PROCEEDINGS OF THE FIFTH SYMPOSIUM ON THE NATURAL HISTORY OF THE BAHAMAS

Edited by
Lee B. Kass

Conference Organizer
Daniel R. Suchy

Bahamian Field Station, Ltd.
San Salvador, Bahamas
1994

Cover photo by
Sandra Buckner

Printed in USA by Don Heuer

ISBN 0-935909-52-4

MECHANISMS FOR PHOSPHATE CYCLING IN SEAGRASS BEDS

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ABSTRACT

Seagrasses grow in a wide variety of coastal marine environments, many of which are phosphorus limited. In this study we investigated phosphorus recycling by examining phosphatase activity associated with root-rhizome segments and rhizoplane bacterial isolates. General phosphatase activity was assayed using pure culture isolates on a glycerol artificial seawater medium (GASW) containing phenolphthalein diphosphate. Root-rhizome segments were assayed for acid and alkaline phosphatase activity over time. Root-rhizome segments displayed variable rates among the three species tested, although all species did show some activity. The tropical root-rhizome segments showed ten-fold higher rates of alkaline phosphatase activity over acid phosphatase. Bacteria associated with *Syringodium* and *Halodule* segments showed higher acid and alkaline phosphatase activity compared to *Thalassia* for samples collected in 1991 and 1993. *Halodule* samples from North Carolina had higher acid and alkaline phosphatase rates than those taken from San Salvador. These data indicate that phosphatase activity may play an important role in recycling phosphorus in tropical and temperate seagrass systems.

INTRODUCTION

Seagrass meadows form the nutritional base of complex coastal marine ecosystems. These plants protect coastlines, are at the base of the food web, and act as nursery grounds for many fish species (Kikuchi and Peres, 1977; Zieman, 1982; Thayer *et al.*, 1984). Therefore, the growth and maintenance of healthy seagrass beds is of concern (Robblee *et al.*, 1991).

Nutrient uptake mechanisms of seagrasses have been studied for a number of years (McRoy and Barsdate, 1970; Patriquin and Knowles, 1972), particularly those nutrients which may limit seagrass productivity. Both nitrogen and phosphorus occur in low concentrations in many coastal waters where seagrass meadows are found. Nitrogen limitation, in some cases, may be compensated by nitrogen fixation by the associated microflora (O'Donohue *et al.*, 1991; Short *et al.*, 1990). Phosphorous, on the other hand, is not generated *de novo*, but is recycled primarily from detrital material in the sediment. In addition, carbonate sediment competes with seagrass roots for phosphate ions by absorption of these ions into the carbonate matrix (DeKanel and Morse, 1978; Short *et al.*, 1985).

Phosphate availability to seagrasses growing in tropical, oligotrophic, carbonate sediments, can be increased by the rhizoplane microflora. For example, Craven and Hayasaka (1982) showed that acid producing rhizoplane bacteria could solubilize apatite. However, most of the sediment phosphorous in carbonate environments is organic rather than mineral (Short *et al.*, 1990) and, therefore, subject to mineralization by microbial phosphatases. The purpose of this study was to determine the prevalence of microbial phosphatases on the rhizoplane of three seagrass species growing in oligotrophic waters and carbonate sediments of San Salvador Island, Bahamas.

MATERIALS AND METHODS

Core samples were collected from Graham's Harbor on San Salvador Island, Bahamas. The root-rhizome systems of the

three seagrasses (*Thalassia testudinum*, *Halodule wrightii*, and *Syringodium filiforme*) were removed aseptically and rinsed in sterile seawater, to remove adhering sediment. Some root-rhizome segments were assayed directly for phosphatase activity while others were plated onto GASW (glycerol artificial seawater) medium (Smith and Hayasaka, 1982) to obtain pure cultures of rhizoplane isolates.

Bacterial isolates were assayed for phosphatase activity using three different methods. The first was a general phosphatase (GP) assay, used to screen isolates for the presence of the enzyme. Cultures were inoculated onto the surface of GASW plates containing 0.01% sodium phenolphthalein diphosphate. The cultures were allowed to grow for three days and then exposed to a drop of ammonium hydroxide on the bottom of an inverted plate. GP positive cultures produce a red color (Gerhardt *et al.*, 1981).

Both bacterial isolates and root-rhizome segments were tested for acid phosphatase (AP) and alkaline phosphatase (BP) activity. Pure bacterial suspensions or root-rhizome segments were added to 60 ml serum vials containing 30 ml of 3.2% sterile artificial seawater. Subsamples of 1.5 ml were removed at 24 hour intervals for up to seven days and assayed for AP and BP using the disodium p-nitrophenyl phosphate method (Gerhardt *et al.*, 1981). Production of p-nitrophenyl was monitored at 405nm on a spectrophotometer. Results from root segments are given on a per gram root-rhizome tissue basis.

RESULTS

All of the seagrass rhizoplane isolates tested had GP activity (Figure 1, A-D), however, some appeared to be more active than others. AP activity, among the isolates was not noticeable before two days incubation (Figure 2A). Strains SSS1, SST10, and SST6 showed higher AP activities than strains SSH6, SSH7, and SSS9 (the third letter in the strain designation corresponds to the seagrass species from which it was isolated). BP activity was apparent for the *Thalassia* strains upon inoculation, and for strain SSS1 after four days (Figure 2B) incubation. Isolates from *Halodule* showed very little activity. Colony counts (Figure 3) for the bacterial communities did not increase greatly after day 1.

AP activity of root-rhizome segments from June of 1991 was higher for *Halodule* and *Syringodium* than *Thalassia* (Figure 4A). *Syringodium* segments from March of 1993 had only slightly higher rates than *Halodule* and *Thalassia* (Figure 4C). The differences in AP activity among species, in 1991 and 1993, became more distinct when root-rhizome segments were aged for seven days at 4° C (Figure 4B&D). Activity for aged root-rhizomes was also higher than for fresh for each season, and aged 1991 samples had higher rates than 1993 samples. AP activity for fresh and aged North Carolina samples (Figure 6) was higher than that for fresh and aged San Salvador samples.

A pattern similar to that of AP was observed for BP activity (Figure 4&5). Fresh root-rhizome samples from June 1991 and March 1993 had BP activity that was higher for *Halodule* and *Syringodium* than *Thalassia*. BP activity for aged root-rhizomes from 1991 and 1993 showed a clearer distinction between species and an increase in activity. BP activity was higher, however, for aged 1991 samples compared to aged 1993 samples. BP activity for root-rhizomes from North Carolina (Figure 7) was higher than that for root-rhizomes from San Salvador.

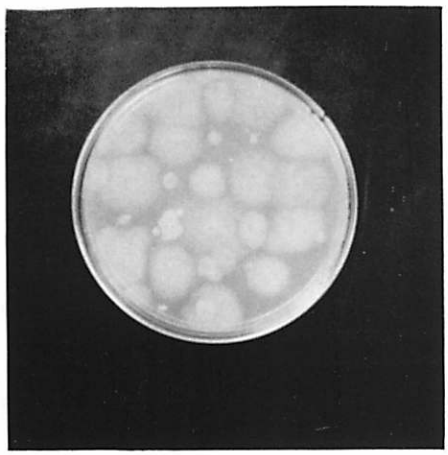
In June 1991, BP activity was ten-fold higher than AP activity, for both fresh and aged segments. Results from March of 1993 also showed BP activity to be ten-fold higher than AP activity. BP activity of *Halodule* samples from North Carolina was higher than AP activity. Fresh and aged North Carolina *Halodule* samples had higher AP activity than fresh or aged *Halodule* samples from San Salvador, as was also true for BP activity.

DISCUSSION

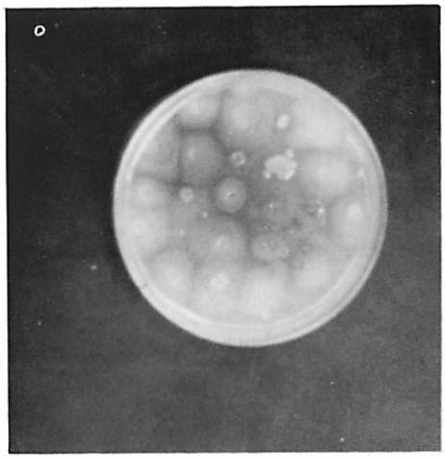
Although the GP assays indicated that all of the seagrass rhizoplane isolates, from San Salvador, could produce phosphatase (Figure 1), the quantitative AP and BP assays (Figure 2, 4&5) showed that rates were not the same for all isolates. Colony counts (Figure 3) indicate that the increase in activity over time

Figure 1. General Phosphatase Activity of Seagrass Rhizoplane Isolates Showing Progressive (A. to D.) Development of the Phenolphthalein (Red) Indicator.

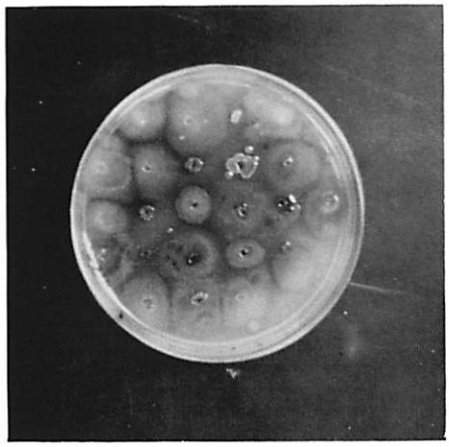
A.



B.



C.



D.

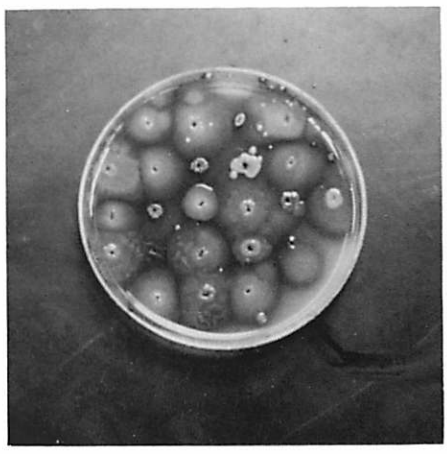


Figure 2. Acid and Alkaline Phosphatase Activity of Thalassia (Thal), Syringodium (Syn) and Halodule (Hal) Isolates.

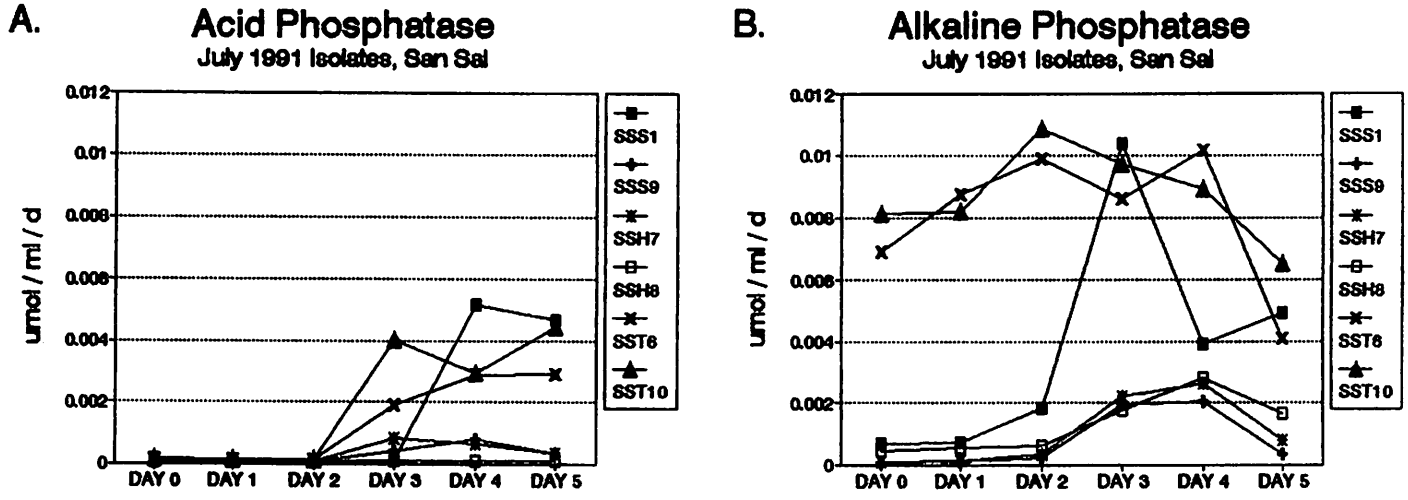


Figure 3. Colony Counts for Halodule (Hal), Syringodium (Syn), and Thalassia (Thal) Root-Rhizomes from San Salvador.

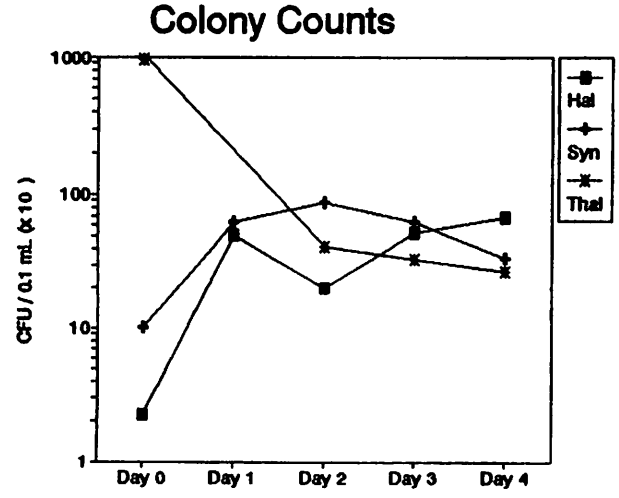


Figure 4. Acid Phosphatase Activity for Fresh and Aged Root-Rhizomes from San Salvador Island.

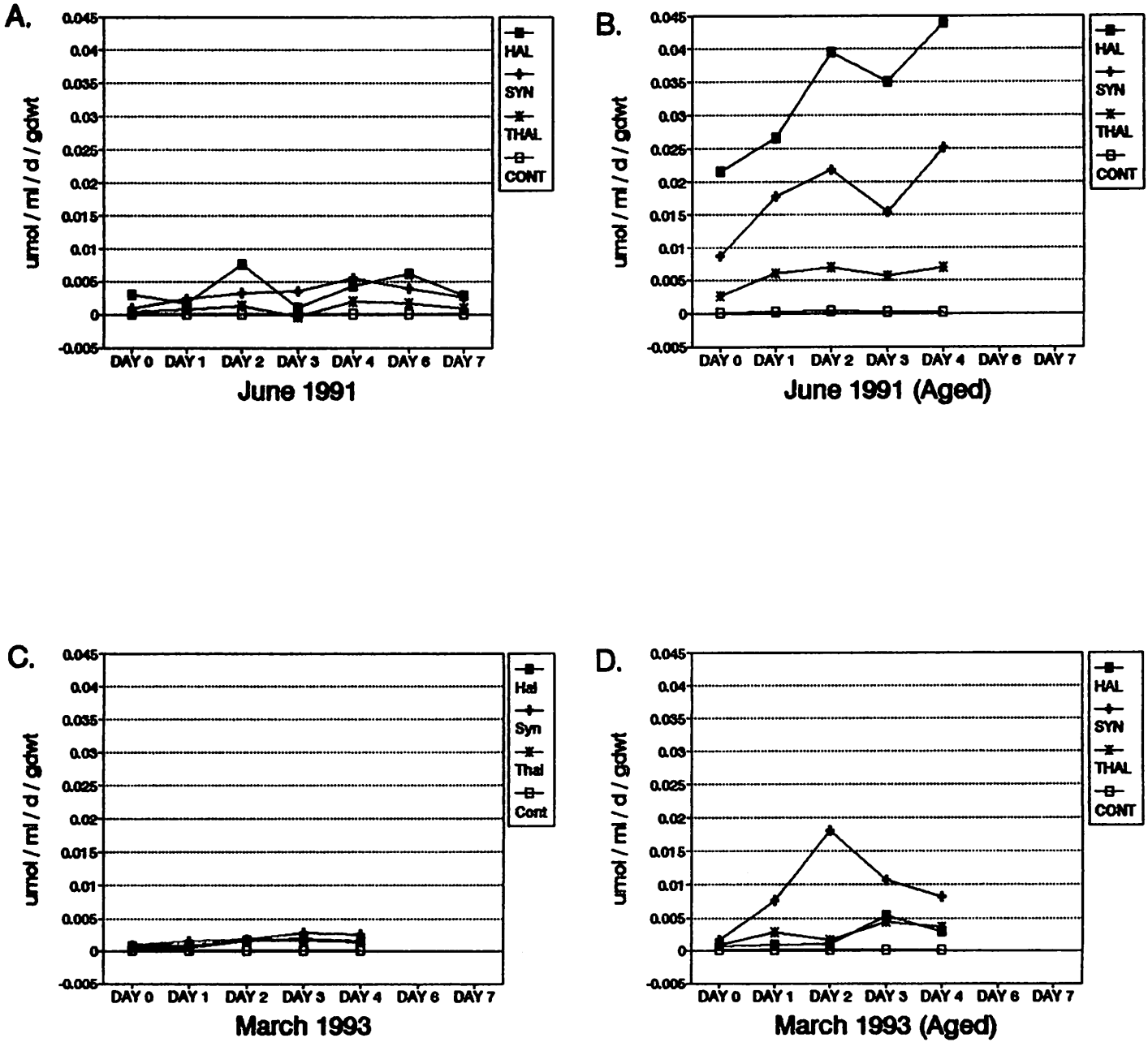


Figure 5. Alkaline Phosphatase Activity for Fresh and Aged Root-Rhizomes from San Salvador Island.

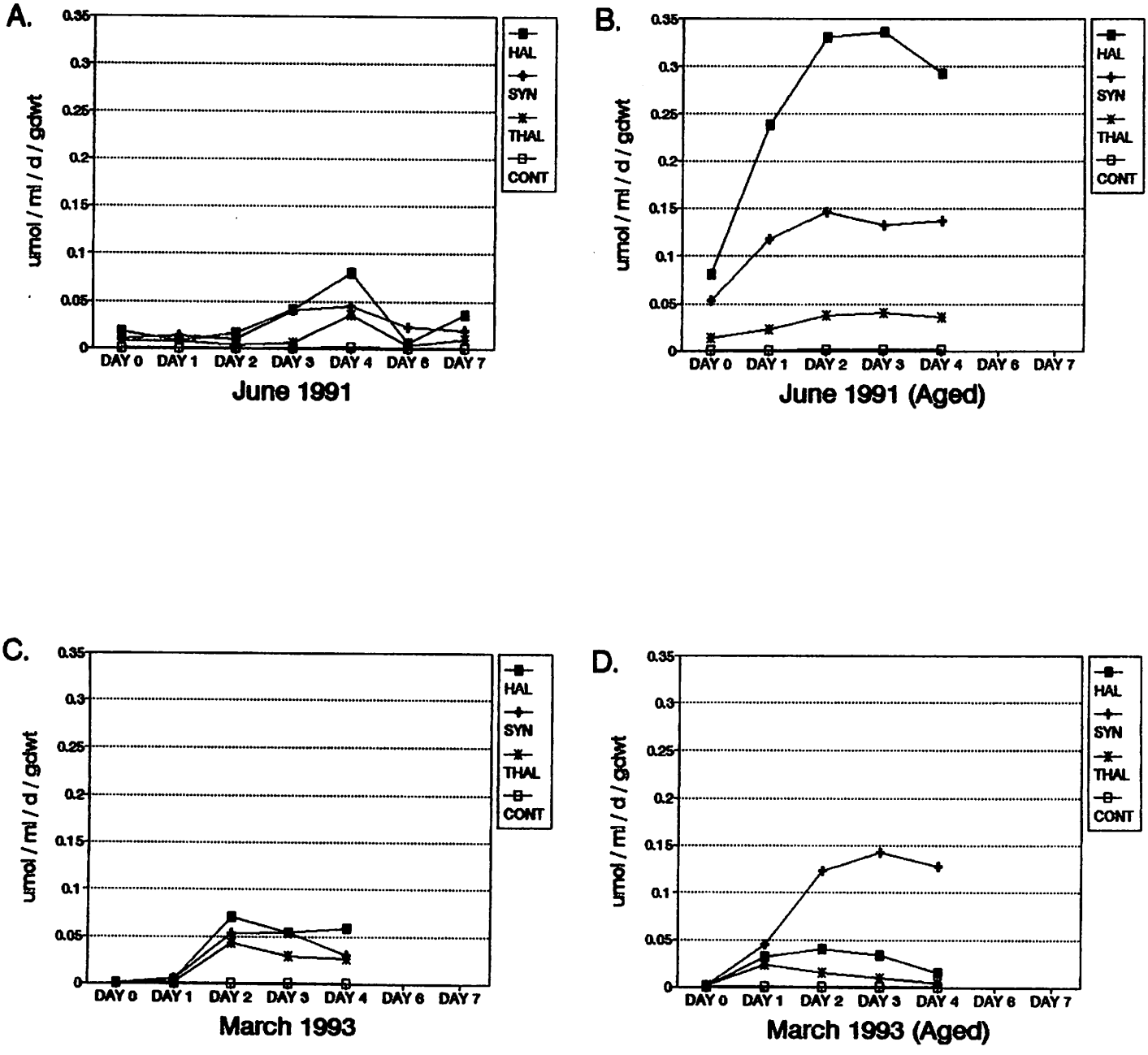


Figure 6. Acid Phosphatase Activity of Halodule (Hal) Root-Rhizomes from North Carolina.

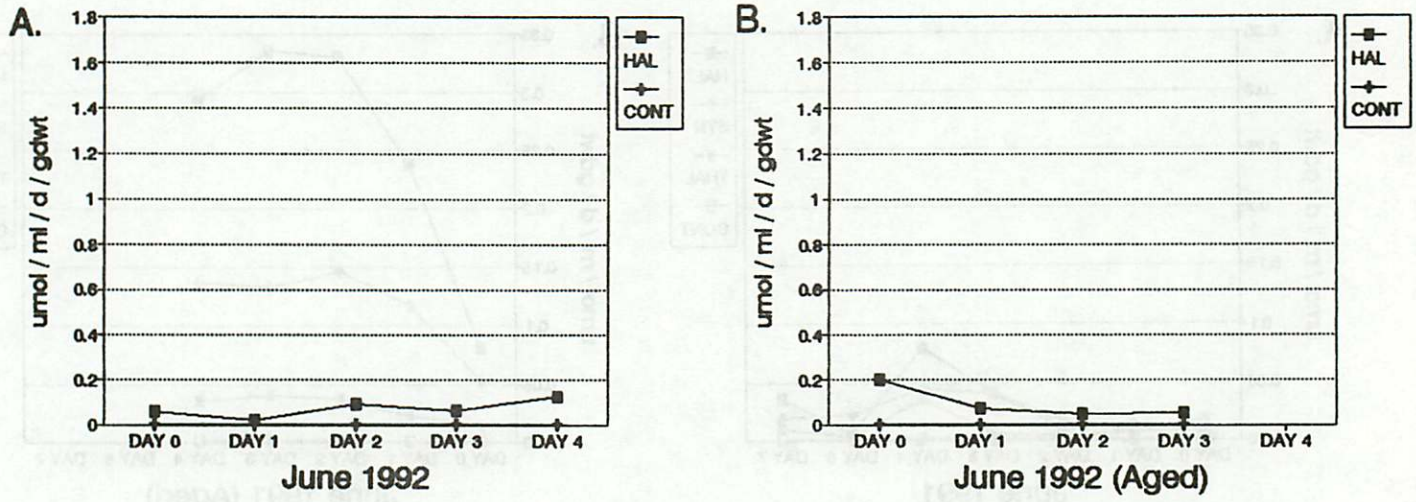
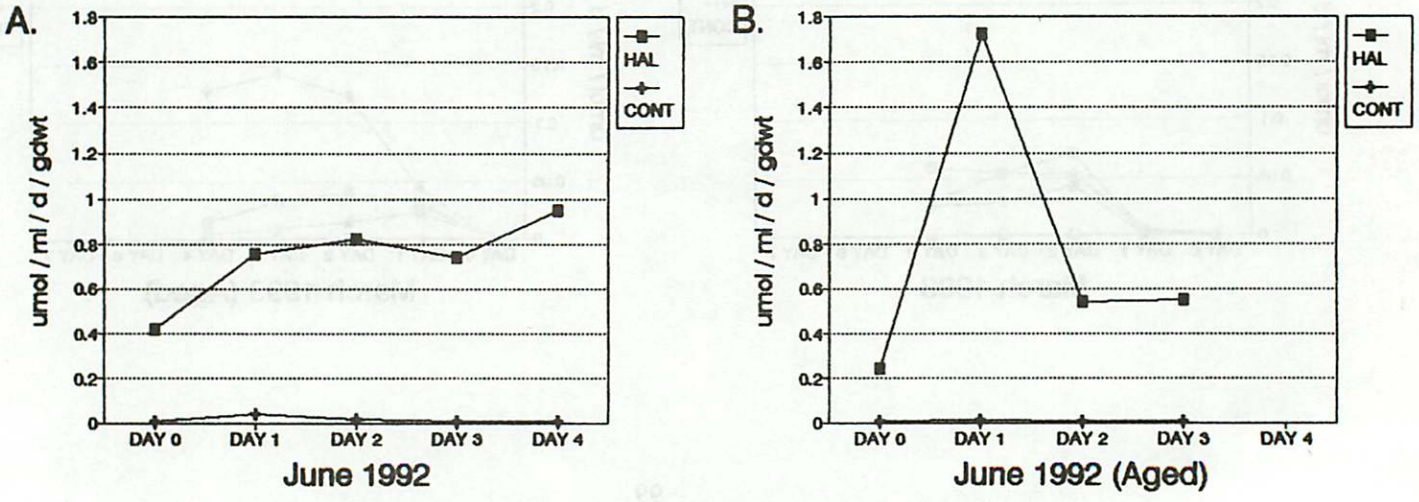


Figure 7. Alkaline Phosphatase Activity of Halodule Root-Rhizomes from North Carolina.



and the variance among rates of different species are not influenced by fluctuations in colony growth. Based on results from pure cultures, one may predict that *Thalassia* roots should exhibit the highest associated rates of both AP and BP activity and that *Halodule* roots should be lowest. The opposite was true (Figure 4&5), for the aged root-rhizome segments (Figure 4 B&D, 5 B&D). However, only six of the isolates were tested and, even if many more were tested, only those heterotrophs that would grow on the GASW medium, would be represented. Although the GASW medium was used because it is relatively nonselective, all media are selective to some degree, and may not represent the actual microflora.

Halodule samples from North Carolina showed AP and BP activity higher than those from San Salvador. This is surprising since the terrigenous sediments and mesotrophic waters around North Carolina contain labile phosphate compounds and phosphatase should not be required to make phosphates available from the environment.

Another interesting observation was that BP activity was about ten-fold greater than AP activity for root-rhizome segments from both San Salvador and North Carolina. Although this could be expected based on the alkaline pH of the sediments (approx. 8.2), the rhizoplane is subject to organic acid exudation by both the roots and microflora and, therefore, may have a lower pH than the surrounding sediment.

Both acid and alkaline phosphatase activity were observed in association with *Halodule, Syringodium* and *Thalassia* in San Salvador and with *Halodule* in North Carolina and their microflora. BP activity was higher than AP activity, for San Salvador samples, and was greatest for *Halodule, Syringodium* and *Thalassia* root-rhizomes, respectively. Results indicate that phosphatase activity by the rhizoplane microflora of tropical seagrasses may play an important role in providing phosphate to the plants.

ACKNOWLEDGEMENTS

This work was supported by a Research and Productive Scholarship Grant from the University of South Carolina, grants from The Center for Field Research, and The Bahamian Field Station, and a NSF grant to the Ruth Patrick Science Education Center. We would like to thank Dr. James R. Yates for scientific insight and criticism. This is contribution no. 999 of the Belle W. Baruch Institute of Marine Biology and Coastal Research.

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