Vol. 101: 163-167, 1993 | MARINE ECOLOGY PROGRESS SERIES
Mar. Ecol. Prog. Ser. | Published November 4

Does $CaCO_3$ in food deter feeding by sea urchins?

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ABSTRACT: Calcified algae typically rank low in preference to generalist herbivores; however, this correlation is confounded by the fact that many calcified algae are also chemically defended. Few studies have experimentally examined the role of $CaCO_3$ in deterring herbivory. $CaCO_3$ could deter herbivory by increasing either the toughness or the mineral content of the algal thallus. By incorporating powdered $CaCO_3$ (calcite and aragonite) into an artificial food, we decouple these 2 possibilities and focus on the second. *Diadema setosum* and *Echinometra* sp. fed preferentially on food containing powdered calcite; *Mespilia globulus* fed similarly on experimental and control foods. Powdered aragonite deterred feeding by *D. setosum*, may have deterred feeding by *M. globulus* (depending upon whether data were analyzed as mass eaten or volume eaten), and had no effect on feeding by *Echinometra* sp. The absence of large negative effects of powdered $CaCO_3$ on feeding by sea urchins is consistent with the fact that most urchins have a neutral gut pH and regularly ingest some carbonate material. The most likely explanation for the more negative effect of aragonite than calcite is that the aragonite particles were larger than particles of the commercially available calcite. Further work needs to evaluate the relative importance of mineral content and toughness to see which is more important in rendering calcified algae unpalatable to a variety of herbivores.

INTRODUCTION

Many species of marine algae are heavily calcified. Calcified algae usually rank low in preference to fish (Littler et al. 1983, Hay 1984, Lewis 1985, Paul & Hay 1986, Wylie & Paul 1988) and other generalist herbivores (Littler & Littler 1980, Pennings 1990, Pennings & Paul 1992; see Steneck 1982, Paul & Van Alstyne 1988 for 2 small herbivores that are specialists on calcified algae), leading to a general consensus that calcification is an effective defense against herbivory. However, many calcified algae are also chemically defended, making it difficult to assess how important $CaCO_3$ alone is in rendering calcified algae unpalatable (Hay 1984, Paul & Hay 1986).

The role of calcification in deterring herbivory was questioned by Padilla (1985, 1989), who demonstrated that calcified tissue was not necessarily difficult to remove with the radula of a limpet. Whether these results extend to other herbivores that feed in other ways is not clear. Penetrometer measurements of 20 species of tropical Pacific marine algae and seagrasses ranked the 4 calcified species that were measured intermediate in toughness (Pennings & Paul 1992), suggesting that herbivores that remove tissue by biting might find at least some noncalcified plants easier to consume than calcified plants. Regardless, focusing only on toughness ignores the possibility that $CaCO_3$ may deter herbivores simply by dramatically increasing the mineral content of their food.

$CaCO_3$ occurs in marine algae in 2 crystalline forms, calcite and aragonite, at concentrations ranging from $\leq 50\%$ (e.g. *Padina* spp.) to over 80 or 90% (e.g. *Halimeda* spp.) (Bohm 1973, Borowitzka 1977, Multer 1988, Padilla 1989, Pennings & Paul 1992). Calcite and aragonite differ in the arrangement of the CO_3 groups within the crystalline lattice; as a result, aragonite is slightly harder and denser than calcite (Hurlbut & Klein 1977). Both calcite and aragonite have been shown to deter feeding by herbivores when incorporated as powders into artificial, carrageenan-based foods (Pennings & Paul 1992, Schupp & Paul in press). The effect of $CaCO_3$ on fishes was variable, depending upon the fishes' gut pH and natural diet: some fishes with fairly neutral gut pH were not deterred from feeding by $CaCO_3$; others, with more acidic guts, were strongly deterred (Schupp & Paul in press).

Here we extend these results by examining the effects of powdered $CaCO_3$ on feeding by 3 species of sea urchins. The genus *Diadema* contains long-spined, large, mobile urchins with very generalized diets. They prefer to eat filamentous algae, but often consume some calcareous material, either indirectly as they scrape algae from coral rubble, or directly as they consume crustose coralline algae or living corals (Birkeland 1989). The genus *Echinometra* contains short-spined, relatively sedentary urchins which tend to live in burrows that they excavate in the reef substratum. They eat drift algae, but also regularly consume carbonate material as they graze on encrusting or boring algae, and can be very important bioeroders of coral reefs (McLean 1967, Birkeland 1989). *Mespilia globulus* is a short-spined, motile urchin which attaches debris to its test. It is not thought to be an important bioeroder, but may ingest some calcareous sediment as it feeds on detritus (Birkeland 1989). Because sea urchins have a fairly neutral gut pH (around 6.3 to 7.8; Lawrence 1982) and often ingest calcareous material, we would predict, following Schupp & Paul (in press), that they would be deterred from feeding only slightly, if at all, by powdered $CaCO_3$ in their diet.

METHODS

Diadema setosum (Leske, 1778) and *Mespilia globulus* (Linne, 1758) (henceforth *Diadema* and *Mespilia*) were collected from the field in Guam (13° 25′ N, 144° 55′ E), taken to the University of Guam Marine Laboratory, and used in experiments within 5 d. *Echinometra* type A [sensu Arakaki & Uehara 1991; white tips on the spines, of *E. mathaei* (de Blainville, 1825) species complex] (henceforth *Echinometra*) were obtained from a laboratory population maintained at the Marine Laboratory.

Urchins were offered artificial foods similar to those used by Pennings & Paul (1992) and Schupp & Paul (in press). We mixed 2 g corn starch, 6 g carrageenan and 12 g powdered, freeze-dried *Enteromorpha clathrata* (Roth) [a green alga that is highly palatable to most herbivores (Wylie & Paul 1988, Paul et al. 1990, Pennings & Paul 1992)] with 200 ml tap water, heated the mixture in a microwave oven, and poured it into a shallow plastic dish. Once the mixture had cooled and set, it was cut into cubes. Food containing $CaCO_3$ was prepared similarly, except that 60 g powdered $CaCO_3$ was included in the recipe. Addition of $CaCO_3$ increased the volume of the artificial food only slightly, but increased food density about 20 % and dramatically decreased % nutritional content on a dry mass basis. Reagent grade calcite was obtained from Sigma Chemical Company. We obtained powdered aragonite by placing the calcified green algae *Halimeda* spp. in bleach to remove organic matter, flushing with water to remove bleach residues, grinding the remaining calcareous skeleton in a mortar and pestle, and passing the ground aragonite through a 600 µm mesh screen. Over 75 % of the calcite was composed of particles 63 to 125 µm in diameter whereas the aragonite was primarily composed of particles between 125 and 500 µm in diameter (Table 1).

Table 1. Particle size distributions of powdered calcite and aragonite. Samples were shaken through a graded series of sieves for 5 min. Data (range of 3 replicate subsamples) are the percent of the total mass of the samples that were represented by particles from each size class

Particle size class	Calcite (%)	Aragonite (%)
>1 mm	0.12–0.74	0.04–0.11
500 µm–1 mm	0.07–0.12	14.22–19.59
250–500 µm	0.10–0.13	40.68–44.17
125–250 µm	7.85–12.10	23.57–26.72
63–125 µm	75.02–76.22	12.97–15.70
38–63 µm	12.27–14.81	1.07–2.10
<38 µm	0.13–0.24	0.09–0.17

Urchins (n = 8 to 19) were placed individually in 20 l (*Diadema, Echinometra*) or 4 l (*Mespilia*) flow-through plastic cages submerged in a large aquarium with running seawater. Each urchin was offered 1 food cube containing $CaCO_3$ and 1 lacking $CaCO_3$. To determine the change in mass of the food cubes in the absence of herbivory, pairs of cubes (n = 8 to 14) were placed in flow-through containers that lacked urchins in the same large aquarium (see Peterson & Renaud 1989 for a discussion of the design and analysis of feeding preference experiments). Food cubes were weighed before and after the experiments, which ran for 1 or 2 nights (all 3 urchins are nocturnal). The smaller urchins (*Echinometra* and *Mespilia*) were fed smaller cubes and were allowed to feed longer than were the larger *Diadema*. Each individual urchin was used only once.

We compared the difference in the change of mass of calcified and noncalcified foods for the treatments with and without urchins (see Peterson & Renaud 1989). Data were then converted to volumetric units and reanalyzed. In only 1 of 6 cases did this reanalysis cause the outcome of a test (significant or not) to change. Because the data fit the distribution assumptions of parametric tests very poorly, we used the nonparametric median test for all our analyses.

RESULTS

Diadema ate significantly more mass of food containing calcite than control food (Fig. 1A, p = 0.0005). How-

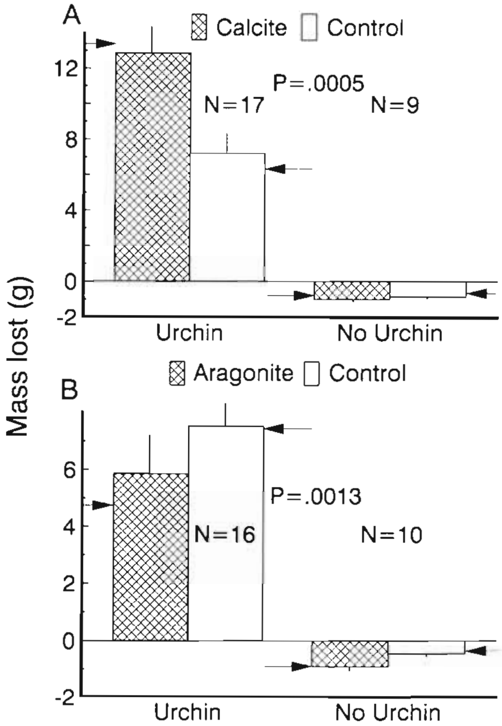

Fig. 1. *Diadema setosum.* Effect of powdered (A) calcite and (B) aragonite on feeding. Data are mean mass loss (+ 1 SE) of artificial food cubes, with and without added $CaCO_3$, in the presence and absence of urchins. Arrows indicate the median of each sample. Sample sizes for urchin and no-urchin trials and p-values (median test) are given. Qualitatively similar results obtain if data are converted to volumetric units (calcite preferred, p = 0.039; aragonite avoided, p = 0.0001). See 'Methods' for details of analysis

ever, when offered control food and food containing aragonite, they preferred the control food (Fig. 1B, p = 0.0013). When data were converted to volumetric units and reanalyzed, qualitatively similar results were obtained (calcite preferred, p = 0.039; aragonite avoided, p = 0.0001).

Echinometra also ate a greater mass of food containing calcite than of control food (Fig. 2A, p = 0.046). However, when offered control food and food containing aragonite, they ate both without displaying any preference (Fig. 2B, p = 0.55). When data were converted to volumetric units and reanalyzed, qualitatively similar results were obtained (calcite preferred, p = 0.046; no preference for aragonite, p = 0.71).

Mespilia ate a similar mass of control food and food containing calcite (Fig. 3A, p = 0.72). They showed a nonsignificant trend toward preferring control food over food containing aragonite (Fig. 3B, p = 0.13). When data were converted to volumetric units and reanalyzed, the first result was qualitatively similar (no preference for calcite, p = 0.72), but the second test attained significance (aragonite avoided, p = 0.0005).

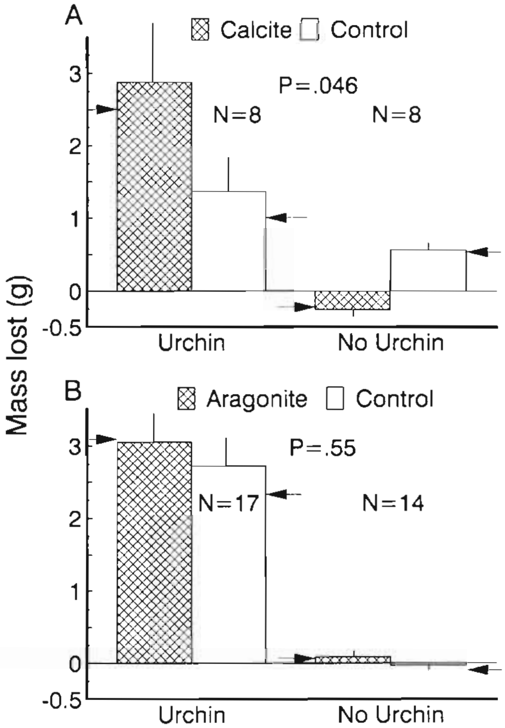

Fig. 2. *Echinometra* sp. type A. Effect of powdered (A) calcite and (B) aragonite on feeding. Data as in Fig. 1 Qualitatively similar results obtain if data are converted to volumetric units (calcite preferred, p = 0.046; no preference for aragonite, p = 0.71). See 'Methods' for details of analysis

DISCUSSION

Calcium carbonate had variable effects on feeding by the urchins we studied. Although it could be debated whether volume or dry mass of food eaten is the appropriate measure for comparing herbivory (Belovsky & Schmitz 1991), our conclusions were affected in only 1 of 6 cases by the variable chosen for analysis. Food containing calcite was ingested more than control food by 2 of 3 species of urchins, and was never significantly avoided. Food containing aragonite was never ingested more than control food. Aragonite deterred feeding by 1 species of urchin, and may or may not have deterred feeding by a second species, depending upon how the data were analyzed. Similarly, in field feeding assays with a mixed assemblage of reef fishes, calcite had no significant effect but aragonite significantly deterred feeding (however, consumption did not differ when directly compared; Schupp & Paul in press). Two hypotheses could explain the different effects of calcite and aragonite. First, $CaCO_3$ might be a more potent feeding deterrent when crystallized as aragonite than when crystallized as calcite. We consider this hypothesis unlikely to be true, since we doubt that herbivores could detect dif-

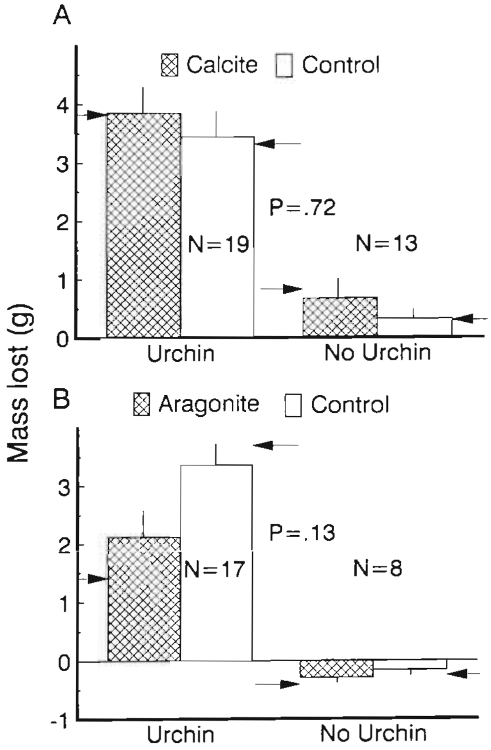

Fig. 3. *Mespilia globulus*. Effect of powdered (A) calcite and (B) aragonite on feeding. Data as in Fig. 1. If data are converted to volumetric units, the results for calcite are qualitatively similar (no preference, p = 0.72), but aragonite is significantly avoided (p = 0.0005). See 'Methods' for details of analysis

ferences in the crystalline structure of $CaCO_3$. Second, since we (like Schupp & Paul) did not grind aragonite into as fine a powder as the commercially available calcite (Table 1), the larger sizes of the aragonite particles may have been responsible for the more negative effect of aragonite on feeding. We suspect that this second hypothesis is correct, and are conducting a series of experiments to test it.

It is not clear why food containing powdered $CaCO_3$ should be preferred over control food by some herbivores (this study, Schupp & Paul in press), although it is possible that these herbivores associate the taste or texture of $CaCO_3$ with food. Although $CaCO_3$ deterred or showed a trend toward deterring feeding in 2 experiments, this negative effect on feeding (approximately a 1/3 reduction in mass eaten versus control food) was minor compared with the dramatic negative effect $CaCO_3$ had on feeding by several susceptible fishes (Schupp & Paul in press). The lack of strong negative effects of $CaCO_3$ on feeding by urchins is consistent with the fact that urchins have a fairly neutral gut pH (Lawrence 1982), commonly ingest some carbonate material while feeding (Birkeland 1989) and are able to assimilate organic material very well despite the addition of large amounts of inorganic material to their diet (Lowe & Lawrence 1976, Lawrence et al. 1989, Lares & McClintock 1991). Consequently, we concur with Schupp & Paul (in press) that the effect of powdered $CaCO_3$ on feeding by a herbivore is predictable based upon the gut pH and feeding ecology of the herbivore.

At the same time, our hypothesis that particle size may be important forces us to return to a major issue of the 'Introduction': does $CaCO_3$ affect feeding by increasing the mineral content of food or by increasing its toughness? Several studies have now shown that increasing the mineral content of food by adding powdered calcite and/or aragonite can strongly deter feeding by some herbivores without dramatically affecting food toughness (Pennings & Paul 1992, Schupp & Paul in press). However, few calcified algae incorporate small loose crystals of $CaCO_3$ into their thallus. In many cases (most dramatically the branching corallines and the *Halimeda* spp.), the $CaCO_3$ crystals fuse to form a larger skeleton which remains intact even if organic material is removed (Multer 1988). This skeleton may have an additional negative effect on feeding by increasing the toughness of the algal thallus. In order to understand fully the effect of calcification on feeding by herbivores, we must experimentally address both potential mechanisms.

Acknowledgements. We thank S. Amesbury, C. Birkeland, A. Kerr, S. Nelson, V. Paul, G. Paulay, T Pitlik, B. Pyle and R. Richmond for ideas and assistance. S. Nelson, V. Paul, G. Paulay and 2 reviewers made valuable comments on the manuscript. S. Pennings acknowledges NSF (OCE-9116307) to V. Paul for financial support. This is contribution number 343 from the University of Guam Marine Laboratory.

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This article was presented by C. Birkeland, Mangilao, Guam

Manuscript first received: April 2, 1993
Revised version accepted: July 14, 1993