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SNAIL GRAZING EFFECTS ON THE COMPOSITION AND
METABOLISM OF BENTHIC DIATOM COMMUNITIES AND
SUBSEQUENT EFFECTS ON FISH GROWTH

by

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B.S., Stanford University
(1974)

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WOODS HOLE, MASS.
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SUBMITTED IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

AT THE

MASSACHUSETTS INSTITUTE OF TECHNOLOGY

AND THE

WOODS HOLE OCEANOGRAPHIC INSTITUTION

June, 1980

Signature of Author
Massachusetts Institute of Technology/ Woods Hole
Oceanographic Institution, June, 1980

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Massachusetts Institute of Technology/ Woods Hole
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Submitted to the Biology Department in June, 1970
in partial fulfillment of the requirements for the
Degree of Doctor of Philosophy in Biological Oceanography

ABSTRACT

Eastern mud snails (Ilyanassa obsoleta) in densities of zero, six or twelve snails were placed in flow-through laboratory microcosms (765 cm²) and incubated for five weeks. Other tanks were raked daily to a depth of 10 mm. Grazing by low densities of snails significantly increased chlorophyll standing stock, respiration and gross photosynthesis as measured by light and dark exchange of oxygen and carbon dioxide compared to untreated tanks. The standing stocks of algal pigments, respiration and photosynthesis were depressed in the microcosms which received the 12-snail or the raking treatments. Simulating snail excretion by fertilization with ammonium increased chlorophyll standing stock by a similar magnitude, but this effect could be inhibited by raking the sediments daily. At low densities Ilyanassa's acceleration of nutrient cycling stimulates algal growth, but this effect is overwhelmed at higher densities by overgrazing and stirring inhibition.

The dominant benthic algal group in the containers were pennate diatoms. Grazed containers contained a larger percentage of the non-motile classes of diatoms, as compared to the motile forms which predominated in the untreated microcosms. The snails are able to selectively graze these mobile species. Their gut contents are enriched in carbon, nitrogen and algal pigment content by 20-40 times over the surface sediments.

A small, non-significant, growth effect can be seen in the snails' response to density changes, but another marsh consumer, Fundulus heteroclitus, grows faster at low snail densities when snails are absent.

Name and Title of Thesis Supervisor: John M. Teal, Senior Scientist

ACKNOWLEDGMENTS

I thank all members of my thesis committee (Penny Chisholm, John Farrington, Fred Grassle, John Hobbie and Francois Morel) and particularly my supervisor, John Teal for their suggestions and help during the course of this work. In addition I received valuable criticism from Ivan Valiela and statistical assistance from Woolcott Smith. Financial support for my research was provided by the WHOI Education Department, the Pew Memorial Trust and the Department of Commerce, NOAA Office of Sea Grant 04-8-MOI-149 and 04-7-158-44104.

Donna Keyser drafted most of the figures. Sue Volkmann taught me how to use the computer for data analysis. Phil Clarner did the CHN analysis. Bob Edgar got me started in diatom taxonomy.

I received many ideas and much support from other students in Woods Hole including Chris Werme, Anne Giblin, Bob Howarth, Sue Vince, Ken Foreman, Joy Geiselman, Larry Brand, Cathy Cetta, Bob Binder, Anne Critz, Russell Cuhel, Tom Jordan and Jerry Cheney.

I dedicate this thesis to my family whose love and support enabled me to attend graduate school in the first place.

CHAPTER 1

GENERAL INTRODUCTION

Many similar mechanisms have been proposed for the grazing stimulation of the two opposite components of life's processes, primary production and decomposition. The speed-up of decompositional processes by grazing has the longer history. Since Darwin's tribute to the earthworm, soil fauna have been linked to enhanced soil fertility. More recently decomposition rates have been shown to be a function of the density and types of detritivores, animals feeding on the matrix of non-living and microbial carbon in soils and sediments (summarized in Dickinson and Pugh, 1974).

It is thought that bacterial growth in soils and sediments is generally slow with doubling times of two or three weeks (Hissett and Gray, 1976). Most individual bacterial cells seem to be inactive. Rapid growth is rare, often associated with the initial colonization of fresh substrates. Grazing could promote colonization and eliminate this bacteriostasis by removing senescent colonies or antibiotic producing cell types (Satchell, 1974). Alternatively, excretion by the soil fauna may release some specific growth-promoting agent or create patches high in nutrients which will overcome non-specific bacteriostasis (MacFayden, 1961). Through mechanical means, the detritivores can distribute cells and spores to more suitable growth sites, or their stirring of the soil itself could increase contact between enzymes on cellular surfaces and the substrate (MacFayden, 1961; Satchell, 1974).

Experiments with protozoans in aquatic microcosms have been useful in demonstrating many of the effects of bacteriovores on decomposition rates (Fenchel, 1968, 1979). While ciliates and small zooflagellates at natural

densities will reduce bacterial numbers by half and are capable of turning over the entire bacterial population in 24-36 hours, decomposition is actually stimulated to a consistently high rate (Fenchel and Harrison, 1976). Systems without protozoans have a lower rate of decomposition.

Similar to their effects in terrestrial systems, deposit feeders in aquatic systems have been shown to speed decomposition. Many mechanisms for this stimulation have been suggested, including fragmentation, exposure of fresh surfaces for microbial activity, reduction of particle size and selection for fast-growing cells (Hargrave, 1970; Welsh, 1975; Fenchel, 1970; Fenchel and Harrison, 1976; Lopez et al., 1977). These mechanisms are almost identical to those cited above for terrestrial processes, the major difference being the reduced flux of gases through water as compared to air.

This stimulation in decompositional rate by one set of detritivores can be passed along to other consumers (Brinkhurst et al., 1972; Tenore et al., 1977). For instance, the incorporation rate of labelled, aged eelgrass detritus by the polychaete Nephtys incisa was nearly doubled when nematodes were added to the culture chamber (Tenore et al., 1977). In streams, feeding by one group of detritivores is often necessary for the efficient feeding of another group. Alder leaf comminution by chewing insects increases the uptake rates of leaf litter by downstream, filter-feeding insects by 2-8 times (Short and Maslin, 1977).

Many of the successional mechanisms cited above for the increase in decomposition rates by detritivores may also explain the stimulation of primary production by herbivores. In his summary of the ways in which consumers regulate ecosystems, Chew (1974) proposes two successional hypotheses for the stimulation of productivity by grazing herbivores: they can increase the production to biomass ratio, or they can delay senescence. A related argument was made by Mattson and Addy (1975). They claimed that insect grazers act as cybernetic regulators of forest primary productivity ensuring the consistent and optimal output of plant production by maintaining forest growth in an early successional state. Pruning studies tend to support this view. They show that photosynthetic rates can be optimized by selectively removing some of a plant's older leaves (Sweet and Wareing, 1966; Jameson, 1963).

Variations on these hypotheses suggest that herbivores can affect the nutrient levels available to the plants they graze. Owen and Wiegert (1976) speculated that insect grazers stimulate productivity because their excretion of honeydew increases bacterial activity and nitrogen fixation in the soil under a tree. Similarly, bovine saliva is thought to provide trace nutrients which stimulate grass growth (Reardon et al., 1974).

The above examples are all taken from terrestrial systems where much of the focus has been on how individual plant fitness can be maximized by grazing. Because aquatic plants are comparatively short-lived and capable

of being entirely eaten in a single grazing episode, those who study grazing maximization of terrestrial plant productivity have hypothesized that this sort of mutualism would not be found in aquatic systems (Owen and Wiegert, 1976, p. 491; Stenseth, 1978, p. 315).

However, there are several examples where different types of aquatic grazers stimulate the production of benthic algae. Periphyton production can be increased by crayfish in lakes (Flint and Goldman, 1975) or damselfish living on Baja California coral reefs (Montgomery, in press). These workers theorize that low densities of these herbivores keep the algal systems in highly productive, early successional stages. Benthic microepiflora can also be stimulated by the grazing of amphipods (Hargrave, 1971), fish (Cooper, 1973), hydrobiid snails (Fenchel and Kofoed, 1976), and brine flies (Collins et al., 1976).

And as Tenore demonstrated with decompositional processes, the effects of a stimulation in primary production by one consumer can be exploited by other consumers. In the Serengeti plains, gazelles forage preferentially in areas cropped the previous month by migratory wildebeest, whose grazing and trampling change the characteristics of the vegetation (McNaughton, 1976).

This thesis documents the above patterns for a salt marsh mudflat community which has been manipulated in laboratory experiments. These mudflats contain a large community of benthic diatoms which are grazed by the common Eastern mud snail, Ilyanassa obsoleta. The other consumer to benefit from snail grazing is the common mummichog, Fundulus heteroclitus.

Chapter 2 explains the methods used to measure benthic algal standing stocks and rates. My extraction procedures for estimating sediment pigment biomass are discussed as well as my method for measuring photosynthesis and respiration in experimental microcosms by monitoring light and dark changes of oxygen and carbon dioxide concentrations by gas chromatography. Finally I describe the microcosms I used for much of the subsequent experimental work and present data regarding patchiness observed in the microcosms using these various measures.

Chapter 3 documents the grazing effects of snails on the rates of photosynthesis and respiration and the structure of the benthic diatom community in experimental microcosms. I show that the effect of snail grazing on algal production is density dependent. Low grazing pressure stimulates production while a higher level of grazing depresses production. The presence of snails also changes the groups which dominate the benthic diatom community. I also show that ammonia excretion by the snails provides sufficient nitrogen to account for their stimulation of benthic production. This chapter has been submitted to Ecology.

Chapter 4 examines the ways in which snail grazing affects the nitrogen budget of a sediment system. I quantify nitrogen consumption and excretion by Ilyanassa and the effect of snails on nitrogen fixation rates.

Chapter 5 explores the selective nature of snail feeding more closely to determine the extent to which snails pick appropriate food items from the sediment matrix. Their selection can be seen for such sediment parameters as percentage carbon and nitrogen, the biomass of photosynthetic pigments and percentage composition of diatom species. This chapter has been submitted to Oecologia.

Chapter 6 summarizes all the different components of the benthic sediment system which are affected by snail grazing and shows how these first order changes affect the growth of the snails and another benthic consumer, the mummichog Fundulus heteroclitus.

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CHAPTER 2

THE MEASUREMENT OF ALGAL PIGMENTS AND METABOLISM IN
SALT MARSH SEDIMENTS

INTRODUCTION

Previous attempts at measuring the effects of grazing on benthic algae have been plagued by spatial variability (Pace, et al., 1979; Nichols and Robertson, 1979). During my own preliminary experiments, I found that I would need to modify my experimental system to reduce this variability. This chapter will characterize the spatial variation of chlorophyll biomass and the microcosms I developed to minimize this variance. I will also describe my methods for determining pigment biomass and rates of photosynthesis and respiration.

MEASURING PIGMENT CONCENTRATIONS

Triplicate cores for pigment analysis were taken in several experiments. I pushed 3 cc syringe barrels (9 mm diameter) one centimeter into the sediment, a depth sufficient to sample most of the pigment biomass (see below).

After extrusion the cores were extracted with 5 ml of cold, 90 o/o methanol after the method of Fenchel and Straarup (1971). Holm-Hansen and Riemann (1978) have shown that methanol is preferable for pigment extraction to acetone; I found that both solvents yielded identical peak heights.

The samples were shaken and stored overnight in the dark at 40C. The next day I centrifuged them and decanted the supernatant into spectrophotometer cuvettes. Sample absorbance was scanned from 400 nm - 800 nm against a methanol blank. Peak height minus baseline was determined for the absorbance peaks. These peaks corresponded to the

maximum absorbance of four photosynthetic pigments; 760 nm (bacterial chlorophyll), 666 nm (chlorophyll a), 615 nm (phycocyanin) and 440 nm (phaeopigments). I converted these absorbances to a concentration per square centimeter using extinction coefficients from the literature (Table 1). There was a good correlation over the ranges of cell densities in these experiments between this calculated chlorophyll concentration and the number of live diatom cells in the same core (Figure 1).

MEASURING COMMUNITY METABOLISM

I measured community metabolism in laboratory microcosms (765 cm², described more fully below) after removing the snails and draining and sealing the microcosms. Oxygen and carbon dioxide concentrations were measured in duplicate gas samples at the beginning and end of 12-20 hour dark and light incubations. If the duplicates differed by more than 0.1 o/o oxygen (molecular fraction), then a third sample was taken. Using a standard computer program, I fitted a regression line to these points and calculated the rate of gas exchange. Both oxygen and carbon dioxide exchange rates were linear during the incubation times (Figure 2).

The gas samples were analyzed using the thermal conductivity detector on a Hewlett-Packard Gas Chromatograph (Model 5730A). Liquid carbon dioxide was used to cool the columns. I found that I could satisfactorily resolve the nitrogen and oxygen peaks by following an oven temperature program of two minutes at 30°C and increasing the temperature at a rate

of 320°C per minute until the oven temperature reached 210°C. The oxygen and nitrogen peaks came at the end of the first two minutes. Carbon dioxide peaked at 160°C. The higher temperature was necessary to drive all the water from the column.

I calibrated the gas chromatograph daily by making four serial injections of laboratory air from which a standard curve for nitrogen and oxygen concentrations could be drawn. From these regressions, I calculated the amounts of nitrogen and oxygen in my sample injection, and normalized them to the injection volume of 100 μ l.

Figure 3 demonstrates the repeatability of the technique. The standard errors of the oxygen values calculated from the regression equation averaged about 0.14 o/o by molecular fraction (Figure 3a). Since the normalization takes into account the relation between the oxygen and nitrogen peak heights, actual agreement of duplicates was much better, averaging about 0.07 o/o (Figure 3b). Carbon dioxide concentrations were computed from a calibration curve made for the above temperature regime (Figure 4). The curve showed very little daily variation.

The gas concentration changes I was measuring during the course of these experiments were about five to ten times this average deviation. Since measurement error was the same during the sampling, the size of the coefficient of variation was mostly a function of the magnitude of the rates of respiration and photosynthesis. The coefficient of variation for oxygen was 12-18 o/o; for carbon dioxide, 5-7 o/o.

SPATIAL VARIATION IN CHLOROPHYLL BIOMASS

Table 2 shows how chlorophyll varied in a 5 x 5 grid of samples collected by coring with 25 of drinking straws. Because drinking straws are not a very satisfactory method for sampling fluid sediments, I did more extensive sampling comparing the coefficient of variations for coring tubes of three different diameters (Table 3). The smallest diameter cores had a slightly larger coefficient of variation than the two larger cores. Field samples showed more variability than cores from the microcosms due to the various ways I pre-treated the microcosm sediments (as discussed below).

Besides horizontal variation in the concentration of pigments, there were also differences due to depth. As shown in Figure 5, absorbance due to phaeopigments (440 nm), phycocyanins (615 nm) and chlorophyll (666 nm) was 2-10 times greater in the 0 to 2 mm section than in the 7 to 10 mm section. Bacterial chlorophyll, however, showed a different pattern. Since these organisms need less light energy to oxidize H_2S , their pigment concentration peaks 3 or 4 mm below the sediment surface. This was about the depth of the redox discontinuity layer in these sediments.

EXPERIMENTAL MICROCOSMS

I used a variety of systems for my early experiments, beginning with aerated gallon jars and then overflowing plastic wash basins. I finally settled on Plexiglas, airtight containers which I could sample for gas exchange measurements with a syringe.

The containers were 29.2 cm x 26.2 cm x 14.2 cm high. Two holes, which could be plugged by serum stoppers and allow the flow of water in and out of the boxes, were offset from one another on opposite sides to encourage good exchange. The top could be sealed with a lid fitted over a silicone-lubricated rubber gasket and secured by wing nuts to the overhanging lip. Leakage or solubility of the gases into the Plexiglas or lubricant was checked by following the disappearance of carbon dioxide or methane injected into the sealed box. This flux proved to be negligible, only a small fraction of a percent of the rates being measured.

Several months before beginning an experiment, I collected sediments from approximately the top 5 cm of a creek bottom, mudflat habitat in Great Sippewissett Marsh, Massachusetts. These were sandy, silty sediments with an organic content of approximately 2 o/o carbon. After collecting the sediment I treated it in several different ways to remove the majority of the macrofauna and meiofauna (Table 4).

The most effective sediment treatment was to sieve the sediments through a 2 mm screen and then repeatedly freeze and thaw them over a period of several months. A few weeks before the experiment the sediment was again passed through a 2 mm screen and placed on a large aquarium table and mixed with a paddle in a folding motion for an hour. At this time the sediment had a uniformly greyish color rather than the pockets of blacks and browns with which it started. Measured volumes of these sediments were removed serially to fill the different Plexiglas containers until the desired volume per container was reached.

Over these sediments flowed filtered seawater from the Woods Hole Oceanographic Institution seawater system. I used an AMF Cuno Aqua-Pure water filter containing Honeycomb nominal filters (Larco Corp., Newton Highlands, Massachusetts) which are rated to filter 95 o/o of the particles greater than 5 μm .

The filtered water entered a dark plastic container (150 l) through a hose in the lid. This barrel acted as a large settling chamber and contained an overflow valve to maintain a constant head. Five centimeters below the waterline were eight holes filled with tubing connectors and sealed with a ring of silicone adhesive. These holes provided an outflow to the aquaria through equal lengths (2 m) of black latex 1.3 cm tubing.

Each microcosm had an overflow hole on the opposite corner from the inflow hole and 4.5 cm above it. I adjusted the flow rate of the system to about 100 ml min⁻¹, equivalent to a turnover rate of approximately two hours for these containers. Fouling by particles and interrupted flow necessitated constant maintenance. The water filters were changed daily. All tubing and connectors received a thorough brushing weekly.

The microcosms were placed under an array of four rows of fluorescent "grow" lights which gave an average illumination of 30 $\mu\text{E m}^{-2} \text{ sec}^{-1}$, approximately the saturation light intensity for several benthic diatoms reported by Admiraal (1977). They were incubated on a 14-10 hr light-dark cycle.

Placement under the lights resulted in a slight difference among tanks. Those containers on the outside corners received 26 o/o less light than those on the inside, and containers were assigned so that this difference was shared equally among all treatments.

The improvements caused by these progressively more complex pre-treatments of the sediments and seawater can be seen in Table 4. Through improved pre-treatment, I could reduce the coefficient of variation of pigment determinations by a factor of three.

CONCLUSION

This laboratory microcosm system was designed to lessen the variability of these community metabolism parameters, and thereby enable me to conduct experiments on the effects of snail grazing on benthic diatom communities. The extent of this field patchiness must be kept in mind when extrapolating from the laboratory experiments reported in the succeeding chapters. Nevertheless, the explanation of the responses of the sediment algal communities in the microcosms to grazing is consistent with the mosaic pattern seen in the field measurements (e.g. Table 2). This interrelation is discussed in Chapter 6.

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TABLE 1. Values used for the computation of algal pigment concentration.

Pigment	Absorbance Peak	Baseline Used	Extinction Coefficient	Reference
Bacterial chlorophyll	770 nm	800 nm, 720 nm	387	Cohen-Bazire et al., 1957
Chlorophyll a	666 nm	720 nm, 630 nm	72	Vollenweider, 1969
Phycocyanin	615 nm	630 nm, 570 nm	77	Chapman, 1973

TABLE 2. Chlorophyll absorbance (10^{-3} Absorbance Units) of methanol extracts of a grid of 6 mm diameter cores from the field.

83	77	66	70	77
84	70	90	85	88
65	66	87	111	94
62	78	73	90	100
84	94	88	118	120

TABLE 3. Mean coefficient of variation of twelve triplicate samples using different diameter cores for chlorophyll absorbance.

Diameter	o/o Coefficient of Variation $\pm$ 1 S.E.		
21 mm	7.4	$\pm$	1.4
9 mm	6.9	$\pm$	1.2
6 mm	9.5	$\pm$	0.8
6 mm (field)	12.6	$\pm$	2.5

TABLE 4. The coefficients of variation of different sediment parameters for experiments from May 78 -- May 79.

DATE	SEDIMENT TREATMENT	SEAWATER SYSTEM	PIGMENT ABSORBANCES				GAS PRODUCTION RATES			
			MEAN COEFFICIENT OF VARIATION (o/o)	440 nm	615 nm	666 nm	760nm	Median	COEFFICIENT OF VARIATION (o/o)	
								O ₂ dark	O ₂ light	CO ₂ light
V 78	frozen, stirred, once	Unfiltered	21.0	21.6	24.9	16.1				
VI 78	frozen twice sieved, stirred	Unfiltered	10.3	10.8	14.3	12.1				
IX 78	frozen, sieved, stirred twice	Unfiltered						16.6	18.6	5.8 6.9
III 79	frozen, sieved, stirred twice	Filtered 5 μ	6.7	6.9	6.9	6.5		15.7	12.5	5.6 6.9

FIGURE 1

Chlorophyll concentration and diatom cell counts from core samples.

FIGURE 2

Microcosm gas transfer rates in the dark measured over time.

FIGURE 3

The extent of measurement error in determining oxygen concentrations by gas chromatography.

- A. The standard errors of regression equations which have been fitted to the daily calibrations.
- B. The difference between percent oxygen of duplicate samples determined by normalizing the regression equation to a sample volume of 100 μ l.

FIGURE 4

Calibration curve for carbon dioxide used for these experiments.

FIGURE 5

Pigment concentrations at different depths in cores from the experimental microcosms. Horizontal lines denote two standard errors of triplicate samples.

DEPTH (mm)

CHAPTER 3

THE EFFECT OF GRAZING BY MUD SNAILS (ILYANASSA OBSOLETA) ON
THE STRUCTURE AND METABOLISM OF A BENTHIC ALGAL COMMUNITY

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RUNNING HEAD: SNAIL GRAZING AFFECTS A DIATOM COMMUNITY

ABSTRACT

Eastern mud snails (Ilyanassa obsoleta) in densities of zero, six or twelve snails were placed in flow-through laboratory microcosms (765 cm²) containing 5 cm of frozen and sieved sediments. Other microcosms were raked once daily to a depth of 10 mm. All these containers were incubated for five weeks and regularly sampled for plant pigments and light and dark transfer of oxygen and carbon dioxide. Grazing by low densities of snails significantly increased chlorophyll standing stock. Respiration and gross photosynthesis increased by an even greater percentage compared to the ungrazed controls. The standing stocks of algal pigments, respiration and photosynthesis were depressed in the microcosms which received the 12-snail or the raking treatments.

The dominant benthic algal group in the containers were pennate diatoms. Grazed containers contained a larger percentage of the non-motile classes of diatoms, as compared to the motile forms which predominated in the untreated microcosms. The other treatments behaved intermediately.

When sediments were fertilized with ammonium at a rate equivalent to the excretion of six snails, chlorophyll content increased by about the same amount as in the 6-snail treatment. Daily raking inhibited this fertilization effect.

We conclude that low densities of Ilyanassa obsoleta stimulate algal growth by accelerating nitrogen cycling and selectively removing specific components of the diatom community. At high snail densities these effects are overwhelmed by overgrazing and inhibition due to sediment stirring.

Keywords: grazing, productivity, stimulation, benthic algae, mudflat, microcosms, snails, Ilyanassa obsoleta, diatoms, community structure.

INTRODUCTION

Grazing herbivores can have dramatic effects on plant community biomass and species composition in both terrestrial (Harper, J.L. 1969) and marine environments (Connell, 1975). Studies of the effects of grazing herbivorous marine gastropods show that they have little direct influence on established macroalgae (Dayton, 1975; Lubchenco, 1978), but there is evidence that they can exert a significant grazing pressure on the microflora thereby influencing algal succession in the rocky intertidal (Castenholz, 1961; Lubchenco, 1978). Recently, it has been demonstrated that these marine gastropods can selectively feed on certain diatom species thereby influencing the composition of the benthic microflora. Nicotri (1977) found that those "canopy" species which form long chains of moderate-sized cells protruding from the rock surface are selectively grazed in preference to those cells tightly attached to the rock. Snails grazing on soft substrates can also change the composition of the microflora. Fenchel and Kofoed (1976) found a shift in the size distribution of diatom cells towards smaller cells in grazed sediments after eight days.

While heavy grazing pressure can drastically reduce the standing stocks of plant communities, it has been suggested that some grazers can also stimulate the primary production of their grazed plant communities. Chew (1974) cites a number of such cases in terrestrial systems. In aquatic systems low levels of grazing by crayfish (Flint and Goldman, 1975) or damsel fish (Montgomery, in press) stimulates the production of

periphyton. Benthic microepiflora can also be stimulated by the grazing of amphipods (Hargrave, 1970), fish (Cooper, 1973) and hydrobiid snails (Fenchel and Kofoed, 1976).

Decomposition of litter has long been known to be stimulated by grazing, and marine benthic microbial processes also show this stimulation as summarized in Fenchel (1972). He proposes that this stimulation could be caused by the physical stirring of the sediment community during the detritivore's feeding activity which may increase the availability of nutrients to the bacterial cells.

Pace et al. (1979) tested the ability of the common Eastern mud snail Ilyanassa obsoleta (= Nassarius obsoletus) to stimulate the benthic microbial community of a Georgia salt marsh at densities of 480 and 1580 animals m^{-2} . They concluded that these densities of grazers led to reduced productivity. However, an experimental removal of 5-10 o/o of the standing chlorophyll daily by their migration into lens paper increased microflora productivity (Pace et al., 1979).

At Great Sippewissett Marsh, the density of Ilyanassa ranges from 0 to 700 m^{-2} so we conducted our experiments at about one-third the density used by Pace et al. The sediments in other microcosms were stirred daily to mimic snail movement. To reduce the extreme variability found in field experiments, we did our density manipulations in laboratory microcosms. Sediments from marsh creeks were frozen and sieved two times to eliminate the macrofauna and meiofauna. We present data here concerning the density dependent effects of Ilyanassa grazing on sediment metabolism, pigment standing stocks and the composition of the benthic diatom community.

Ilyanassa obsoleta is one of the predominant grazers of intertidal and subtidal sandflats, mudflats, and salt marshes along much of the Atlantic coast of the United States and in isolated Pacific Coast bays. It is a deposit-feeder which subsists mainly on benthic algae although it is also known to scavenge larger dead animal remains (Scheltema, 1964; Wetzel, 1977; and Brown, 1969). Labelling experiments have shown that Ilyanassa ingests and assimilates sediment bacteria and algae, but not Spartina detritus (Wetzel, 1977).

The benthic algal community in Great Sippewissett Marsh, Massachusetts is dominated by pennate diatoms, which exhibit two life forms. The motile, free-living forms of the epipelon migrate daily to the sediment water interface, while the epipsammon are mostly attached to sand particles (Round, 1971). The migratory species belong to the section Biraphidineae. Raphe systems on both valves allow them to move by extruding mucus threads. The extent of movement exhibited by these species can be quite variable, ranging from relatively immobile Amphora spp. (Round, 1979) to diurnally migrating Hantzschia virgata (Palmer and Round, 1967).

The other two sections (Araphidineae and Monoraphidineae) of pennate diatoms are mostly non-migratory, though M.A. Harper (1969) has shown that they can move slowly from sand grain to sand grain. They are found in the salt marsh either attached to sand grains or epiphytic on the green alga, Enteromorpha.

Studies of salt marsh diatoms to date have mostly ignored these non-migratory forms. They have been presumed unimportant due to the continual deposition of sediments by the tides (Sullivan, 1975; Williams, 1962; Pace et al., 1978). The method commonly used for diatom collection (Eaton and Moss, 1966) depends on the migration of the diatoms into fine netting spread on the sediment surface overnight. Yet when methods are used which sample both epiphytic and epipsammic species, the latter are found in abundance in salt marshes (Lee et al., 1975), shallow bays (Rao and Lewin, 1976), and tidal flats (Riznyk, 1973). These araphidaceous and monoraphidaceous diatoms could have an important role at Great Sippewissett Marsh. The yearly accretion of sediment is 1.5 mm (Valiela and Teal, 1979), and the top centimeter of the surface sediments is frequently reworked by resident invertebrates, lessening the problem of burial.

METHODS

All the sediments and animals for this experiment were collected from creek bottom and mud flat habitat in Great Sippewissett Marsh, Massachusetts. The sediment is a mixture of sand and silt with an organic content of 2 o/o carbon by dry weight. After collection the sediment was passed through a 2 mm sieve and repeatedly frozen and thawed to remove the macrofauna and meiofauna. Subsequent microscopic examination of the sediment failed to show the presence of these organisms.

We conducted the experiments in gas-tight, flow-through, plexiglass microcosms (29.2 x 26.2 x 14.2 cm) which are described elsewhere (Chapter 2). We added homogenized sediment to each container to give a depth of 5 cm and added 5 μ -filtered seawater to give a final depth of 10 cm. The water was continuously renewed at a rate of one turnover every two hours. We placed the microcosms under an array of fluorescent "grow" lights to stimulate photosynthesis. The chambers received an average illumination of $30 \mu\text{E m}^{-2}\text{sec}^{-1}$, approximately the saturation light intensity for several benthic diatoms reported by Admiraal (1977). The microcosms were allowed to incubate for two weeks on a 14-10 hr light-dark cycle before the experiment began. During the course of the experiment, chlorophyll content averaged $17.1 \mu\text{g cm}^{-2}$, comparable to ranges seen in Great Sippewissett Marsh (Estrada et al., 1974).

We sampled the microcosms three times during the first week before we began treatments. Four duplicate treatments were then begun. Zero, six or twelve snails were added to two containers each, and two containers were raked once daily to a depth of 5-10 mm with tines which were 10 mm apart (Table 1). We sampled the microcosms four times during the next 15 days. To eliminate possible biasing effects of the containers, we switched snails between the 0- and 6-snail treatments and also switched the 12-snail and raking treatments. The microcosms were then sampled four more times in the next 14 days.

We collected Ilyanassa obsoleta from Great Sippewissett Marsh, using snails between 20 and 25 mm in length which averaged 2.4 g in weight. The different density treatments are within the range of densities of Ilyanassa found grazing at this marsh, between 0 and 700 m⁻². The treatments are equivalent to 0, 80 and 240 snails m⁻², respectively. Preliminary experiments showed us that higher densities inhibited production as found by Pace et al. (1979). The stirring regimen was chosen as typical of the daily movement equivalent to a similar range of snail densities used above. By marking the position of snails every five minutes in a pilot experiment, we calculated an average movement of 4.3 ± 0.6 cm, which is equivalent to about 50 cm per hour, and a path width of 0.5 cm. A snail therefore crawls over about 25 cm² each hour, a value comparable to movement rates for Ilyanassa from other areas (Edwards, 1979). As a result of its movement and burrowing when not foraging (Crisp, 1978), Ilyanassa bioturbates the top centimeter (L. Boyer, personal communication) so our raking treatment was equivalent to the movement of about 4-12 snails per microcosm.

At each sampling date, the snails were removed and the microcosms drained and sealed. Community metabolism was then measured by light and dark incubations for determination of oxygen and carbon dioxide concentrations. Duplicate gas samples were taken from each microcosm at the beginning and end of 12-20 hr dark and light incubations. Gas concentrations were analyzed using the thermal conductivity detector of a

Hewlett Packard gas chromatograph (Model 5730A). At the end of the incubations we measured sediment temperature and pH and resumed the treatments.

At each sampling triplicate cores 9 mm in diameter and 1 cm deep were taken from each microcosm and extracted with 5 ml of 90 o/o methanol after the method of Fenchel and Straarup (1971). The samples were shaken and stored overnight in the dark at 4°C. We then centrifuged the samples and scanned the absorbance spectra from 400 to 800 nm. Peak height minus baseline was determined for the four absorbance peaks, 760 nm (bacterial chlorophyll), 666 nm (chlorophyll), 615 nm (phycocyanin) and 440 nm (phaeopigments), and converted to pigment biomass per square centimeter (Chapter 2).

For some of these sample days we took surface cores (0-1 cm) for determining the composition of the benthic diatom community. The cores were oxidized with sulfuric acid, potassium permanganate and oxalic acid (Hasle and Fryxell, 1970). We then washed the cleaned frustules and mounted them in Hyrax. Diatom populations in each sample were identified to the level of genus and enumerated from a random sample of between 500 and 1000 valves which were observed using oil-immersion, phase-contrast optics at a magnification of 1000x. The permanent slides have been deposited at Hellerman Diatom Herbarium, North Dartmouth, Massachusetts: HD SM 1551-1586.

We also measured the effect of nitrogen fertilization and stirring on algal growth in nine plastic containers (9.3 x 9.3 cm) filled with sieved, frozen, stirred sediments to a depth of 3 cm. To these sediments were added 125 ml of 5 μ -filtered seawater and the containers were

incubated for one week under "grow" lights. The containers were sampled daily for the next five days for algal pigments. At this point we began three treatments in triplicate using a randomized blocks design: control, fertilized and fertilized with daily raking. All containers received 10 ml of filtered seawater daily. Both fertilization treatments also received 35 μM of NH_4Cl , an amount equivalent to the excretion rate of six snails ($5.7 \mu\text{g N cm}^{-2}$; Chapter 4). Three of the six fertilized containers were also raked once daily to a depth of 5-10 mm with tines 10 mm apart. We sampled all the containers four times in the next week for algal pigments.

Pigment concentrations were assayed as before and normalized by subtracting pre-treatment means for each container. The normalized data were subjected to a 3-Way ANOVA (Treatment x Tank x Day).

In the course of designing this experiment, three preliminary experiments were done. The results of those experiments are presented in Appendix 1, and are consistent with those presented here.

RESULTS

Community Metabolism

There was a slight difference in the visual appearance of the microcosms. The untreated tanks were covered by a brownish film. When snails were present or the sediments were stirred, this film was absent. The redox discontinuity layer was at about 6 mm in all containers.

Since we switched treatments to eliminate microcosm effects, we have considered the temporal trends in the microcosms during the experiment by pooling all containers (Figure 1). On the sampling days before the treatments started (Days 0-6), the different microcosms' pigment standing stocks and gas exchange rates showed the same trends. Light and dark oxygen rates were high at first, then dropped precipitously. During the treatment period, these rates were less variable for all the microcosms. Chlorophyll gradually built up in the microcosms during the experiment, and dark oxygen uptake also showed a gradual increase as the containers aged.

Carbon dioxide exchange rates followed a very different pattern with both respiration and photosynthesis declining over the sampling period. This decline corresponded with a rising pH in the tanks during the experiment. When the average carbon dioxide light and dark exchange rates for the microcosms are plotted against average sediment pH, there is a significant correlation between the two (Figure 2; $P < 0.05$), as expected from carbonate equilibrium calculations. The predominance of aqueous carbon dioxide is proportional to the hydronium ion concentration, and bicarbonate will predominate as the pH rises. Both sulfate reduction and denitrification commonly occur in marsh sediments, and these processes probably caused the rise in pH and also changed the alkalinity thereby confounding bicarbonate calculations.

We have compared the deviations of individual treatments from these general trends in two ways. The community metabolism and pigment data were analyzed by a mixed-model, three-way ANOVA (Treatment x Tank x Day)

on data normalized by subtracting the tank averages before treatments began. In this way we were able to remove the systematic variation due to initial tank differences.

We were particularly interested in comparing the behaviour of the microcosms containing six snails to the untreated microcosms. Since the treatments were switched in the middle of the experiment, we plotted these four microcosms separately (Figures 3-5). At nearly every sampling period, the microcosms containing six snails had higher gas transfer rates or chlorophyll standing stocks. In the figures this appears as a flip-flop in the behavior of individual tanks. In both instances, the response of photosynthetic and respiration rates to snail addition was rapid, peaking after one week and then declining (Figure 3). Chlorophyll concentrations showed no clear temporal pattern (Figure 5).

Grazing by six snails significantly ($P < 0.05$) increased the rates of respiration and gross photosynthesis compared to those containers without snails. These low densities of snails had a much larger effect on the rates of photosynthesis and respiration than on pigment standing stocks. The 6-snail microcosms had significantly more dark oxygen uptake (an average of 41 o/o higher than the 0-snail containers during the 28 days of the experiment), 38 o/o more dark carbon dioxide release, 42 o/o higher light oxygen release and 35 o/o more light carbon dioxide uptake, but chlorophyll biomass was only 9 o/o higher (Figure 5) and phycocyanins only 7 o/o higher (Figure 6) in the tanks with 6 snails as compared to the untreated tanks. All these differences were significant at $P < 0.05$.

Figure 6 also summarizes the response of pigment concentrations and ratios to the 12-snail and raking treatments. Phycocyanin and chlorophyll concentrations were depressed below those untreated values for the raking treatment and particularly the heavily grazed 12-snail treatment. Both densities of snail grazing and the raking treatment reduced bacterial chlorophyll levels below those of the untreated tanks. The ratio of absorbance at 666 nm to the other absorbance peaks was always greater in the 6-snails treatment, and in the case of 666 nm/440 nm, significant at $P < 0.05$. None of the other pigment ratios for any of the four treatments differed statistically from each other.

As with the standing stock measurements, doubling the snail densities reduced photosynthetic and respiration rates below those of the untreated tanks as measured by oxygen exchange (Figure 7). The raking treatment had the same effect as 12-snails. Dark oxygen uptake was slightly depressed, and gross photosynthesis was significantly depressed ($P < 0.05$) compared to the untreated tanks.

The carbon dioxide data are not so clear-cut, mainly because the changing rates are confounded by the pH effect. Generally the untreated tanks had a slightly higher pH than all the treated tanks, particularly the raked containers, where the pH was often 0.1-0.2 units lower than all the other tanks. As explained above the ratio of aqueous carbon dioxide to bicarbonate is higher at low pH. Consequently, light and dark carbon dioxide exchanges were higher in all the treated microcosms, but these rates are only a small percentage of the rates as measured by oxygen exchange.

Community Structure

Although blue-green algae were present, pennate diatoms were by far the dominant benthic algal group. Microscopic observations showed that approximately 65-80 o/o of the diatom cells were viable. Cell counts averaged 5×10^9 diatoms cm^{-2} .

The clearest pattern in community composition emerged when the diatoms were grouped by class, the highly migratory Naviculineae (Biraphidineae) compared to the mostly attached classes of Achnantheae (Monoraphidineae) and the Fragilarineae (Araphidineae). Both grazing treatments resulted in a much higher proportion of non-migratory cells, especially very small (2-10 μm) Fragilaria pinnata, than in the untreated containers. The raking treatment had an intermediate proportion of the migratory biraphidaceous species (Figure 8).

Fertilization

The fertilized, unstirred containers had significantly higher ($P < 0.05$) standing stocks than the controls of all the photosynthetic pigments measured (phaeopigments, phycocyanin, chlorophyll and bacterial chlorophyll: Figure 9). Those fertilized containers which were also raked showed an opposite trend. Compared to the controls, they contained significantly lower standing stocks of all the pigments except bacterial chlorophyll, for which their levels equalled the unstirred, fertilized containers.

DISCUSSION

Our results show a stimulation of sediment algal productivity and standing stock by low levels of snail grazing and an inhibition at higher snail densities. We can cause these parameters to flip-flop by the addition or removal of snails. Flint and Goldman (1975), Cooper (1973) and Montgomery (in press) all hypothesized a successional mechanism for the stimulation of periphyton or edaphic algae by low grazing intensities. They suggest that older, senescent plants are removed from the substrate so that the community remains in an early successional, highly productive state. At higher grazing intensities, the primary producers can no longer compensate for the increased removal of cells.

Chew (1974), in his review of the mechanisms by which grazing may stimulate the productivity of terrestrial plants makes a similar argument. Two of his proposed mechanisms of effects at the individual plant level relate closely to the successional hypothesis above; grazing increases the production to biomass ratio or grazing delays senescence.

Such a successional argument could be made for this system. Besides the increased productivity in the lightly grazed containers, another indication of a successional change is that the grazed containers have a significantly higher ratio of 666 nm absorbance to 440 nm absorbance than the controls. Margalef (1967) has characterized this ratio as an indicator of phytoplankton succession. An increasing proportion of absorbance at 440 nm indicates that the community is in a later successional stage or contains more senescing cells.

We found other indications of a change in algal community composition due to grazing. The ratio of 666 nm absorbance to the two other photosynthetic pigments was slightly increased by the 6-snail treatment. Within the pennate diatoms, the dominant benthic algal group, grazing caused a shift from the larger, migratory, epipelagic forms to the smaller, non-migratory forms. This change could consist of two components, selective removal of the large migratory cells and increased growth by the uneaten cells. Snails do selectively feed on migratory cells (Chapter 5), however the decreased percentage of Naviculineae in the 6-snail as compared to the 12-snail treatments suggests that both processes are occurring (Figure 8). Otherwise, there should be fewer migratory cells in the 12-snail microcosms.

This community shift to smaller, non-migratory cells could increase production in two ways. Growth rate is related to cell size and volume, smaller cells generally growing faster than larger ones (Round, 1971). In addition, the investment of a large portion of the carbon budget of migratory cells for mucus strands that allow movement should decrease the growth efficiency of these cells compared to non-migratory cells. Besides devoting less resources to photon capture, a thick polysaccharide mat will inhibit photosynthesis below it and produce a canopy effect similar to forest trees. Removal of a senescent canopy by grazing will allow increased light penetration for the growth of non-migratory species.

Besides these successional grazing changes, snails affect nutrient regeneration. Given sufficient light, edaphic diatom communities show seasonal nitrogen limitation (Sullivan and Daiber, 1975; Van Raalte et al., 1976). The regeneration of nitrogen by snail grazing and excretion increases the flux of nutrients available to the benthic diatoms and can stimulate their growth. This effect can easily be seen by comparing the nitrogen budget of the 0- and 6-snail treatments to the fertilization experiment (Table 2). Oxygen uptake rates were $3.75 \mu\text{l cm}^{-2}\text{hr}^{-1}$ for the 0-snail treatment and $5.35 \mu\text{l cm}^{-2}\text{hr}^{-1}$ for the 6-snail treatment. Assuming a photosynthetic ratio of 1 and a C/N molar ratio of 6.63 (Redfield et al., 1963), the difference in daily production is $3.7 \mu\text{g N cm}^{-2}$. Approximately $5.7 \mu\text{g N cm}^{-2}$ is available daily from snail excretion (Chapter 4). Fertilizing containers at that rate under the same light and temperature regimes increased chlorophyll concentration by an average of $1.5 \mu\text{g cm}^{-2}$ in seven days, the same amount of average chlorophyll increase found in the 6-snail microcosms versus the 0-snail treatments during a 29 day incubation. The amount of nitrogen excreted by low densities of grazing snails, then, is sufficient to account for the increased production.

The increase in chlorophyll concentration in the grazed microcosms may either be due to an increase in the chlorophyll to carbon ratio, characteristic of improved growth (Eppley and Renger, 1974), or a function of the snails' mobilization of non-algal nitrogen pools. Alternatively, the algal community changes discussed above could influence the chlorophyll standing stock or ratios.

At some grazing intensity this stimulation due to nutrient regeneration and changes in algal community composition must be inhibited by simple overgrazing, but the inhibition at 12-snail densities may not be due solely to the primary grazing effect of the removal of individual diatoms from the substrate. As we have shown above, it is important to separate primary effects of herbivores from secondary effects. Besides excretion, stirring is an important secondary effect of Ilyanassa's activity.

Raking the sediments daily inhibited photosynthesis, respiration and the standing stock of chlorophyll and also inhibited the chlorophyll stimulation due to fertilization. Pace et al. (1979), using a slightly different raking method, found a similar depression. Stanley (1976) suggests that this sediment disturbance by grazing benthic invertebrates may limit the productivity of epipelagic algae in tundra ponds. Live cells are mixed below the surface and receive less light. Mathematical simulations of epipelagic productivity suggested to him that this process was removing more cells than simple grazing.

Lopez and Levinton (1978) suggest that the grazing pressure of hydrobiid snails will select for strong attachment by benthic diatoms to the sediment particles. An opposing selective force is the importance of light to benthic diatoms. Strongly attached diatoms are at a disadvantage compared to migratory cells which can escape burial unless the shifting of the sediment is as frequent as the speed at which the diatoms move. Snail grazing itself also provides a selective pressure in that direction through the secondary effect of sediment stirring during grazing and movement.

Other workers have documented the impact of Ilyanassa sediment disturbance on the benthic meiofauna and macrofauna. In the absence of grazing, algal mat formation can make the sediments quite stable. Laboratory experiments have shown that the mucilage-secreting diatoms significantly reduced resuspension and retarded laminar flow over the sediments (Holland et al., 1974). By its movement Ilyanassa disrupts this structure and reduces the densities of associated fauna such as nematodes (Nichols and Robertson, 1979) or the fauna associated with polychaete tubes (Grant, 1965). Yet, while the snails are disrupting the previous sediment structure, they are excreting mucus themselves as a result of movement and fecal packaging.

Dayton (1975) has previously noted the similarity between molluscan grazers in rocky intertidal systems and mammalian grazers in terrestrial systems. The similarity becomes even more striking on soft bottoms where the secondary effects of grazers are more apparent. Harper (1969) cites trampling and dung deposition as being two important secondary effects of grazing herbivores. They create heterogeneity in the physical environment and increase vegetational diversity. As we have seen, both have correlates in the grazing of snails which stir and fertilize the sediments as part of their feeding behavior.

One of the most catastrophic examples of secondary herbivore effects is the change of the East African landscape from forest to grassland by herds of elephants (Laws, 1970). Like the elephants, Ilyanassa is orders of magnitude larger than the resource it is exploiting. Their stirring effects (trampling) as they move through the environment are substantial.

Surface cells are buried, large migratory cells are eaten, and at high snail densities, community production can be significantly reduced. By stirring alone, six snails can overturn the top centimeter of soil in the entire microcosm every day. Their total chlorophyll consumption represents only 5-10 o/o of the daily standing stock of chlorophyll, about the same level as daily productivity.

These calculations demonstrate the differences between grazing in soft surface sediments and terrestrial or rocky intertidal systems. Because of substrate fluidity and the short distances involved in mixing plant cells below the photic zone, Ilyanassa obsoleta's secondary grazing processes are disproportionately important in benthic soft bottom communities. As a result, benthic diatoms are presented with a variety of selective pressures by snail grazing. Snails selectively feed on unattached, migratory cells which affects community succession. Their movement can bury surface cells or bring deeper cells to the surface, while their mucus trail probably affects the resuspension of sediments below it. Surviving cells are provided a rich source of nutrients by snail excretion which can stimulate their growth.

The variety of components that make up grazing by Ilyanassa probably ensures that the overall effects on community structure and metabolism reported here are a complicated function of snail density. At low densities the acceleration of nutrient cycling by the snails' grazing and excretion stimulate photosynthesis. At high densities nutrient cycling is overwhelmed by stirring inhibition and simple overgrazing.

ACKNOWLEDGEMENTS

This study was supported by the WHOI Education Department, the Pew Memorial Trust and the Department of Commerce, NOAA Office of Sea Grant 04-8-MOI-149 and 04-7-158-44104. R.K. Edgar provided assistance with the diatom identifications. G. Lopez, J. Hobbie and F. Morel provided helpful critiques of an earlier manuscript. We thank Salt Pond Sanctuaries and the late A.B. Gifford and his wife for access to their property at Great Sippewissett Marsh. Woods Hole Oceanographic Institution Contribution Number 4593.

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TABLE 1. Experimental design for determining the effect of four treatments on sediment metabolism in laboratory microcosms.

		DAY NUMBER										
		0	4	6	11	15	18	21	25	28	32	35
TANK 1						0-Snails					6-Snails	
TANK 2						6-Snails					0-Snails	
TANK 3	Pre-treatment					6-Snails					0-Snails	
TANK 4						0-Snails					6-Snails	
TANK 5	Incubation					Raked					12-Snails	
TANK 6						12-Snails					Raked	
TANK 7						12-Snails					Raked	
TANK 8						Raked					12-Snails	

TABLE 2. A comparison of the nitrogen budgets for the stimulation of algal photosynthesis by Ilyanassa grazing and by fertilization with ammonium chloride.

<hr/>		
	Daily production	
6 snails	12.2 $\mu\text{gN cm}^{-2}$	
0 snails	8.5 $\mu\text{gN cm}^{-2}$	
<hr/>		
Difference	3.7 $\mu\text{gN cm}^{-2}$	
	5.7 $\mu\text{gN cm}^{-2}$	Daily excretion (6 snails)
	Average Chlorophyll Standing Stock	
6 snails	19.3 $\mu\text{g cm}^{-2}$	
0 snails	17.8 $\mu\text{g cm}^{-2}$	
<hr/>		
Difference (29 Day Average)	1.5 $\mu\text{g cm}^{-2}$	
	1.5 $\mu\text{g cm}^{-2}$ Difference Between Fertilized (at 5.7 $\mu\text{gN cm}^{-2}$ day ⁻¹) and Unfertilized Containers (7 Day Average)	
<hr/>		

FIGURE 1

Average chlorophyll standing stocks and rates of photosynthesis and respiration for all experimental microcosms during the experiment.

FIGURE 2

The effect of pH on light and dark carbon dioxide transfer in flow-through experimental microcosms.

PHOTOSYNTHESIS
 $r^2 = 0.87$

RESPIRATION
 $r^2 = 0.85$

FIGURE 3

The effect of snail grazing on respiration (dark oxygen uptake) and photosynthesis (light - dark oxygen production) in laboratory microcosms. The deviations from the daily and tank means are plotted. Average tank means are respiration, $2.37 \mu\text{l cm}^{-2}\text{hr}^{-1}$ and photosynthesis, $4.55 \mu\text{l cm}^{-2}\text{hr}^{-1}$.

FIGURE 4

The effect of snail grazing on respiration (dark carbon dioxide production) and photosynthesis (light - dark carbon dioxide uptake) in laboratory microcosms. The deviations from the daily and tank means are plotted. Average tank means are respiration, $0.65 \mu\text{l cm}^{-2}\text{hr}^{-1}$ and photosynthesis, $1.01 \mu\text{l cm}^{-2}\text{hr}^{-1}$.

FIGURE 5

The effect of snail grazing on sediment chlorophyll concentrations in laboratory microcosms. The deviations from the daily and tank means are plotted. Average tank mean is $18.6 \mu\text{g cm}^{-2}$.

FIGURE 6

The effect of four treatments on sediment pigment concentrations and ratios in laboratory microcosms. Plotted are the pooled data for two tanks sampled in triplicate four times during a fifteen day period and, after treatments were switched, four more times during a fourteen day period. All values have been normalized by subtracting tank pre-treatment means. Horizontal lines denote two standard errors computed from the interaction component of a 3-Way ANOVA (Day x Treatment x Tank). Average pre-treatment tank means are phycocyanin, $1.39 \mu\text{g cm}^{-2}$; chlorophyll, $17.1 \mu\text{g cm}^{-2}$; bacterial chlorophyll, $0.37 \mu\text{g cm}^{-2}$; 666/440, .183; 666/615, 11.7 and 666/760, 9.33.

NORMALIZED PIGMENT CONCENTRATION ($\mu\text{g cm}^{-2}$)

NORMALIZED ABSORBANCE RATIOS

FIGURE 7

The effect of four treatments on respiration and photosynthesis in laboratory microcosms. Plotted are the pooled data for two tanks sampled four times during a fifteen day period and, after treatments were switched, four more times during a fourteen day period. All values have been normalized by subtracting tank pre-treatment means. Horizontal lines denote two standard errors computed from the interaction component of a 3-Way ANOVA (Day x Treatment x Tank). Average pre-treatment tank means are CO_2 respiration, $1.35 \mu\text{l cm}^{-2}\text{hr}^{-1}$; photosynthesis, $1.50 \mu\text{l cm}^{-2}\text{hr}^{-1}$, O_2 respiration, $2.11 \mu\text{l cm}^{-2}\text{hr}^{-1}$ and photosynthesis, $3.72 \mu\text{l cm}^{-2}\text{hr}^{-1}$.

NORMALIZED OXYGEN EXCHANGE ($\mu\text{l cm}^{-2} \text{hr}^{-1}$)

NORMALIZED CARBON DIOXIDE EXCHANGE ($\mu\text{l cm}^{-2} \text{hr}^{-1}$)

FIGURE 8

The effect of four treatments on the dominance of the benthic diatom community in laboratory microcosms by migratory diatoms. Vertical bars show the range from duplicate treatments.

FIGURE 9

The effect of three treatments on sediment pigment concentrations in laboratory microcosms. Plotted are the normalized means of three tanks sampled in duplicate four times during a seven day period. Horizontal lines denote two standard errors computed from the interaction component of a 3-Way ANOVA (Treatment x Tank x Day). Average pretreatment means for all tanks are 440 nm absorbance, 3.4 cm^{-2} ; phycocyanin, $1.7 \mu\text{g cm}^{-2}$; chlorophyll, $22.7 \mu\text{g cm}^{-2}$ and bacterial chlorophyll, $.39 \mu\text{g cm}^{-2}$.

CHAPTER 4

A SIMPLE NITROGEN BUDGET FOR ILYANASSA OBSOLETA

INTRODUCTION

Nitrogen input of grazers has been implicated in their stimulation of the plants they graze. The purpose of these experiments was to determine the various inputs of nitrogen due to the grazing of the snail Ilyanassa obsoleta and to determine the effect of these nitrogen inputs on the growth of benthic diatoms.

Three terms of the snails' nitrogen budget were quantified: consumption, excretion and the stimulation of nitrogen fixation by the snails' stirring of the sediments. I suspected that nitrogen fixation might be important in these sediments since it has been shown that sea urchins can enrich their nitrogen-poor macroalgal food through nitrogen fixation by gut flora (Guerinot et al., 1977) and that nitrogen fixation is an important regulator of nitrogen-poor lake systems (Liao and Lean, 1978). Nitrogen is thought to be a limiting nutrient in salt marshes (Valiela and Teal, 1979), too, the habitat for Ilyanassa obsoleta.

EXPERIMENTAL SYSTEM

All the sediments and animals for these experiments were collected from creek bottom and mud flat habitat in Great Sippewissett Marsh, Massachusetts. The sediment is a mixture of sand and silt with an organic carbon content of 2 o/o carbon. After collection the sediment was passed through a 2 mm sieve and repeatedly frozen and thawed to remove the macrofauna and meiofauna. Subsequent microscopic examination of the sediment failed to show the presence of these organisms.

I conducted the experiments in gas-tight, flow-through Plexiglas microcosms (29.2 x 26.2 x 14.2 cm) which are described elsewhere (Chapter 2). I added homogenized sediment to each container to give a depth of 5 cm and added 5 μ -filtered seawater to give a final depth of 10 cm. The water was continuously renewed at a rate of one turnover every two hours. The microcosms were placed under an array of fluorescent "grow" lights to stimulate photosynthesis. The chambers received an average illumination of 30 $\mu\text{E m}^{-2}\text{sec}^{-1}$, and were incubated on a 14-10 hr light-dark cycle.

CONSUMPTION

I calculated the capacity of the snails' stomachs by holding animals of different sizes in filtered seawater for 48 hours, then allowing them to feed on laboratory-cultured sediments for one or two hours. The snails were immediately frozen and their stomachs dissected out and weighed. Unfed snails were treated in the same manner. Carbon and nitrogen content were also measured for the gut contents and feces of these animals (Chapter 4).

Plotting full stomach weight over time against snail weight shows that the snails are able to fill their stomachs in one hour (Figure 1). The difference between stomach weights of fed snails and starved snails yielded a regression for this size range of adult snails of:

$$\text{Gut Contents (dry wt mg)} = 0.81 (\text{Snail Dry Weight (mg)}) - 40$$
$$(r^2 = 0.83).$$

Brown (1969) has shown that the clearance rate of sand and mud from the stomachs of natural populations of Ilyanassa obsoleta is approximately 12 hr. In her behavioral studies of Ilyanassa, Crisp (1978) has found activity and rheotactic response to be depressed for two days after feeding. Taken together those observations suggest a range of 12-48 hrs between successive meals. For my calculations, I have assumed that Ilyanassa obsoleta fills its stomach once a day.

I can then calculate the daily consumption of a hypothetical adult snail 20 mm long weighing 110 mg, which was the average size of the individuals I used for many of these experiments. This snail would consume 47 mg dry weight of food each day. Carbon and nitrogen analyses show a difference of 35 o/o carbon and 5.6 o/o nitrogen between Ilyanassa's ingested food and egested feces. This 20 mm snail would then consume 16 mg C and 2.6 mg N daily. Cammen (1980) has proposed an equation for deposit-feeding invertebrates which predicts a daily consumption of 12 mg C for this hypothetical snail, comparable to these measured values.

EXCRETION

Nitrogen excretion was measured by allowing the previously starved snails to feed on laboratory-cultured sediments for 48 hrs, then placing groups of three snails in 125 ml Erlenmeyer flasks containing 50 ml of 16° C, filtered, autoclaved seawater. Samples of seawater were withdrawn at hourly intervals for three hours and assayed with a modification for small samples of the method of Solorzano (1969).

Excretion rates agreed quite well with those calculated for *Ilyanassa obsoleta* in marshes further south and other marine prosobranchs (Table 1). Comparing these values to consumption rates for our hypothetical 20 mm adult snail, we find this snail ingests $2.6 \text{ mg N day}^{-1}$. Excretion amounts to about $0.6 \text{ mg N day}^{-1}$. Data for *Ilyanassa obsoleta* in Connecticut suggests that its assimilation efficiency is about 37 o/o, a finding consistent with these results (Edwards, 1979).

I also tried to determine if snails could mobilize non-living nitrogen from the substrate on which they feed by measuring excretion rates of snails feeding on sediments containing 8.5 or $24 \mu\text{g}$ chlorophyll cm^{-2} and on autoclaved sediments rinsed several times with autoclaved seawater. Excretion levels for fed snails were three times higher than those for starved snails (Figure 2). There was no difference between snails feeding on sediments containing two different levels of chlorophyll, but snails feeding on the autoclaved sediments had significantly higher levels of ammonia excretion (Figure 2, top). These sediments had a rich, organic smell which suggested there may have been a great many small molecules freed by autoclaving and available for easy assimilation. This explanation is consistent with the changes in ammonia excretion over time. For snails feeding on these sediments, ammonia excretion dropped quickly in the second and third hours following feeding, but nitrogen excretion for the snails feeding on natural sediments increased gradually over time (Figure 2, bottom).

Snail excretion and presumably consumption are insensitive to changes in the standing stock of algae in these containers, at least during the short run. If grazing rates were dependent on plant biomass, it would be more difficult to see a stimulation of production by monitoring standing stock.

STIMULATION OF NITROGEN FIXATION

I measured nitrogen fixation in the laboratory microcosms by the acetylene reduction method of Patriquin and Denike (1978) before and after adding different densities of snails. Zero, three, six or twelve snails were added to two microcosms each after a pre-incubation of three days. During the next nine days, I sampled the microcosms seven times for acetylene reduction activity. At each sampling date I took triplicate 9 mm diameter cores, 1 cm deep which were extruded into a 7 ml serum vial and flushed with a gas mixture of 86 o/o nitrogen, 4 o/o oxygen and 10 o/o acetylene. These vials were incubated in the dark at 20° C. I withdrew samples at 24 and 48 hours to determine the rate of production of ethylene using the flame ionization detector of a gas chromatograph. I also attempted to measure C_2H_2 reduction activity in whole snail incubations as suggested by Guerinot et al. (1977), but could find no activity.

Acetylene reduction declined in all the microcosms during the experiment. Throughout the experiment the 6-snail treatment showed the highest levels of acetylene reduction followed by 3-snail, 12-snail and 0-snail, respectively (Figure 3). The differences between the 0-snail and 6-snail treatments are statistically significant at $P < 0.05$.

CONCLUSION

Acetylene reduction is an especially poor way to measure nitrogen fixation in anaerobic systems, but in this case it is sufficient to demonstrate that nitrogen fixation is not a significant source of nitrogen in these containers. Assuming a 3:1 molar ratio of acetylene reduced to nitrogen fixed ---- a very generous assumption for natural reducing systems where the ratio can vary from 4 to 170 (Liao, 1977; Witty, 1979)---- the daily difference between the 0- and 6-snail treatments is $0.014 \mu\text{g N cm}^{-2}$. Excretion can account for a daily difference between the two treatments of $5.7 \mu\text{g N cm}^{-2}$ (Table 2), approximately 400 times the difference due to nitrogen fixation.

To distinguish the relative importance of fertilization and successional hypotheses in explaining Ilyanassa's grazing stimulation, it is necessary to determine the source of the excreted nitrogen. My results show that the snails are capable of mobilizing nitrogen from autoclaved sediments, but the nitrogen budget suggests that most of the excreted nitrogen is coming from algal nitrogen. Therefore the source of nitrogen used to stimulate new algal cell production must mainly be other algal cells.

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TABLE 1. Excretion by marine prosobranch snails ($\mu\text{g N (g shell-free dry weight)}^{-1} \text{ hr}^{-1}$).

SPECIES	EXCRETION RATE	REFERENCES
<u>Acmaea scutum</u>	30.2	Duerr (1968)
<u>Acmaea digitalis</u>	2.9	Duerr (1968)
<u>Calliostoma ligatum</u>	60.3	Duerr (1968)
<u>Fusitriton oragensis</u>	14.6	Duerr (1968)
<u>Littorina sitkana</u>	43.4	Duerr (1968)
<u>Thais lima</u>	19.3	Duerr (1968)
<u>Thais lamellosa</u>	21.7	Duerr (1968)
<u>Nassarius obsoletus</u> (= <u>Ilyanassa obsoleta</u>)	19.2	Nixon et al. (1976)
<u>Ilyanassa obsoleta</u>	9.8-40.6	Edwards (1979)
<u>Ilyanassa obsoleta</u>	14	This study

TABLE 2. A comparison of nitrogen inputs due to nitrogen fixation and excretion for different densities of Ilyanassa obsoleta in 765 cm² laboratory microcosms.

NUMBER OF SNAILS	NITROGEN FIXATION (ng N cm ⁻² day ⁻¹)	SNAIL EXCRETION* (μg N cm ⁻² day ⁻¹)
0	13	0
3	20	2.8
6	27	5.7
12	17	11.3

*For these experiments average snail length was 23 mm.

FIGURE 1

Dry weights of stomachs of fed and starved Ilyanassa obsoleta.

FIGURE 2

Ammonia excretion of Ilyanassa obsoleta. Error bars denote two standard errors and were calculated from three experiments with twenty-seven measurements of excretion rates each.

Top. Average hourly excretion for three hours following grazing on different substrates.

Bottom. Hourly excretion rates as a function of time following removal from the different grazing substrates.

FIGURE 3

The effect of snail density on acetylene reduction rates in laboratory microcosms. Rates have been normalized by subtracting pre-treatment means. The plots are treatment means of duplicate tanks sampled in triplicate. Bar graphs show the average for all days with two standard errors computed from the interaction component of a 3-Way ANOVA (Treatment x Tank x Day). Average pretreatment mean for all tanks is 2.8 nmoles C_2H_2 day⁻¹.

CHAPTER 5

Selective grazing by the mud snail Ilyanassa obsoleta

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RUNNING HEAD: MUD SNAIL SELECTIVE FEEDING

ABSTRACT

Mud snails (Ilyanassa obsoleta) starved for 48 hr were allowed to feed on sediments in laboratory microcosms. Sediment cores sliced at 2 mm intervals were compared to snail stomach contents for per cent carbon and nitrogen, plant pigment contents and species composition of benthic diatoms. Concentrations of carbon, nitrogen, phaeopigments, phycocyanin and chlorophyll were enriched in the top 2 mm of the sediments compared to 7-10 mm depth by a factor of 2-10. In turn, these materials were 20-40 times more concentrated in snail guts than in the surface sediments. Snail feces were enriched for carbon and nitrogen by 5-7 times over the surface sediments. Bacterial chlorophyll peaked at about 3-4 mm in the sediments and was not detectable in the snail stomach contents. The C/N ratio of the snail stomach contents was only 6 compared to a ratio of 8.5 for their feces and 12 for the surface sediments.

The percentage of migratory diatoms (e.g. Nitzschia and Navicula) decreased with depth where non-migratory species, such as Fragilaria pinnata, dominated. These migratory species were more common in the snails than in the sediments on which they were feeding.

A comparison of daily ingestion rates to the animal's energy budget shows that this selective ingestion is sufficient to meet Ilyanassa's energy needs.

Keywords: selectivity, grazing, snails, benthic diatoms, mudflat, Ilyanassa obsoleta.

INTRODUCTION

Do detrital consumers utilize dead organic matter directly or must it first be converted to microbial tissue before assimilation? Since 1938, when ZoBell and Feltham (1938) demonstrated that bacteria could be assimilated by marine organisms, this question has remained unanswered.

Newell (1965) attempted to distinguish between the feeding types "detritivore" and "microbivore" using the molluscs Hydrobia and Macoma. Both animals use only a small part of the organic carbon contained in their ingested food. This ingested material is identical to the nitrogen-rich component of the food. Newell and later Darnell (1967) suggested that animals feeding on detritus could be feeding on the associated microflora and fauna rather than the detritus itself. Subsequent microscopic work following microbe density through the digestive tracts of a variety of marine invertebrates has shown that bacteria, fungi, ciliates and some algae are very efficiently removed from the sediment matrix on which they feed (Hargrave, 1970; Fenchel, 1972; Chua and Brinkhurst, 1973; Pitts and Cowley, 1974; Hylleberg, 1975; Lopez and Levinton, 1978).

The microbial portion of detritus should be easier to digest and more nutritious than the structural carbohydrates which make up the bulk of plant detritus. Bacterial cells (13-88 o/o protein, 12-28 o/o carbohydrates and 1-41 o/o lipids; Kofoed, 1975) represent a valuable source of food for an organism. Microalgae, particularly the diatoms which do not have the vast reserves of structural carbohydrates seen in terrestrial plants, contain a high percentage of protein (17-50 o/o) and low (3-6) C/N ratios (Darley, 1977).

Labelled substrate studies of detritivore assimilation show high efficiencies for microbial carbon and very low efficiencies for sterile plant material (Kofoed, 1975; Wetzel, 1977; Lopez et al., 1977).

Enzymatic data provide no clear answer to this question. Most aquatic molluscs and crustaceans have at least a weak cellulase activity (Yokoe and Yasumasu, 1964; Hylleberg, 1972; Elyakova, 1972; Monk, 1976), but there seems to be no correlation between the presence of structural carbohydrases and feeding type. For instance, the enzymatic activity spectrum of a carnivore was similar to that of a detritivore (Hylleberg, 1972).

While microorganisms are certainly nutritious food, there is some question whether their density in the sediments is sufficient to allow organisms to use them. Cammen et al. (1978) have compared the ingestion rates of a suite of deposit-feeding invertebrates to their rates of oxygen consumption. Using normal standing stocks of bacteria and algae, they conclude that microbial carbon could account for less than 10 o/o of the metabolic needs of these animals.

It is also possible that these animals are benefitting from symbiotic gut bacteria. Many invertebrates do have a resident gut fauna (Johannes, 1964; Johannes and Satomi, 1966). Guerinot et al. (1977) have even suggested that such microorganisms can fix nitrogen within sea urchins when the echinoderms are feeding on nitrogen-poor algae.

Such considerations yield the following antipodes; either detritivores are capable of selecting food enriched in living carbon, or they derive most of their nutrition from assimilating dead matter.

For one prominent eastern Atlantic detritovore, Ilyanassa obsoleta, both mechanisms have been proposed. Based on labelling experiments, Wetzel (1977) states that Ilyanassa ingests and assimilates sediment bacteria and algae, but not Spartina alteriflora detritus. However, by calculating the numbers of microorganisms found in the sediments on which Ilyanassa feeds, Cammen et al. (pp. 75-76) conclude that Ilyanassa's carbon budget can only be balanced by its feeding on non-living carbon. A high degree of food selectivity could explain both of these results, and we have investigated that possibility in this study.

Ilyanassa obsoleta is one of the predominant grazers of intertidal and subtidal sandflats, mudflats, and salt marshes along much of the Atlantic coast of the United States and in isolated Pacific Coast bays. It is a deposit-feeder which subsists mainly on benthic algae although it is also scavenges larger dead animal remains (Scheltema, 1964; Wetzel, 1977; Brown, 1969). It possesses the hydrolytic enzymes necessary for metabolizing some of the principal components of algae as well as the plant polysaccharides contained in salt marsh grasses (Brown, 1969).

METHODS

Experiments were conducted in 765 cm² laboratory microcosms which had been previously used to determine the effects of snail grazing on benthic diatom community structure and metabolism (Chapter 3). The microcosms contained 5 cm of sediment collected from Great Sippewissett Marsh, Massachusetts, from which the meiofauna and macrofauna had been removed by sieving and repeated freezing and thawing.

These sediments were incubated in the microcosms with filtered seawater under "grow" lights. Standing stocks of pigment biomass and percent carbon and nitrogen in the sediments were assayed by taking cores to a depth of 1 cm with a coring barrel 21 mm in diameter. The cores were carefully extruded and sectioned at 2 mm intervals, about the thinnest section we could slice with a razor blade. After taking these cores from the containers, we added six mud snails, Ilyanassa obsoleta, which had been collected 48 hours earlier at Great Sippewissett Marsh and held in filtered seawater. The snails were allowed to graze on the sediments for an hour, then immediately frozen. Their stomachs were dissected out and the gut contents extruded. Gut contents from several snails were pooled for each of the analyses. Another group of snails was allowed to feed for 24 hr, then held in filtered seawater and their feces collected.

Carbon and nitrogen content were determined for the various samples with a Perkin-Elmer 240 CHN Analyzer after oven-drying (48 hr at 650 C) to constant weight and grinding the dried sample. Pigment contents were determined by extracting with 5 ml of 90 o/o methanol overnight at 40C and determining peak height minus baseline for phaeopigments (440 nm), phycocyanin (615 nm), chlorophyll (666 nm) and bacterial chlorophyll (760 nm). Pigment concentrations were calculated (Chapter 2) and normalized to the wet weight of the sample.

Sample contamination by fragments of stomach tissue would have had a minimal effect on these measures. We measured the percentage carbon and nitrogen of the stomachs of unfed snails and the absorbance of their extracted tissues. The dry weights of the stomachs of unfed snails were

only 5-10 o/o those of fed snails. The average percentage carbon and nitrogen from these tissues were slightly below those of the stomach contents.

The stomach pH of Ilyanassa ranges from 6.0-6.5, with buffering due to crystalline style material (Brown, 1969). Complete digestion in these snails takes about twelve hours (Brown, 1969). Our estimates of chlorophyll in the stomach contents may be slightly low, but we saw no additional peaks in the spectra of snail stomach contents which might result from pigment breakdown products. In addition, the ratio of absorbance at 666 nm to that to 440 nm was higher in the stomach contents than in the surrounding sediment, suggesting that chlorophyll breakdown was not far advanced.

The diatoms in the stomachs of the snails and in the surrounding sediments were compared in two stages. Initially, a random sample of 400 to 650 diatom frustules was determined to genus from three sliced sediment cores and the pooled stomach contents of two groups of five snails. Secondly, a more detailed taxonomic comparison based on approximately 300 frustules per sample was made between a sample of the pooled 0-2 mm sections of two sediment cores and samples of the pooled stomach contents of two groups of six snails. The diatoms were prepared for microscopic examination by oxidation of the organic matter in the samples, repeated washing of the cleaned siliceous frustules and final

mounting in Hyrax on permanent slides (Hasle and Fryxell, 1970).

Taxonomic determinations were made with oil-immersion, phase-contrast optics at a magnification of 1000x. The permanent slides have been deposited in the Hellerman Diatom Herbarium: HDSM 1540-1550.

RESULTS

The per cent carbon and nitrogen declined with increasing depth in the sediment (Figure 1, top). Per cent carbon and nitrogen content of the surface 2 mm was nearly twice that of sediment between 7 and 10 mm. Phaeopigments (440 nm absorbance), phycocyanin and chlorophyll were enriched by about a factor of five in the top 2 mm (Figure 2, top). Bacterial chlorophyll, which requires less light energy for the oxidation of H_2S , peaked below the surface at about 3-4 mm, the approximate depth of the redox discontinuity layer.

While snails could enrich their food slightly by feeding only in the top 2 mm of sediment, there was an even greater enrichment in the snail stomach contents compared to these surface sediments. Their stomachs contained 20-40 times more carbon and nitrogen, and their feces contained 5-7 times more carbon and nitrogen than the surface sediments by per cent dry weight (Figure 1, bottom). The snails' stomach contents were enriched for phaeopigment, phycocyanin and chlorophyll concentrations by about the same extent as carbon and nitrogen, but we could not detect any bacterial chlorophyll in their stomach contents (Figure 2, bottom).

Several of these parameters were enriched differentially by the snails. Chlorophyll and phycocyanin concentrations were increased over surface sediment levels by twice as much as the phaeopigments and nitrogen by twice as much as carbon (Table 1). The C/N ratio of the surface sediments was about 12 while the C/N ratio of the snails' stomach contents was only 6 and that of their feces about 8.5.

We found it useful for ecological interpretation to group the diatom taxa into migratory versus non-migratory species, approximated by the distinction between epipsammic (sand-associated) and epipelagic (silt-associated) assemblages and the most frequent association of these diatom taxa with each (Round, 1971; McIntire, 1977). Such a classification of diatoms with respect to their vertical migratory behavior in sediments is not a stringent one, especially for comprehensive taxa such as families and genera which are likely to be heterogeneous in this respect. We classified problematic taxa based on diatom size and relative migratory ability (Harper, 1969). Taxa in the Centrales, Fragilariaceae and Achnantheaceae (generally this last family was characterized by small individuals in our samples) have been considered non-migratory; all other pennate taxa have been treated as migratory. The most abundant species of non-migratory diatoms in the samples was Fragilaria pinnata. Individuals of Nitzschia and Navicula predominated among the migrating groups.

In the initial set of observations confined to examining only the genera of diatoms in the sediment profile and the snails, the relative abundance of non-migratory taxa in the 0-2 mm level was approximately 10 o/o less than that in the subsurface levels (Figure 3). Snail stomachs contained 10 o/o less of the non-migratory groups than the 0-2 mm section.

In our second set of observations comparing the diatoms in the stomachs of the top 2 mm of sediment, non-migratory taxa were about 15 o/o less frequent in the snails than in the surrounding sediments. Migratory taxa accounted for 40-45 o/o of the snails' stomach contents, but represented only 28 o/o of the total sediment diatom assemblage (Table 2). In particular, Nitzschia was almost three times more abundant in the snails than in the surface sediments.

DISCUSSION

These data show that Ilyanassa obsoleta exhibits a high degree of selectivity for the particles it eats. Not only does it select for high organic content and living plant pigments, as demonstrated by its preference for chlorophyll over phaeopigments, but it also takes a specific fraction of the benthic diatom community.

The most selected food are the migratory species of diatoms which are concentrated in the surface sediments by their daily migrations to the sediment-water interface, which allows for easy capture by the snails. Selective feeding on specific types of benthic diatoms has also been demonstrated for grazing gastropods in the rocky intertidal by Nicotri

(1977), who found that intertidal limpets and littorines could not effectively graze on diatoms which were tightly attached to rock surfaces. The grazers more efficiently removed those individuals which extended up above the substrate.

Assimilation of these epipellic species may be easier because the non-motile, epipsammic forms are attached quite strongly to inorganic particles of no nutritional benefit (Harper, 1969). Ilyanassa may assimilate these species in two ways. Lopez and Kofoed (in press) have shown that hydrobiid snails will take small particles into their buccal cavity, scrape off the attached microorganisms and spit out the particle, a process they call epistrate browsing. It is also possible that the crystalline style, which Ilyanassa develops only when deposit-feeding (Brown, 1969), aids in the detachment of microorganisms from particles as a preparation step for digestion (Lopez, in press).

Is Ilyanassa's selectivity for carbon and chlorophyll sufficient to balance the snails' energy budget. Edwards (1979) has done extensive work on the energy budget of Ilyanassa obsoleta living in Connecticut salt marshes. For a hypothetical adult 0 mm snail with an shell-free dry weight of 107 mg, daily respiration at 20°C would require 0.7 mg C, production 0.2 mg C and mucus and DOM secretion 3.2 mg C. We have estimated ingestion rates for Ilyanassa (Chapter 4). A snail this size ingests at least 47 mg dry wt of sediment daily. If we take the difference in per cent carbon between stomach contents and feces, this snail would assimilate 16 mg C daily, easily sufficient to meet its metabolic needs. Chlorophyll assimilation would be approximately 37 ug daily.

An enrichment of microbial food items on the order of 10-20 times their standing stock in the sediments has been generally the amount calculated as necessary to meet metabolic needs solely through living material (Fenchel, 1972; Baker and Bradnam, 1976; Cammen et al., 1978). Our calculations for the amount of organic carbon ingested daily by Ilyanassa agree well with Cammen's (1980) model for benthic invertebrate deposit feeders. He predicts our 107 mg snail should eat 12 mg C daily. Without selective feeding his model predicts that this same snail should need to eat 361 mg of sediment, almost nine times the amount the snails actually eat.

The literature is replete with examples of different means of selective feeding by detritivores (Fenchel and Jorgensen, 1977). Some choose specific particle types which would contain preferred foods. Coull (1973) reported that meiofauna will select organically-coated over plain sand, and in some cases will select for specific species of bacteria. Particle size selection is common among deposit-feeding detritivores (Fenchel et al., 1975; Hylleberg and Galluci, 1975) and filter-feeding ones (Winter, 1978). Selection of feeding depth is also common (Whitlatch, 1974), especially selection for surface sediment particles which are finer and probably greatly enriched in bacteria.

Another kind of sediment selection which is widespread among benthic fauna is coprophagy, the ingestion of fecal pellets. Several animals can survive on a diet of fecal pellets (Johannes and Satomi, 1966; Frankenberg and Smith, 1967), which are enriched in bacteria and have a

higher oxygen consumption than their surrounding sediment (Hargrave, 1976). Because the nutritional value of a fecal pellet changes with microbial colonization, coprophagy can depend on time after egestion (Levinton and Lopez, 1977).

Animals can also sort sediments to enrich their diet in microbial fauna. Pitts and Cowley (1974) have found that the crab Uca pugilator selectively removes yeast cells, Rhodotorula mucilaginosa, from the surrounding sediment. The mullet Mugil cephalus uses pharyngeal filtering to select the fine particles richer in bacteria (Odum, 1968). By comparing plant pigment concentrations in the surrounding sediment to the contents of the mullet's stomach, Odum (1970) calculated that a mullet must filter 100 g of sediment to get one gram of stomach material.

We have shown that Ilyanassa obsoleta has the capacity to select for plant pigments at about one-quarter of the extent of mullet, but from these data we cannot determine if this selectivity is due to an active sediment sorting or Ilyanassa's choosing particularly rich feeding areas. We do know, however, that if Ilyanassa is simply choosing rich microhabitats, its capabilities are much better than our selective strategy of sampling the top 2 mm. The absence of bacterial chlorophyll and the increased abundance of migratory diatoms in the snails' stomach contents would suggest that they must be feeding at the very edge of the sediment water interface.

These diatoms represent a high quality food in a specific microhabitat. The same adaptations which allow migratory diatoms optimal light capture at the sediment surface also provide an enriched microhabitat suitable for exploitation by grazers. In an environment which provides a small amount of high quality food diluted by large amounts of low-quality non-living carbon and inorganic mineral grains, these nutritionally-enriched microhabitats must play a large role in a deposit feeder's overall nutrition. While Ilyanassa may be capable of assimilating non-living organic matter, its marked selective ingestion for carbon, nitrogen, chlorophyll and specific species of diatoms suggests that living material is more important to the snail's growth and nutrition.

ACKNOWLEDGMENTS

This study was supported by the WHOI Education Department, the Pew Memorial Trust and the Department of Commerce, NOAA Office of Sea Grant 04-8-MOI-149 and 04-7-158-44104. G. Lopez, J. Hobbie, J. Teal and I. Valiela provided helpful criticism of an earlier manuscript. We thank Salt Pond Sanctuaries and the late A.B. Gifford and his wife for access to their property at Great Sippewissett Marsh. Woods Hole Oceanographic Institution Contribution Number 4592.

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TABLE 1. Selective feeding by Ilyanassa obsoleta as demonstrated by a comparison of its stomach contents to the sediment surface layer.

SEDIMENT CHARACTER (g wet wt) ⁻¹	0-2 MM SEDIMENT	SNAIL GUT CONTENT	SNAIL SELECTIVITY (gut conc./sediment conc.)
440 NM ABSORBANCE	0.656	7.93	12
μg PHYCOCYANIN	1.0	28.0	28
μg CHLOROPHYLL	13.8	265.0	20
μg BACTERIAL CHLOROPHYLL	0.11	0	0
PER CENT CARBON	2.04	45.4	22
PER CENT NITROGEN	0.17	6.85	40

TABLE 2. The percentage abundance of vertically migrating and non-migrating benthic diatoms in the top 2 mm of surface sediment and in the stomach contents of two pooled groups of Ilyanassa obsoleta feeding on those sediments. Only diatom species with greater than 2 o/o relative abundance in at least one sample have been listed within families. Two standard errors for the percentages is indicated.

DIATOM TAXA	SEDIMENTS	SNAILS	
		GROUP A	GROUP B
Coscinodiscaceae			
Fragilariaceae	64.3	39.5	54.7
<u>Fragilaria pinnata</u> Ehr.	64.3	38.1	54.3
Achnanthaceae	6.8	15.3	4.9
<u>Achnanthes hauckiana</u> Grun.	2.6	4.3	1.7
<u>Achnanthes lemmermanii</u> v. <u>obtusa</u> Hust.	0	9.0	0.7
TOTAL NON MIGRATING	72.3	55.9	59.6
Naviculaceae	21.9	22.8	26.1
<u>Amphipleura</u> sp.	0.3	2.6	0.7
<u>Navicula cf. diserta</u> Hust.	3.2	5.7	7.0
<u>Navicula pygmaea</u> Kuetz.	6.8	3.2	2.8
<u>Navicula cf. salinarum</u> Grun.	4.8	3.6	6.6
Gomphonemaceae	0	0	0.3
Cymbellaceae	1.3	9.6	2.8
<u>Amphora coffeiformis</u> (Ag.) Kuetz.	1.0	8.5	2.1
Epithemiaceae	0.3	0.3	0.3
Bacillariaceae	4.2	11.4	11.1
<u>Nitzschia frustulum</u> (Kuetz.) Grun.	1.6	2.9	2.8
<u>Nitzschia laevis</u> Hust.	0.6	4.6	0.3
TOTAL MIGRATING	27.7 (+2.5)	44.1 (+3.0)	40.4 (+2.9)

FIGURE 1

Carbon and nitrogen content of sediments and the stomach contents and feces of Ilyanassa obsoleta feeding on those sediments.

Top. Distribution of per cent carbon and nitrogen with core depth in laboratory microcosms. Bars denote two standard errors of triplicate samples.

Bottom. Percentages carbon and nitrogen in Ilyanassa's stomach contents and feces compared to the sediment surface layer. Bars denote two standard errors of five samples.

FIGURE 2

Plant pigment concentrations of sediments and the stomach contents and feces of Ilyanassa obsoleta feeding on those sediments.

Top. Distribution of plant pigments with core depth in laboratory microcosms. Bars denote two standard errors of triplicate samples.

Bottom. Plant pigment concentrations in Ilyanassa's stomach contents and the sediment surface layer. Bars denote two standard errors of triplicate samples.

FIGURE 3

Percentage abundance of benthic diatom groups found in Ilyanassa's stomach contents and at different sediment depths in laboratory microcosms. Each bar represents counts of 400-650 diatom frustules.

CHAPTER 6

DENSITY DEPENDENT EFFECTS OF SNAIL GRAZING ON BENTHIC ALGAL PRODUCTION
AND THE GROWTH OF TWO CONSUMERS

INTRODUCTION

In the preceeding chapters I have presented evidence that low mud snail (Ilyanassa obsoleta) densities stimulate respiration, gross photosynthesis, chlorophyll standing stocks and acetylene reduction rates of sediments in laboratory microcosms (Figure 1). I hypothesize that this stimulation is a result of the selective removal of slower growing benthic diatoms by the grazing snails and a fertilization of the remaining species by snail excretion of ammonia. Fertilizing sediments with ammonium at these rates of snail excretion increases the sediment chlorophyll content by an amount more than sufficient to explain the stimulation due to low densities of snails.

Doubling these snail densities produces a decline in all the above parameters of community metabolism below the levels of ungrazed controls, with the exception of CO₂ exchange rates which were confounded by the pH effect discussed earlier (Chapter 3). Much of this decline is probably due to sediment stirring, a by-product of the snails' movement. Sediments which have been raked daily show rates of community metabolism very similar to the heavily grazed sediments and, the stimulation of chlorophyll production by fertilization can be totally erased by also raking these sediments daily (Chapter 3).

Given these clear effects of Ilyanassa obsoleta on the productivity of the benthic diatoms they eat, I conducted experiments to see if this stimulation was reflected in the growth of the snails themselves at different densities. I have also attempted to determine if another marsh consumer, the striped killifish, Fundulus heteroclitus, is affected by

the changes in the diatom community. Fundulus is an omnivore, ingesting mostly benthic animals and some plant fragments and diatoms (Bigelow and Schroeder, 1953; Kneib and Stiven, 1978). During some seasons of the year its preferred foods are scarce, and benthic diatoms can constitute an important part of its diet.

METHODS

To determine the effect of snail density on snail growth, three densities of snails (three, six or twelve) were added to two 765 cm² flow-through aquaria each. The snails were weighed initially and after 19 and 38 days.

A second experiment cross-classified three densities of fish (1,2 and 3) with three densities of snails (0,3 and 6). Eighteen one-gallon, wide-mouth glass jars served as the experimental aquaria. The bottom of each jar was filled to a depth of 3 cm with sediments which had been previously frozen and sieved after collection from Great Sippewissett Marsh, Massachusetts. The jars were filled with 800 ml of 5 μ -filtered seawater and continuously aerated. They were set in a 20° C. seawater bath under fluorescent "grow" lights on a 14-10 hr light-dark cycle. Five days later 3 cm-long Fundulus heteroclitus and 20-25 mm-long Ilyanassa obsoleta were added to the jars. Each density combination was run in duplicate, and positions in the seawater bath were assigned in a randomized blocks design. Snail stocking densities ranged between 0 and 400 m⁻², the normal range of Ilyanassa found within Greater Sippewissett Marsh. Fish densities ranged from 0 to 200 m⁻², quite a high density for all but the tightest aggregations in the field. After 11 days the snails and fish were removed and weighed.

Growth in the fish and snails was determined by following changes in the animals' wet weights. Excess water was shaken from the fish for five seconds before they were put on the scale. The snails were blotted dry with paper towels for five minutes before weighing. Reweighing the same fish and snails showed a measurement error of less than 2 o/o. Reweighed fish were always lighter, probably because they lost some of their outer mucus covering during handling. This may have also made the fish more susceptible to infection. Four fish died the day after the initial weighing forcing an imbalance in the experimental design..

RESULTS AND DISCUSSION

In the snail growth versus density experiment, snail wet weight declined overall during the first 19 days of incubation, but the lower density treatments showed an increase in weight during the second period, averaging 0.02 percent day⁻¹. At 12-snail densities, the animals lost weight during the entire experiment (Figure 2).

In the second experiment, the high density of fish in these containers often resulted in weight loss. Nevertheless, in all instances, fish in containers with low densities of snails weighed more than fish in containers without snails. At very high snail densities, growth decreased to levels below that of the controls (Figure 3). Fish density also had a significant effect on fish growth. A two-way ANOVA of fish growth as a function of fish and snail densities for the 0- and 3-snail densities showed a significance level of $P < 0.05$ for both manipulations. Snail growth in these containers was quite variable, and there was no significant difference between any of these containers ($P > 0.50$).

Since both Fundulus and Ilyanassa eat the same sorts of food, one would expect fish growth to decrease with increasing snail densities just as fish growth declines with increasing fish densities. At low snail densities, though, I find that Fundulus grows faster than when snails are absent. There is some evidence that the snails themselves benefit from this grazing stimulation, but the slowness at which they grow and the difficulty in obtaining accurate wet weights precludes their being a sensitive indicator of this grazing stimulation. The negative effect of increasing Fundulus densities on growth in these experiments is most probably due to the larger grazing effect of a fish compared to a snail. While the average dry weight of these organisms was similar, a comparison of their respiration and excretion rates shows that one fish is metabolically equivalent to 8-20 snails (Edwards, 1979; Nixon et al., 1976; Maloeuf, 1937). For these densities, then, the fish are constantly overgrazing the sediments.

CONCLUSION

The interactions between Fundulus heteroclitus and Ilyanassa obsoleta could be classified as mutualism (++), commensalism (+0), amensalism (-0) or competition (--) depending on the relative densities of each of these animals. The complex, density-dependent nature of these interactions, mediated through a third party, the benthic diatoms, are indicative of a class of interaction labelled "higher-order". Such non-additive interactions among species have been shown to be important in competitive

(Wilbur, 1972; Neill, 1974; and Lynch, 1978) or predator-prey interactions (Menge, 1978). They also often arise in mutualistic situations as documented by Smith's (1968) case of cowbird egg mimicry of oropendolas.

Much of the time these interrelationships between Ilyanassa and Fundulus are mutualistic, commensalistic or amensalistic. For the most part ecologists have overlooked these classes of interactions in preference to competition and predation. Yet mutualistic and commensalistic interactions abound with species providing one another with habitat, food or protection. Examples include obligate understory plants in the forest or rocky intertidal, pollinator attraction and seed dispersal by plants, cleaner wrasses associated with larger fishes, owls roosting in abandoned woodpecker holes, the supply of shells to hermit crabs, heterospecific flocks of birds or groups of animals including monkeys, antelopes and coral-reef fishes, and a variety of microbial, symbiotic relationships from the microalgae cultured within radiolarians and foraminiferans to the intestinal biota of grazing mammals.

The emphasis on predator-controlled systems in terrestrial and rocky intertidal environments has led to a number of such experiments in soft-bottom communities. Most communities are subjected to disturbance followed by selection, and much of the predator's function is as a disturbance mechanism which creates free space.

In soft-bottom surface communities, though, rates of disturbance are much more frequent in comparison to terrestrial or hard substrate communities where competition for space predominates. Soft-bottom

environments are constantly being reworked by tidal and current activities in addition to bioturbation. Organisms can either adapt themselves to different rates of sediment turnover or try to stabilize their environment by burrowing more deeply into the sediments. For short-lived organisms in the sediment-surface community, the patchiness of bioturbation will create a mosaic of shifting "hot spots", where the process of succession begins anew. An organism's response to sediment instability will determine whether its response to the range of bioturbation interactions is classed as mutualism, commensalism or amensalism.

Most investigators of mutualism have concerned themselves with systems of parasitism or pollination, tightly coupled systems where organisms depend on one another. But strictly speaking, a positive interaction term merely indicates a positive effect on the other individuals fitness, which in this case is represented by growth. The Ilyanassa-Fundulus system is a very loosely coupled one, and I think generally representative of the types of interactions between organisms occurring on soft bottoms. These interactions probably develop as a result of the large inefficiencies which can exist with trophic transfer. Slobodkin (1972) argues that ecological efficiency cannot be constrained or have a limit on theoretical grounds. At the population level the amount of ingestion is an extensive or global value which individual population constituents cannot do anything about. For example, the growth efficiency of polychaetes in experimental microcosms is a function of the presence of meiofauna and ciliates and also the daily nitrogen inputs (Tenore and Rice, in press).

The various density-dependent ways in which Ilyanassa obsoleta and Fundulus heteroclitus can interact are all sediment-mediated via Ilyanassa's grazing effect. Throughout this thesis I have tried to analyze this grazing effect in terms of three of its constituents; stirring, fertilization and selective consumption. Of those terms stirring has the largest potential to act as a disturbance mechanism on the benthic algal community, and it is a general effect of deposit feeding invertebrates in surface sediment communities. Experiments to determine the extent of free-space creation and response to free-space in soft-bottom environments would be valuable in explaining the ecology of these communities.

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FIGURE 1

The effect of snail density on several different sediment parameters. This figure summarizes the measurements made in Chapters 3 and 4 of chlorophyll standing stocks, acetylene reduction, photosynthesis (Light O_2 and CO_2) and respiration (Dark O_2 and CO_2). Values for the different densities of snails are expressed as a percentage of the controls. All measures at 6-snail densities are significantly greater ($P < 0.05$) than at 0-snail densities.

FIGURE 2

Growth of Ilyanassa obsoleta at three densities in flow-through aquaria. Means and two standard errors of snails in duplicate containers is plotted.

FIGURE 3

Growth of Fundulus heteroclitus in 14 cm diameter jars with different densities of mud snails, Ilyanassa obsoleta. Means and two standard errors of three different densities of fish in duplicate containers is plotted.

APPENDIX 1

FURTHER EXPERIMENTS DEMONSTRATING A SNAIL GRAZING EFFECT

In Chapter 3 I reported the results of the final set of experiments designed to measure the grazing effects of Ilyanassa obsoleta on a benthic algal community. In the course of developing the techniques for those experiments, I did a number of preliminary experiments. I used similar methods to those reported in Chapters 2 and 3 except that the intensity of raking in the experiment reported here was only half that reported in Chapter 3. These experiments are summarized in Table 1.

In both instances chlorophyll was significantly higher ($P < 0.05$) in containers with low densities of snails than in the controls (Figures 1 and 2). The magnitude of the difference --- 16 o/o higher in the first experiment, 10 o/o higher in the second --- are similar to the stimulation seen in Chapter 3. The ratios of absorbance at 666 nm (chlorophyll) to the absorbance at other pigment peaks was always higher in the grazed tanks. This difference was significant at $P < 0.05$ in the first experiment only.

Pigment concentrations in the raked containers did not differ significantly from the controls, but their absorbance ratios were always higher than the controls.

Measurements of photosynthesis rates also showed stimulation by snails (Figure 3), but daily variation was high in this experiment.

Table 1. Experiments for measuring snail grazing effects on benthic algae.

Date	Exptl. Chambers	Sediment Treatment	Seawater System	Treatments	Characteristic Measured	Number of Sampling Days	
						Before	After
V 78	dish trays	frozen, stirred once	Unfiltered	Control, Low snail, Raked	Pigments	2	8
VI 78	dish trays	frozen, sieved, stirred	Unfiltered	Control Low snail	Pigments	5	8
IX 78	plexi microcosm	frozen, sieved, stirred	Unfiltered	Control Low snail	Light-dark O ₂ exchange	3	5
III 79	plexi microcosm	frozen, sieved, stirred, twice	Filtered 5 μ	Control, Low snails, High snails, Raked	Pigments Light-dark O ₂ , CO ₂ Exchange	3	8

FIGURE 1

The effect of snail grazing and sediment raking on sediment pigment concentrations and ratios in laboratory microcosms. Plotted are the normalized means of two tanks sampled eight times during a 20 day period. Horizontal lines denote two standard errors computed from the interaction component of a 3-Way ANOVA (Day x Treatment x Tank). May 1978.

Pre-treatment tank means are phycocyanin, $0.59 \mu\text{g cm}^{-2}$; chlorophyll, $6.3 \mu\text{g cm}^{-2}$ and bacterial chlorophyll, $0.17 \mu\text{g cm}^{-2}$.

NORMALIZED PIGMENT BIOMASS

($\mu\text{g cm}^{-2}$)

PHYCOCYANIN

CHLOROPHYLL

BACTERIAL CHLOROPHYLL

ABSORBANCE RATIOS: 666/440

666/615

666/760

FIGURE 2

The effect of snail grazing on sediment pigment concentrations and ratios in laboratory microcosms. Plotted are the normalized means of two tanks sampled eight times during a 21 day period. Horizontal lines denote two standard errors computed from the interaction component of a 3-Way ANOVA (Day x Treatment x Tank). June 1978. Pre-treatment tank means are phycocyanin, $0.59 \mu\text{g cm}^{-2}$; chlorophyll, $6.0 \mu\text{g cm}^{-2}$ and bacterial chlorophyll, $0.24 \mu\text{g cm}^{-2}$.

NORMALIZED PIGMENT BIOMASS
($\mu\text{g cm}^{-2}$)

FIGURE 3

The effect of snail grazing on respiration and photosynthesis in laboratory microcosms. Plotted are the normalized means of two tanks sampled five times during a 33 day period. Horizontal lines denote two standard errors computed from the interaction component of a 3-Way ANOVA (Day x Treatment x Tank). September 1978. Pre-treatment tank means are respiration, $0.21 \mu\text{l O}_2 \text{ cm}^{-2}\text{hr}^{-1}$ and photosynthesis, $1.7 \mu\text{l O}_2 \text{ cm}^{-2}\text{hr}^{-1}$.

NORMALIZED OXYGEN EXCHANGE ($\mu\text{l cm}^{-2}\text{hr}^{-1}$)

APPENDIX 2

DIATOM COUNTS FROM LABORATORY MICROCOSM
SEDIMENTS SUBJECTED TO FOUR TREATMENTS

Chapter 3 summarizes the effects of snail grazing or raking on edaphic diatoms by presenting a graph of percentage migratory Naviculineae (Biraphidineae) during the experiment (Chapter 3, Figure 8). That figure is based on a more extensive identification of the diatom groups which is presented here (Table 1).

TABLE 1. Percent abundance of edaphic diatoms from laboratory microcosms subjected to four treatments.

DIATOMS	TREATMENT TANK	DAY -7							
		0-Snails		6-Snails		12-Snails		Raked	
		A	B	A	B	A	B	A	B
<u>Fragilaria spp.</u>		52.0	55.2	51.6	52.6	53.3	53.5	51.4	53.9
Total Fragilarineae		55.5	58.7	55.3	55.0	57.1	56.3	55.8	58.1
<u>Achnanthes spp.</u>		5.7	4.0	5.1	3.7	5.5	4.5	5.9	5.2
Total Achnanthineae		5.7	4.0	5.1	3.7	5.5	4.5	5.9	5.2
<u>Navicula spp.</u>		32.1	32.2	35.0	36.1	30.6	34.6	31.7	31.8
<u>Nitzschia spp.</u>		4.9	3.8	3.3	3.3	4.3	2.9	4.3	3.7
<u>Amphora spp.</u>		1.2	0.9	1.0	1.6	1.2	1.0	1.4	0.8
Total Naviculineae		38.8	37.3	39.6	41.3	37.5	39.2	38.2	36.8
Number valves counted		652	821	669	513	510	691	625	601

DIATOMS	TREATMENT TANK	DAY 4							
		0-Snails		6-Snails		12-Snails		Raked	
		A	B	A	B	A	B	A	B
<u>Fragilaria spp.</u>		38.1	38.9	40.0	36.3	38.9	40.1	39.1	39.3
Total Fragilarineae		43.6	43.7	44.1	41.7	42.5	44.3	41.5	41.3
<u>Achnanthes spp.</u>		6.9	6.2	5.7	6.3	8.7	7.6	7.2	8.3
Total Achnanthineae		6.9	6.3	5.7	6.3	8.7	7.6	7.2	8.3
<u>Navicula spp.</u>		41.3	44.6	43.4	45.6	41.7	43.6	46.7	45.6
<u>Nitzschia spp.</u>		4.9	2.2	2.8	2.9	3.4	2.8	1.8	2.6
<u>Amphora spp.</u>		1.5	0.9	1.2	1.1	1.4	0.6	0.8	0.8
Total Naviculineae		49.4	50.0	50.2	52.0	48.8	48.1	51.2	50.3
Number valves counted		720	772	775	619	725	881	884	888

DIATOMS	TREATMENT TANK	DAY 15							
		0-Snails		6-Snails		12-Snails		Raked	
		A	B	A	B	A	B	A	B
<u>Fragilaria spp.</u>		41.8	46.6	58.4	70.2	57.4	54.7	52.5	53.9
Total Fragilarineae		44.2	50.2	61.7	74.2	60.8	58.4	54.6	57.8
<u>Achnanthes spp.</u>		7.2	4.5	5.4	3.8	6.5	5.9	6.3	3.5
Total Achnanthineae		7.2	4.5	5.4	3.8	6.5	5.9	6.3	3.5
<u>Navicula spp.</u>		43.6	36.7	27.8	16.7	28.0	30.1	33.0	32.4
<u>Nitzschia spp.</u>		2.4	5.1	2.4	3.0	2.7	3.6	2.3	3.5
<u>Amphora spp.</u>		2.2	1.3	1.3	1.4	0.6	0.6	1.5	0.9
Total Naviculineae		48.6	45.3	32.9	22.0	32.7	35.7	39.1	38.6
Number valves counted		459	839	627	504	786	810	476	740

DIATOMS	TREATMENT TANK	DAY 18							
		0-Snails		6-Snails		12-Snails		Raked	
		A	B	A	B	A	B	A	B
<u>Fragilaria spp.</u>		39.1	37.4	50.3	59.8	49.2	53.7	45.5	45.0
Total Fragilarineae		43.0	42.0	55.8	65.5	52.3	55.3	47.9	50.2
<u>Achnanthes spp.</u>		7.9	5.6	9.7	5.0	8.7	8.8	3.8	8.8
Total Achnanthineae		7.9	5.7	10.2	5.3	8.7	8.8	3.8	8.8
<u>Navicula spp.</u>		43.8	46.2	28.4	23.8	34.2	30.8	44.9	36.1
<u>Nitzschia spp.</u>		1.8	2.6	2.1	2.0	1.7	1.4	1.6	1.6
<u>Amphora spp.</u>		0.9	1.4	1.4	0.7	0.7	1.2	0.8	1.4
Total Naviculineae		49.1	52.3	34.1	29.2	39.0	35.9	48.2	41.0
Number valves counted		926	1005	719	684	597	432	365	634

DIATOMS	TREATMENT TANK	DAY 35			
		0-Snails		6-Snails	
		A	B	A	B
<u>Fragilaria spp.</u>		57.0	58.0	60.2	70.6
Total Fragilarineae		61.1	62.5	64.6	73.5
<u>Achnanthes spp.</u>		5.3	4.5	3.8	5.0
Total Achnanthineae		5.4	4.5	3.8	5.2
<u>Navicula spp.</u>		28.5	28.8	25.5	16.4
<u>Nitzschia spp.</u>		2.5	1.9	3.5	2.6
<u>Amphora spp.</u>		1.2	1.2	2.0	1.4
Total Naviculineae		33.5	33.0	31.6	21.4
Number valves counted		945	891	819	697

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PROFESSIONAL INTEREST

Providing technical information and assistance to communities interested in developing their natural resources in an ecologically sound manner with an awareness of the political implications of such development.

EDUCATION

Massachusetts Institute of Technology/
Woods Hole Oceanographic Institution
Ph.D., June 1980, Joint Program in Biological Oceanography. Thesis titled Snail grazing effects on the composition and metabolism of benthic communities and subsequent effects on fish growth (supervisor, John M. Teal). Research interests include nutrient cycling at the mud-water interface, plant-animal interactions and their effect on the growth of cultured organisms.

Stanford University
B.S., June, 1974. Biology with Distinction. Phi Beta Kappa. Broad background in natural and physical sciences plus economics and journalism.

WORK EXPERIENCE

1975-present. Graduate school projects.

Technical consultant to the Abenaki Indian Self-Help Association's Aquaculture Project. Prepared a feasibility study on the cage culture of trout in Vermont lakes. Represented ASHAI in meetings with the Vermont Departments of Fish and Wildlife and Environment. 1978-

Technical consultant to the Institute for Local Self-Reliance and the National Children's Island Appropriate Technology Park for a feasibility study of the suitability of the Anacostia River for raising fish. 1979

Prepared a proposal with the Isan Film Group of Hong Kong to make a documentary film about aquaculture in China. 1979-

Consultant panelist to the State Review Board for the Department of Energy's Small Grants Program in Appropriate Technology. 1979

Provided technical information and prepared information pamphlets on the biological effects of microwaves for the Cape Cod Environmental Coalition, Falmouth, MA. 1978

Regional contact for the National Center of Appropriate Technology. 1977

Organized a seminar to study the effects of offshore oil drilling on the Georges Bank fishery. Presented findings at public hearings. Participated as a lobbyist for a coalition of environmentalists and fishermen advocating stricter drilling safeguards. 1976-1978

Reviewed environmental statements for the Massachusetts Secretary of the Environment. 1976

1974-1975. Co-director, Institute for Local Self-Reliance

Major responsibilities for this policy/consulting group were with the Urban Food Project. Helped coordinate, organize, troubleshoot, etc. ten community gardens on vacant city lots. Started a 2 tons/mo. composting project using the vegetable wastes from neighborhood food stores. Produced pamphlets posters and articles about urban food issues and travelled to other cities to help them organize various food projects.

1972-1973. Volunteers in Asia

Teacher at a Korean high school which stressed community development and self-help in Hong-dong, South Korea. During that time, did research for a book chapter, "South Korea" which appeared in On Your Own: Air Siam's Student Guide To Asia, VIA Press, 1976.

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Pamphlets and posters for ILSR. "Composting in the city," "Large-scale sprouting as a cottage industry," "Gardening for health and nutrition," "The urban farmer".