# Reports

## Transport of Calcareous Fragments by Reef Fishes

*Abstract.* The weight of sand, coral scrapings, algal fragments, and other calcareous materials which pass through the intestines of reef fishes was calculated on a hectare-per-year basis. It was found that browsing omnivorous reef fishes which rely, in part, on a plant diet ingested and redeposited at least 2300 kg of such material on a 1-hectare study reef near Bermuda. Reasons are presented why this estimate, certainly in order of magnitude, should be applicable to coral reefs in general.

Over a nongeologic time span coral reefs represent relatively stable ecological associations. Live coral colonies can accumulate limestone at remarkable rates (*1*), while wave and tide erosion as well as animal activity are responsible for the breakdown of the dead coral. Burrowing annelid worms, mollusks, and crustaceans riddle the coral rock and weaken it for the attack of mechanical forces, while some fishes either scrape on corals (parrot fishes, Scaridae) or nibble on coral branches (filefishes, Monacanthidae). Many calcareous algae occur on the reef with its calcium-rich water, and these are eaten by certain reef fishes (surgeonfishes, Acanthuridae, and others). Coralline and foraminiferous sands are also frequently found in large quantities in the digestive tracts of many families of reef fishes (*2*). These materials may be eaten by the fishes for various reasons; some fragments are certainly ingested accidentally in the quest for small attached animals and algae; shells are crushed and swallowed, but, at least in the case of parrot fishes and surgeonfishes, sand serves as a milling agent for algal cell walls and perhaps even contributes to the buffering of intestinal juices.

I have attempted to arrive at an estimate of the amount of calcareous material, shell fragments, coral scrapings and branches, coralline algae, and sand that passes through the digestive tract of reef fishes to be redeposited for further displacement by waves or tides. My calculations and observations indicate that between 2 and 3 metric tons of calcareous material per hectare are redeposited on a typical Bermuda reef after passage through the digestive tract of fishes. Cloud (*3*) mentions that several tons of sand and fine gravel per hectare (1.100 to 1.600 metric ton/mi$^2$ are moved by fishes on Pacific atolls but cautions that this figure is based on some assumptions and wholesale estimates; there are no methods given.

Atlantic and Pacific reef-fish faunas are reasonably alike, at least in the feeding adaptations of their members, and estimates of total fish biomass for Bermuda and for certain Pacific reef environments are also comparable (*4*); it may therefore be safe to extend my estimates to reefs in general, at least in order of magnitude.

Estimates of the amounts of calcareous material (not differentiated as to type or provenience) which went through the fishes were reached as follows.

1) The stomachs and intestines of many reef fishes were examined for calcareous material.

2) The numbers of individuals of different species present were taken from a standing-crop estimate on a 1-hectare Bermuda reef (*4*).

3) The digestive tracts of about 200 individuals were weighed wet and oven-dried; full and empty intestines were used, to arrive at the relative weight of the gut and its contents. Water content of 75 to 80 percent was assumed for algal and invertebrate remains.

4) The passage time of materials through the intestine was determined by introducing a small amount of charcoal or vital dye with a blunted large injection needle past the pharynx of each fish while it was under MS-222 anesthesia. After separation of different species and size groups into various tanks, the time of the first appearance of colored feces was recorded. These passage times were checked against the time of natural emptying when the fishes were not fed.

5) Scuba and skindiving observations were made, in the field, of the time certain fishes spent in feeding during periods of daylight.

Thus, from knowledge of the numbers of coral- and sand-ingesters present, their rate of feeding and digestion, and the weight of calcareous fragments in the gut at any one time, a fair estimate of yearly deposition of such materials could be made.

The following families, given roughly in the order of their importance, were, in the main, found to carry sand, coral fragments, and other calcareous matter in their stomachs and intestines [see also Hiatt and Strasburg (*2*)]: parrot fishes (Scaridae) and surgeonfishes (Acanthuridae) (consistently large amounts of sand, coral fragments, and limestone powder present); butterfly fishes (Chaetodontidae) and damselfishes (Pomacentridae); gobies (Gobiidae); triggerfishes (Balistidae); goatfishes (Mullidae); wrasses (Labridae); and puffers (Tetraodontidae) (frequent to occasional findings of the above materials).

Small fishes and juveniles (10 to 50 g) passed colored fecal pellets after 1 to 2 hours, while medium-sized omnivores which feed predominantly on algae (250 to 500 g) retained ingested food for 3 to 5 hours—findings which indicate that the gut is filled thrice daily, or more often, in small fishes and twice daily in the medium-sized group. Fishes weighing over 1000 g are not likely to turn over their intestinal content more than once a day. Parrot fishes and surgeonfishes spent from two-thirds to four-fifths of their time feeding when they were observed during 5- to 10-minute periods scattered throughout the day.

There were 55 kg of small and young browsing fishes per hectare on the Bermuda study reef; they belonged to all the groups listed above with the exception of the triggerfishes (Balistidae). The dry, nonnutritive material found in their intestines amounted to between 2 and 4 percent of their wet body weight, and they turned over their food at least three times a day.

If they are fully active for 8 months

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*Instructions for preparing reports.* Begin the report with an abstract of from 45 to 55 words. The abstract should *not* repeat phrases employed in the title. It should work with the title to give the reader a summary of the results presented in the report proper.

Type manuscripts double-spaced and submit one ribbon copy and one carbon copy.

Limit the report proper to the equivalent of 1200 words. This space includes that occupied by illustrative material as well as by the references and notes.

Limit illustrative material to *one* 2-column figure (that is, a figure whose width equals two columns of text) or to *one* 2-column table or to *two* 1-column illustrations, which may consist of two figures or two tables or one of each.

For further details see "Suggestions to Contributors" [*Science* **125**, 16 (1957)].

(240 days), if they have three feeding cycles a day, and if calcareous fragments in the gut constitute 3 percent of the body weight, then the fishes must redeposit 1080 kg (roughly 1 ton) of calcareous material per hectare per year (3 percent of 55 kg × 3 × 240).

Analogous calculations have indicated that one or two daily fillings of the digestive tracts yield about 700 kg of material per hectare per year from surgeonfishes and 600 kg from parrot fishes. The larger filefishes were disregarded because they were present in relatively small numbers on the study reef. Where they occur in greater numbers they should be included in such an estimate because they feed almost exclusively on the tips of branching corals (*2*).

Adding the weight of calcareous material, purposely or accidentally ingested, which passes through the gut of small, medium-sized, and larger browsing reef fishes, one arrives at a total weight of at least 2300 kg/hectare yr.

Possible sources of error in this study lie in the following factors.

1) The estimate of numbers of fishes present. This source of error was discussed in a previous publication (*4*), where it was suggested that the estimate was on the low rather than on the high side.

2) The indicated effect of temperature on feeding. While such effects may be considerable in Bermuda, where corals and certain reef fishes live at the northern margin of their range, they should not be noticeable in truly tropical circumequatorial reef regions.

3) The omission of certain additional groups of fishes which only rarely ingest sand and coral or calcareous algal fragments (for example, jacks, Carangidae). For Bermuda this omission would tend to counteract an error on the high side resulting from reductions in feeding during the winter months, but for more tropical regions the omission would make my estimate of the amount of redeposited material too low.

4) The occurrence of periods of nonfeeding—for instance, during spawning. I do not believe this to be a large consideration because (i) parrot fishes were observed to feed in the spawning season, on one occasion even between successive pairing acts, and (ii) the nutritive content of attached algae forces fish which feed largely or partly on such materials to take in substantial quantities of food to sustain themselves, to say nothing of growing (*5*, *6*).

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**References and Notes**

1. H. B. Moore, *Marine Ecology* (Wiley, New York, 1958), p. 331.
2. R. W. Hiatt and W. Strasburg, *Ecol. Monographs* **30**, 65 (1960).
3. P. E. Cloud, Jr., *U.S. Geol. Survey Profess. Papers No. 280-K* (1959), p. 398.
4. J. E. Bardach, *Limnol. Oceanogr.* **4**, 77 (1959).
5. D. W. Menzel, *J. conseil, Conseil permanent intern. exploration mer.* **24**, 308 (1959).
6. This study was carried out at the Bermuda Biological Station for Research and at the Lerner Marine Laboratory at Bimini. I thank the directors and staffs of both stations for help received. The investigation was supported by the Horace Rackham School of Graduate Studies of the University of Michigan and by the Lerner Marine Laboratory. This report is contribution No. 277 from the Bermuda Biological Station for Research.

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## Facilitation of Infection of Monkey Cells with Poliovirus "Ribonucleic Acid"

*Abstract.* The plaque titer of poliovirus "ribonucleic acid" on monkey kidney cells cultured in vitro is greatly increased by depleting these cells of calcium and treating the "ribonucleic acid" inoculum with a suspension of any one of several poorly water-soluble substances before inoculation. These undissolved substances apparently facilitate infection by serving as solid vehicles for the "ribonucleic acid."

Intact ribonuclease-stable poliovirus is changed by phenol into an infective unit destructible with ribonuclease; this ribonuclease-labile poliovirus unit is called poliovirus "ribonucleic acid (RNA)" (*1*). When standard plaque assay techniques are used poliovirus "RNA" manifests a plaque titer on monkey kidney tissue cultures of only about $10^{-6.5}$ of the titer of the intact virus from which it was prepared (*2*). In this report we show how the titer of poliovirus "RNA" on such kidney cells can be greatly increased (*3*).

Poliovirus "RNA" was obtained by one treatment of intact wild-type virus of the Brunhilde strain (antigenic type 1) at 0° with 7 percent water-saturated phenol (*2*); this method is a modification of method of Gierer and Schramm (*4*). Kidney cells were grown and maintained and poliovirus stocks were obtained as described previously (*2*).

The combination of two specific procedures results in a large increase in the number of plaques produced by poliovirus "RNA." These two procedures are (i) addition to the "RNA" of any one of several compounds of low solubility in water, and (ii) depletion of the kidney cells of calcium (Table 1). Poorly soluble substances which facilitate infection of calcium-depleted cells with "RNA" include, besides $CaHPO_4 \cdot 2H_2O$ (*5*) and $Cr_2O_3$ (Table 1), $Al_2O_3$, $CaCO_3$, $CaSO_4$, $Co_2O_3$, $Fe_2O_3$, Fuller's earth, $MgCO_3$, $MgF_2$, $MgHPO_4 \cdot 3H_2O$, $Mg_3(PO_4)_2 \cdot 5H_2O$, $Mg_3Si_4O_{11} \cdot H_2O$, NiO, and ZnS. Two of these facilitators were tested for their capacity to adsorb poliovirus "RNA": at a facilitator concentration of 0.25 percent, $CaHPO_4 \cdot 2H_2O$ adsorbed 56 percent and $Mg_3(PO_4)_2 \cdot 5H_2O$ adsorbed 67 percent of the "RNA."

With $CaHPO_4 \cdot 2H_2O$ as facilitator, and calcium-depleted cells, the number of plaques formed is dependent on the tonicity both of the medium used for the "RNA" and of the medium used for the cells (Table 1) (see *6*). When the medium for the cells is isotonic fewer plaques are formed when the medium for the "RNA" is hypertonic than when it is isotonic or hypotonic. When the medium for the "RNA" is slightly hypotonic, peak plaque production is obtained when the medium for the cells is slightly hypotonic.

With $CaHPO_4 \cdot 2H_2O$ as facilitator, and calcium-depleted cells, the number of plaques produced by the "RNA" was dependent on the duration of the post-inoculation incubation at 37°C before layering with the nutritional agar maintenance medium. The results of three experiments suggest that the largest number of plaques is obtained when this duration approximates 1 hour. The moderately large variation among these experiments, however, suggests

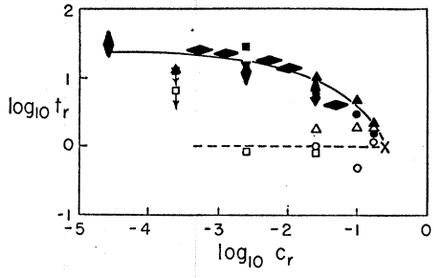


Fig. 1. The relationship of calculated plaque titer of poliovirus "RNA" with "RNA" concentration inoculated. Symbol "$c_r$" means relative concentration of "RNA" inoculated, based on assigning unity concentration value to "RNA" concentration in undiluted "RNA" preparation. Symbol "$t_r$" means calculated relative titer of "RNA," based on assigning unity titer value to titer obtained when $c_r = 0.25$. For $c_r = 0.25$, denoted by *X*, and for all solid symbols inoculum contained 0.25 percent $CaHPO_4 \cdot 2H_2O$; for all open symbols inoculum contained <0.25 percent $CaHPO_4 \cdot 2H_2O$ since these inocula were obtained by dilution of the mixture of "RNA" and $CaHPO_4 \cdot 2H_2O$ into diluent(s) without $CaHPO_4 \cdot 2H_2O$. All symbols of same shape from same experiment, of different shape from different experiments. Arrows denote maximal points; that is, no plaques were found at inoculum "RNA" concentration indicated.