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Effect of Snails (Elimia clavaeformis) on Phosphorus Cycling
in Stream Periphyton and Leaf Detritus Communities

by

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A technical report submitted to the faculty of the University of North Carolina at Chapel Hill in partial fulfillment of the requirements for the degree of Master of Science in Public Health in the Department of Environmental Sciences and Engineering, School of Public Health.

Chapel Hill, 1993

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ABSTRACT

ELIZABETH A. JAY. Effect of Snails (Elimia clavaeformis) on Phosphorus Cycling in Stream Periphyton and Leaf Detritus Communities (Under the Directions of Dr. DONALD E. FRANCISCO and Dr. PATRICK J. MULHOLLAND)

In this study, I examined the effect of grazing on phosphorus cycling in stream periphyton and leaf detritus communities using the snail Elimia clavaeformis. Phosphorus cycling fluxes and turnover rates were measured in a laboratory and in a natural stream, respectively, using radioactive tracer techniques.

Snails increased phosphorus fluxes and turnover rates from the communities. However, this effect was only significant for turnover rates from detritus, perhaps because higher fecal production rates occurred when snails grazed on detritus compared with periphyton. When the phosphorus concentration in water was increased in algal studies, cycling fluxes were not significantly affected when snails were present, but were significantly reduced when snails were absent.

My results indicate that although snails may not strongly affect phosphorus cycling, snails can enhance phosphorus turnover from certain substrates such as leaf detritus. In addition, the effect of snails on phosphorus cycling from leaf detritus depends on the fate of the large flux of phosphorus into fecal matter. Because the experimental design of my studies involved substrates commonly grazed by snails, I have been able to observe how phosphorus cycling in streams is influenced by snails.

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ACKNOWLEDGMENTS

It is necessary to thank many people for helping me complete this technical report and keep my sanity simultaneously. Without their assistance, this production would have required several more episodes of frustration and anxiety!

I am grateful for the aide of several personnel affiliated with the Environmental Science Division of ORNL. As a scientific advisor, Pat Mulholland helped plan, prepare, and perform experiments as well as presented suggestions regarding data analysis and report editing. Tony Palumbo also served on my committee, and offered advice concerning this report and computer problems. Al Steinman, Ramie Wilkerson and Bonnie Lu gave technical assistance during various phases of the research. Erich Marzolf answered many computer questions and helped prepare overheads for presentations of the experiments. Gloria Lomax located several important journal articles. Finally, Kathy Ketner made paperwork involved in conducting research at a location far away from school much easier to handle.

I wish to thank many people involved with my graduate program at UNC-Chapel Hill for their aide, too. Don Francisco, my academic advisor, offered advice ranging from the best classes to take, to interesting areas of research to pursue, to possible alterations to make on this report; he also offered much encouragement and assistance with the red tape of school policies. Fred Pfaender, another

committee member, helped improve this report. C. L. Lassiter and Carol Carden answered several questions and provided correct forms concerning the "important stuff" (registration, grades, graduation, etc.). Various teachers gave helpful suggestions in times of confusion. Finally, fellow classmates made the adjustment to school and life in North Carolina easier than expected.

I am grateful for the relationships I have had with families and friends over the years, also. My parents have almost always been supportive of my goals, and have enabled me to pursue these goals via school and various experiences; they also fostered the drive for success that I now possess. The Carnell family has always made me feel like one of their family. Cindy Tsai has been a good listener during good and bad times. Dobie Gnash has consistently greeted me with warmth, sweetness, and a tail wag. Finally, to Bill Kountis, to whom this work is dedicated, I can not express my appreciation: he has been extremely supportive of my work, has made me smile, and let me share, life's roses, and has soothed me through many times of moodiness, tears and tension.

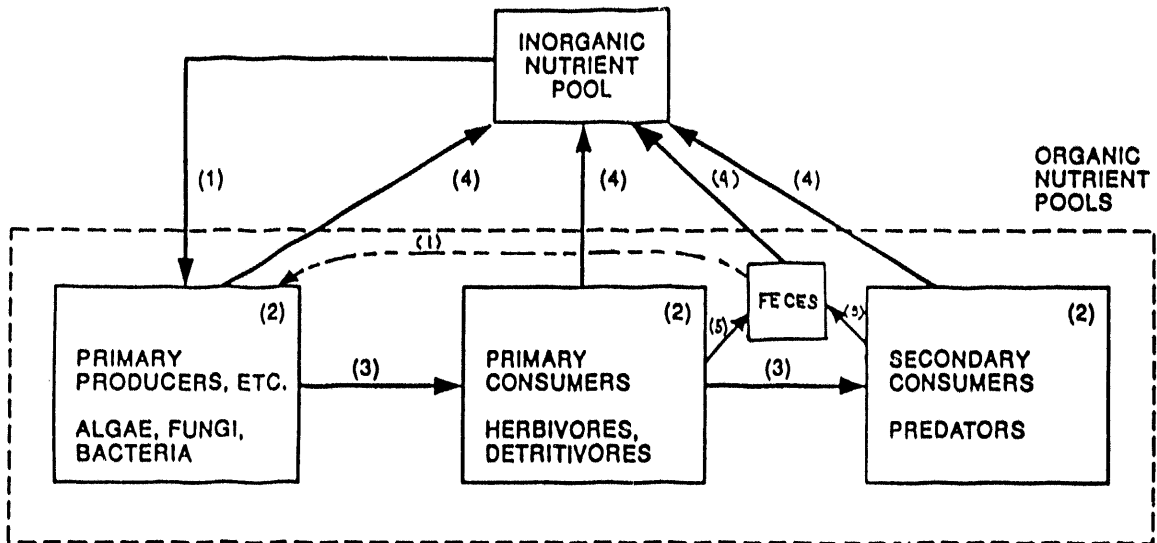
This technical report is based on work performed in the laboratory graduate participation program under contract number DE-AC05-76OR00033 between the United States Department of Energy and Oak Ridge Associated Universities.

INTRODUCTION

Nutrient cycling in aquatic systems is of interest because, together with nutrient inputs, it determines the supply of nutrients to organisms and hence the productivity of nutrient limited ecosystems. In addition, the form and amount of a nutrient available to producers and consumers is determined by how rapidly nutrients cycle as well as by external inputs. Finally, the transport of a nutrient is controlled by nutrient cycling in flowing water ecosystems along with other characteristics of those systems, e.g. flow rate and biota present.

Nutrient cycling is the uptake, assimilation, trophic transfer, regeneration, loss, and reuptake of a nutrient by organisms in an ecosystem (Figure 1). Nutrients are initially taken up in inorganic form by plants and microbes. Nutrients are assimilated and transferred through the trophic web as animals consume plants, microbes, or other animals. Nutrients are regenerated by organisms via excretion, because the supply of nutrients in food exceeds nutrient demand, because of unavoidable loss resulting from cell metabolic processes, or as result of inefficient feeding. Nutrients are regenerated when plants, microbes, and animals die and cells lyse or decompose, too. Nutrients are also regenerated and lost by consumers via

FIGURE 1. NUTRIENT CYCLING IN AQUATIC SYSTEMS

**NUTRIENT CYCLING PROCESSES:**

- (1) NUTRIENT UPTAKE
- (2) ASSIMILATION OF NUTRIENT
- (3) TROPHIC TRANSFER OF NUTRIENT
- (4) REGENERATION OF NUTRIENT
- (5) LOSS OF NUTRIENT AS FECES

feces.

Nutrient cycling is often quantified by measuring nutrient regeneration and/or turnover rates. Nutrient regeneration is the release of a nutrient in dissolved inorganic form from an organic pool. Nutrient turnover is the loss rate of a nutrient from an organic pool or sum of pools relative to storage of a nutrient in that pool or pools.

LITERATURE REVIEW

Although many nutrients are important in freshwater ecosystems, phosphorus has been shown to be the limiting nutrient in several freshwater streams throughout the world (Jurgens and Gude 1990, Elwood et al. 1981). In other words, phosphorus has been shown to be the nutrient in lowest supply relative to its demand by biota in many freshwater systems. Therefore, the focus of my study is phosphorus.

Studies of phosphorus cycling in aquatic systems have involved a variety of plant and microbial communities and their consumers. Macrophytes, algae and microbes which live attached to underwater surfaces have been studied. In addition, primary consumers, such as grazers which feed upon the plant and microbial communities mentioned above, as well as secondary consumers (predators) have been studied. Consumers commonly used in studies of nutrient cycling include protozoans, zooplankton, aquatic insects, snails, and fish. The role of consumer organisms in the phosphorus cycle involves feeding and assimilation of organic forms of phosphorus (trophic transfer) and regeneration of inorganic phosphorus.

1. Bacteria and algae

The role of bacteria and algae in phosphorus cycling in aquatic systems is twofold: bacteria and algae take up and compete for phosphorus in inorganic dissolved form (Johannes 1968, Bloem et al. 1988), and bacteria and algae release nutrients to the water in dissolved organic and inorganic forms (Barsdate et al. 1974, Taylor 1982). For algae, uptake is generally much more important than regeneration (Pomeroy 1970). However, the importance of regeneration versus uptake activities of bacteria depends on the relative availability of organic carbon and inorganic phosphorus in a system as well as the phosphorus requirement of the bacteria. Therefore, this theory leads to the conclusion that if a water body has a low available concentration of inorganic phosphorus, sufficient available organic carbon, and contains bacteria which have a high phosphorus requirement, uptake and retention of phosphorus by the bacteria will dominate over regeneration of phosphorus by the bacteria.

The size of an algal or bacterial cell can also affect phosphorus regeneration. Because larger planktonic bacteria may have higher phosphorus to carbon ratios than smaller planktonic bacteria, more phosphorus may be retained in the bodies of larger planktonic bacteria than smaller planktonic bacteria (Jurgens and Gude 1990). However, in general, bacteria and algae act more as agents for uptake of dissolved inorganic phosphorus than as agents of

regeneration (Berman et al. 1987, Bloem et al. 1989).

Although algae and bacteria have similar roles, the role of bacteria is more complex because, unlike algae, they can use dead organic matter (detritus) as a source of phosphorus. If the detritus contains enough phosphorus, additional inorganic phosphorus from the water may not be needed to satisfy the phosphorus demanded by microbes growing on it. However, often the phosphorus content of detritus is lower (higher carbon to phosphorus ratio) than that needed by attached microbes and the phosphorus deficiency must be overcome by uptake from the water. This situation is particularly true for leaves from deciduous terrestrial vegetation that fall into aquatic systems, especially streams. Most of the phosphorus originally present in the leaf is leached rapidly after the leaf has fallen into the water (Webster and Benfield 1986). As the aquatic microbes colonize the leaf surface and utilize the organic carbon within the leaf, they must take up phosphorus from the water, and in doing so, they increase the phosphorus content of the detritus (dead leaf-microbe complex) as a whole (Mulholland et al. 1984). In this way, bacteria improve leaf detritus as a food source for higher consumers. Thus, the higher consumers (detritivores), not the bacteria themselves, are responsible for the regeneration of most phosphorus from the detritus (Johannes 1968). Bloem et al. (1988) found this to be the case with microbial utilization of dissolved organic matter, too.

Nevertheless, it has also been shown that bacteria in the presence of detritivores can regenerate considerable amounts of phosphorus from detritus (Barsdate et al. 1974).

However, it is important to keep in mind that the nutrient content of the leaf relative to that in bacteria, as well as the effectiveness of bacteria in forming bacterial matter from dead leaf matter, determines whether bacteria attached to leaf detritus will regenerate phosphorus (Jurgens and Gude 1990).

2. Consumers

Consumer organisms also need phosphorus, but their mechanisms for uptake and release are different from those of bacteria and algae. Consumers cannot obtain phosphorus from the water in inorganic dissolved form; they must obtain phosphorus from the food they consume in particulate, organic form (Taylor 1982). Consumers release phosphorus back to the water in dissolved inorganic (regeneration) and organic form, as well as in particulate form as feces (Andersson et al. 1985, Andersen et al. 1986, Taylor 1982, Taylor and Lean 1981). Consumers can influence phosphorus cycling directly by assimilation of phosphorus and indirectly by altering the rates of algal and bacterial uptake. In this report, I will focus on two types of primary consumers in stream ecosystems, grazers (consumers that eat periphyton) and detritivores (consumers that eat detritus).

2.1. Alteration of bacterial and algal uptake rates by consumers

Consumers can modify the biomass, metabolic rates and species composition of bacterial and algal communities. Therefore, consumers can change the amount and/or time required by these communities to consume phosphorus.

2.1.1. Effects on biomass

Although most studies have shown that grazers cause reductions in biomass of bacteria and algae (Mulholland et al. 1983, Sumner and McIntire 1982, Mulholland et al. 1991, Hill and Knight 1987, Lamberti and Resh 1983, Barsdate et al. 1974), other studies have shown that grazers have no significant effect or even a positive effect on biomass of microbes and periphyton (Marks and Lowe 1989, Gallepp 1979). The impact of grazing on biomass may change as nutrient concentrations, grazer density and/or algal and bacterial size change. For instance, Bergquist and Carpenter (1986) found that, especially for nutrient depleted algae, growth rates of algae < 30 μm in size declined at higher zooplankton biomass and increased at lower zooplankton biomass levels. Growth rates of algae > 30 μm did not change or increased as grazer biomass increased.

2.1.2. Effects on metabolic rates

Although some studies report that total phosphorus uptake rates by bacteria and algae are reduced by grazing

across all grazer densities due to reduction in bacterial and algal abundance (Lehman 1980a, Mulholland et al. 1985, Mulholland et al. 1983), the rate of phosphorus uptake per bacterial or algal cell can remain high in the presence of grazers (Mulholland et al. 1984). By consuming algal and bacterial material, grazers can increase the metabolism of that material by preventing self-limiting population densities (Mulholland et al. 1984, Barsdate et al. 1974). Decomposition of dead organic matter is also enhanced by consumers because microbial metabolism is increased via consumption and regeneration of available nutrients, and the surface area of leaf detritus available for microbial activity is increased (by consumption) as well (McDiffett and Jordan 1978).

2.1.3. Effects on species composition

Grazing can cause changes in phosphorus consumption by bacterial and algal communities by causing shifts in species composition of the communities. Different species of algae and bacteria are known to have different uptake rates of phosphorus (Vanni and Findlay 1990). Therefore, grazers, by selecting certain species, can indirectly alter phosphorus uptake (Cuker 1983b, Sumner and McIntire 1982). This selection by grazers is due to differences in edibility, accessibility, and digestibility of various algae and bacteria (Marks and Lowe 1989, Porter 1976). In many cases, grazing selects for the algal or bacterial species that have

the highest growth rate (Taylor 1982). However, recently Rosemond et al. (in press) have shown that grazing can result in a shift toward slower growing algal species.

2.2. Storage/Retention

Consumers, especially those with relatively long life spans, may reduce fluctuations in the amount of phosphorus cycled in a system due to their capacity for phosphorus storage in biomass (Kitchell et al. 1979, Taylor 1982). For example, because storage of phosphorus can lessen downstream transport, it can decrease the loss of phosphorus from a system (Merritt et al. 1984). However, such storage can also reduce phosphorus cycling rates by reducing regeneration back to water (Berman et al. 1987). Thus, nutrient accumulation in consumer biomass can have either a positive or negative effect on nutrient cycling within a system.

2.3. Regeneration

Consumers stimulate phosphorus cycling because they are very important agents of phosphorus regeneration back to water (Andersen et al. 1986, Bloem et al. 1989, Jurgens and Gude 1990, Johannes 1968). However, as with bacteria, the rate of phosphorus regeneration by consumers is dependent on phosphorus and carbon availability in their food relative to their physiological needs for phosphorus (Bloem et al. 1988).

Sterner (1990) has argued for a ratio approach for examining nutrient regeneration by aquatic consumers. This approach assumes that the nutrient which is in shortest supply in the food relative to its need is regenerated by the consumer at the lowest rate. In addition, because different consumers have somewhat different chemical compositions, they have different nutrient demands. Thus, nutrient regeneration can vary greatly for different types of consumers even when the same food is utilized. For example, Kairesalo and Koskimies (1987) found that oligochaetes regenerated almost three times as much of their ingested phosphorus as did snails when both grazers fed upon an epiphyte community.

The size of a consumer may also influence its phosphorus regeneration rate. In general, because smaller consumers have higher rates of metabolism, they can have higher phosphorus regeneration rates per unit biomass than larger consumers (Lehman 1980b, Vanni and Findlay 1990). However, Taylor and Lean (1981) showed that microzooplankton did not release phosphorus faster than larger zooplankton. Several other studies have reported that larger aquatic insects have a greater effect on phosphorus regeneration rates than smaller ones (Gardner et al. 1981, Taylor 1982). Therefore, it is unclear if larger or smaller organisms have higher regeneration rates.

2.4. Feces

Although the importance of feces in nutrient regeneration has been highly debated, it appears that feces production does not result in significant amounts of phosphorus regeneration. Taylor and Lean (1981) found that release of phosphorus via zooplankton feces was more important to phosphorus regeneration than direct release of P to water. However, grazers feeding on algae can excrete feces capable of nearly the same photosynthetic activity as the ungrazed algae, and thus the feces would "consume" bioavailable phosphorus rather than release it via leaching (Cuker 1983a). In addition, it is probable that microbes attached to feces, as well as many other organisms, could consume bioavailable phosphorus in the feces, too (Shepard and Minshall 1984). Finally, the formation of phosphorus-iron complexes in feces in well oxygenated water may result in retention of phosphorus within fecal matter (Fukuhara and Sakamoto 1987).

2.5. Snails

The role of snails as primary consumers in stream ecosystems is twofold. Snails are scrapers, a type of grazer, because they feed (scrape) on periphyton adhering to rock surfaces, and snails are detritivores because they consume leaf detritus (Hom 1982, Mulholland et al. 1985). Algal and microbial communities often adhere to organic and inorganic surfaces, or substrates, such as macrophytes,

leaves which fall into the water from terrestrial trees, and
fragments of bedrock. Thus, snails are commonly found
feeding on periphyton and microbially-colonized rocks and
leaves.

2.6. Food chain effects (predators)

Predators can alter rates of phosphorus regeneration to water. Predators can increase phosphorus regeneration via release to water, but can also decrease regeneration indirectly as a result of decreasing grazer numbers or activity (McCormick 1990). In addition to lethal effects, predators decrease grazer activity by causing changes in feeding behavior or by confining grazers to certain microhabitats (Power et al. 1985). Predators may consume grazers of certain sizes, and as a result, change the average size of grazers in a system, also (Shahady et al. in press).

3. Research objectives

In this study, I examine the role of snails in phosphorus cycling in stream periphyton and leaf detritus communities. More research regarding the role of snails in phosphorus cycling is necessary for several reasons. This research should be conducted mainly because a positive effect of consumers on phosphorus cycling processes has been explicitly demonstrated for a few types of organisms but not snails (McDiffett and Jordan 1978). In addition, phosphorus

has been shown to be the limiting nutrient for microbial leaf decomposition and algal reproduction occurring in streams worldwide (e.g. Southeastern United States, Federal Republic of Germany) (Elwood et al. 1981, Jurgens and Gude 1990); thus, the role of primary consumers in phosphorus cycling is crucial for algal production and microbial leaf decomposition. Many past studies on phosphorus have presented turnover and regeneration rates, but few have evaluated all of the major fluxes involved in phosphorus cycling in attached algal and microbial communities in streams, also. Finally, snails are numerically abundant in many streams. Because they are the dominant herbivore in most streams on the Oak Ridge reservation, which was the site of my research in eastern Tennessee, the impact of snails on phosphorus cycling in these streams may be high.

My objectives were:

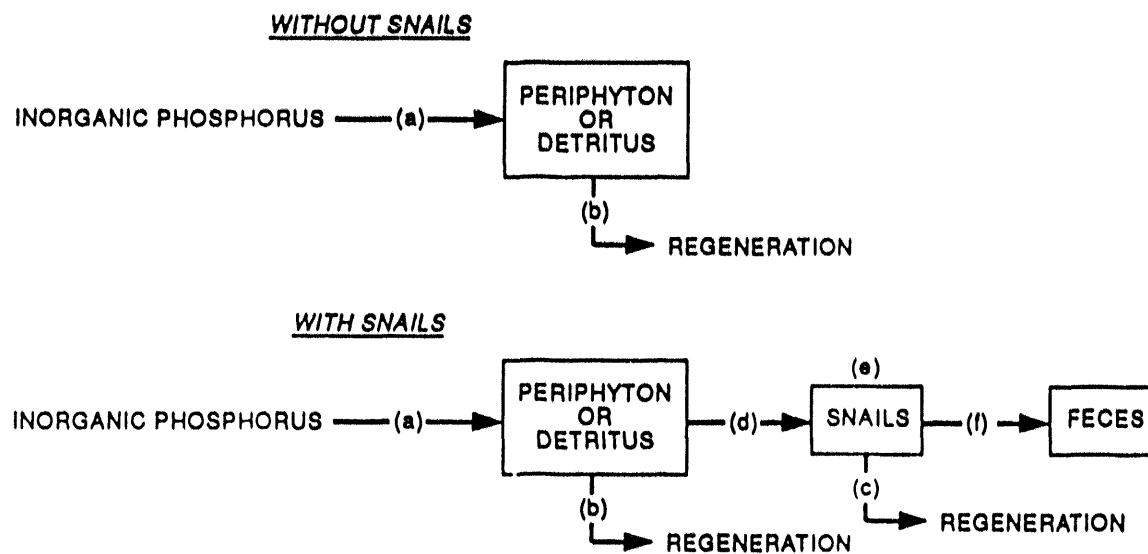
1. To determine the effect of snails on phosphorus cycling fluxes and turnover in streams;
2. To determine the effect of different types of substrates (periphyton, leaf detritus) on phosphorus cycling fluxes in streams; and
3. To determine the effect of water phosphorus content on phosphorus cycling fluxes in streams.

METHODS

1. General approach

Phosphorus cycling fluxes were studied in the laboratory while phosphorus turnover was studied in the field. Both phosphorus cycling fluxes and phosphorus turnover were measured using radioactive tracer techniques. Periphyton and leaf detritus were labelled with ^{33}P by exposing each substrate to $^{33}\text{PO}_4$ in short term laboratory incubations. Labelled substrates were then transported to the field or placed in beakers in the laboratory. Snails were excluded from half of the labelled substrates, and added to the rest of the labelled substrates, at both study locations. In the laboratory studies, the release of ^{33}P from ^{33}P -labelled algae or leaf detritus back to water was measured. In addition, ^{33}P and total phosphorus content of the substrates, snails, and feces were measured. Phosphorus fluxes were then calculated from the ^{33}P data and $^{33}\text{P} / \text{TP}$ ratio in the substrate. In the field studies, ^{33}P content of the substrates was measured several times over a period of days and phosphorus turnover rates were calculated from these data (Figure 2).

FIGURE 2. PHOSPHORUS FLUXES IN THE PRESENCE AND ABSENCE OF SNAILS IN PERIPHYTON AND LEAF DETRITUS ^{33}P LABELLED COMMUNITIES



FLUXES:

- (a) UPTAKE OF ^{33}P FROM WATER BY PERIPHYTON OR LEAF DETRITUS
- (b) RELEASE OF ^{33}P FROM PERIPHYTON OR LEAF DETRITUS TO WATER
- (c) RELEASE OF ^{33}P BY SNAILS TO WATER
- (d) ^{33}P IN FECES AND IN SNAIL BIOMASS
- (e) ^{33}P RETAINED IN BIOMASS OF SNAILS GRAZING ^{33}P LABELLED PERIPHYTON OR LEAF DETRITUS
- (f) ^{33}P IN FECES PRODUCED BY SNAILS GRAZING ^{33}P LABELLED PERIPHYTON OR LEAF DETRITUS

2. Description of research areas

2.1. Field site

The field studies which investigated the effect of snails on phosphorus turnover were performed in the West Fork of Walker Branch, a first order woodland stream in the Oak Ridge National Environmental Research Park which is located in eastern Tennessee (Newbold et al. 1983b). A detailed description of the system is provided by Curlin and Nelson (1968).

The stream drains a 38.4-ha forested watershed within the research park and is fed primarily by springs and seeps that arise in dolomitic limestone. The stream bottom consists mostly of cobble and gravel-size chert and outcrops of the parent dolomite (Newbold et al. 1983b). The stream is comprised of small pools and riffle areas with low summer baseflows of 3 - 5 L/s and occasional winter and spring storm flows which surpass 50 L/s (Mulholland et al. 1990). Stream gradient averages 55.9 m/km and mean annual discharge is 10.5 L/s. Water temperatures vary from winter lows of 4 - 5 °C to summer highs of 17 °C. During baseflow, the average depth is 5 - 10 cm and the average width is 3 m (Mulholland et al. 1990).

A forest canopy consisting mostly of oak (Quercus spp.) and hickory (Carya spp.) covers the stream from May through September (Elwood and Nelson 1972). Stream algae are dominated by basal cells of the green alga Stigeoclonium, and are maintained at low biomass levels by intense

herbivory (Rosemond et al., in press, Steinman 1992).

Although stream fauna is composed of over sixty species of benthic macroinvertebrates, the snail Elimia clavaeformis is by far the dominant stream invertebrate organism (Hom 1982, Mulholland et al. 1985, Elwood and Nelson 1972). This species of snail represents more than 90% of the total biomass of the stream, and average snail density ranges from 1000 snails/m² to 1500 snails/m².

Stream chemistry reflects the parent dolomite. Alkalinity is 2 - 3 meq/L and streamwater pH is 7.4 - 8.2 (Mulholland et al. 1990). Dissolved oxygen levels are at or close to saturation levels throughout the year (Mulholland 1992). Concentrations of soluble reactive phosphorus (SRP) range from <1 to 6 ug/L (Mulholland 1992). Concentrations of ammonium are <5 ug N/L and concentrations of nitrate range from <5 to 60 ug N/L (Mulholland 1992). Studies involving long-term phosphorus and nitrogen enrichments have shown that algal growth and leaf decomposition in Walker Branch are phosphorus limited (Newbold et al. 1983a, Elwood et al. 1981).

2.2. Laboratory

The experiments on phosphorus regeneration were conducted in 1 L beakers. The beakers were kept in a large tank which served as a constant temperature water bath (12 - 20 °C) and the oxygen concentration of the water in the beakers was maintained close to saturation level. The water

as well as the substrates and/or snails in the beakers originated from the natural stream (described above) or an artificial laboratory stream. The artificial laboratory streams are supplied with snails and periphyton from the natural stream and once-through flow from a spring-fed pond. These streams are illuminated by overhead metal halide lamps providing approximately $100 \text{ umole quanta m}^{-2}\text{s}^{-1}$ (or $100 \text{ uEinstein m}^{-2}\text{s}^{-1}$) at the water surface. The temperature of the artificial streamwater is $12 - 15 \text{ }^{\circ}\text{C}$. The SRP concentration of the artificial streamwater is generally $4 - 6 \text{ ug/L}$, and the oxygen concentration is close to saturation levels (Mulholland et al. 1991).

3. Focus and hypothesis of each experiment

3.1. Experiment 1

The effect of snail grazing on phosphorus cycling fluxes in periphyton communities as well as the effect of water phosphorus content on these fluxes was investigated in the laboratory. It was hypothesized that snail grazing would increase phosphorus cycling fluxes, especially in algal communities which had been exposed to water with a high phosphorus content.

3.2. Experiment 2

Experiment 2 was performed to investigate the effect of snail grazing on phosphorus turnover in periphyton communities in the field. It was expected that the presence

of snails would increase turnover rates of phosphorus from periphyton.

3.3. Experiment 3

The third experiment was very similar to Experiment 1 except that it used leaf detritus. The hypothesis of the experiment was that snails would have a positive influence on phosphorus cycling fluxes in leaf detritus communities.

3.4. Experiment 4

The effect of snail grazing on phosphorus turnover in leaf detritus communities in the field was determined in this experiment. As in Experiment 2, I hypothesized that the presence of snails would increase the turnover rate of phosphorus from a community, but I used leaf detritus rather than periphyton in this experiment.

4. Specific experimental methods

4.1. Experiment 1

4.1.1. Preparation of substrates before labelling

Experiment 1 used periphyton communities grown on tiles (exposed surface area per tile equalled 9.5 cm^2) in large laboratory streams for approximately 6 months. The tiles were unglazed, solid, ceramic, and cylindrical (1.5 cm high by 1.5 cm wide) (DU-CO Ceramics "ceramic cylinders"). In addition, half of the periphyton covered tiles were grown in a section of a laboratory stream containing water that had

an enriched SRP concentration. This concentration was 5 - 7²¹ times the ambient, or in stream, SRP concentration of the streamwater. To elevate streamwater SRP concentration, a carboy delivered drops of a phosphate solution to a section of the stream for 18 days, and the SRP concentration increased from 4 - 5 ug/L to about 27 ug/L. The function of the two sections of the stream was to provide two different samples of algae. In other words, the algae in the SRP enriched section of the stream would contain more phosphorus than a similarly sized sample of algae found in the ambient SRP section of the stream.

4.1.2. ³³P labelling and uptake

Six groups of 17 tiles from the ambient SRP section of the stream and 6 groups of 17 tiles from the SRP enriched section of the stream were placed into recirculating chambers containing 1 liter of filtered streamwater from the appropriate section of the stream. The water in the chambers was recirculated by small pumps attached to the chambers, and the water was filtered through Gelman type A/E glass fiber filters, 1 um pore size. The chambers were placed in a large tank that served as a constant temperature water bath (15 - 20 °C). Approximately 0.5 uCi of carrier-free ³³PO₄ was added to each chamber and 1 ml samples were taken at varying intervals over a 6 - 23 hour period from each chamber and assayed for ³³P activity by liquid scintillation. (The time period allowed for labelling, 6 -

23 hours, was chosen on the basis of past research (Newbold et al. 1983b)). Rates of phosphorus uptake were computed from the first-order uptake rate coefficient and the SRP concentration in water. The rate coefficient was determined from the decline in ^{33}P in water during the first 30 - 60 minutes of the experiment. SRP was measured by the standard molybdate blue ascorbic acid method using a Perkin-Elmer dual path length spectrophotometer (APHA, 1989).

4.1.3. Phosphorus cycling fluxes

After the tiles were labelled with ^{33}P , they were placed in streamwater for 1 hour (in order to remove any ^{33}P adsorbed to the surface of the algae). Then 5 tiles from each of the 12 groups of 17 tiles were collected for determination of algal incorporation of ^{33}P . Each of the 12 remaining groups of 12 tiles was placed into 1 of 12 600 ml beakers containing 315 ml or 415 ml of filtered (as described above) streamwater from the appropriate section of the stream. Approximately 100 $\mu\text{g/L}$ PO_4 was then added to each beaker containing water and tiles from the ambient SRP section of the stream to prevent reuptake of ^{33}P released to water by algae. This phosphate addition did not alter the P : C ratio in the algae because a change in this ratio would require a much longer time period than that of the experiment. Snails collected from Walker Branch and acclimated for 24 hours to streamwater from the artificial stream were added in groups of 10 to 6 of the 12 beakers

containing tiles from each stream section. All beakers were covered with pieces of plastic film (11.5 inches wide by approximately 9 - 14 inches long by 0.0005 inches thick, Indusol, Inc. "Stretch-tite" plastic sheet household wrap) to prevent snails from leaving the beakers and retard evaporation. Then the beakers were placed in a large tank which served as a constant-temperature water bath that maintained the beakers at 12 °C. Air was continuously supplied to each beaker to mix the water and keep dissolved oxygen concentrations at nearly saturated levels.

Five ml samples were collected from each of the beakers in triplicate at varying intervals over a 3 - 5 hour period and filtered using Nucleopore membrane filters of 0.45 um pore size. The samples were added to 15 ml of Ecolume (ICN Biomedicals Corporation), and assayed for ^{33}P activity by liquid scintillation counting whereas the filters were discarded. Tiles were then removed from the beakers, rinsed in artificial streamwater, and dried at 80 °C for 24 - 48 hours. Snails were transferred to fresh streamwater in beakers for an additional 24 hours to allow for clearance of gut contents. Snails were then collected, rinsed, their shells cracked, and dried at 80 °C for 24 - 48 hours. Snail feces which were produced during the experiment and during the time allotted for clearance of gut contents were removed from each beaker using a glass pipet, rinsed, and dried at 80 °C for 24 - 48 hours. All samples of dried material were weighed to obtain dry mass, combusted at 500 °C overnight,

and reweighed to obtain ash mass. Ash free dry mass (AFDM)²⁴ was computed as the difference between dry mass and ash mass.

4.1.4. TP and ^{33}P analysis of periphyton, snails, and feces

After the samples had been combusted, the ^{33}P and total phosphorus were leached from the fecal and snail samples with 3 ml of 2N HCl and from the periphyton samples with 25 - 40 ml of 2N HCl for 24 hours. The leachates were diluted 1 : 1 with distilled water. Then 1 ml of the diluted algal leachates and 3 ml of the diluted fecal and snail leachates were added to 15 ml of Ecolume and assayed for ^{33}P by liquid scintillation. In addition, 1 ml of the diluted leachates was added to 50 ml of distilled water and assayed for SRP as described above (Figure 3). Both water and organic matter ^{33}P measurements were corrected for background activity and isotopic decay. The ^{33}P of the background samples (initial conditions) were measured in the same manner as the ^{33}P of the experimental samples. Phosphorus regeneration rates were computed from the measured increases in ^{33}P concentration in the water in each beaker and the ^{33}P : total P ratio of the periphyton used in the study. Incorporation of P into snails and feces phosphorus production were computed from the measurements of snail and feces ^{33}P and the ^{33}P : total P ratio of periphyton. These flux rates were calculated for each of the beakers used in the study.

Figure 3. Procedures for TP and ^{33}P Analysis of Samples in Experiment 1

Dry samples



Ash samples



Add 3 ml of 2N HCl to fecal and snail samples and 25 - 40 ml of 2N HCl to algal samples



Wait 24 hours (for ^{33}P and P to leach out of samples)



Dilute leachates 1 : 1 with distilled water (amount of water added should equal amount of acid added previously)



Subsample 1 ml of diluted algal leachates and 3 ml of diluted fecal and snail leachates into 15 ml of scintillation cocktail (Ecolume)



Assay for ^{33}P by liquid scintillation



Subsample 1 ml of diluted leachates into 50 ml of distilled water



Assay for SRP using standard molybdate ascorbic acid method

4.2. Experiment 2

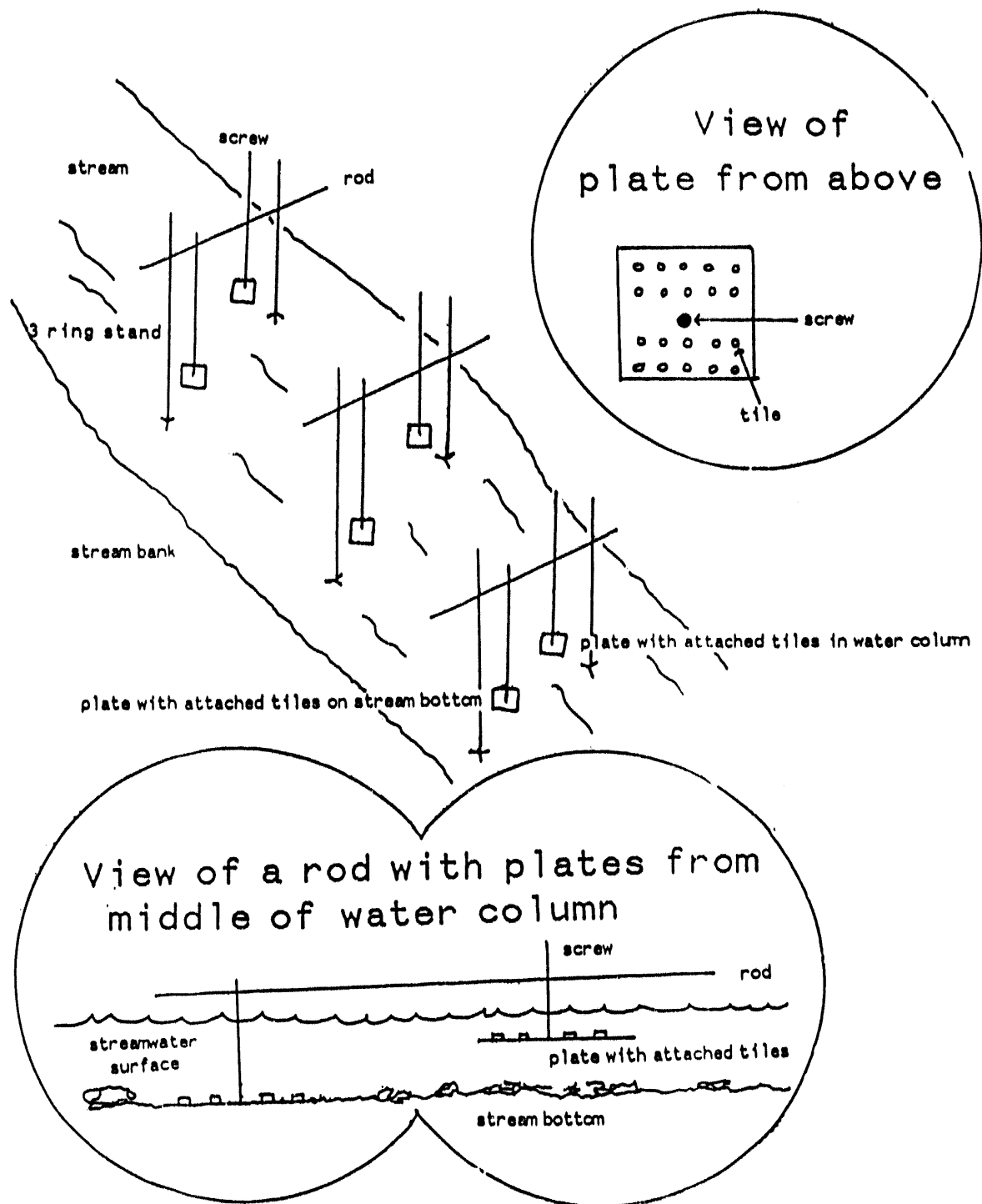
4.2.1. Preparation of substrates before labelling

Experiment 2 used periphyton covered tiles prepared as in Experiment 1. However, the SRP concentration of the streamwater in which periphyton grew (on tiles) was not altered.

4.2.2. ^{33}P labelling and uptake

The same basic techniques for labelling algae as used in Experiment 1 were utilized in this experiment except that periphyton colonized tiles and water came from Walker Branch rather than from the laboratory streams. In addition, 6 groups of 20 tiles were labelled over a 3 hour period rather than 12 groups of 17 tiles which were labelled over a 6 - 23 hour period. The ^{33}P labelled tiles were transported to the field and each group of 20 tiles was attached to a 20.5 cm X 20.5 cm plexiglass plate with silicone cement. Each plate had a screw bolted through its center that allowed it to be clamped to a horizontal rod suspended over the stream. Each plate could be suspended off the stream bottom to prevent snail access or placed on the stream bottom to allow snail grazing of the attached tiles. The 6 plates with attached tiles were placed into 3 groups, each group containing two plates. One plate of each group was suspended about 5 cm off the bottom (no snail treatment), and the other plate was placed next to it on the bottom (snail treatment) (Figure 4). The 3 sets of plates occupied a 2 m stream run.

Figure 4. Field Design of Experiment 2



****Picture is not drawn to scale****

Approximately 60 snails were placed on each of the plates on the bottom to initiate grazing at a level similar to natural snail densities in Walker Branch. Snail densities on all plates were checked twice per day (mid-morning and mid-afternoon). The number of snails on tiles were counted as well as the total number of snails on each plate. If fewer than 60 snails were present on the plates, snails were added to the plates to reestablish the correct snail density. Finally, any grazers present on the no snail treatments (suspended plates) were removed.

4.2.3. Phosphorus turnover

Five tiles were collected from each plate on days 0 (2 hr after placement in stream), 4, 8, and 14. The tiles were dried at 80 °C, weighed, combusted at 500 °C, and reweighed, and AFDM was calculated as described for Experiment 1. ^{33}P in the periphyton on each tile was then extracted in 5 ml of 2N HCl and assayed as described in Experiment 1. Total phosphorus in the samples was not measured. The ^{33}P counts were corrected for background activity and radioactive decay. The ^{33}P counts for each tile were log-transformed and turnover rate for each treatment replicate (each plate) was calculated as the slope of the regression between time and the natural logarithm of ^{33}P .

4.3. Experiment 3

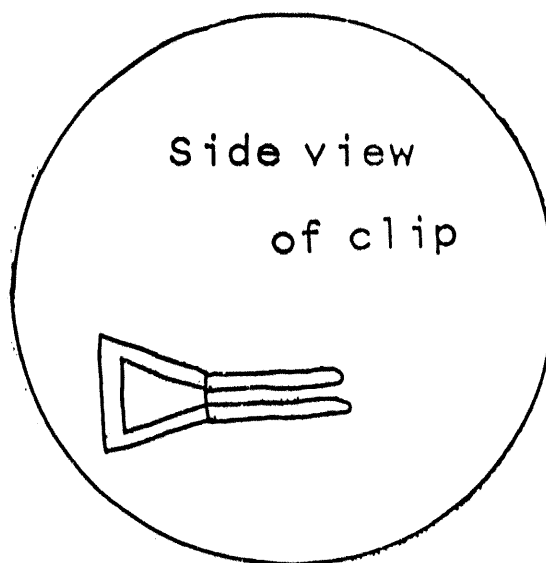
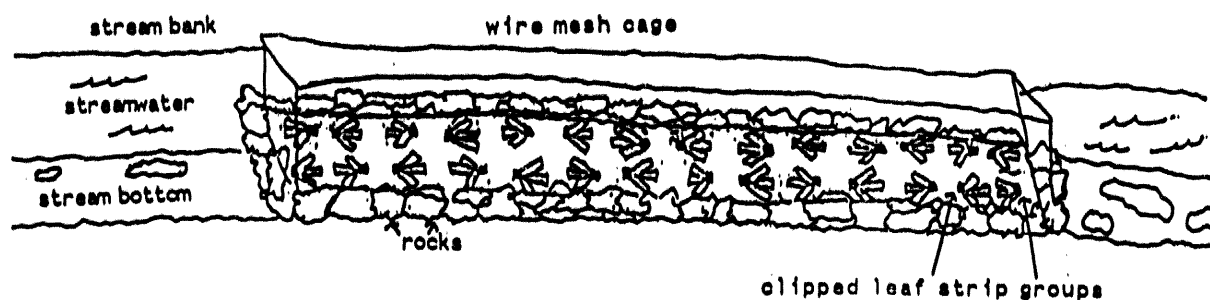
4.3.1. Preparation of substrates before labelling

Experiment 3 used leaf detritus communities grown on leaf strips rather than periphyton communities grown on tiles. Autumn-shed White Oak leaves, which had been collected from the local area, air dried, and maintained in a dry state for 5 years, were soaked overnight in distilled water and then cut into 2.25 cm X 7 cm leaf strips. The strips were attached in sets of 3 to "Plastick Binder" plastic clips, and placed in a topless, wire mesh rectangular cage (115 cm long by 17 cm wide by 9 cm high) in Walker Branch for 10 days. 28 clipped sets of leaves were placed in an alternating pattern within 2 rows, 14 clipped sets of leaves per row, for efficient use of space in the cage. The wire cage was placed on a flat bridge of rocks to allow the top of cage to rise a few centimeters above the water surface. The base of the cage was surrounded by rocks to keep the cage from floating downstream (Figure 5). The leaf strips were placed in the stream to allow for microbial colonization (exposed surface area per leaf strip equalled 16 cm²). The time allowed for microbes to grow on the leaf strips (10 days) was chosen in order to establish a moderate level of bacterial colonization on the leaves (Mulholland et al. 1984).

4.3.2. ³³P labelling and uptake

The strips were labelled with ³³P as described for

Figure 5. Microbial Colonization of Leaf Strips in Walker Branch for Experiment 3



Picture is not drawn to scale

Experiment 1, except that the time of exposure to ^{33}P was chosen to be 5 hours, instead of 6 - 23 hours, based on previous research (Mulholland et al. 1988). Three clipped leaf strip groups were placed in each 1-L recirculating chamber, resulting in 9 leaf strips. In addition, total surface area of the 8 leaf strips placed in each beaker (128 cm^2) was similar to the total surface area of the 12 tiles placed in each beaker (114 cm^2) used in Experiment 1.

4.3.3. Phosphorus cycling fluxes

After the leaf strips were labelled with ^{33}P , they were placed in streamwater overnight in order to remove any ^{33}P adsorbed to the surface of the algae. Then 1 leaf strip from each group of 9 strips was collected for determination of algal incorporation of ^{33}P and total phosphorus. Each of the 6 remaining groups of 8 leaf strips was placed into 1 of 6 1000 ml beakers containing 365 ml of filtered (as described above) streamwater.

Procedures involving snails, beakers in the tank, and sampling for water followed in this experiment were as described for Experiment 1 except that water samples were collected over a 2 hour period rather than over a 3 - 5 hour period. At the end of the experiment, leaf strips, snails, and feces were removed from beakers, and AFDM, ^{33}P , and total phosphorus content measured as described for Experiment 1 except that 5 ml, rather than 25 - 40 ml, of 2N HCl was added to the combusted leaf detritus samples to

leach out ^{33}P and total phosphorus.

4.4. Experiment 4

4.4.1. Preparation of substrates before labelling

Experiment 4 used leaf detritus strips prepared as in Experiment 1.

4.4.2. ^{33}P labelling and uptake

Four sets of three leaf strips were clipped to the sides of each of the 6 plexiglass plates used in Experiment 2, resulting in 12 strips per plate. Total surface area of the 12 leaf strips "clipped" to each plate (192 cm^2) was similar to the total surface area of the 20 tiles attached to each plate (190 cm^2) used in Experiment 2. The plates with attached leaf strips were placed in Walker Branch just prior to a 90 - minute injection of 1.5 uCi of ^{33}P . The injection was made by pumping a ^{33}P mixture (1.5 uCi of ^{33}P and 4 L of streamwater) from a carboy into the stream at a continuously slow rate of 44 ml/min..

4.4.3. Phosphorus turnover

After the ^{33}P labelling, the plates were transferred to the same site used in Experiment 2 and 4 strips were removed from each plate for ^{33}P assay on days 0 (2 hr after placement in stream), 4, and 8. Leaf strips were dried, weighed, combusted, and reweighed, and ^{33}P was extracted from the strips into 2N HCl as described for Experiment 2.

Snail density on all plates was monitored daily.

4.5. Statistical analysis

The effect of snails on phosphorus cycling and turnover rates was analyzed by use of a one-way ANOVA (SAS/STAT 1988). If P was less than 0.10, the effect under consideration was deemed not significant. If P was less than 0.05, the effect being analyzed was significant. If P was less than 0.01, the effect under scrutiny was highly significant.

RESULTS

1. Experiments 1 and 3

1.1. Phosphorus uptake

The rate of phosphorus uptake by periphyton was about 40% higher in the ambient streamwater phosphorus treatment than in the high (enriched) streamwater phosphorus treatment (Figure 6). The effect of treatment on algal phosphorus uptake rate was marginally significant ($F = 3.55$, $P = 0.0888$, $df = 1,10$) (Figure 6).

The phosphorus uptake rate was only about 10% higher in the leaf detritus study than in the periphyton study, and this effect of substrate type on uptake rate of phosphorus was not significant (Figure 7). The substrate surface areas used in the periphyton and leaf detritus experiments were similar as described above. Streamwater SRP concentrations were slightly greater for the periphyton study (4.4 ug P/L) than for the leaf detritus study (3.5 ug P/L).

1.2. Phosphorus regeneration

The rate of regeneration of phosphorus from periphyton back to water in the absence of snails was about 50% higher under the ambient streamwater P treatment than under the high streamwater P treatment, and this effect of streamwater phosphorus treatment on regeneration rate was significant

Figure 6.

Effect of Streamwater Phosphate Concentration on Phosphorus Uptake and Regeneration to Water in Periphyton Communities

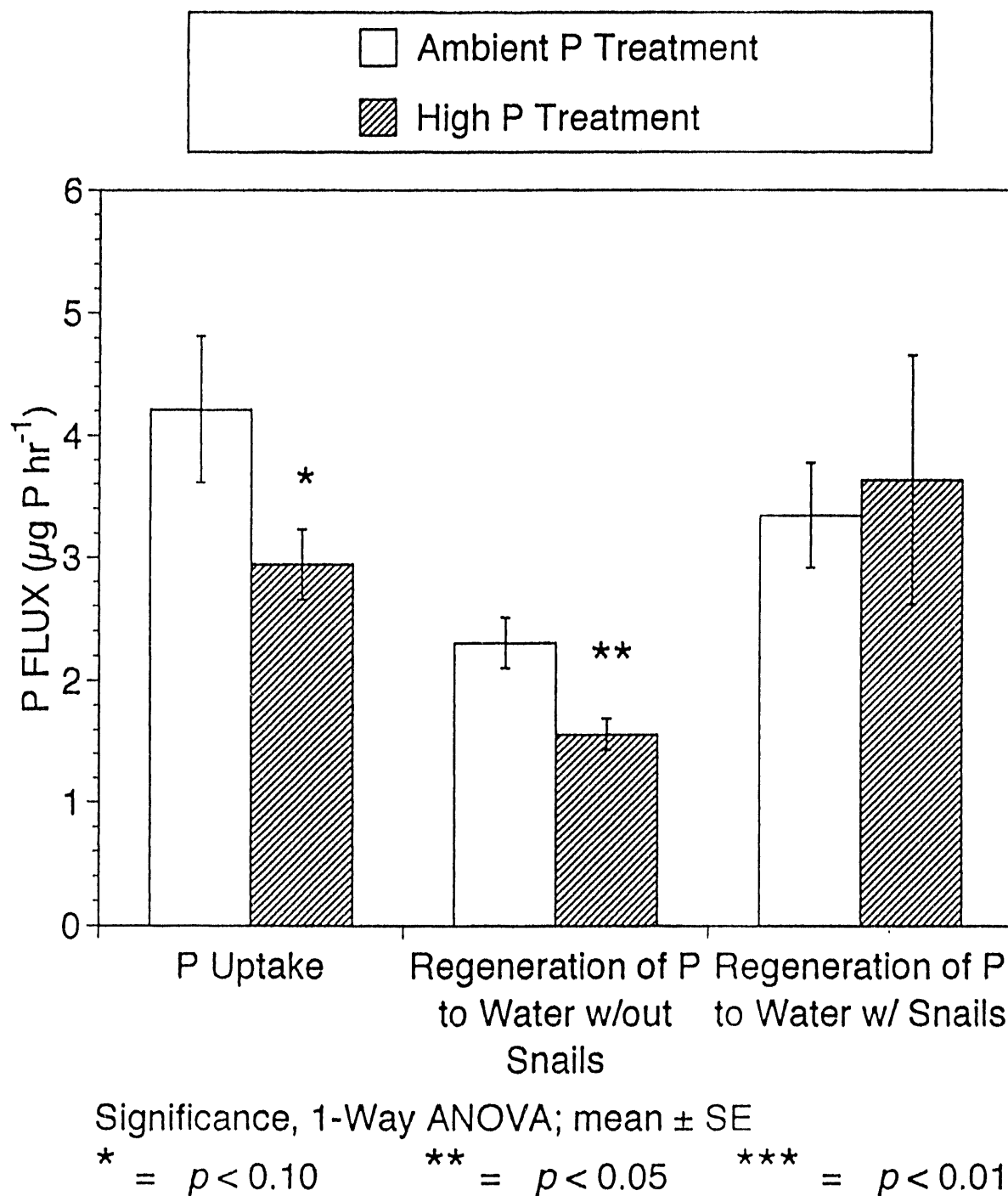
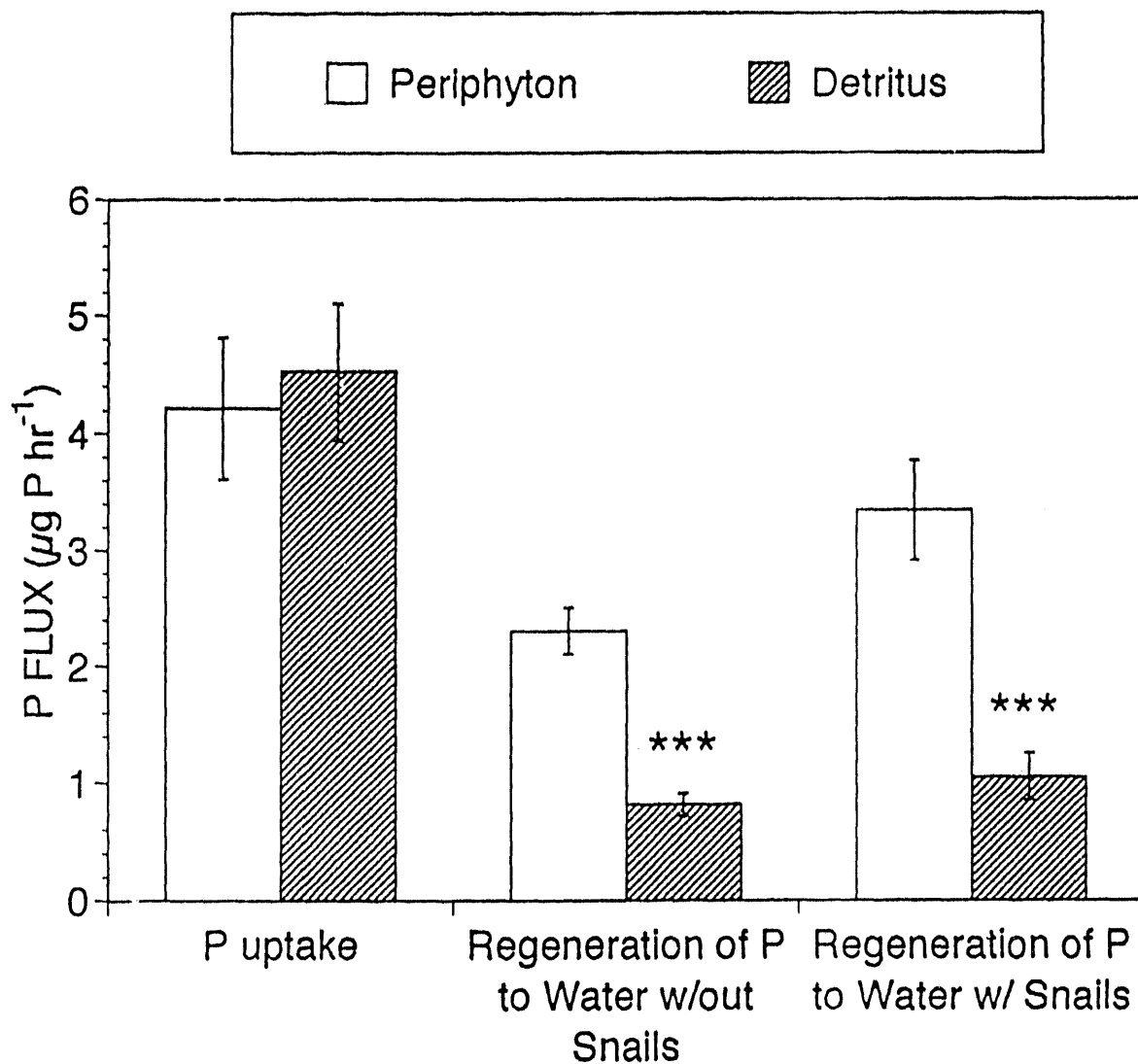


Figure 7.
Effect of Substrate Type (Periphyton, Detritus) on Phosphorus Uptake and Regeneration to Water



Significance, 1-Way ANOVA; mean \pm SE

* = $p < 0.10$

** = $p < 0.05$

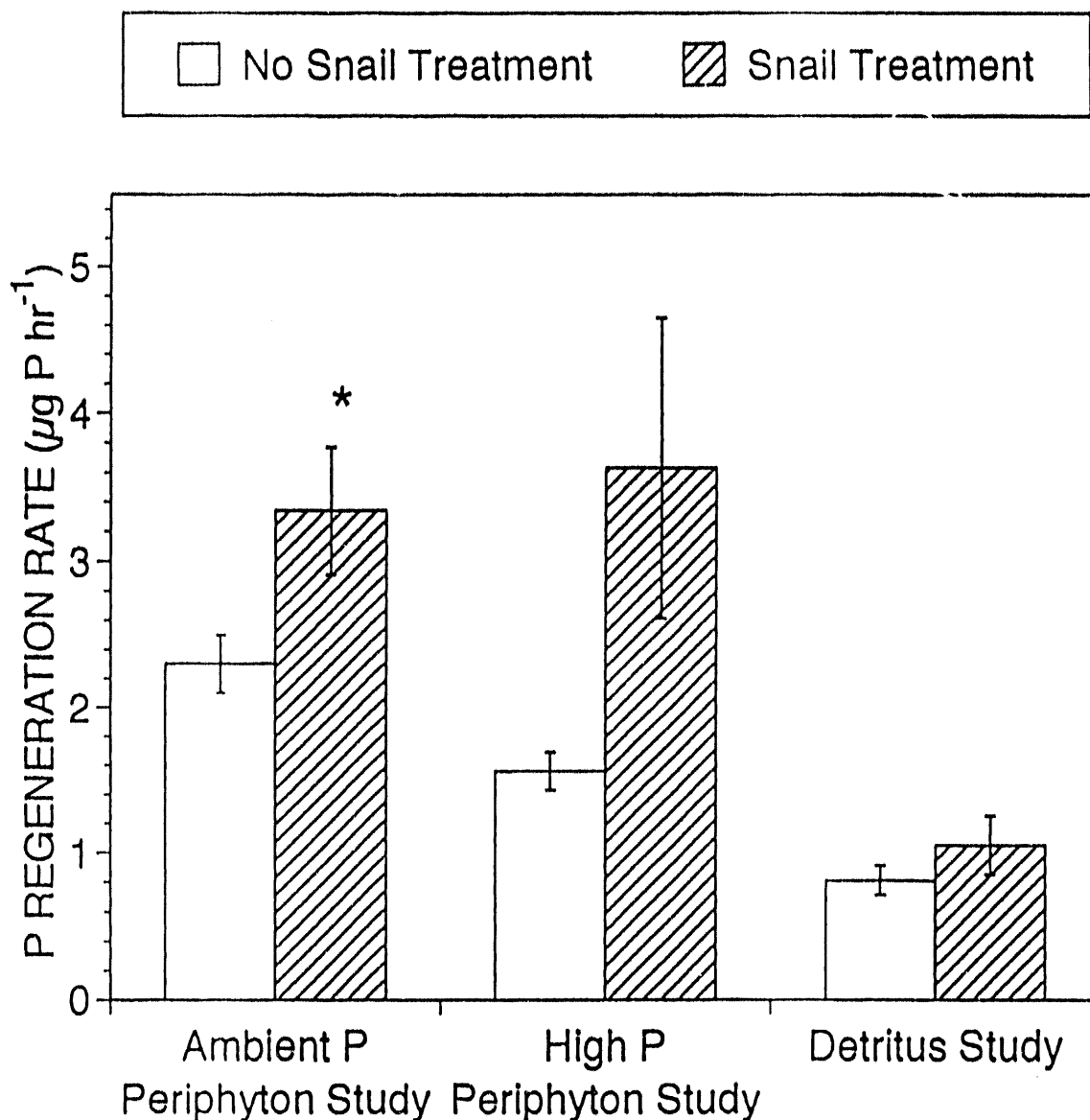
*** = $p < 0.01$

(Figure 6). However, the regeneration rate was only about 10% greater in the high streamwater P treatment as compared to the ambient streamwater P treatment in the presence of snails, and this effect of streamwater phosphorus treatment was not significant (Figure 6). Regeneration rates were about 55% of the uptake rate in the ambient and high streamwater phosphorus treatments in the absence of snails. However, regeneration rates were approximately 80% of the uptake rate in the ambient streamwater phosphorus treatment and >100% of the uptake rate in the high streamwater phosphorus treatment with snails (Figure 6).

Regeneration rates in the leaf detritus study were approximately 30% of the regeneration rates in the periphyton study, in both the presence and absence of snails. This effect of substrate type on rates of phosphorus regeneration to water was significant (Figure 7). The significant effect of substrate type on phosphorus regeneration rate is in contrast to the lack of effect of substrate type on phosphorus uptake rate. This indicates that phosphorus cycled more rapidly in the periphyton system.

The rate of phosphorus regeneration to water from periphyton was increased in the presence of snails under both ambient and high streamwater phosphorus treatments (Figure 8). Although the regeneration rate was increased by about 45% in the ambient phosphorus treatment by snails and more than doubled in the high phosphorus treatment by

Figure 8.
Effect of Snails on P Regeneration to Water
from Periphyton and Detritus Communities



Significance, 1-Way ANOVA; mean \pm SE (n = 3)

* = $p < 0.10$

** = $p < 0.05$

*** = $p < 0.01$

snails, the increase was only marginally significant for the ambient phosphorus treatment ($F = 5.06$, $P = 0.0876$, $d, f = 1, 4$) and not significant for the high phosphorus treatment ($F = 4.10$, $P = 0.1128$, $d, f = 1, 4$) (Figure 8). In the leaf detritus experiment, snails increased the rate of phosphorus regeneration by about 30%, but this effect was not significant (Figure 8).

1.3. Phosphorus incorporation into snail biomass and feces production

Although the rate of phosphorus incorporation into snail tissue was about 50% greater in the ambient streamwater phosphorus treatment than in the high streamwater phosphorus treatment, this effect was not significant (Figure 9). In addition, the feces production rates were very similar in both streamwater P treatments, and total feces production was only 10% higher in the ambient streamwater P treatment as compared to the high P streamwater treatment. This effect of streamwater phosphorus treatment on both measures of feces production was not significant (Figure 9).

Incorporation rates of phosphorus into snail tissue in the periphyton experiment were nearly 50% greater than in the leaf detritus experiment, but this effect was not significant. However, feces production, both in terms of phosphorus and AFDM, was significantly greater when snails fed on leaf detritus than on periphyton (Figure 10). In the

Figure 9.
Effect of Streamwater Phosphate
Concentration on Phosphorus Incorporation
into Snail Biomass and Feces Production in
Periphyton Communities

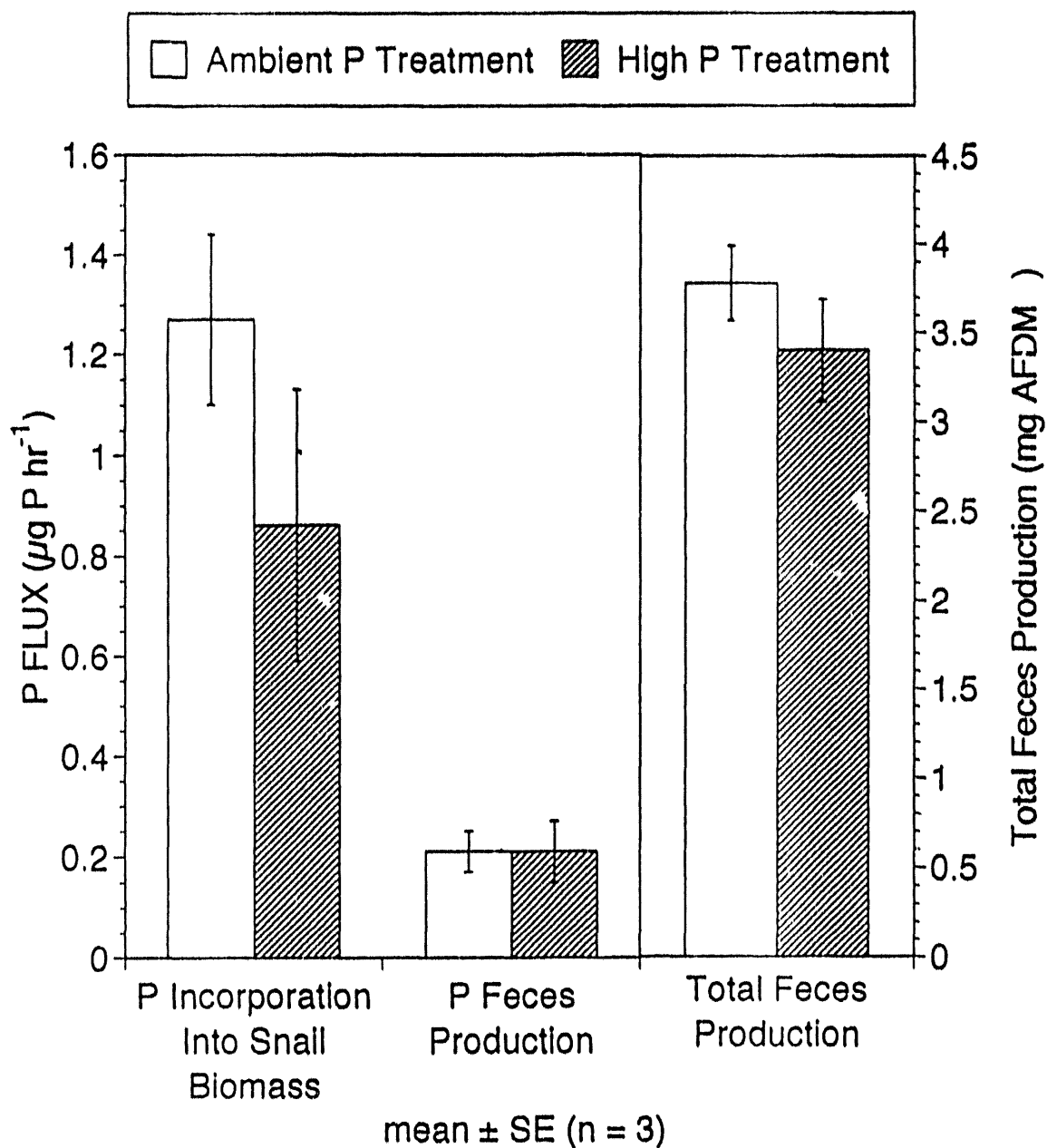
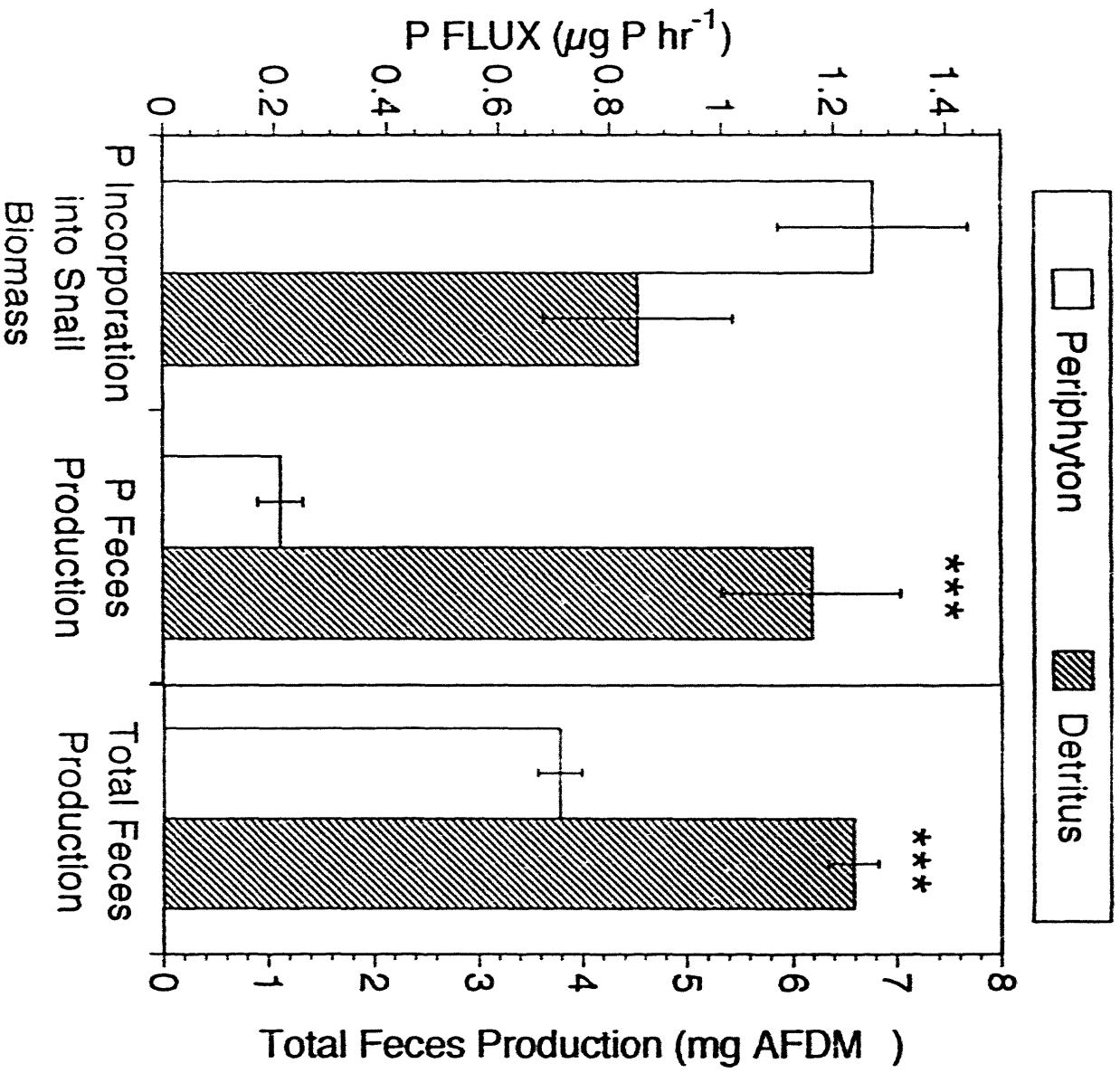


Figure 10.
Effect of Substrate Type (Periphyton,
Detritus) on Phosphorus Incorporation into
Snail Biomass and Feces Production



Significance, 1-Way ANOVA; mean \pm SE (n = 3)

* = $p < 0.10$

** = $p < 0.05$

*** = $p < 0.01$

periphyton study, about 85% of the phosphorus ingested was incorporated into snail tissue, whereas about 15% of the phosphorus ingested went into feces production (Figure 11). However, when snails grazed on leaf detritus, approximately 40% of the phosphorus ingested was incorporated into snail biomass and about 60% of the phosphorus ingested went into feces production (Figure 12).

1.4. Phosphorus content of substrate, snail, and feces

The effect of streamwater phosphorus treatment on substrate P content could not be analyzed for the periphyton in the high streamwater phosphorus treatment because the periphyton AFDM for that high P streamwater treatment was not precisely measured. A balance which could not detect minute changes in weight was mistakenly used for the measurement of dry and ash mass of the high P streamwater treatment algae. However, periphyton AFDM for the ambient streamwater phosphorus treatment was measured. The average AFDM of tiles from an artificial stream which had an ambient concentration of phosphorus was 4.75 ± 0.28 mg/tile (N=6).

When measured as ug P/AFDM, phosphorus content of leaf detritus was only about 10% of algal phosphorus content, and this effect was significant (Figure 13). However, when measured as ug P/cm², the P content of the substrates was very similar and there was no significant difference between substrates. Finally, the phosphorus contents of snails and feces were approximately 15% higher in the leaf detritus

Figure 11.

EFFECT OF SNAILS ON PHOSPHORUS FLUXES (MEAN \pm STD DEV) DURING PERIPHYTON EXPERIMENT. P INGESTION IS CALCULATED AS THE SUM OF P INCORPORATION AND FECES PRODUCTION. OTHER FLUXES WERE MEASURED DIRECTLY.

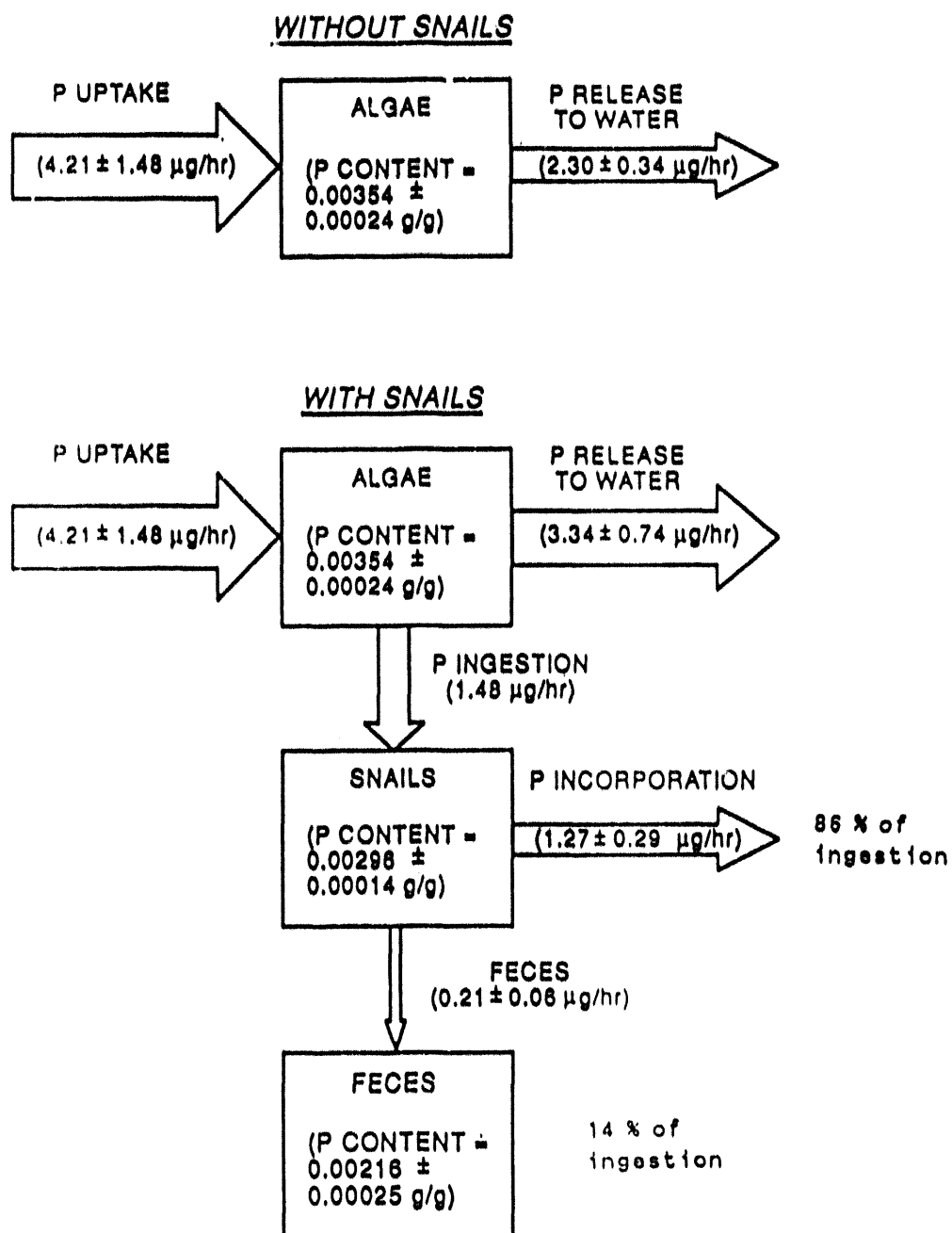


Figure 12.

EFFECT OF SNAILS ON PHOSPHORUS FLUXES (MEAN + STD DEV) DURING LEAF DETRITUS EXPERIMENT. P INGESTION IS CALCULATED AS THE SUM OF P INCORPORATION AND FECES PRODUCTION. OTHER FLUXES WERE MEASURED DIRECTLY.

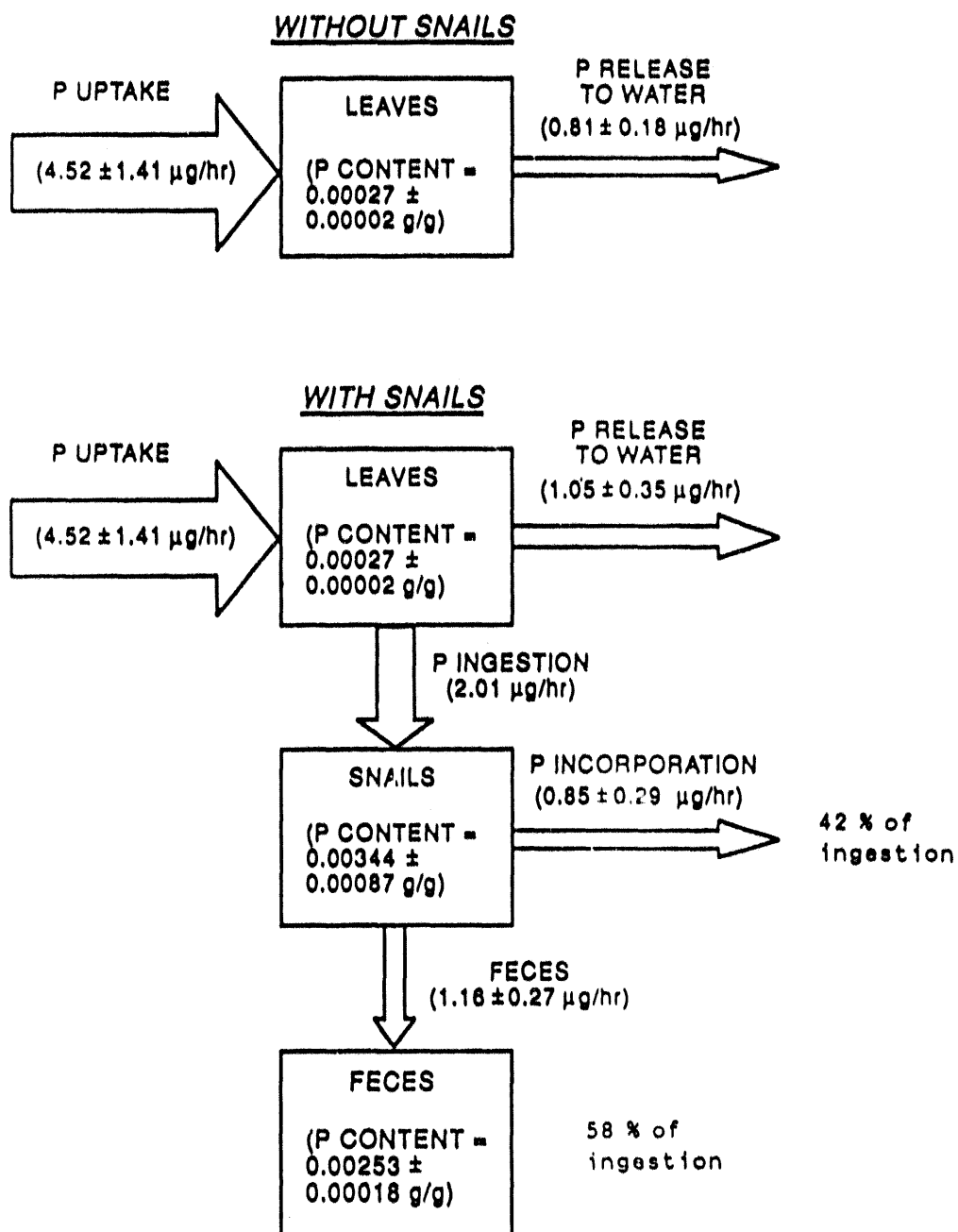
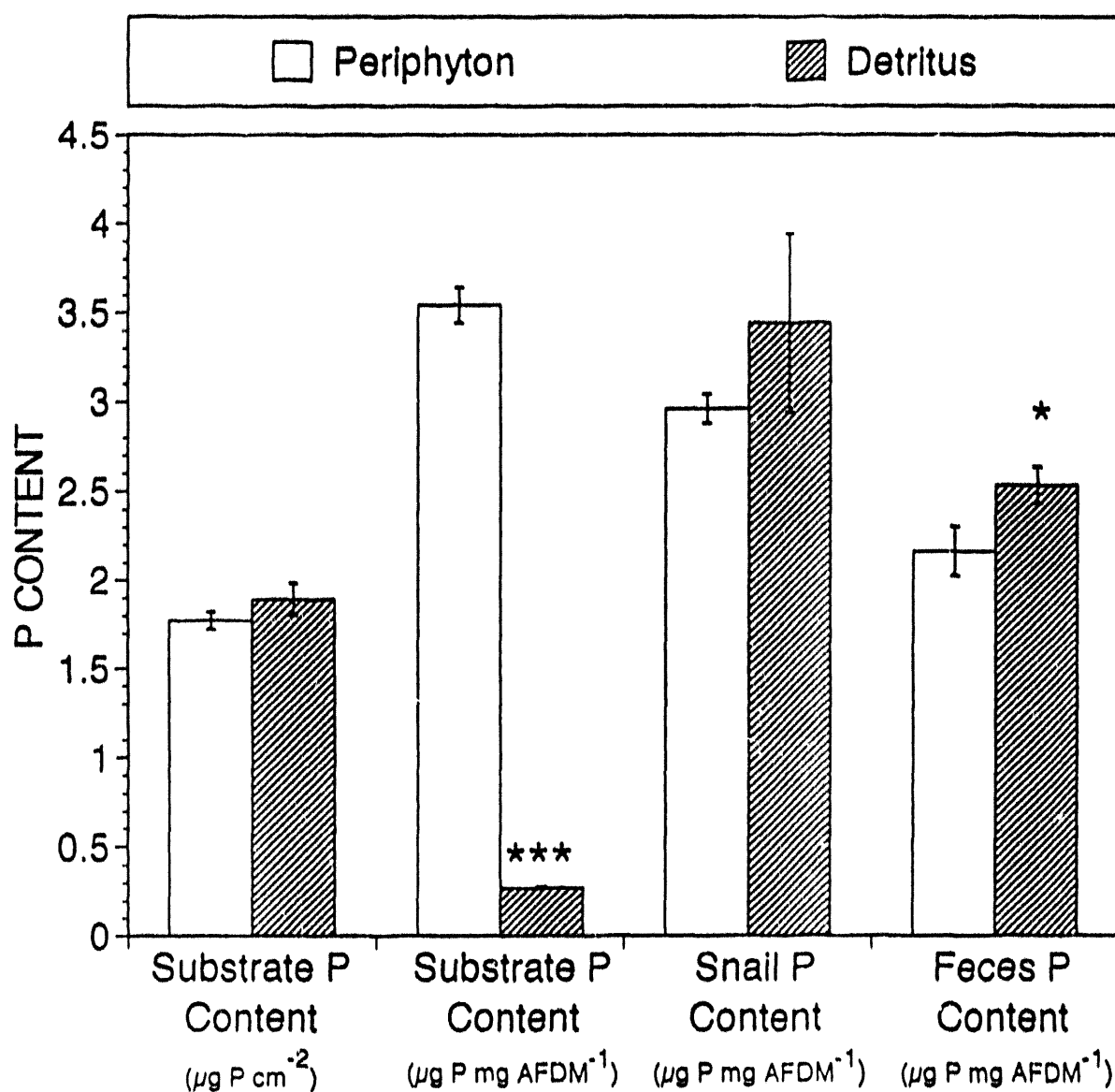


Figure 13.
Effect of Substrate Type (Periphyton,
Detritus) on Phosphorus Content of
Substrate, Snail Tissue and Feces



Significance, 1-Way ANOVA; mean \pm SE

* = $p < 0.10$

** = $p < 0.05$

*** = $p < 0.01$

study than in the periphyton study, but this effect was not significant for snail P content and only marginally significant for fecal P content ($F = 4.65$, $P = 0.0973$, $d, f = 1, 4$) (Figure 13). The minimal effect of substrate type on phosphorus content of the feces is in contrast to its highly significant effect on fecal phosphorus flux. This underscores the importance of the much larger total production of feces in the detritus study compared with the periphyton study.

2. Experiments 2 and 4

2.1. Phosphorus turnover

Grazing rates of snails in the field turnover studies varied considerably by substrate and treatment. Grazing rates of periphyton had more fluctuations over time than grazing rates of leaf detritus in snail and no snail treatments (Figure 14, 15). In addition, the ranges of grazing rate values were smaller for the periphyton experiment than for the leaf detritus experiment (0.00 - 0.13 versus 0.00 - 0.67 in no snail treatments and 0.27 - 0.78 versus 0.92 - 3.58 in snail treatments) (Figure 14, 15). Finally, grazing rates in the snail treatments increased over time in the leaf detritus study compared to the periphyton study (Figure 14, 15).

The turnover rate was increased by about 45% in the periphyton experiment by snails, and by about 70% in the leaf detritus experiment by snails. However, the effect of

Figure 14. Average Grazing Rate versus Time

By Treatment In Periphyton Experiment

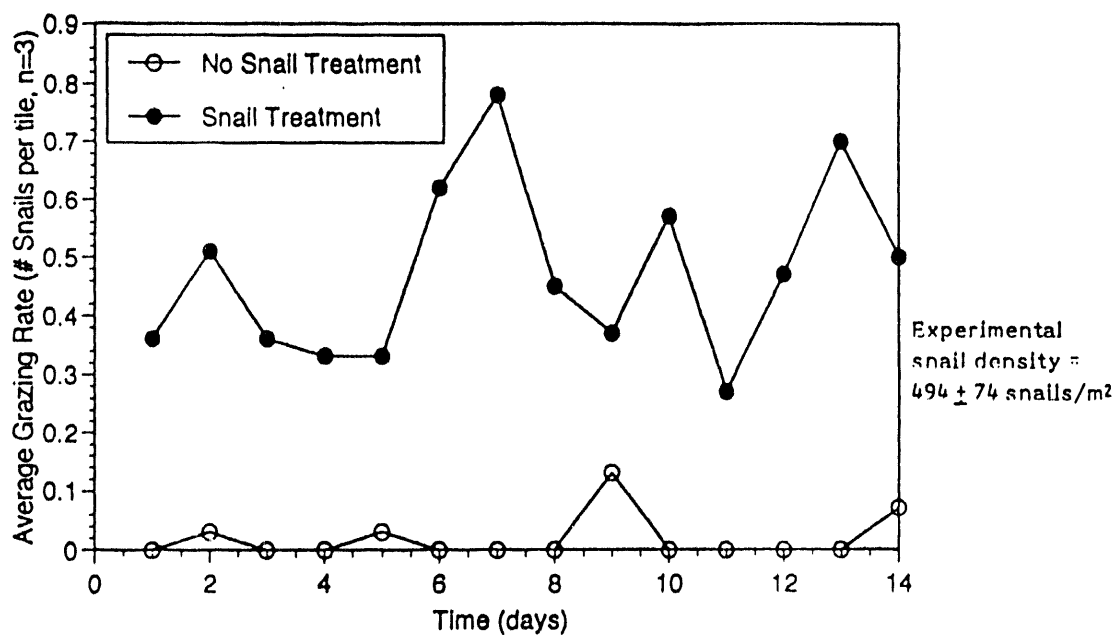
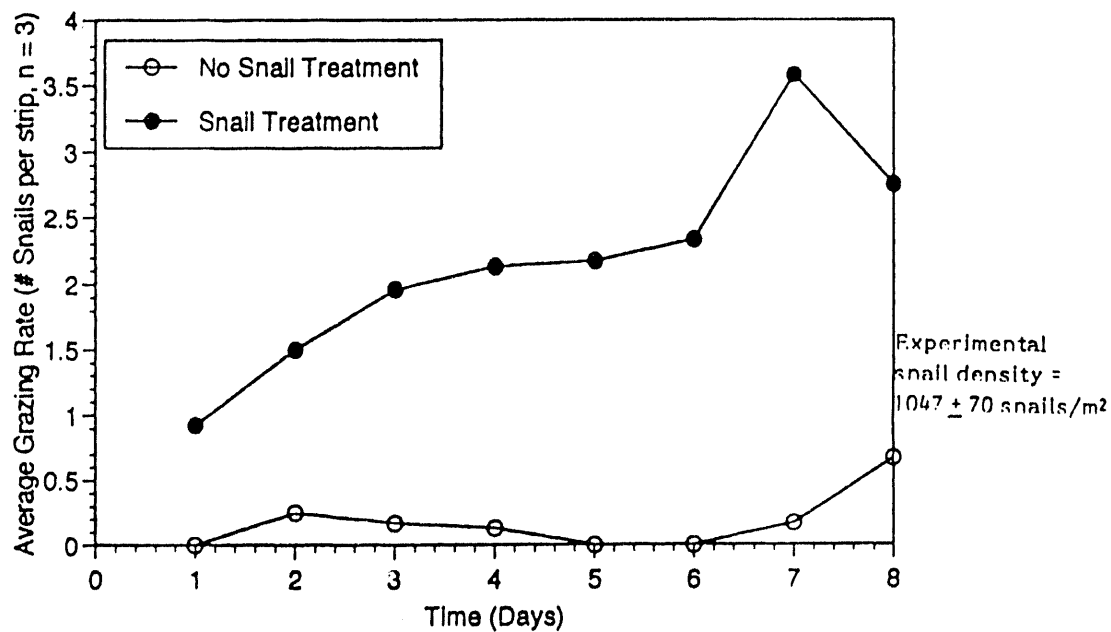


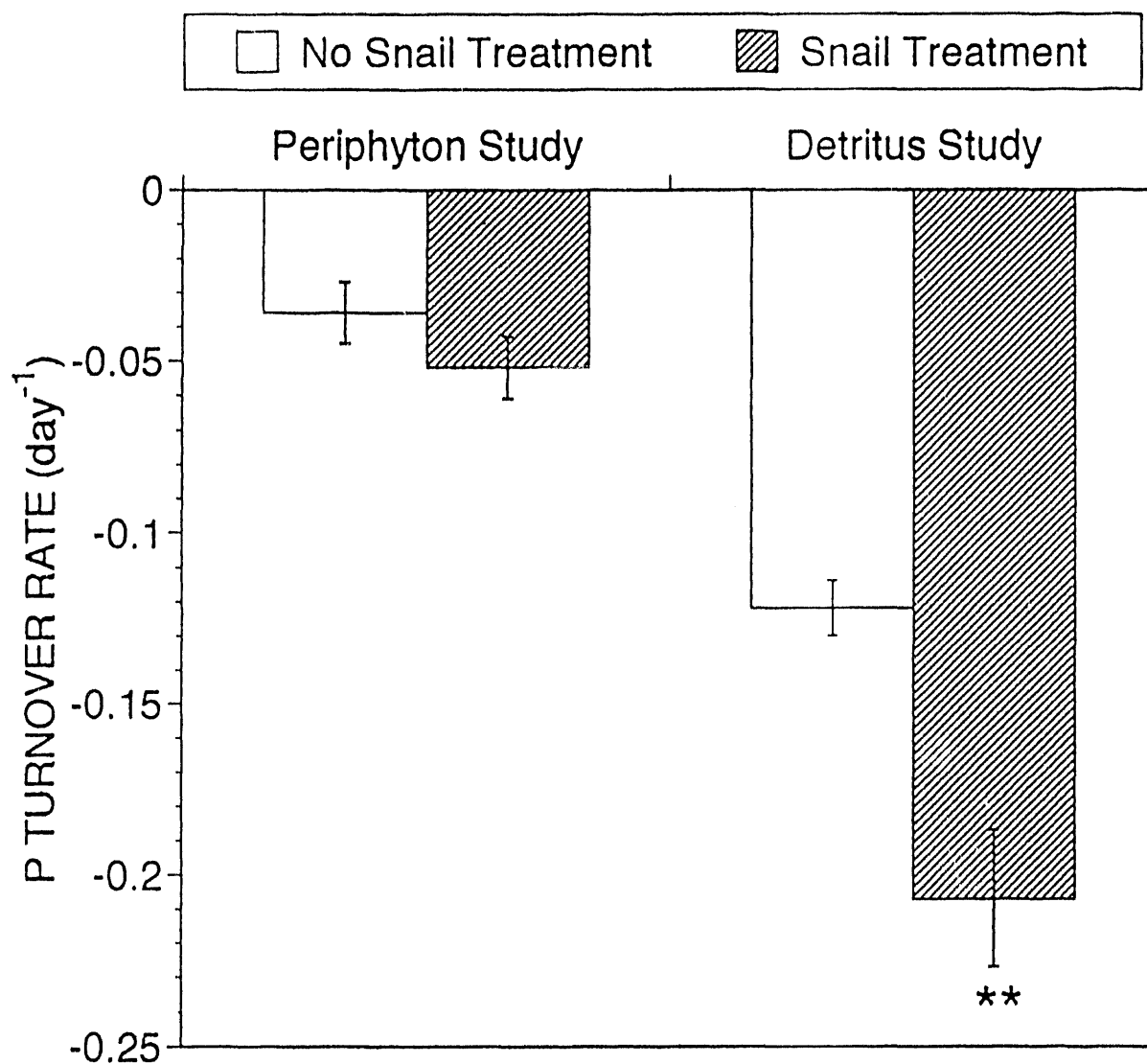
Figure 15. Average Grazing Rate versus Time

By Treatment In Leaf Detritus Experiment



snails on rate of turnover was not significant in the periphyton study and was significant in the leaf detritus study (Figure 16). It is interesting to note that snails significantly affected turnover rates when leaf detritus was used but did not significantly affect rates of regeneration from leaf detritus (Figure 8). Thus, it appears that the major effect of snails when feeding on detritus is the flux of P into snail tissue and feces.

Figure 16.
Effect of Snails on Phosphorus Turnover
in Periphyton and Detritus Communities



Significance, 1-Way ANOVA; mean \pm SE (n = 3)

* = $p < 0.10$

** = $p < 0.05$

*** = $p < 0.01$

DISCUSSION

1. Effect of snails on phosphorus cycling fluxes and turnover

Although there was some evidence of an increase in rates of phosphorus regeneration to water from periphyton and leaf detritus as a result of snail grazing, this effect was only marginally significant for the ambient P streamwater treatment using periphyton, and was not significant for the leaf detritus experiment (Figure 8). The lack of an effect of snails on P regeneration rates might have been the result of similar ratios of P:C in the snail tissue, algae, and bacteria. In this case, there would be little regeneration of P by snails since their cellular needs would be almost met by the nutrient contents of the algae and bacteria. Other studies have also suggested the importance of nutrient to carbon ratios of consumers relative to their prey in regard to nutrient regeneration by consumers (Bloem et al. 1988, Sterner 1990). My results could have also occurred if snails were P-limited. P should be retained more efficiently by P-limited snails than by C-limited snails.

My results are also consistent with other observations indicating that snails do not strongly enhance rates of regeneration of P (McDiffett and Jordan 1978). Mulholland

et al. (1985) found that snails had little effect on leaf detritus mineralization, and this finding is also consistent with my results. In contrast, most other studies on nutrient cycling involving consumer organisms other than snails, such as protozoans, have demonstrated that grazers do significantly increase rates of regeneration from leaf detritus and algae communities (Berman et al. 1987, Barsdate et al. 1974, Taylor 1982).

Turnover rates of phosphorus, which are, in effect, the sum of P regeneration, incorporation into snails, and feces production rates relative to the substrate P pool, did increase in the presence of snails. The effect of snails on turnover rates was significant only for the leaf detritus study (Figure 16). Others have found that the effects of other consumer organisms on phosphorus turnover rates were significant for leaf detritus and periphyton communities (e.g. caddis fly larvae in streams, zooplankton in lakes) (Lamberti and Resh 1983, Lehman 1980a). Since snails did not significantly affect rates of P regeneration, the only other way in which snails could have increased turnover rates was via other P fluxes from the substrate. My research revealed that phosphorus fluxes other than regeneration were created in the presence of snails, specifically incorporation into snail tissue and feces production, and the sum of these fluxes plus the additional regeneration flux was large enough to cause turnover rates to increase, especially in the leaf detritus study (Figure

11, 12).

Not all of the P fluxes involved in P turnover necessarily enhance P cycling. For instance, incorporation of P into snail biomass may actually reduce P cycling because the pool of P in consumer biomass is probably regenerated back to water slowly (Berman et al. 1987). However, incorporation of P into the tissues of consumers could also stabilize cycling of P in streams because (1.) algae and leaf detritus might otherwise be transported downstream, (2.) consumer organisms usually are able to remain in place, and (3.) consumers, particularly those with relatively long life spans, have the ability to retain phosphorus (Kitchell et al. 1979, Taylor 1982, Merritt et al. 1984).

Feces production, like incorporation of P into consumer tissue, may not necessarily stimulate P cycling. Such production may reduce P cycling if feces are readily transported downstream, or if P in feces is unavailable to algae and bacteria, as suggested by Fukuhara and Sakamoto (1987), and is released back to water very slowly. However, feces production could stabilize P cycling if feces act as a food source for other consumers and the consumers in turn regenerate P to water (Shepard and Minshall 1984). Furthermore, feces production could enhance cycling of P if photosynthetic activity of undamaged algae in feces or subsequent attachment of microbes to feces results in elevated P uptake from water or feces (Coker 1983a, Hansson

et al. 1987). Feces production could also stimulate P cycling if P is released from feces back to the water via leaching processes, in amounts equivalent to or exceeding direct release of P to water by consumers (Cuker 1983a, Taylor and Lean 1981).

2. Effect of substrate type on P cycling fluxes

Results from the experiments designed to compare the P cycling fluxes between different communities (periphyton and leaf detritus) are valid although the time allowed for any ^{33}P adsorbed to periphyton to be removed (overnight) was much greater than the time allowed for such removal from leaf detritus (1 hr). One hour of time should have been sufficient to remove any adsorbed ^{33}P to a substrate, and an overnight period was utilized during the periphyton experiment only for the sake of convenience.

The results regarding the effect of substrate type on P cycling fluxes appear to indicate that rates of regeneration from periphyton are higher than those from leaf detritus (Figure 7). This result is understandable since leaf detritus often has a very low ratio of P : C initially. Thus, bacteria and fungi that colonize detritus may be very retentive of P taken up from the water. The leaf detritus used in the experiments had a significantly lower P content ($\mu\text{g P/mg AFDM}$) than did the periphyton that was used (Figure 13). However, much of the carbon in leaf detritus may not be bioavailable. As a result, the calculated P : C ratios

may overestimate the P deficiency of microbes growing on this substrate and detritivores feeding on it. Thus, the calculated P : C ratios may not completely explain the effect of substrate type on regeneration rates.

Although rates of P incorporation into snail tissue were higher in the periphyton experiment than in the leaf detritus experiment and measures of P feces production were higher in the leaf detritus study than in the periphyton study, a significant effect of substrate type was only observed for the measures of feces production (Figure 10). These results suggest that snails were limited by the availability of digestible carbon in the leaf detritus study (Hill et al. 1992). Snails may have also been limited by C in the algae study, but a greater fraction of the substrate which was ingested was incorporated in the periphyton experiment than in the leaf detritus experiment. As a result, snails have a greater demand for the P in periphyton than for the P in leaf detritus, and less P is lost as either regeneration or feces production when snails graze periphyton compared to leaf detritus. Several of my results showed that much more P went into feces produced by snails grazing on leaf detritus than feces produced by snails grazing on periphyton. For example, if the phosphorus content of a substrate measured per mg AFDM is compared to the feces produced by snails feeding on that substrate, this ratio is much lower in the detritus study than in the periphyton study (Figure 13). Thus, because snails consumed

considerable quantities of leaf detritus due to its lack of digestibility, snails consumed considerable amounts of P associated with leaf detritus and had to release much of this P due to lack of assimilable C. In fact, in terms of P, snail feces production in the leaf detritus study was 5 times greater than in the periphyton study, whereas in terms of AFDM, feces production in the leaf detritus study was only twice as great as it was in the periphyton study (Figure 10).

If different internal cellular pools, or compartments, of P exist in the substrates and have different cycling rates, there may have been significant changes in some of those pools that were not detected. Therefore, the interpretation of my results regarding the effect of substrate type on P cycling fluxes may have been inaccurate. This consideration is valid because the substrates were exposed to ^{33}P over a short time period. Thus, the different P pools in the different substrates might have been brought to different states of isotopic equilibrium. The time required for an isotopic equilibrium to be established regarding ^{33}P for periphyton is 1 - 2 days, and I labelled the periphyton covered tiles grown in the ambient streamwater P treatment for 6 hours (Newbold et al. 1983b). Furthermore, the time needed for isotopic equilibrium to be reached with respect to ^{33}P for leaf detritus is 6 hours, and I labelled the microbially colonized leaf strips for 5 hours (Mulholland et al. 1988). Thus, my results concerning

the effect of substrate type on P cycling fluxes might be more reflective of the P pools in the substrates that were brought close to isotopic equilibrium.

3. Effect of streamwater P content on P cycling fluxes

One would expect that phosphorus fluxes would be lower in the ambient streamwater P treatment than in the high streamwater P treatment because there is less P available in the water for uptake in the ambient streamwater P treatment. However, P uptake by algae from the water was significantly higher in the ambient streamwater P treatment than in the high streamwater P treatment, as was regeneration rate to water without snails (Figure 6). In addition, the effect of P treatment was not significant for other fluxes (regeneration to water with snails, incorporation into snails, feces production), and these results did not confirm the above expectation (Figure 6, 9). These results may be explained by the brevity of the experiment (about 48 hours) or perhaps a lack of P limitation in periphyton. Finally, in the high streamwater P treatment with snails, the regeneration rate was 125% of the uptake rate (Figure 6). This result suggests that either the uptake of P by periphyton was underestimated or that the periphyton was losing P due to an experimental system that was not at steady state. The snail density in the study was slightly higher than the snail density in the artificial stream from which the snails and periphyton were collected, and this

difference could have led to a system that was not at steady state with respect to P during the experiment.

4. Grazing treatments during the P turnover studies

In the P turnover studies, the average grazing rates, 494 snails/m² and 1047 snails/m² in the periphyton and leaf detritus experiments, respectively, were somewhat lower than the 1000 - 1500 snails/m² normally found in undisturbed stream areas of Walker Branch (Figure 14, 15). Therefore, the experimental grazing rates were much lower than natural grazing rates in the periphyton P turnover study. The low grazing rates during the periphyton experiment are not surprising because the periphyton covered tiles were small islands of resources on large surfaces (plexiglass plates) that had little available food, and the plates with affixed tiles provided less available food to the snails than an equivalent size of stream bottom. Thus, the effect of snails on phosphorus turnover rate in the algal experiment may have been lower than the effect of snails on phosphorus turnover rate in periphyton which occurs naturally in the stream.

The experimental rates of grazing on algae were also lower than the experimental rates of grazing on leaf detritus (Figure 14, 15). This result may have occurred because snails adhered to the surface of a leaf strip more readily than to the surface of a tile. Thus, the difference in the effect of snails on phosphorus turnover rate between

substrate types may have primarily been a result of differences in snails density (Figure 16).

5. Future research

Several questions have arisen as a result of my research. For instance, the fate of P incorporated into herbivore tissue and especially into feces is unknown and could be very important in P cycling, particularly in leaf detritus systems. In order to understand the role of these fluxes in P cycling, it is necessary to know the residence time and ultimate fate of P in consumer tissue and feces. Also, it is necessary to know how available fecal P is to algae and bacteria that might colonize the feces.

CONCLUSIONS AND RECOMMENDATIONS

1. Snails did not strongly enhance the regeneration of P back to water in periphyton and leaf detritus communities.
2. Snails increased the turnover of P in leaf detritus and periphyton communities. The effect of snails on P turnover was significant in the leaf detritus study, but was not significant in the periphyton study. The difference observed between substrate types may have been the result of a much greater flux of P into feces which occurred when snails fed on leaf detritus compared with periphyton.
3. P regeneration back to water was greater in periphyton communities compared to detritus communities. However, P loss as feces was greater in leaf detritus systems compared to periphyton systems.
4. The enrichment of SRP in water reduced P cycling fluxes if snails were absent in periphyton systems, but this enrichment had no effect on P cycling fluxes if snails were present in periphyton systems.

5. Residence time, bioavailability, and fate of P in snail tissue and feces should be studied in the future, particularly in leaf detritus systems.

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APPENDIX A: TABLES USED TO CREATE FIGURES (BAR GRAPHS)

TABLE 1. EFFECT OF STREAMWATER PHOSPHATE CONCENTRATION ON PHOSPHORUS CYCLING FLUXES IN PERIPHYTON COMMUNITIES

FLUX ($\mu\text{gP/hr}$)		AMBIENT PHOSPHORUS TREATMENT			HIGH PHOSPHORUS TREATMENT			F VALUE $P_r > F$	
		REP	MEAN	STD DEV	REP	MEAN	STD DEV		
P UPTAKE	(a)	4.66 3.15 2.75 3.34 4.58 6.76	4.21	1.48	2.86 4.16 2.72 1.96 2.78 3.19	2.94	0.72	3.55	0.0888
REGENERATION FROM PERIPHYTON TO WATER (WITHOUT SNAILS)	(b)	2.40 1.92 2.57	2.30	0.34	1.74 1.32 1.61	1.56	0.22	10.28	0.0327
REGENERATION FROM PERIPHYTON TO WATER (WITH SNAILS)	(b+c)	4.03 2.57 3.44	3.34	0.74	2.54 5.66 2.69	3.63	1.76	0.07	0.8096
SNAIL REGENERATION TO WATER	(c)	—	1.04	—	—	2.07	—	—	—
SNAIL INGESTION	(d)	—	1.48	—	—	1.07	—	—	—
SNAIL INCORPORATION	(e)	1.59 1.04 1.18	1.27	0.29	0.61 1.40 0.58	0.86	0.48	1.67	0.2644
‡ SNAIL FECES PRODUCTION	(f)	0.27 0.18 0.21	0.21	0.06	0.15 0.32 0.16	0.21	0.10	0.00	1.0000
‡ TOTAL FECES PRODUCTION (mgAFDM)		4.19 3.47 3.68	3.78	0.37	2.91 3.91 3.39	3.40	0.50	1.10	0.3538

MEASURED FLUXES:

- (a) SHORT-TERM UPTAKE OF ^{33}P FROM WATER BY PERIPHYTON ON TILES
- (b) RELEASE OF ^{33}P FROM PERIPHYTON ON TILES TO WATER OVER 1 HOUR (SNAILS ABSENT)
- (b+c) RELEASE OF ^{33}P FROM PERIPHYTON ON TILES TO WATER OVER 1 HOUR (SNAILS PRESENT)
- (e) ^{33}P RETAINED IN SNAIL BIOMASS OF SNAILS GRAZING ^{33}P LABELLED PERIPHYTON FOR 24 HOURS
- (f) ^{33}P IN FECES PRODUCED BY SNAILS GRAZING ^{33}P LABELLED PERIPHYTON FOR 24 HOURS

CALCULATED FLUXES:

- (c) DIFFERENCE BETWEEN (b+c) AND (b); RELEASE OF ^{33}P BY SNAILS TO WATER OVER 1 HOUR
- (d) SUM OF (e) AND (f); ^{33}P IN FECES AND ^{33}P RETAINED IN SNAIL BIOMASS

* SNAIL FECES PRODUCTION IS A FLUX, CALCULATED USING MEASUREMENTS OF FECES ^{33}P , THE ^{33}P / TOTAL P RATIO OF THE SUBSTRATE, AND THE DURATION OF THE EXPERIMENT, AND IS MEASURED IN $\mu\text{g/hr}$.

TOTAL FECES PRODUCTION IS THE INORGANIC WEIGHT, OR ASH FREE DRY MASS, OF THE FECES AT THE END OF THE EXPERIMENT AND IS MEASURED IN mg.

TABLE 2. EFFECT OF SUBSTRATE TYPE (PERIPHYTON, LEAF DETRITUS) ON PHOSPHORUS CYCLING FLUXES

FLUX ($\mu\text{gP/hr}$)		DETRITUS EXPERIMENT			PERIPHYTON EXPERIMENT			F VALUE	Pr > F
		REP	MEAN	STD DEV	REP	MEAN	STD DEV		
P UPTAKE	(a)	3.14 3.71 6.04 3.18 4.76 6.30	4.62	1.41	4.66 3.15 2.75 3.34 4.58 6.76	4.21	1.48	0.14	0.7128
REGENERATION TO WATER (WITHOUT SNAILS)	(b)	0.85 0.98 0.61	0.81	0.18	2.40 1.92 2.57	2.30	0.34	44.34	0.0026
REGENERATION TO WATER (WITH SNAILS)	(b+c)	1.46 0.83 0.87	1.05	0.35	4.03 2.57 3.44	3.34	0.74	23.77	0.0082
SNAIL REGENERATION TO WATER	(c)	---	0.24	---	---	1.04	---	---	---
SNAIL INGESTION	(d)	---	2.01	---	---	1.48	---	---	---
SNAIL INCORPORATION	(e)	1.19 0.71 0.65	0.85	0.29	1.59 1.04 1.18	1.27	0.29	3.13	0.1518
* SNAIL FECES PRODUCTION	(f)	1.46 1.07 0.98	1.16	0.27	0.27 0.15 0.21	0.21	0.06	37.54	0.0036
.....									
* TOTAL FECES PRODUCTION (mgAFDM)		6.10 6.74 6.89	6.58	0.42	4.19 3.47 3.68	3.78	0.37	74.93	0.0010

MEASURED FLUXES:

- (a) SHORT-TERM UPTAKE OF ^{33}P FROM WATER BY SUBSTRATE
 (b) RELEASE OF ^{33}P FROM SUBSTRATE TO WATER OVER 1 HOUR (SNAILS ABSENT)
 (b+c) RELEASE OF ^{33}P FROM SUBSTRATE TO WATER OVER 1 HOUR (SNAILS PRESENT)
 (e) ^{33}P RETAINED IN SNAIL BIOMASS OF SNAILS GRAZING ^{33}P LABELLED SUBSTRATE FOR 24 HOURS
 (f) ^{33}P IN FECES PRODUCED BY SNAILS GRAZING ^{33}P LABELLED SUBSTRATE FOR 24 HOURS

CALCULATED FLUXES:

- (c) DIFFERENCE BETWEEN (b+c) AND (b); RELEASE OF ^{33}P BY SNAILS TO WATER OVER 1 HOUR
 (d) SUM OF (e) AND (f); ^{33}P IN FECES AND ^{33}P RETAINED IN SNAIL BIOMASS

* SNAIL FECES PRODUCTION IS A FLUX, CALCULATED USING MEASUREMENTS OF FECES ^{33}P , THE ^{33}P / TOTAL P RATIO OF THE SUBSTRATE, AND THE DURATION OF THE EXPERIMENT, AND IS MEASURED IN $\mu\text{g/hr}$.

TOTAL FECES PRODUCTION IS THE INORGANIC WEIGHT, OR ASH FREE DRY MASS, OF THE FECES AT THE END OF THE EXPERIMENT AND IS MEASURED IN mg.

TABLE 3. EFFECT OF SNAILS ON PHOSPHORUS REGENERATION RATES TO WATER FROM PERIPHYTON AND LEAF DETRITUS COMMUNITIES

	PHOSPHORUS REGENERATION TO WATER (ugP/hr)				F VALUE	Pr > F
	SNAIL TREATMENT		NO SNAIL TREATMENT			
	MEAN	STD DEV	MEAN	STD DEV		
PERIPHYTON EXPERIMENT						
AMBIENT PHOSPHORUS TREATMENT	3.34	0.74	2.30	0.34	5.06	0.0876
HIGH PHOSPHORUS TREATMENT	3.63	1.76	1.56	0.22	4.10	0.1128
DETRITUS EXPERIMENT	1.05	0.35	0.81	0.18	1.09	0.3549

TABLE 5. EFFECT OF SNAILS ON PHOSPHORUS TURNOVER RATES IN PERIPHYTON AND LEAF DETRITUS COMMUNITIES

	SNAIL TREATMENT			NO SNAIL TREATMENT			F VALUE Pr > F	
	REP	MEAN	STD DEV	REP	MEAN	STD DEV		
PERIPHYTON EXPERIMENT								
NON-AFDM NORMALIZED TURNOVER RATE (d ⁻¹)	-0.070	-0.082	0.016	-0.054	-0.036	0.016	1.60	0.2750
	-0.080			-0.029				
	-0.037			-0.028				
AFDM NORMALIZED TURNOVER RATE (d ⁻¹)	-0.060	-0.067	0.004	-0.074	-0.056	0.016	0.00	0.9468
	-0.082			-0.052				
	-0.056			-0.042				
LEAF DETRITUS EXPERIMENT								
NON-AFDM NORMALIZED TURNOVER RATE (d ⁻¹)	-0.189	-0.207	0.034	-0.138	-0.122	0.014	16.26	0.0157
	-0.246			-0.114				
	-0.186			-0.113				
AFDM NORMALIZED TURNOVER RATE (d ⁻¹)	-0.175	-0.205	0.042	-0.130	-0.097	0.031	13.05	0.0225
	-0.253			-0.070				
	-0.187			-0.090				

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