size of the transport envelope and may increase the potential effectiveness of local marine resources management.

The Western Sambos Ecological Reserve is the largest no-take marine reserve within a larger area zoned for multiple uses in the United States. Long-term monitoring just beginning within the reserve is designed to detect ecosystem structural and functional changes in relation to changes in adjacent areas where fishing and collecting are permitted.

The Western Sambos Reserve is also a natural research laboratory for examination of key features of reserve design, particularly whether marine reserves can export larvae in sufficient amounts to replenish adjacent areas where fishing and collecting are permitted. For example, the concept of “minimum critical population size” has driven theory of reserve size and connectivity in terrestrial conservation biology. In the sea, most marine organisms have external fertilization triggered by chemical cues or complex behavioral interactions. If populations fall below critical minimums, fertilization success can be drastically decreased (6). A second approach is to examine experimentally the export of molecularly tagged larvae from the reserve (7). These approaches, now in the planning phase, may be important in future management decisions.

Although reserves are being advanced all over the world by economic arguments, the key impediments to implementing reserves are political. In both the developed and developing worlds, there is surprising uniformity in the closely held traditions of free use of the ocean commons and in the failure of political will to change these traditions, even in the face of overwhelming evidence of damage to marine resources. By providing an easily understandable assessment of the physics and biology that link ocean regions and countries, Roberts' model will have a great influence on the design and implementation of regional networks of marine reserves.

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