1 W.C. Campbell, R.O. McCracken and L.S. Blair, J. Parasit. *61*, 844 (1975).
2 W.S. Evans, B. Gray and M. Novak, J. Parasit. *65*, 31 (1979).
3 W.S. Evans and M. Novak, J. Parasit. *66*, 258 (1980).
4 D.D. Heath, M.J. Christie and R.A.F. Chevis, Parasitology *70*, 273 (1975).
5 D. Thienpont, O. Vanparijs and L. Hermans, J. Parasit. *60*, 1052 (1974).
6 M. Novak and W.S. Evans, ASP, 54th annual meeting, Minneapolis, Minnesota 1979, abstr. p.34.
7 B.S. Seo, S.Y. Cho, S.Y. Kang and J.Y. Chai, Korean J. Parasit. (1977).
8 Acknowledgments. We are indebted to Mr Douglas W. Ankrom for his superb contribution in the synthesis of these compounds and to Ms Marianne Hardy for excellent technical assistance.
9 Smith, Kline and French Laboratories, Br. Pat. 1,123,317; 14 August 1968.

Mass expulsion of zooxanthellae by heat-stressed reef corals: a source of food for giant clams?

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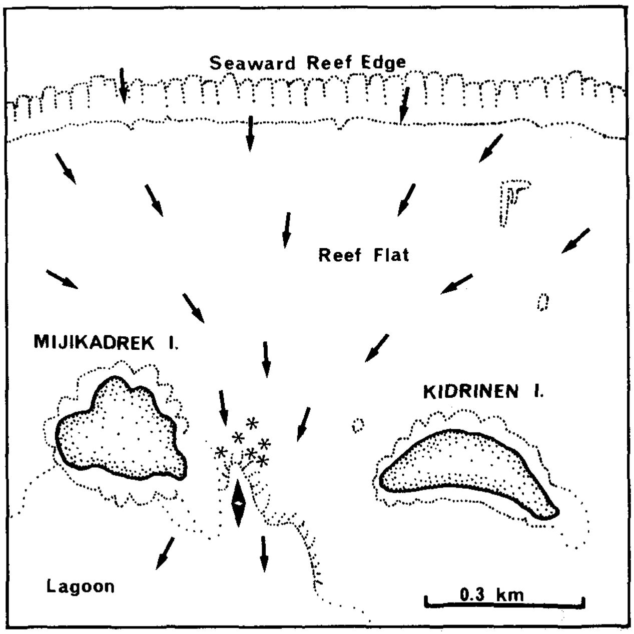
Summary. Symbiontic zooxanthellae found in the stomach of the giant clam *Tridacna gigas* are of exogenous origin. They become available to the clam following their mass expulsion from heat-stressed hermatypic corals. The frequent appearance of these mini-plankton blooms also permits the primary production of zooxanthellae to become an available food source for other filter-feeding reef organisms rather than remaining imprisoned within the tissues of corals.

Giant clams are hosts to endosymbiotic algae called zooxanthellae which inhabit the subepithelial haemal spaces of their hypertrophied siphons. As this animal-plant association is presently understood, the relationship is mutualistic with both participants accruing physiological advantages[1,2]. Zooxanthellae have also been found in the stomachs of giant clams, and it has been assumed that these algae have originated from the clam's blood spaces[3,4]. The more parsimonious explanation, that the material found in guts has been eaten, has been disregarded. In this paper, we report that the reason zooxanthellae occur in the stomachs of giant clams is indeed that they have filtered these algae from the surrounding sea water and eaten them. As to the origin of ingested zooxanthellae, it is evident that they have arisen from cohabitating hermatypic corals which have expelled their algal symbionts in response to the thermal stress of tidal exposure.

To explain the means by which zooxanthellae migrate from siphonal blood spaces to the lumen of the stomach, several ingenious mechanisms have been proposed including a reverse digestion process[3] and the presence of special canals linking the stomach to the siphons[4]. At Enewetak Atoll, Marshall Islands, we have frequently found the stomach of *Tridacna gigas* to be full of zooxanthellae. However, exhaustive thin sectioning of the stomach's digestive diverticula failed to substantiate a build-up of zooxanthellae adjacent to that tissue or the transport of these algal cells into the stomach lumen, which Morton[3] has reported for *Tridacna crocea*. Furthermore, the canals figured by Mansour[4] between the hypertrophied siphons and the stomach of *Tridacna* do not exist[5,6].

Tridacnid clams typically inhabit shallow coral reef waters within the Indo-Pacific geographical region[7]. Like these bizarre molluscs, the vast majority of reef-building corals (the hermatypic Scleractinia) live symbiontically with zooxanthellae[8,9]. However, whereas only 1% or less of the biomass of *Tridacna* consists of zooxanthellae[10], in some hermatypic corals this figure approaches 50%[11]. During mid-day low tides, reef corals are exposed to intense tropical sunlight and may experience temperature elevations exceeding 36 °C; they respond to this stress by expelling masses of algal symbionts into the surrounding sea water[8]. We observed this phenomenon during several mid-day low tide periods in August, 1979 at Enewetak Atoll.

While collecting specimens of *Tridacna gigas* between the islets of Mijikadrek and Kidrinen (figure), we noted the incoming tide, manifested by a line of brown froth, spill over the recently-exposed seaward reef edge, join waters of the reef flat, and advance towards the atoll lagoon. Seen underwater, the tidal flood appeared as a cloudy-green vertical front, about 2 m deep, advancing across the reef flat at 1–2 knots. Underwater visibility was reduced from about 40 m to 3 m as the front passed by. The temperature of the tidal front was conspicuously higher than ambient. In fact, so much so that when free-diving about 100 m to the lee of the reef edge, one of us (P.V.F.) experienced a transient burning sensation as the tidal water touched has skin. Microscopic examination of the sea water revealed the presence of numberous zooxanthellae along with bits of filamentous algae, miscellaneous protozoa, polychaete setae and molted crustacean cuticles. On the following day,



Site of mass expulsion of zooxanthellae by reef corals at Enewetak Atoll. Direction of tidal flooding is indicated by arrows. * Designates a specimen of *Tridacna gigas* while black diamond represents the research vessel Rontak.

(the 3rd day of this low tide series) quantitative surface samples of tidal water were collected adjacent to an aggregation of *T. gigas* upstream of our research vessel (figure). The tidal flood was dramatically less opaque in comparison to the previous day, due to depletion of zooxanthellae in the reef corals. In spite of this, and the dilution factor caused by mixing of the tidal flood with reef flat waters, millipore (0.22 µm) filtered samples revealed 5.1×10^3 zooxanthellae/l of sea water. Giant clams at this site were actively filter-feeding, as indicated by gaping and inhalant water currents. Examination of the stomach contents from specimens of *T. gigas* feeding upon tidal flood waters in comparison to clams transplanted to a zooxanthellae-free lagoon pool revealed numerous zooxanthellae in the former and their conspicuous absence in the latter. These observations confirm that gastric zooxanthellae are of exogenous origin and not derived from symbionts living in the clams' hypertrophied siphons.

With few exceptions, mass expulsion of zooxanthellae by reef-building corals has gone unrecorded[8,12,13]. Yet at Enewetak Atoll, tidal-cycle data[14] and the reports of local observers suggest that this event occurs frequently, particularly during summer months. The significance of expelled zooxanthellae as a resource for reef organisms is an intriguing question. Traditionally, zooxanthellae have been viewed as 'imprisoned phytoplankton'[15] which are unavailable to reef dwelling filter-feeders. We have discovered that *Tridacna* utilizes exogenous zooxanthellae following their release by heat-stressed hermatypic corals, but it is also likely that modest numbers of suspended zooxanthellae are available to reef organisms on a continuing basis. Recent evidence suggests that coral polyps may control the population size of their intracellular zooxanthellae via the ongoing expulsion of the older and less metabolically active algae[9]. These data, and the distinctive habitat of giant clams, always being located on or near reef-forming corals[7], indicate that exogenous zooxanthellae are an important, perhaps obligatory, constituent of nutriment for *Tridacna.*

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2 T.F. Goreau, N.I. Goreau and C.M. Yonge, J. Zool., Lond. *169,* 417 (1973).
3 C.M. Yonge, Sci. Am. *232,* 96 (1975).
4 B.S. Morton, J. Zool., Lond. *185,* 371 (1978).
5 K. Mansour, Nature *157,* 844 (1946).
6 C.M. Yonge, J. Zool., Lond. *123,* 551 (1953).
7 P.V. Fankboner, Ph.D. Thesis, University of Victoria, B.C., 1972.
8 J. Rosewater, Indo-Pacific Mollusca *1,* 347 (1965).
9 C.M. Yonge and A.G. Nicholls, Scient. Rep. Gt Barrier Reef Exped. *V 1,* 177 (1931).
10 T.F. Goreau, N.I. Goreau and T.J. Goreau, Sci. Am. *241,* 124 (1979).
11 H.T. Odum and E.P. Odum, Ecol. Monogr. *25,* 291 (1955).
12 L. Muscatine, in: Biology and Geology of Coral Reefs, vol. 2. Ed. O.A. Jones and R. Endean. Academic Press, New York 1973.
13 D.F. Squires, Nature *195,* 361 (1962).
14 T.F. Goreau, Science *145,* 383 (1964).
15 Admiralty Tide Tables, Pacific Region. H.M. Stationary Office, London 1979.
16 C.M. Yonge, in: Advances in Marine Biology, vol. 1. Ed. F.S. Russel. Academic Press, New York 1963.

Increasing porosity of the incubating alligator eggshell caused by extrinsic microbial degradation[1]

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Summary. The outer densely calcified layer of the alligator eggshell shows progressive crystal dissolution, with the production of concentrically stepped erosion craters, as incubation progresses. This dissolution is caused by the acidic metabolic byproducts of nest bacteria. Extrinsic degradation serves to gradually increase the porosity and decrease the strength of the eggshell.

The nesting American alligator *(Alligator mississippiensis)* lays eggs in the centre of a mound of vegetation[2,3], and after 65 days of incubation the young hatch[4]. During incubation the eggshell becomes stained due to the ingress of microorganisms[5] (as far as the eggshell membrane), extensive cracks develop throughout its length[4] and eventually it flakes off the eggshell membrane a few days before hatching, so that the young alligator has only to slit the latter with its egg caruncle before emerging from the 'egg'. Alligator farmers have successfully incubated eggs in trays of nesting media (mostly Spartina patens) at nest temperatures (28 °C) and humidities (100%)[6]. However if alligator eggs are incubated without nesting media (as in experimental embryological investigations[5,7-9]), the young develop fairly normally but fail to hatch due to an abnormally tough eggshell. In order to investigate this phenomenon and to determine the percentages of minerals that the alligator fetus removes from the egg during incubation (for 'shell less culture' and eggshell windowing experiments) the structure and composition of the alligator eggshell were investigated in 396 specimens removed from eggs at daily intervals throughout the 65-day incubation period (details of methods used given in Ferguson[5]). The alligator eggshell consists from the surface inwards of an outer densely calcified zone (approx. 100-200 µm thick), a honeycomb zone (approx. 300-400 µm thick) and a mammillary zone (approx. 20-30 µm thick) to which is attached the eggshell membrane (approx. 150-250 µm thick). The entire eggshell is composed of small rhombohedral crystals of calcite (figure 1, A) interspersed with a variable amount of organic matrix. In the outer densely calcified zone there is no detectable organic matrix and the calcite crystals are regularly stacked in vertical layers on their a faces (figures 1, A, C-J; 3 and 4) with their c axes at right angles to a tangent to the shell surface at any point (figures 1, C-J; 3 and 4). Contrariwise in the honeycomb zone there is a higher percentage of organic matrix which creates a meshwork of vesicular holes between the calcite crystals; and these holes