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Carbonate Deposits in Marine Fish Intestines: A New Source of Biomineralization

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Abstract—Marine teleostean fish are hypo-osmotic to seawater. As part of a multiorgan osmoregulatory strategy they drink seawater and selectively absorb water and minerals across the intestinal epithelium. Notably, divalent cations (Ca^{2+} and Mg^{2+}) are left behind. We report here that in the gulf toadfish, *Opsanus beta*, the ionic by-products of osmoregulation in the intestine contribute to de novo formation of a carbonate mineral, tentatively identified as calcian kutnohorite. Our data suggest that intestinal mineralization is a general feature of osmoregulation in marine teleosts and that this process is an unrecognized and possibly substantial source of marine carbonate sediments.

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Fish actively recycle and transport marine carbonate sediments through ingesting, pulverizing, and excreting calcified food organisms such as corals, coralline algae, or molluscs (Barnes 1983). While investigating fates of urea C (Mommsen and Walsh 1989) in the gulf toadfish (*Opsanus beta*) we observed soft, white pellets in the intestine and feces. These pellets effervesced upon HCl addition. The striking uniformity of the pellets and the fact that they were found in starved fish led us to suspect that they were not of dietary origin. We considered two other possible origins of this material in the design of the present study. First, our previous hypothesis on the existence of urea recycling through microbial urease (urea → NH_3 and CO_2) in the toadfish intestine (Mommsen and Walsh 1989) suggested that urease-enhanced rates of CO_2 production and NH_3 buffering might contribute to mineralization. Second, the well-documented

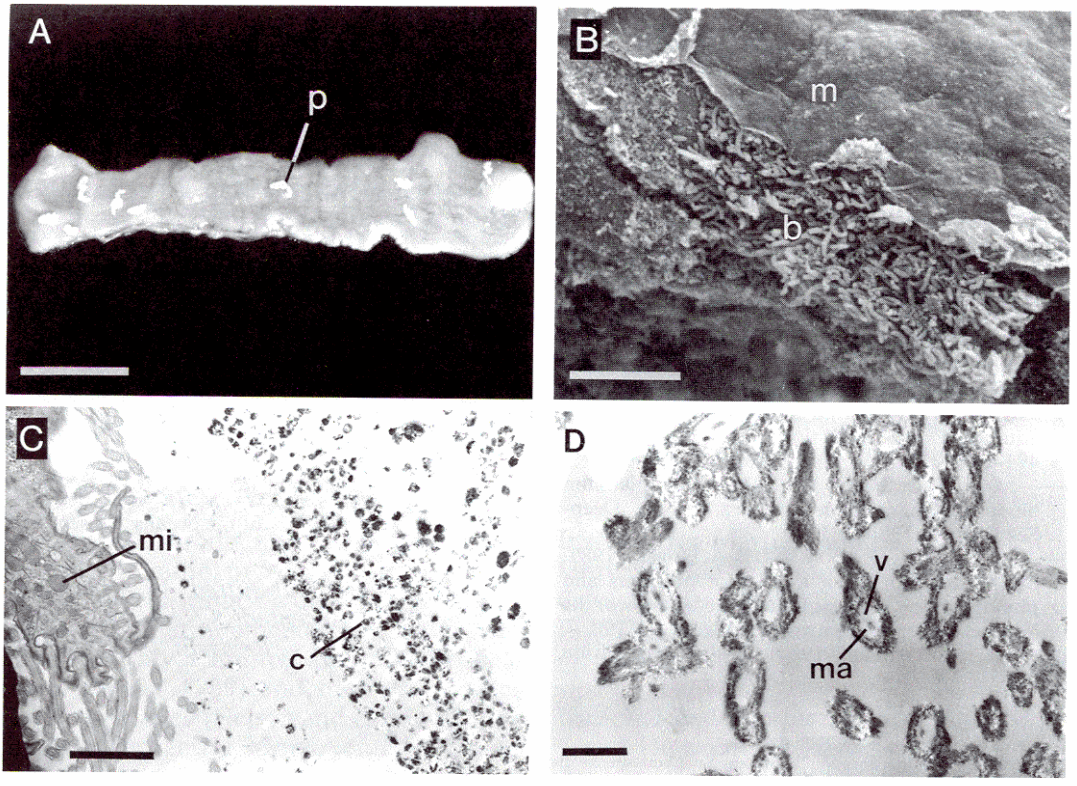

Fig. 1. Carbonate deposits in toadfish intestine. A. Fresh intestine dissected to reveal numerous carbonate pellets (p). Anterior (oral) end is at right. Scale bar—20 mm. B. Scanning electron micrograph of a pellet containing carbonate crystal aggregates. The pellets are covered with a membrane (m) and are rich in bacterialike rods (b). Energy dispersive spectroscopy indicated that the mineral grains are rich in Ca and Mg and low in Mn. Scale bar—40 μm. C. Transmission electron micrograph of a section through the tissue adjacent to the crystal aggregates within the intestine. Note the closely associated mitochondria (mi) and the relationships between the rice-grain-shaped crystal aggregates (c) and layer of organic material between the tissue and crystal aggregates. Scale bar—2 μm. D. Transmission electron micrograph of the crystal aggregates within the intestine lumen. Most of the crystal aggregates are in cross section and the center of each contains a void area (v) and an organic matrix material (ma) which may act as a site for crystal nucleation. Scale bar—0.5 μm.

hydromineral transport capabilities of teleostean intestinal tissues suggested an alternative source of CO_2 (respiration) as well as potential concentrating mechanisms for organic ions (Prosser 1973; Howe and Gutknecht 1978; Schmidt-Nielsen 1983; Groot and Bakker 1988).

We report that the pellets consist of a relatively unusual carbonate mineral tentatively identified as calcian kutnohorite. This mineral is formed de novo in the toadfish intestine and excreted even in the absence of any dietary input. Our experiments suggest that this heretofore unrecognized mode of biomineralization does not intimately involve intestinal microbes, but is a by-product of routine osmoregulation by this species. Our model suggests that this process is a potentially significant source of carbonate mineral in the marine environment.

Toadfish (5–500 g) were maintained in seawater aquaria without access to food, gravel, air-stones, or other foreign substances for a minimum of 3 weeks, unless noted. Regardless of sex or size, intestines of all toadfish contained substantial numbers of pellets (Fig. 1A; Table 1) identical to those first observed in freshly caught specimens and contained no other visible particulate materials. Although calcified or-

Table 1. Effects of antibiotics and low salinity on rates of carbonate sediment production in the gulf toadfish. Values are means ±1 SE. Toadfish were held in aerated 9-liter plastic tubs and water was changed and excreted pellets were harvested every 12 h for 2 d. Excretion for each of four 12-h increments was averaged to yield one value for each fish. Antibiotic-treated toadfish were treated with 50 ppm each of sisomycin and ampicillin (Danulat 1986) which reduced bacterial counts from 7.7×10^7 per intestine in control fish to 2.2×10^4 per intestine. Low-salinity-treated toadfish were exposed to 25% seawater (diluted with distilled water) for 1 week. After 2 d no further pellet excretion was observed. At the end of 1 week, only two fish showed a few strands of diffuse white material in their rectum (the posterior 10% of the digestive tract), which could not be accurately weighed. Values for low-salinity-treated fish are significantly different from control and antibiotic-treated fish which are not statistically different, $P < 0.05$, Student's *t*-test.

	Standing stock		Excretion rate
	[(mg dry wt) (100 g fresh body wt)$^{-1}$]	(pellets fish^{-1})	[(mg dry wt)(100 g fresh body wt)$^{-1}$ (12 h)$^{-1}$]
Control ($N = 6$)	16.0±7.0	13.3±3.0	1.0±0.4
Antibiotic treated ($N = 6$)	14.2±4.0	10.7±3.7	2.4±0.8
Low-salinity treated ($N = 4$)	<0.1	0	0

ganisms comprise a portion of the normal varied carnivorous diet of toadfish, our treatments ensured that none was present in these specimens. The pellets were either loosely associated with intestinal tissue or floating freely in intestinal fluids. We often observed strands of material (up to 1 cm long) and we could tease the pellets apart into strands, which suggests that the pellets were formed from these strands as they moved along the intestine. Closer examination in the scanning electron microscope (SEM) revealed an external coating and numerous bacterialike rods (Fig. 1B).

After fixation and preparation for the transmission electron microscope (TEM), we observed individual "rice-grain"-shaped crystal aggregates (Fig. 1D) that were not found intracellularly in the microbes or cells of the toadfish intestine (Fig. 1C). The crystals were intimately associated with the intestinal tissue and appeared to form on matrix material produced in this area (Fig. 1D). We judged the matrix material to be organic because energy dispersive spectroscopy (EDS) during SEM examination did not reveal minerals and because the matrix did not dissolve when selected samples were decalcified by uranyl acetate. EDS during SEM examination indicated high Ca and Mg contents (and no detectable Mn) which were later confirmed by TEM and elemental analysis (Table 2). A pooled sample of pellets from several fish was subjected to X-ray diffraction analysis, and the peak spacings of the spectrum (Table 3) coincided with the JCPDS No. 19-234 spectrum for calcian kutnohorite [$Ca_{0.74}(Mg, Mn)_{0.26}CO_3$] (Bayliss et al. 1986).

We found that toadfish intestinal fluids provide a suitable microenvironment for in situ carbonate mineralization, in that they are quite alkaline and have high CO_2 content (Table 2). In contrast, the pH and [CO_2] of toadfish blood plasma are lower (Table 2). Marine fish, including toadfish, routinely drink seawater (Prosser 1973; Howe and Gutknecht 1978; Schmidt-Nielsen 1983; Groot and Bakker 1988), so a Ca^{2+} and Mg^{2+} supply is readily available (Table 2).

To identify potential sources of CO_2, we analyzed bile, which in mammals is rich in HCO_3^- (Ganong 1983). However, the total [CO_2] and pH of toadfish bile were low (Table 2), so we can rule out bile as a CO_2 source for mineralization. Furthermore, given the pH and low CO_2 content of imbibed seawater (Table 2), we conclude that intestinal tissue respiration per se is a more likely source of CO_2. This notion is corroborated by the abundance and high density of mitochondria (Fig. 1C), which conceivably support energetically costly transport activities in this tissue. One final potential source of CO_2 relates to the fact that toadfish belong to a small subset of teleostean fish synthesizing substantial quantities of urea as a nitrogenous end product (Mommsen and Walsh 1989). Because the toadfish intestines contain substantial urease activity

Table 2. Ca, Mg, and CO_2 contents (mM) and pH in toadfish body fluids, seawater, and intestinal sediments. Values are means ±1 SE (N). Pellets were solubilized in HCl. Measurements were performed on a Perkin-Elmer 2380 atomic absorption spectrometer, a Radiometer capillary pH electrode and PHM 84 pH meter, and a Corning 965 CO_2 analyzer. Mn was not analyzed by these means because it was not detectable by EDS.

	[Ca]	[Mg]	[CO_2]	pH	Ca content	Mg content
Body fluids						
Plasma	2.17±0.24(11)	1.7–2.5*	2.7*	7.70*		
Intestinal fluid	7.70±1.12(12)	99.80±4.05(16)	68.1±3.6(5)	8.61±0.03(5)		
Intestinal fluid—25% SW	1.32±0.74(4)†	7.89±4.67(4)†				
Bile	16.00±1.29(13)	7.07±0.65(13)	0.4±0.2(12)	5.11±0.02(12)		
Bladder urine	1.50±0.25(2)*	149.80±9.30(6)*				
Seawater	12.03±0.10(3)	52.36±0.20(3)	2.0±0.1(3)	7.94±0.02(3)		
Intestinal pellets						
% of dry wt					20.72±1.52(8)	10.78±1.23(8)
% of acid-labile wt					29.30±5.70(8)	15.24±3.70(8)
Predicted for kutnohorite					31.30	6.30

* Values from previous studies (Prosser 1973; Howe and Gutknecht 1978; Walsh 1989).
† These four fish correspond to the four fish in Table 1. Two fish with gut material had a mean [Mg] of 15.8 mM; [Mg] was below the limit of detection in the two fish lacking material in their intestines. Values for 25%-seawater-treated fish are significantly different from 100%-seawater-treated fish, $P < 0.05$, Student's t-test.

Table 3. X-ray diffraction analysis of toadfish intestinal crystals (Ca-Mn-$MgCO_3$) in comparison to published values for JCPDS No. 19-234 (Bayliss et al. 1986). Peaks chosen for comparison had intensity ratios >40 in known standard.

Intestinal crystals		JCPDS No. 19-234 $Ca_{0.74}(Mn, Mg)_{0.26}CO_3$	
d (Å)*	I/I_1†	d (Å)	I/I_1
3.80	6	3.78	40
2.98	54	2.98	100
2.45	6	2.46	40
2.25	11	2.25	50
2.06	6	2.06	50
1.87	9	1.87	50
1.84	11	1.84	60

* d-spacing of atoms in Angstroms.
† Relative intensity.

(presumably of microbial origin), we reasoned that intestinal microbes were breaking down urea to $2NH_3 + CO_2$ as part of a nitrogen conservation shuttle (Mommsen and Walsh 1989). If microbial urease supplies all or part of the elevated [CO_2] needed for carbonate mineralization, reduction of intestinal microbial populations should lead to reductions in pellet standing stock and excretion. Treatment of toadfish with antibiotics, as in prior studies of cod (Danulat 1986), decreased intestinal microbe populations by 1,000-fold but exerted no effect on pellet formation or excretion (Table 1). We conclude that microbial urease activity and carbonate mineral formation are not intimately related.

We next examined pellets for Mg and Ca and compared their contents with fluids to identify possible sources of these ions. The Ca content of the pellets (Table 2) agrees well with stoichiometries for calcian kutnohorite when corrected for material not soluble in HCl. The Mg content was 2.4-fold higher than the maximum predicted stoichiometrically and may stem from the high Mg content of surrounding intestinal fluids trapped in the pellets (Table 2). With the exception of intestinal fluid, all body fluids examined were much lower in Mg and Ca than seawater (Table 2). When a ready source of divalent cations was removed by experimental exposure to 25% seawater—a condition occasionally experienced by toadfish in nature—the fish did not form or excrete carbonate pellets (Table 1) and failed

to show the above concentration effects on Mg^{2+} in intestinal fluids (Table 2).

These results fit well with some previous measurements (Prosser 1973; Howe and Gutknecht 1978) and current paradigms of osmoregulation in marine teleostean fish (Prosser 1973; Schmidt-Nielsen 1983; Groot and Bakker 1988). Marine teleosts, being hypo-osmotic to seawater, are faced with a continual net influx of salts and dehydration. Because many marine teleosts cannot produce a sufficiently hyperosmotic urine, a major osmoregulatory role is assumed by other organs, including the intestine, which absorbs water from imbibed seawater and selectively leaves behind certain ions, particularly Mg^{2+} (Prosser 1973; Howe and Gutknecht 1978; Schmidt-Nielsen 1983; Groot and Bakker 1988).

Viewed in this context, our findings point to the conclusion that the carbonate mineral formed in the marine toadfish intestine is a by-product of intestinal osmoregulation. The likely origin for CO_2 is respiration and the high concentration of divalent cations originates from seawater as water is removed in the intestine. Because CO_2 would decrease the pH of intestinal fluids and we measured a high pH, we infer that active transport of monovalent ions raises pH (e.g. Na^+-H^+ exchange). Conversely, freshwater teleosts, being hyperosmotic to their environment, normally do not drink for osmoregulatory purposes (Prosser 1973). However, when acclimated to seawater in the laboratory they can be induced to drink, and in preliminary studies Ca-rich intestinal precipitates were detected in seawater-acclimated rainbow trout (*Oncorhynchus mykiss*) (Shehadeh and Gordon 1969).

What is the geological significance of intestinal carbonate as a potential source of sediment production? The crystals did not dissolve when held in filtered normal seawater for 24 h, but dissolution and possible recrystallization to more common carbonate forms must be studied more closely and over longer time periods before conclusions regarding the mineralogical fate of the crystals can be drawn. The relative importance of in situ production rates also remains open. With the measured excretion rate averaged for both control and antibiotic-treated groups (Table 1), a representative density of toadfish in Florida Bay (2.0 toadfish m^{-2}) (Sogard et al. 1987), and an average body mass of 100 g, the annual production of pellets is 2.5 g m^{-2} yr^{-1}. This annual production rate is low compared to champion marine carbonate producers (e.g. coral reefs at 4.2 kg m^{-2} yr^{-1}) (Barnes 1983), but it is well above estimates of the $CaCO_3$ "rain" rate from the pelagic water column (1.5 g m^{-2} yr^{-1})—a major source of deep-sea carbonate sediments (Broecker and Peng 1987).

Of course the relative importance of these rates depends on area covered, and use of the relative biomass of all pelagic teleost species (a more difficult number to estimate) would provide a fairer comparison to the rate of pelagic $CaCO_3$ rain. However, our estimate for *one* fish species may be amplified, if, as our results suggest, intestinal carbonate mineralization is a common by-product of osmoregulation in other marine teleosts. In this regard we have recently observed similar carbonatelike pellets in unfed cod (*Gadus morhua*), rockfish (*Sebastes caurinus*), quillback rockfish (*Sebastes maliger*), and midshipman (*Porichthys notatus*). Further studies that document the presence and origins or absence of carbonate minerals in the intestines of other marine fish species would test the proposed generality of our findings.

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Validation of the alkalinity anomaly technique for investigating calcification and photosynthesis in coral reef communities

Abstract—Experiments were conducted to establish the validity of the alkalinity anomaly technique for investigating rates of calcification and photosynthesis by coral reef-building organisms. Rates of $CaCO_3$ precipitation by whole colonies of the scleractinian coral *Pocillopora damicornis* (L.) were estimated under light and dark conditions with two different methods: complexometric titration of Ca with EGTA and acid titration of total alkalinity, with and without correction for alterations in the concentrations of ions other than carbonate species. The two techniques provided equivalent estimates of light-enhanced and dark calcification, irrespective of whether corrections were applied to the total alkalinity data for changes in nutrient concentration. These results confirm that the assumptions of the alkalinity anomaly technique are fundamentally correct and that it is not necessary to correct total alkalinity data for changes in nutrient concentration because the corrections which apply are smaller than the variability observed in calcification data.

Physiological investigations of calcification by reef-building organisms, primarily the hermatypic corals and calcareous red algae, began a little over 30 yr ago. The evidence which has accumulated clearly indicates that the rapid rates of calcification which characterize reef development are largely attributable to processes associated with algal photosynthesis (Barnes and Chalker 1990). Although the measurement of photosynthesis, at least in terms of oxygen production, is a relatively straightforward procedure with modern polarographic or galvanic oxygen electrodes, the measurement of calcification is more complicated.

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