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## Fish Schools: An Asset to Corals

**Abstract.** *Schools of juvenile haemulid fish feed in sea grass beds at night. By day they rest over coral heads, where they excrete substantial quantities of ammonium and particulate nitrogen and phosphorus into the nutrient-poor waters. The percentages of these nutrients contributed by the fish were comparable to those from other sources. Coral heads with resident fish schools grew faster than those without resident schools, indicating that fish may be more beneficial to the corals than has been assumed.*

Fish schools resting over and in coral heads are a well-known feature of coral reefs throughout the world, and the benefits of such shelter to the fish have long been recognized (1). We found that corals may also benefit from the association. Excretory and fecal products from the fish were a substantial source of nitrogen and phosphorus in the nutrient-poor waters of a coral reef, and coral heads with fish had higher growth rates than those without fish.

It has been suggested that when fish migrate between resting and feeding areas they transfer energy and nutrients to their resting areas (2, 3). Although this phenomenon has been widely reported in birds (4), only carbon transport by fish in a temperate rocky reef community has been described (3). A coral reef is an ecosystem characterized by high biomass and productivity despite low nutrient content of the water. In such an ecosystem it is particularly critical to understand the pathways by which nutrients are cycled, and thereby enhance productivity. We investigated whether migrating fish could be a source of nutrient enrichment for reefs. Numerous tropical fish species migrate daily to feed (5); we studied heterotypic schools of juvenile French and white grunts (*Haemulon flavolineatum* and *H. plumieri*) in Teague Bay, St. Croix, U.S. Virgin Islands. These fish (30 to 120 mm total length) aggregate over coral heads during the day and migrate at sunset to surrounding sea grass beds, where they feed on benthic invertebrates; their guts are full in the morning and empty by late afternoon (6). The fish use the same migration routes and return to the same coral heads each morning (6-8). Schools

have been observed on the same head for more than 3 years (8).

To determine whether fish excretory products were enriching the water around a coral head, we sampled water within a school resting in a large *Acropora palmata* head (9). When fish were present,  $\text{NH}_4^+$  concentrations up to 0.9  $\mu\text{M}$  were recorded; concentrations remained at 0.2  $\mu\text{M}$  in an adjacent head without fish. No statistically significant differences in concentrations of molybdate-reactive phosphorus (MRP) were detected between the sites (10).

We also examined the possibility that fish feces were providing an additional nutrient source for the benthic community associated with the coral head. Sediment traps were placed in an *A. palmata* head with grunts and in a head without fish (11). A significantly greater quantity

of nitrogen and phosphorus as well as particles with significantly higher percentages of these nutrients were collected from traps under fish schools (12), suggesting that the excretory products were providing a supplement to the coral head community.

To determine the amounts of nutrients contributed by fish, we monitored the biomass of grunts on six coral heads and measured nitrogen and phosphorus excretion rates of fish in the laboratory. Fish biomass was estimated on three *Porites furcata* and three *A. palmata* heads at 4-month intervals from April 1980 to December 1981. Numbers of fish and the size-frequency distribution were determined for each school from photographs (13). To assess the accuracy of this technique, photographs were taken just before all fish were removed from two heads. Photographic estimates of biomass were 67 and 71 percent of the biomass of captured fish. Hence, the data are probably underestimates of the nutrient contribution by fish. We present data only for grunts although the coral heads harbored squirrelfish and cardinal fish, species that also feed away from the head.

Daily generation of  $\text{NH}_4^+$ , MRP, total dissolved nitrogen and phosphorus (14), and particulate nitrogen and phosphorus (15) were measured on fish in the laboratory (16). Regressions of the rate of nutrient generation on body weight were developed from these data and combined with the biomass and size-frequency data from the photographs to estimate the daily production of nitrogen and phosphorus by the resident population on each coral head (Table 1).

Corals are known to use other sources of nitrogen and phosphorus, namely, dis-

Table 1. Amounts of nutrients deposited by juvenile French and white grunts resting over two species of coral heads. The nutrient contribution is also expressed as a percentage of nutrients available from other sources. Values are the means  $\pm$  95 percent confidence intervals from three heads of each species measured on three to five different dates. Fish biomass was  $39 \pm 13$  g/m<sup>2</sup> (dry weight) on *Porites furcata* ( $N = 12$ ) and  $172 \pm 29$  g/m<sup>2</sup> on *Acropora palmata* ( $N = 12$ ).

| Nutrient           | Contribution to                 |                |                                 |               |
|--------------------|---------------------------------|----------------|---------------------------------|---------------|
|                    | <i>Porites furcata</i>          |                | <i>Acropora palmata</i>         |               |
|                    | mmole/m <sup>2</sup><br>per day | Percent        | mmole/m <sup>2</sup><br>per day | Percent       |
| Nitrogen           |                                 |                |                                 |               |
| $\text{NH}_4^+$    | 2.4 $\pm$ 0.7                   | 30 $\pm$ 10    | 7.3 $\pm$ 2.2                   | 48 $\pm$ 14   |
| Dissolved          | 3.0 $\pm$ 0.8                   | 0.8 $\pm$ 0.2  | 7.4 $\pm$ 1.2                   | 1.1 $\pm$ 0.3 |
| Particulate        | 0.9 $\pm$ 0.2                   | 41 $\pm$ 13    | 2.2 $\pm$ 0.6                   | 59 $\pm$ 17   |
| Total              | 3.9 $\pm$ 0.6                   | 1.0 $\pm$ 0.3  | 9.6 $\pm$ 2.8                   | 1.4 $\pm$ 0.4 |
| Phosphorus         |                                 |                |                                 |               |
| Molybdate-reactive | 0.05 $\pm$ 0.02                 | 3.0 $\pm$ 1.2  | 0.21 $\pm$ 0.06                 | 6.6 $\pm$ 1.9 |
| Dissolved          | 0.07 $\pm$ 0.02                 | 1.0 $\pm$ 0.4  | 0.30 $\pm$ 0.09                 | 2.2 $\pm$ 0.7 |
| Particulate        | 0.12 $\pm$ 0.02                 | 68 $\pm$ 20    | 0.31 $\pm$ 0.09                 | 94 $\pm$ 29   |
| Total              | 0.20 $\pm$ 0.05                 | 2.6 $\pm$ 0.18 | 0.62 $\pm$ 0.8                  | 4.4 $\pm$ 1.3 |

Table 2. Growth of fingers from three *Porites furcata* heads on patch reef one, Teague Bay, St. Croix, U.S. Virgin Islands. Values are the means  $\pm$  1 standard error of determinations of weight of  $\text{CaCO}_3$  deposited over the periods indicated. Head W had grunts, head WR had grunts until they were removed in late December 1980, and head WO had no grunts. Means from the same sampling period that are significantly different are followed by a different letter (analysis of variance, Duncan's multiple range test,  $\alpha = .05$ ). An average monthly growth rate for each finger determined in the December 1979–December 1980 data set was used to calculate an expected growth rate over an 8-month period. Only the observed growth rate on head WR was significantly different from the expected [ $t(18) = 2.69$ ].

| Head | December 1979–<br>December 1980 |                   | December 1980–August 1981 |                   |                        |
|------|---------------------------------|-------------------|---------------------------|-------------------|------------------------|
|      | Fingers<br>(N)                  | Growth<br>(g)     | Fingers<br>(N)            | Growth<br>(g)     | Expected<br>growth (g) |
| W    | 13                              | 5.43 $\pm$ 0.60 A | 8                         | 3.45 $\pm$ 0.42 C | 3.62 $\pm$ 0.40        |
| WR   | 12                              | 5.19 $\pm$ 0.61 A | 8                         | 1.91 $\pm$ 0.35 D | 3.46 $\pm$ 0.41        |
| WO   | 13                              | 3.18 $\pm$ 0.63 B | 11                        | 2.87 $\pm$ 0.20 C | 2.12 $\pm$ 0.42        |

solved nutrients in the water column and zooplankton (17). The magnitude of these sources was determined for calm conditions by multiplying average concentrations, approximate cross-sectional area of the coral head, and a current speed of 0.3 cm/sec (18). Fish excretion is expressed as a percentage of these other sources in Table 1. Clearly fish can provide a sizable supplement of  $\text{NH}_4^+$ , a readily available nitrogen form for corals (19). Fish feces may also provide a large portion of particulate nitrogen and phosphorus. Although the coral may not ingest fish feces, the feces will leach nitrogen and phosphorus and should enrich the benthic community associated with the coral head.

The greater the water movement the more rapidly excreta will be diluted; however, nutrients remain for at least short periods of time, particularly in the bay under study, where a strong surge is more common than a high current. On a rough day when currents were approximately 4 cm/sec, we injected dye into a fish school in an *A. palmata* head. It took 6.5 minutes for the dye concentration to be reduced to 10 percent of its initial value. The fish are in close proximity to the corals, and some evidence indicates that algae are able to respond to and rapidly assimilate nutrients from microscale patches (20); zooxanthellae may have similar uptake capacities.

We also compared fish excretion rates with measured rates of nitrogen fixation, an important nitrogen source on some coral reefs (21). The highest rate of nitrogen fixation recorded from a coral reef community is 12.8 mmole/m<sup>2</sup> per day on a blue-green algae dominated reef flat (21). The amount of nitrogen supplied to the coral heads each day by the fish is 30 to 75 percent of that maximum nitrogen fixation rate (Table 1).

To determine whether increased rates of nutrient supply or some other factor

associated with the presence of grunts were conferring some advantage to coral, we measured growth rates of three heads of *P. furcata* within 25 m of each other on the same patch reef. Two heads had grunts, and one did not. At least ten coral fingers on each head were stained with alizarine; after 1 year, they were harvested and the weight of new  $\text{CaCO}_3$  produced was determined (22). Growth rates were significantly greater on the two coral heads with grunts (Table 2).

To examine the alternative explanation that environmental factors other than the presence of fish were responsible for the higher growth rates, we compared expected and actual growth rates of a head for an 8-month period after removal of grunts (23). Growth on the head from which grunts were removed was significantly different from the expected rate (Table 2). Although other environmental factors influence coral growth and can overshadow the effect of grunts (24), our data indicate that the presence of resident grunt schools contributes, to more rapid coral growth.

Thus it appears that juvenile grunt schools may provide an important supplement of some nutrients to the coral heads over which they rest and that this supplement, or some other factor associated with the presence of the fish, may increase the growth rate of the coral. Although juvenile grunt schools are not found on all coral reefs, fish in at least 14 other families also feed away from and then rest in or over coral heads (5, 25). They probably also serve as a nutrient source for these corals and as an important link between the ecosystems in which they rest and feed.

JUDY L. MEYER

ERIC T. SCHULTZ\*

GENE S. HELFMAN

Zoology Department and Institute  
of Ecology, University of  
Georgia, Athens 30602

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9. Tubing was placed in the coral head where the fish congregated, and on four calm days, divers sampled water by drawing it through the tubing with a syringe ( $N = 6$ ).
10. For  $\text{NH}_4^+$  analysis technique, see L. Solorzano, *Limnol. Oceanogr.* **14**, 799 (1969); for MRP analysis, see J. Murphy and J. P. Riley, *Anal. Chim. Acta* **27**, 31 (1962).
11. Traps were 16 plastic bottles (opening, 15.9 cm<sup>2</sup>; depth, 16 cm) taped together.
12. Nested analysis of variance,  $\alpha = .05$ .
13. Meter sticks were placed in a school for scale so that fish length (millimeters) could be measured from the photographs. Fish dry weight (grams) was calculated with a regression ( $N = 108$ ):  $\ln(\text{weight}) = 2.669 + 0.0496 \times \ln(\text{standard length})$ .
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15. Modified Kjeldahl digestion: Technicon Auto-Analyzer II, industrial method No. 329-47 W/B, Technicon Industrial Systems, Tarrytown, N.Y.
16. Fish were captured at dawn; a seine was placed across the migration route, and the fish were collected in a 1-m<sup>3</sup> clear plastic bag with screening at one end. During transfer to experimental chambers, fish were out of water for less than 5 seconds and showed no damage from handling. The chambers were either 3-liter plastic beakers or 20-liter plastic buckets with 2 or 5 liters of filtered seawater, respectively, and one to five fish (0.3 to 13.5 g, dry weight). Experiments continued from dawn to dusk, the period that the fish would spend in the coral head. Water samples were removed with a syringe at 2-hour intervals and feces were removed every hour. Chambers were maintained at ambient water temperature in baths of flowing seawater and were reoxygenated each hour by drawing water into a 50-ml syringe and forcefully reinjecting it into the chamber. After the first 5 minutes, the fish remained relatively immobile near the bottom of the chamber, although they would dart away from the syringe used for sampling. No signs of respiratory stress were observed.
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18. Mean concentrations of  $\text{NH}_4^+$ , MRP, and total dissolved nitrogen and phosphorus were determined from 16 to 22 water samples taken during January, April, and August 1980. Particulate nitrogen and phosphorus were determined on subsamples from tows of a diver-held net (153- $\mu\text{m}$  mesh, 0.5-m diameter) along a 60-m transect. During May and August 1980 and January 1981, 39 tows were taken between 0500 and 2100 hours. A mean daily concentration was calculated from these data by determining daytime and nighttime means with an assumed 14-hour day and a 10-hour night. Approximate cross-sectional area of each coral head was calculated as average height of the head times the diameter of a circle with area equal to the area of the head.
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22. Fingers were stained by injecting 2 ml of a 0.5 percent alizarine solution into Whirlpac bags that were secured over the fingers for 36 hours. After harvest, corals were bleached, dried, and cut along the growth axis, and  $\text{CaCO}_3$  deposited before staining was filed away and the new

growth weighed. This weight was corrected for material cut away by the saw, generally 10 percent of the weight.

23. Because grunt schools consistently associate with the same coral heads and because recruitment from the plankton was low over this period, < 30 grunts were found on this head both 4 and 8 months after the original school of 304 was removed.
24. Over the 8-month period, mean growth rate of the head with fish (W) was higher than the control head (WO), but the difference was not statistically significant. A decreased number of grunts on head W and environmental factors other than the presence of grunts (weather-related factors or simply more squirrelfish on head WO over this period) were obscuring the impact of grunts.

25. Families include acanthurids, apogonids, carangids, chaetodontids, holocentrids, lutjanids, mullids, pempherids, pomacentrids, priacanthids, scarids, sciaenids, serranids, and siganids.
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\* Present address: Department of Biological Sciences, University of California at Santa Barbara, Santa Barbara 93106.

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## Rapid Flow Cytometric Analysis of the Cell Cycle in Intact Plant Tissues

**Abstract.** Mechanical chopping of plant tissues in the presence of mithramycin released intact nuclei representative of the cells within the tissues. The amount of nuclear DNA in the homogenates of monocotyledonous and dicotyledonous plants was accurately and rapidly determined by flow microfluorometry, and the distribution of nuclei involved in the cell cycle was charted for tissues selected from different physical locations or developmental stages.

Flow cytometric methods have several advantages over conventional microscopic procedures for determining the cell cycle status of eukaryotic cells. In particular, they are exceptionally rapid, accurate, convenient, and sensitive (1). A disadvantage is the requirement for

single-cell suspensions. Although some animal cell types exist as single cells in vivo, and many other animal tissues can be converted into single-cell suspensions by proteolytic digestion, higher plant cells commonly exist as complex three-dimensional tissue structures. Further-

more, these structures often display a gradation in cellular differentiation as a function of position, particularly in terms of distance from a meristematic region.

It is clear that cell cycle activity is intimately connected with differentiation and that an understanding of plant cell cycle control is central to an understanding of the establishment of plant form during growth. Less clear is the means for establishing a method to accurately and conveniently determine the status of the cell cycle in higher plant tissues, as reflected in the cellular nuclear DNA contents. Investigators have been using quantitative microphotometry with Feulgen staining or DNA-specific dyes such as Hoechst 33258 (2, 3). The slowness and inaccuracy of such techniques have hindered the satisfactory resolution of questions on the cell cycle in higher plant tissues. Although the preparation of protoplasts by the use of cell wall-digesting enzymes can be an appropriate first step for the analysis of the plant cell cycle (4), in that protoplasts represent single-cell populations, it is difficult to determine the precise prior location of the cells in the tissue from which the protoplasts are obtained. Furthermore, many plant tissues do not readily yield satisfactory preparations of protoplasts. Finally, it cannot be determined whether a proto-

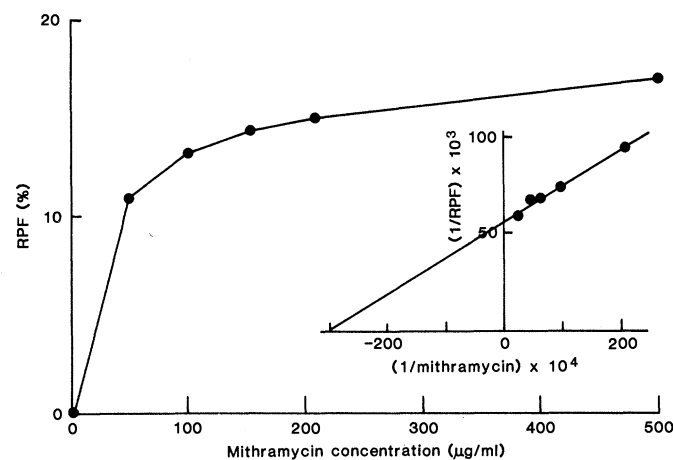
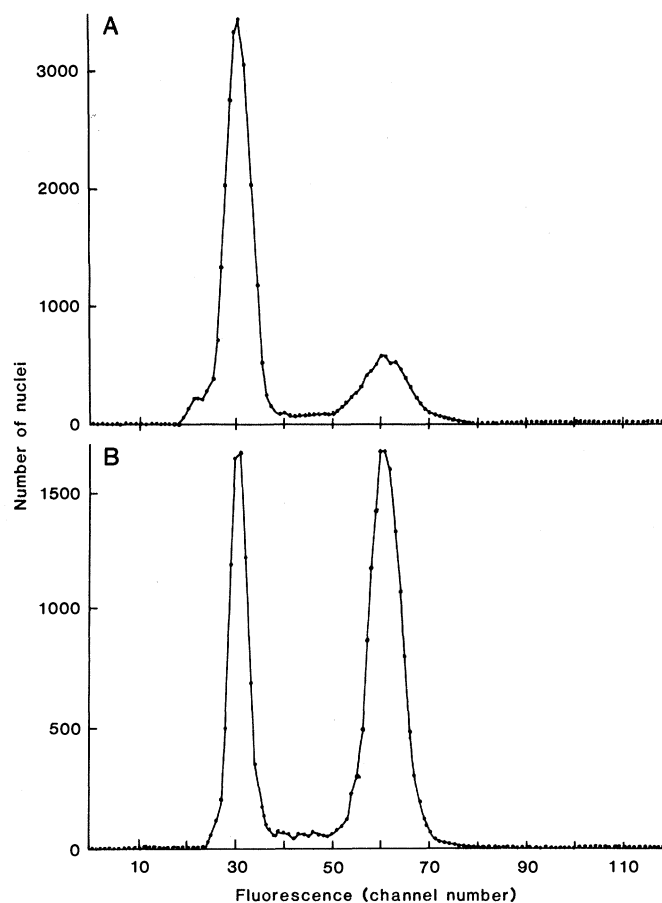


Fig. 1 (left). Flow cytometric analysis of nuclei released by chopping of leaf (A) or terminal root (B) tissue of *N. tabacum*. The nuclei were stained with mithramycin before analysis. The coefficients of variation for the  $G_1$  peaks were 8.1 and 6.0 percent, respectively, and the profiles represent (A) phase  $G_1$ , 71.1 percent; S, 6.6 percent; and  $G_2$ , 22.3 percent; and (B)  $G_1$ , 30.5 percent; S, 13.6 percent; and  $G_2$ , 55.9 percent.

Fig. 2 (right). Binding characteristics of the mithramycin-DNA interaction in nuclei released by chopping *N. tabacum* leaf tissue. The degree of fluorescence of the  $G_1$  peak is expressed as the relative peak fluorescence (RPF), a value obtained by dividing the channel number of the  $G_1$  peak by the channel number of the peak fluorescence of Fullbright fluorescent microspheres, which were included in each flow cytometric determination as internal standards.