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Herbivory in Asymbiotic Soft Corals

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A zooxanthellae-free soft coral from the Red Sea feeds almost exclusively on phytoplankton, a mode of nutrition so far unknown for corals. Herbivory was also found in three other azooxanthellate soft corals. In tropical oligotrophic waters, phytoplankton biomass density may be an order of magnitude higher than that of zooplankton. Use of this resource allows these azooxanthellate cnidarians to be highly productive in flow-exposed oligotrophic reef waters.

Soft corals are an important group of sessile marine invertebrates in tropical and temperate waters. They are the second most common benthos component in coral reefs of the Indo-Pacific and the Red Sea, in which their abundance can be higher than that of hard corals (*1*). Their feeding organs are characterized by relatively poorly developed stinging cells (nematocysts) (*2*), and their tentacles are branched so that rows of narrowly spaced pinnules are arranged in a comblike structure around each of the eight polyp tentacles. Thus, the surface area used for passive suspension feeding in soft corals is much larger than in stony corals, whose tentacles do not carry pinnules.

We have studied the diet of the common reef-inhabiting soft coral *Dendronephthya hemprichi* from the northern Red Sea and assessed the composition of its food and rates of food intake in field experiments. Most reef-inhabiting corals live in symbiosis with unicellular algae (zooxanthellae), which translocate enough photosynthetically fixed carbon to the host to fully cover the host's carbon demand in its characteristically nutrient-depleted environment (*3*). *Dendronephthya hemprichi* does not contain zooxanthellae but is successful in coexisting with or even outcompeting symbiotic reef corals. The arborescent colonies embody dense filters with up to eightfold ramification, and the pinnules are the smallest filter elements, with diameters of only 45 to 55 μm. Gap width between the pinnules is 60 to 80 μm. These structures seem more suitable for suspension feeding than for predatory capture of prey. We demonstrate here that suspension feeding on phytoplankton is the principal mode of nutrition that fuels this rapidly growing (*4*) soft coral.

Three lines of evidence indicate that *D. hemprichi* feeds on phytoplankton: (i) Epifluorescence microscopy of the gastrovascular cavity of freshly collected *D. hemprichi* showed high concentrations of small (3 to 20 μm) phytoplankton cells (*5*). (ii) Chlorophyll a degraded to phaeopigments in actively feeding colonies, a process indicative of phytoplankton digestion (*6*). (iii) Phytoplankton gradually accumulated in starved corals after their reintroduction to natural seawater.

The observations under the epifluorescence microscope confirmed that *D. hemprichi* was free of autofluorescence and epiphytic algae and did not contain zooxanthellae. A great majority of the ingested algae were eukaryotes, whereas very few blue-green algae were taken in. This contrasts to the great proportion of blue-green algae in cell numbers and biomass in phytoplankton populations of tropical waters (*7*) and may be related to the small size of blue-green algae cells (<3 μm).

The concentrations of phytoplankton pigments (chlorophyll a and its degradation products, phaeopigments) in the corals were quantified in order to estimate rates of phytoplankton intake and decomposition. Concentrations were determined fluorometrically, after a standard acetone-extraction technique (*8*), in colony branches with a known number of polyps (*9*). For the experiments, colonies 4 to 5 cm tall growing on small polyvinyl chloride plates were kept in a flow chamber (18 cm by 15 cm in cross section) in continuously replaced seawater. The plates were suspended on metal-free wire away from the glass walls in such a way that each colony was exposed to unobstructed laminar flow of 4 to 10 cm/s (*10*).

The chlorophyll a gradually decomposed to phaeophytin in the gastrovascular cavities of the colonies. Ten colonies were kept in natural seawater in the flow chamber. After 3 days of feeding on the natural phytoplankton, the ratio of chlorophyll a to total photopigments in the colonies was 0.23 (±0.04 SD), as compared with 0.69 ± 0.02 SD in the seawater. The seawater in the flow chamber was then replaced by filtered water (filter pore width was 0.7 μm), and the changes in concentrations of plant-derived pigments in the colonies were recorded over 48 hours by random sampling of branch tips of the colonies for pigment extraction. Within the first 14 hours, chlorophyll concentrations decreased at a rate of 3.5% per hour in these starving colonies, whereas phaeopigment concentrations did not change. Around 14 hours after the ini-

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tiation of the measurements, the ratio of chlorophyll a to total photopigment reached an average value of 0.13 ± 0.04 SD (Fig. 1A). From this time on, both chlorophyll and phaeopigment concentrations dropped at similar rates. The decrease in chlorophyll a in starved corals and its degradation to phaeopigments are clear indicators of digestion of phytoplankton trapped in the gastrovascular cavity (*11*).

To determine rates of phytoplankton intake, successive pigment measurements were carried out on 20 actively feeding colonies kept in the flow chamber with continuously replaced natural seawater. Before the experiments, the colonies were kept in filtered seawater for 3 days, hence their gastrovascular systems were free of phytoplankton at the beginning of the measurements. Rates of chlorophyll a intake depended on the flow environment of the colonies (Fig. 1B). Within the first 10 to 12 hours, intake rates were 0.0063 ± 0.0032 μg of chlorophyll a per polyp per hour (± SD) in colonies exposed to a flow of 4 to 5.9 cm/s (linear regression analysis over the first 10 hours: $N = 60$, $R^2 = 0.79$). At a flow rate of 6 to 7.9 cm/s, intake rates were higher (0.0104 ± 0.0029 μg per polyp per hour) ($N = 60$, $R^2 = 0.92$), and at a flow rate of 8 to 10 cm/s, intake rates were 0.0291 ± 0.0079 μg per polyp per hour ($N = 90$, $R^2 = 0.93$). These rates equal 9.0 ± 4.8, 15 ± 4.1, and 41.0 ± 11.4 μg of carbon per polyp per day, respectively, if a chlorophyll a to phytoplankton carbon mass conversion factor of 1:60 is assumed (*12*). Ambient chlorophyll a concentrations in the seawater averaged 0.25 mg/m^3(15 mg of carbon per cubic meter) during the experiments. The phytoplankton clearance efficiency, calculated by normalization of phytoplankton intake rates by the flux through an imaginary plane with the area of the polyp's cross section (9.6 ± 1.2 mm^2), increased from 1.7% (at a flow rate of 4 to 5.9 cm/s) to 4.5% (at a rate of 8 to 10 cm/s).

In contrast to the high rates of intake of phytoplankton, we found very little zooplankton prey in the polyps of *D. hemprichi* (*13*), with an average of less than 0.02 items per polyp (mostly planktonic mollusks and copepods). Mean prey size was 508 μm, and the rate of zooplankton carbon intake was 0.21 μg of carbon per polyp per day. Phytoplankton filtration supplied two orders of magnitude more carbon to the diet of *D. hemprichi* than did zooplankton capture. The concentration of zooplankton carbon in the seawater during the experiment averaged 3.5 mg of carbon per cubic meter (*14*).

A microscopic examination of three other reef-inhabiting asymbiotic octocorals (*D. sinaiensis*, *Scleronephthya corymbosa*, and *Acabaria* sp.) showed that the gastrovascular cavities of these species also contained large quantities of phytoplankton cells. Mechanisms of phytoplankton intake by octocorals and biochemical adaptations to this diet are still unknown (*15*). To date, cnidarians have been considered carnivorous (*16*). Our work clearly shows that this generalization is incorrect.

A major question addressed by tropical reef studies is how do the corals maintain their high levels of biological productivity in a nutrient-impoverished environment (*17*). One explanation is the symbiotic association with zooxanthellae. Efficient retention of food particles carried over the reef is a second mode of energy supply. Zooplankton, and detritus with bacteria attached, have been discussed as main nutrient sources for the predominately filter-feeding coral reef inhabitants (*18*), whereas phytoplankton has until now been neglected in most trophic studies of coral reefs and reef invertebrates, despite its great biomass. It is now imperative to evaluate the extent of herbivory among cnidarians and other filter-feeding reef benthos in order to assess the contribution of phytoplankton to the high gross productivity of reefs.

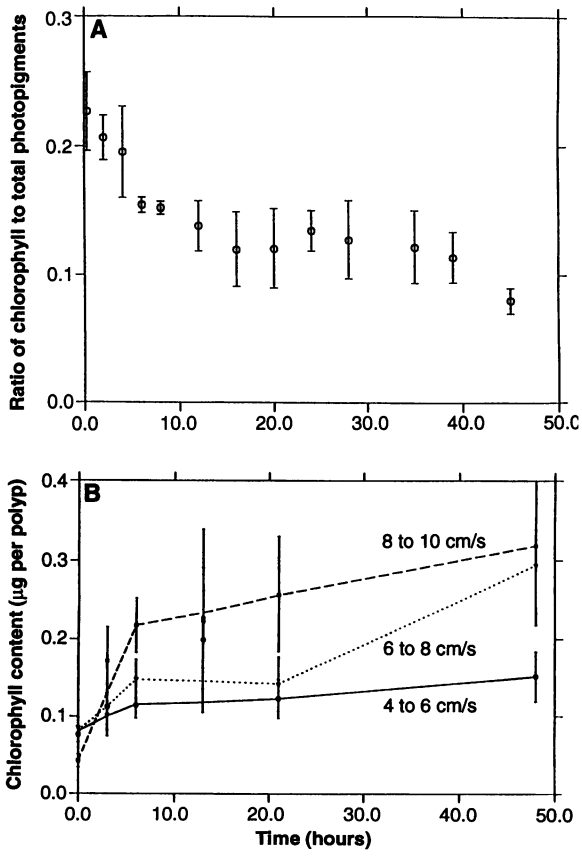

Fig. 1. (**A**) Decrease in the ratio of chlorophyll a to total plant-derived photopigments in polyps of *D. hemprichi* over time. The curve shape is the result of a steady decrease in chlorophyll a concentrations within the polyps, due to decomposition to phaeopigments. Photopigment concentrations remained initially constant but began to drop after about 12 to 15 hours. The decreasing ratio of chlorophyll a to total photopigments is evidence for the digestion of the ingested phytoplankton by this soft coral. Each data point averages six measurements from a colony kept in the flow chamber in filtered water (filter pore width, 0.7 μm). Error bars indicate 1 SD. (**B**) Increasing chlorophyll a concentrations in actively feeding colonies of *D. hemprichi* after a period of starvation. Colonies were in natural seawater at a flow rate of 4 to 10 cm/s. Each data point averages 36 samples from six colonies (24 samples from four colonies after 14 hours). Data points are connected with Lowess Smoothing, a nonparametric regression function. Error bars represent 1 SD. The asymptotic shape of the chlorophyll curves suggests a steady state after about 12 hours, in which the chlorophyll a ingestion rates equalled the chlorophyll a decomposition to phaeopigments.

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14. A known volume of water was pumped from 40 m offshore and 5 m water depth into a reservoir ashore and then gently passed through a 60-μm plankton net. Items were counted and body sizes determined by means of video image analysis. Zooplankton carbon values are most probably lower in yearly average than during the experiment (which was done in May), whereas phytoplankton carbon concentrations (measured in October) are higher at other seasons.
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Native *Escherichia coli* OmpF Porin Surfaces Probed by Atomic Force Microscopy

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Topographs of two-dimensional porin OmpF crystals reconstituted in the presence of lipids were recorded in solution by atomic force microscopy (AFM) to a lateral resolution of 10 angstroms and a vertical resolution of 1 angstrom. Protein-protein interactions were demonstrated on the basis of the AFM results and earlier crystallographic findings. To assess protein-lipid interactions, the bilayer was modeled with kinked lipids by fitting the head groups to contours determined with AFM. Finally, two conformations of the extracellular porin surface were detected at forces of 0.1 nanonewton, demonstrating the potential of AFM to monitor conformational changes with high resolution.

The outer membrane protects the *Escherichia coli* cell against hostile agents and facilitates the uptake of nutrients (*1*). The latter activity is mediated by porins (*2*), of which matrix porin [OmpF (*3*)] is a major species. X-ray crystallography has resolved the atomic structure of this trimeric channel protein (*4*). The strands of the β barrel forming the pores are connected by short turns on the periplasmic surface and by loops of variable length on the extracellular surface. When reconstituted in the presence of phospholipids, OmpF porin assembles into various two-dimensional (2D) crystal forms. Electron microscopy of negatively stained, double-layered trigonal and rectangular crystals led to the 3D structure of the channel at a resolution of 20 Å (*5*).

In pursuit of our goal to acquire subnanometer-scale surface information of a membrane protein embedded in the lipid bilayer, we imaged 2D OmpF-phospholipid crystals adsorbed to freshly cleaved mica in buffer solution by AFM (*6*). Double-layered rectangular OmpF crystals expose only the periplasmic surface to the aqueous environment (*7*). In a manner similar to the dissection of gap junctions (*8*), mechanical displacement of the top layer of crystals with the AFM stylus revealed the extracellular surface of the lower layer. Most likely as a result of electrostatic forces mediated by divalent cations, this lower layer remained firmly attached to the negatively charged mica surface (*9*). Steps at which the top layer broke off revealed both the corrugated extracellular surface (Fig. 1, left side) and the smooth periplasmic surface (Fig. 1, right side) of the porin trimer, as well as the packing of the two layers with respect to each other.

The periplasmic surface contains regularly arranged triplet channels that protrude less than 5 Å from the lipid surface. This surface was rather resistant to deformation by the stylus, reflecting the tight packing of the protein in the lipid bilayer. Thus, periplasmic surfaces could be scanned at least 10 times at forces below ~0.3 nN (*9*). Unprocessed topographs exhibit fine structural details (see Fig. 2A, left panel) that are enhanced by averaging (inset). The lateral resolution was determined to 8 Å by the Fourier ring correlation function (*9*). Quantitative analysis of the root-mean-square (rms) deviation of each pixel has revealed deviations smaller than 1 Å over the lipid moiety and the threefold symmetry axis of the trimer (*9*). To assess the accuracy of these topographical data, we generated a model of the 2D crystal taking into account the atomic structure of the porin trimer (*4*) and the position and orientation of the trimers within the unit cell as determined by AFM, without including the lipid bilayer. Averaged surface contours determined by AFM could thus be directly compared to the protein structure from x-ray analysis. As displayed in Fig. 2A (right panel), subtle features such as the three protrusions clustered about the threefold symmetry axes or

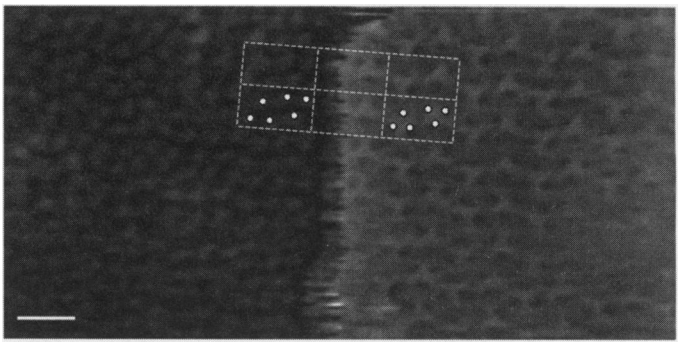

Fig. 1. Steps of the OmpF porin sheets imaged by AFM. The corrugated extracellular surface (left side) and the smooth periplasmic surface (right side) were visualized simultaneously at a loading force of 0.1 nN, after careful displacement of the upper layer. The topograph was recorded with a Nanoscope III (Digital Instruments, Santa Barbara, California) and oxide-sharpened Si_3N_4 tips on a 100-μm-long cantilever with a spring constant k = 0.1 N/m (Olympus Ltd., Tokyo, Japan). Rectangular unit cells (a = 135 Å and b = 82 Å) that comprise two trimers are framed. Small circles mark the trimers in unit cells on both extracellular and periplasmic surfaces. Scale bar, 10 nm. Topographs were recorded in 20 mM Hepes (pH 7.0), 100 mM NaCl, and 2 mM $MgCl_2$.

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