Life History of Symbiont-Bearing Giant Clams from Stable Isotope Profiles

DOUGLAS S. JONES, DOUGLAS F. WILLIAMS,
CHRISTOPHER S. ROMANEK

Stable isotopic and shell-growth banding studies of the symbiont-bearing giant clam *Tridacna maxima* reveal the existence of two growth phases related to sexual maturity that can be discerned in the shells of extinct and extant mollusks. The changeover from the first to second growth phase at an age of approximately 10 years is accompanied by a decrease in rate of calcification and suggests a reordering of energy priorities between biomineralization and reproduction. The carbon-13 to carbon-12 ratio of *Tridacna maxima* is systematically depleted relative to symbiont-barren mollusks, making it possible to determine the importance of algal-molluscan symbiosis to the functional morphology and paleoecology of mollusks in the geologic record.

GIANT CLAMS OF THE FAMILY TRIDACNIDAE are prominent members of the Indo-Pacific coral reef community and are among the largest bivalves to have existed. They inhabit shallow waters of the euphotic zone where their corrugated shell margins are directed upward so that symbiotic algae (zooxanthellae) living in the hypertrophied siphonal tissues receive maximum exposure to sunlight (*1*). There is disagreement about the true nature of the symbiotic relation between the zooxanthellae and the giant clams. Although similar relations are common in other calcifying taxa, such as corals and foraminifera, they are rare and poorly characterized among the mollusks. We used stable oxygen and carbon analyses of shell carbonate combined with shell growth increment studies (sclerochronology) of one species of giant clam, *Tridacna maxima*, to explore the influence of zooxanthellae on calcification processes and the unusual shell growth rates of this group.

Integration of geochemical and sclerochronological analyses of living and fossil mollusks provide information on life histories and the environment that cannot be obtained by conventional approaches (*2*). For example, when serially sampled in a detailed fashion, systematic oxygen isotopic changes in molluscan shells accurately reflect the annual seasonal changes in water temperature (*3*). The correlation of isotopic records with shell structural changes provides information on age, growth rate, season of calcification, and season of death (or harvest in archeological contexts). The carbon isotopic composition of shells, in conjunction with oxygen isotopic and growth increment records, can potentially be used

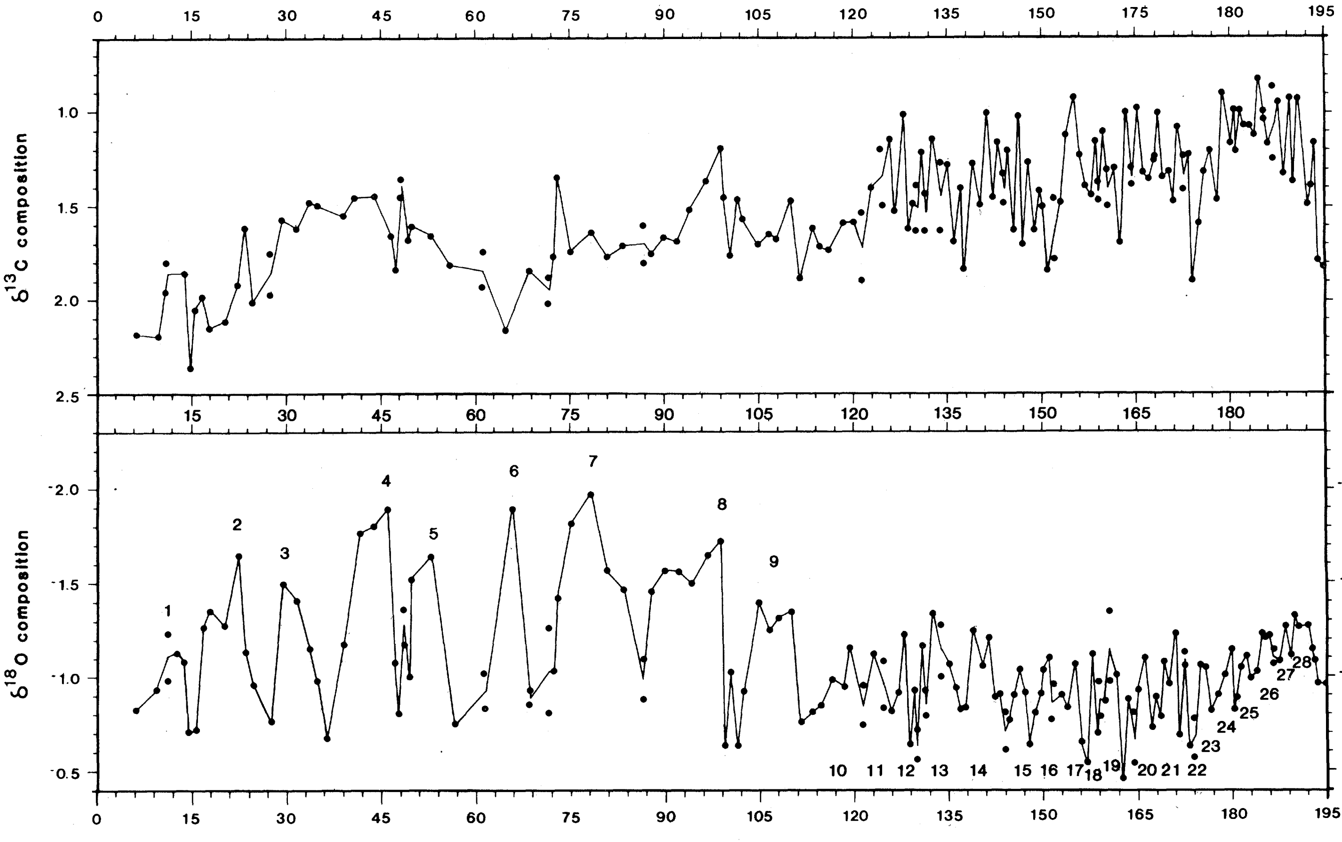

Fig. 1. Carbon and oxygen isotopic profiles for an adult specimen (195 mm curvilinear shell height) of *Tridacna maxima* from Rose Atoll. Numbers on the δ^{18}O profile reflect annual cycles inferred from the character of the δ^{18}O record and corroborated from sclerochronology. According to Jameson (*15*), this specimen would have reached sexual maturity at approximately 110 mm, which corresponds to an age of approximately 10 years. Differences between duplicate analyses are split by the isotopic profile curves.

D. S. Jones, Florida State Museum, University of Florida, Gainesville 32611.
D. F. Williams, Department of Geology, University of South Carolina, Columbia 29208.
C. S. Romanek, Department of Geology, University of Florida, Gainesville 32611.

to estimate changes in productivity and nutrient concentrations in both modern and ancient marine environments (*4*) as well as to monitor the pathways of calcification.

Our objective was to determine whether the presence of photosynthetic zooxanthellae has a systematic effect on the $\delta^{18}O$ or $\delta^{13}C$ values (*5*) of shell carbonate in modern mollusks. Such an effect could be used to investigate fossil species that might have hosted photosymbionts. Work on hermatypic corals and larger symbiont-bearing foraminifera has shown that the presence of zooxanthellae has an effect on the calcification rate, utilization of metabolic CO_2, inorganic carbon pool, nutritional requirements of the host, and possibly the removal of metabolic waste products (*6*).

We also investigated the relative effects of environmental variables on the isotopic composition of the shell and the life history of *T. maxima*. The genus *Tridacna* is the subject of considerable research as a potential food source in Pacific mariculture experiments. It is also useful as an analog for understanding the paleobiology of larger mollusks, particularly with coarsely plicate, interdigitating commissures and in studies of the magnitude and timing of glacioeustatic sea-level fluctuations as recorded by uplifted Quaternary coral reefs (*7*). Our investigation has implications in all these areas (*8*).

Aragonitic shells of *T. maxima* were obtained from live specimens collected in 3 to 10 m of water at Rose Atoll (14°30'S, 168°10'W), the easternmost possession of American Samoa. The annual temperature variation is approximately 7°C, ranging from 28°C in the winter (June–September) to 34°C in summer. The salinity is much less variable, averaging 35.38 per mil. Seawater at this locality has a mean $\delta^{18}O$ value of 0.52 per mil (*9*) and the $\delta^{13}C$ of ΣCO_2 is 2.13 per mil (*10*). With the use of the paleotemperature equation for biogenic aragonite (*11*), the $\delta^{18}O$ composition of shell aragonite in equilibrium with such waters should range from -0.86 (at 28°C) to -1.98 per mil (at 34°C) (*12*). The $\delta^{13}C$ equilibrium composition of shell material should average 4.86 per mil (*12*). For comparison of the isotopic records of *Tridacna* with those of a symbiont-barren or typical mollusk, gastropods (most notably *Terebra areolata*) were also collected from the same site. All shells were sampled in a manner to produce an ontogenetic sequence of isotopic determinations (*13*).

The $\delta^{18}O$ profile for a mature *T. maxima* (Fig. 1) shows large amplitude (up to 1.2 per mil), systematic cycles throughout the initial 100 mm of shell growth. The nature of the cycles and the measured values are consistent with the observed annual water temperature changes at Rose Atoll and the predicted $\delta^{18}O$ values of aragonite formed in oxygen isotopic equilibrium (-0.86 to -1.98 per mil). Nine years of shell growth are thus recorded in the interval 0 to 110 mm. The isotopic record from the next 110 to 195 mm of shell contrasts dramatically with that of the initial portion. Higher frequency and lower amplitude (less than 0.5 per mil) fluctuations occur. The offset of the absolute $\delta^{18}O$ values in the positive direction indicates that during this latter growth phase, minimal calcification occurred during the warmer parts of the year. Though not as well defined as in the first 110 mm, seasonal $\delta^{18}O$ cycles suggest the possibility that as many as 19 years of shell growth are recorded in this latter portion of the record; this would yield a total age of 28 years for this specimen. This age estimate is supported by sclerochronological counts of major (annual?) shell growth increments revealed in cross sections of the valve (*12*) as well as by census studies of living populations of *T. maxima* (*14*).

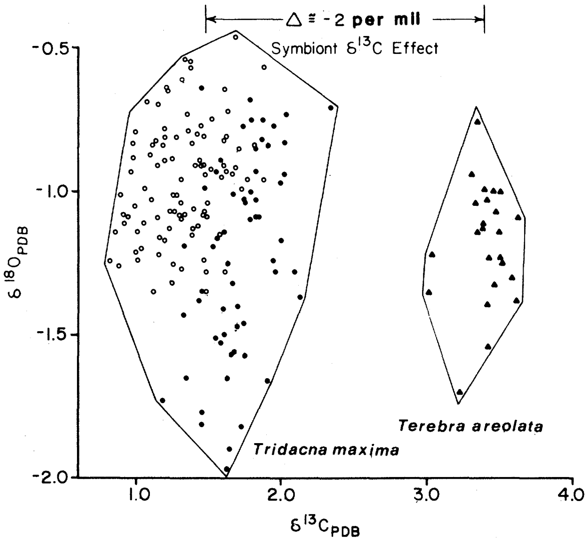

Fig. 2. Carbon isotopic values plotted as a function of oxygen isotopic values for the giant clam, *Tridacna maxima* (mean $\delta^{18}O$, -1.06 per mil; mean $\delta^{13}C$, 1.48 per mil) and the gastropod *Terebra areolata* (mean $\delta^{18}O$, -1.18 per mil; mean $\delta^{13}C$, 3.41 per mil). Closed circles represent the early growth phase of *T. maxima* and open circles the later phase after sexual maturity. Variations in $\delta^{18}O$ values of both specimens reflect seasonal temperature changes. Differences in carbon values between the two species are attributed to the presence of zooxanthellae in the giant clam.

A somewhat similar offset is noted in the $\delta^{13}C$ profile. The initial portion (0 to 110 mm of the shell) of the $\delta^{13}C$ record is characterized by changes with no systematic pattern. The mean value is 1.7 per mil and the range is 1.2 to 2.4 per mil. After 110 mm, the $\delta^{13}C$ record becomes systematically more negative (mean, 1.1 per mil) and more variable, exhibiting high-frequency fluctuations on the order of 0.5 per mil over sample intervals of less than 5 mm. The entire $\delta^{13}C$ record is depleted with respect to an average value of aragonite (4.86 per mil) formed in carbon isotopic equilibrium with seawater at Rose Atoll.

Both the oxygen and carbon profiles reveal significant changes at approximately 110 mm, dividing the shell isotopic record into two distinct parts or growth phases. Jameson (*15*) has shown that *T. maxima* reaches sexual maturity at this size. Reconciling this observation with the $\delta^{18}O$ profile suggests that calcification is fairly rapid (large cycle length) and year-round (large cycle amplitude) until sexual maturity is attained at approximately 10 years of age (110 mm). Thereafter the shell growth rate slows and is limited to the cooler seasons, perhaps in response to the increased energy requirements associated with gametogenesis and spawning during warmer seasons. The erratic and increasingly depleted $\delta^{13}C$ values of the second growth phase also seem to be associated with the changeover that occurs at this time, though the influence of other factors such as rapid and irregular feeding by *T. maxima* and thus a decrease in the role of the symbionts cannot be dismissed.

A comparison of the carbon and oxygen isotopic values for the specimen of *T. maxima* used in Fig. 1 is made with values obtained from a specimen of the gastropod *T. areolata* collected from the same site (Fig. 2). This species also possesses an aragonitic shell but does not contain symbionts. The 24 data points for the gastropod represent the final 1 or 2 years of shell growth. The $\delta^{18}O$ values of the giant clam and gastropod overlap and do not differ significantly whereas the means of the $\delta^{13}C$ distributions are offset by approximately 2 per mil. The carbon values of both organisms are depleted with respect to equilibrium, indicating that both are combining different amounts of light carbon derived from metabolic processes with environmental carbon to make shell carbonate. We suggest, however, that the more depleted $\delta^{13}C$ composition of *T. maxima* is best explained by the incorporation of excess metabolic CO_2 (*12*) originating from a zooxanthellae-enhanced metabolic rate in the host. According to the Erez model of calcification (*16*), zooxanthellae enhance the production of metabolic CO_2 by the host which cannot be removed and the carbon pool becomes diluted, leading to a depletion of the $\delta^{13}C$ record in skeletal carbonate. The $\delta^{13}C$ record in Fig. 1 suggests the CO_2 removal problem becomes particularly acute after *T. maxima* reaches sexual maturity and energy allocation between calcification and reproduction changes.

This isotopic evidence reveals (i) the existence of two distinctly different growth phases in a molluscan species that are associated with the onset of sexual maturity and (ii) that the onset of sexual maturity is reflected in the character of both the $\delta^{18}O$ and $\delta^{13}C$ records. Such pronounced changes have not been described before in mollusks (*2–4*), perhaps because the few temperate species studied to date reach sexual maturity comparatively early, in their first or second year of life. It appears that for *Tridacna* the energetic priorities change upon maturity when energies normally expended on calcification during summer months may be diverted to gametogenesis and spawning. Annual cycles in the $\delta^{18}O$ profile record a marked reduction in the growth rate of *T. maxima* at this point and reveal a life-span of several decades. The $\delta^{13}C$ profile also shows a marked change with the onset of sexual maturity suggesting that with the change in energy allocation comes an increased utilization of CO_2 from the host-zooxanthellae metabolic relationship. Furthermore, the overall carbon profile indicates that zooxanthellae have the effect of depleting the skeletal $\delta^{13}C$ record in mollusks that host symbionts.

These results may have implications for paleobiological studies because the same techniques may be used to interpret the records of fossil shells (*17*), such as fossil tridacnids (their fossil record begins in the Eocene), and to study changes in growth rate, longevity, and onset of sexual maturity, important factors in gauging the role of heterochrony as an agent of evolution (*18*). Analyses of the carbon records of fossil tridacnids in particular may reveal when the symbiotic relationship with the zooxanthellae began. Other fossil bivalve genera thought to host algal symbionts (certain rudists, inoceramids, and cardiids) may show similarly depleted $\delta^{13}C$ records. Such genera, which need to be in the euphotic zone, could be valuable paleodepth indicators in sedimentary sequences. Finally, because oxygen isotopic analyses of fossil *Tridacna* are used in investigations of ancient sea levels from raised coral terraces (*7*), it is important to understand that these organisms may or may not calcify during an entire seasonal range of paleotemperatures, depending on sexual maturity, and therefore that ontogenetic considerations are required in sampling of the shell for isotopic studies.

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13. The left valves of clams were radically sectioned from umbo to shell margin along the axis of maximum growth. Encrustations and scales were ground away so that uncontaminated, sequential samples of aragonite powder could be obtained from the outer shell layer across the entire specimen. Consecutive grooves were then ground with a 0.5-mm dental burr, parallel to external growth ridges, and 0.5-mg powdered samples were recovered. Samples from *Terebra areolata* were obtained in a similar manner, by grinding shallow consecutive grooves in the external shell layer parallel to lines of growth on the body whorl near the aperature. Carbonate powders were roasted in vacuo for 1 hour at 375°C, then reacted with orthophosphoric acid at 60°C, and CO_2 gas was extracted and purified by fractional freezing. Gases were analyzed on either a VG 602D Micromass or VG SIRA 24 isotope ratio mass spectrometer according to established procedures [D. F. Williams, M. A. Sommer, M. L. Bender, *Earth Planet. Sci. Lett.* **36**, 391 (1977)].

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Osmotic Adaptation by Gram-Negative Bacteria: Possible Role for Periplasmic Oligosaccharides

KAREN J. MILLER, EUGENE P. KENNEDY, VERNON N. REINHOLD

The cyclic (1 → 2)-β-D-glucans produced by species of *Agrobacterium* and *Rhizobium* resemble the membrane-derived oligosaccharides of *Escherichia coli* in their periplasmic localization, intermediate size, and (1 → 2)-β-D-glucan backbones. The regulation of the biosynthesis of cyclic (1 → 2)-β-D-glucan by *Agrobacterium tumefaciens* is now shown to parallel the osmotic regulation of membrane-derived oligosaccharide biosynthesis in *Escherichia coli*. This result suggests a general role for periplasmic oligosaccharides in the osmotic adaptation of Gram-negative bacteria as ecologically diverse as enteric and soil bacteria.

OSMOTIC ADAPTATION IS A PROBlem for living cells of every kind because the plasma membranes of cells are freely permeable to water, but impermeable to most cytoplasmic solutes. Mammals have solved this problem by developing an elaborate system for the regulation of the osmolarity of the extracellular fluid in which cells are bathed, so that the osmotic differential across the cytoplasmic membrane is minimized. In contrast, many species of bacteria are capable of growth in environments of wide ranges of osmolarity. Very little is known about the molecular basis of osmotic adaptation in bacteria, but the importance of this problem is increasingly recognized and it is the subject of considerable interest and research (*1–4*). An understanding of the fundamental mechanisms of osmotic signaling and adaptation in bacteria may elucidate similar adaptations in higher plants, a topic of agricultural and economic importance (*4–6*).

The cytoplasm of bacteria, like that of other cells, contains essential constituents with a minimum total concentration of about 300 mos*M*. In a medium of low osmolarity, water will tend to flow into the

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K. J. Miller and E. P. Kennedy, Department of Biological Chemistry, Harvard Medical School, Boston, MA 02115.
V. N. Reinhold, Department of Nutrition, Harvard School of Public Health, Boston, MA 02115.