COMBINING NUTRIENT REMOVAL WITH PROTEIN SYNTHESIS USING A WATER HYACINTH-FRESHWATER PRAWN POLYCULTURE WASTEWATER TREATMENT SYSTEM

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ABSTRACT

A two-stage polyculture wastewater treatment system, using water hyacinth, Eichhornia crassipes, in the first-stage and a combination of Azolla and the freshwater prawn, Macrobrachium rosenbergii, in the second-stage, was developed at the pilot plant scale to provide tertiary treatment of municipal wastewater. A conceptual three-compartment nitrogen model was also developed to simulate the first-stage of the system.

The results of the analysis of the first-stage water hyacinth system indicated that for NH_3-N loadings ranging from 11.6 to 76.1 kg NH_3-N/ha-d, NH_3-N removals averaged 76.8 percent. Nitrate and ORG-N removals ranged from 24 to 80 percent and 75 to 95 percent, respectively. The first-stage effluent NH_3-N, NO_3-N, and ORG-N concentrations ranged from 0.3 to 25.0 mg/L, 0.5 to 2.3 mg/L, and 0.3 to 0.8 mg/L, respectively.

The results from monitoring the second-stage Azolla-prawn system indicated that at NH_3-N loadings between 115 to 10,360 gm-N/ha-d, system performance for NH_3-N varied between a 14 percent increase to a 90 percent removal. Nitrate and ORG-N removals in the Azolla-prawn system were highly variable through the system.

Overall performance of the polyculture system for the removal of total COD, TSS, total coliforms (MPN), and turbidity (NTU) indicated removals of 58, 98, 99.9, and 94 percent, respectively. Other parameters for the two stage system were monitored including temperature, Ortho-P, biomass, productivity, alkalinity, pH, and specific conductance. In addition, prawn growth studies were performed in the field and laboratory.

Key words: tertiary wastewater treatment, water hyacinth, nitrogen removal, nutrient removal, Azolla, Machrobrachium rosenbergii, conceptual model, water reuse

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INTRODUCTION

Background

The earth's water supply, some 30 billion cubic miles, viewed in a holistic sense, appears to be an unlimited resource. Yet with this worldwide abundance come demographic shortages apparent today in parts of Africa where widespread drought and famine have resulted in hundreds of deaths and thousands of people being left homeless. Even in North America, which has well-distributed water supplies, recent shortages in south Texas, New York and California may foreshadow the future problems of water supply. As populations and industry increase, so does the demand for water, food and other resources. Water and wastewater treatment continue to be a necessity dictated by the demands for a cleaner and healthier environment. However, as society demands cleaner water, costs associated with construction, maintenance, and operation of more advanced treatment facilities will increase and these costs will be passed directly to the consumer.

Alternatives to these spiraling costs include the implementation of water reuse and water recycling techniques to conserve the quantity and quality of water. In these schemes, nutrients associated with wastewaters become resources which, when coupled with appropriate technology, become sources of useable food protein and fuel. An integrated system for wastewater treatment might include a facility designed to both treat wastewater to a desired quality level and beneficially use the sludge, gas, biomass, etc. produced from the main treatment plant to produce a variety of by-products such as fuel, food and fertilizer. The fuel could be sold to local utilities or used on-site, and the food could be used on-site to support local livestock or sold off-site in the form of high quality protein such as shrimp, fish, shellfish, crayfish, or microalgae. The fertilizer could be used internally or sold locally. Flow streams could be developed for specific by-products, depending on the waste stream characteristics.

These ventures could be financed in several ways: 1) through existing combined state and federal government subsidies, 2) through private capital and private operations looking to profit from surcharges to customers, and 3) from the sales of by-products and the resale of water. Highly nutrient-laiden waste streams would become sought after by entreprenuerial by-products and waste treatment companies, employing high technology systems for process control and production. Computer-controlled operation could provide the margin of profitability to make these systems a reality.

Initial developments of integrated systems can be traced from two widely dispersed points following parallel paths of development. The first, and more recent of the two paths, consisted of the development of conventional wastewater stabilization lagoons, which have been used for over 60 years and still represent cost-effective wastewater pollution control technology (Ferrara and Harteman 1978). These ponds, employing long detention times, use algae to convert nutrients and biochemical oxygen demand (BOD) to separable biological solids. While these lagoons are effective at converting BOD to algal solids and are inexpensive to operate, removal of the algal solids, either to meet effluent standards or for use as a by-product, is difficult and expensive.

The second path of development, which has its roots in ancient China (Bardach, Ryther and McLarney 1972), was the culture of fish in highly enriched rice ponds. Manure and other organic materials were dumped into ponds and algal blooms were allowed to develop. Carp and other fish species would feed on the phytoplankton and the fish would be harvested for sale or direct consumption and, in many cases, these ponds would also be used to grow rice concurrently with the fish. The ponds in effect converted BOD to fish protein but, health risks and effluent discharges were, at the time, not considered to be major problems.

Because of difficulties encountered with either conventional wastewater lagoons or the use of fish in fertilized ponds to remove algal solids in the effluent to acceptable water quality levels, design concepts in the United States shifted to the use of various higher aquatic plants, which grow rapidly to produce biomass while producing significantly improved water quality by shading the water column to prevent the growth of algae.

Biomass production and adequate wastewater treatment are important to integrated systems, but without additional attributes, these systems are no different from conventional wastewater treatment. Since the value of the biomass produced is low, both in terms of energy production and nutritional quality, the removal, processing and use of these materials must be as cost-effective as possible if the operation is to show a profit. The process which can use the biomass directly or indirectly should represent low-cost methods. In the aquaculture process, the low nutritional value of the plant material can be converted to high quality protein in the form of fish, crayfish or prawns. The selection of the species is critical because certain species will aid in the process of wastewater treatment while others could be detrimental to the process.

The idealized concept of an integrated process is to maximize all operational functions to produce highly-treated effluent, large quantities of plant biomass and large yields of the cultured animal species. In design this concept is relatively simple; provide the tanks, air, and food, in sufficient quantities and sizes and growth and production will be maximized. But, in practical operation, optimizing the system can be difficult. Each process reaches a different level of completion over a different span of time. Aquatic animal species are ready for harvest in six months; plant biomass can double in 7 to 10 days; detention times for effective nitrogen removal are three to five days; effluent standards are evaluated daily; within hours, elevated NH_3-N concentrations can kill aquatic animal species; and influent water quality characteristics can change every second. In addition to these dynamics, many processes are poorly understood and difficult to quantify. Furthermore, in a practical sense, a process cannot be too expensive or complicated to operate.

Research Objectives

The specific objectives outlined for the research project were to:

1. Develop a two-stage water hyacinth-*Azolla*-freshwater prawn polyculture wastewater treatment system at a pilot-plant scale of operation.
2. Determine the overall wastewater treatment efficiencies of the first and second stages of this process by monitoring various water quality parameters.
3. Determine the potential to use processed water hyacinth as a food supplement for the freshwater prawn *Macrobrachium rosenbergii*.
4. Develop a conceptual model of the water hyacinth system describing the dynamics of nitrogen removal.

Scope of Study

The system, which became the focus of this investigation, was a two-staged polycultural wastewater treatment process using a water hyacinth-*Azolla*-freshwater prawn combination. Water hyacinth, well known for its potential productivity, was grown in the first-stage to remove the major portion of nutrients, thus producing large quantities of biomass and providing water of sufficient quality to the second-stage to permit the culture of an aquatic animal species. The second-stage consisted of an aquatic fern, Azolla, and the freshwater prawn, Macrobrachium rosenbergii. The Azolla was selected to shade the water column, and thus prevent algae growth and to remove nutrients via plant growth and uptake. The freshwater prawn was selected to consume naturally-occurring detritus and Azolla.

The selection of these species was based on a number of environmental and commercial factors. All three species have optimum growth temperatures between 25 and 30°C (Hayes and Johnson 1980, Lumpkin and Plunkett 1980, Reddy and Sutton 1984). Water hyacinth provides good nutrient removal and has potential to be used either directly or mixed as feed for cattle, poultry, sea mantatees, grass carp and other animals (Bagnall et. al. 1974). The freshwater prawn was selected because of: 1) excellent market value, 2) omnivorous feeding habits, 3) general tolerance to a wide range of environmental factors, and 4) rapid growth at optimum conditions to a marketable size in four to six months. The plant, Azolla mexicana, was selected because it is native to New Mexico and the Southwest.

It was critical for continuous-system operation that the first-stage process remove influent NH_3-N levels to below toxic levels for the freshwater prawn. Water hyacinth is capable of removing 80-90 percent of the influent NH_3-N at concentrations up to 40 mg/L NH_3-N (Hauser 1984, Reddy and Sutton 1984). Concentrations of unionized NH_3-N of 0.1 to 0.3 mg/L have been shown to reduce growth of Macrobrachium rosenbergii juveniles by 30 percent (Colt and Armstrong 1979). Crustaceans have been reported killed at concentrations of unionized NH_3-N between 0.4 to 2.3 mg/L. Thus, the first-stage process had to produce water of quality suitable for the growth of the second-stage species.

To investigate the potential of the proposed polyculture combination, a field facility capable of treating 570 to 760 L/day was constructed. This facility consisted of a first-stage water hyacinth system and the second-stage, Azolla-prawn system. The purpose of operating the field system was to evaluate its potential as tertiary treatment by monitoring the ability of the combination of organisms to remove nitrogen, phosphorus, chemical oxygen demand (COD) and suspended solids from secondary wastewater.

It was not the intent of this research to totally ignore the critical aspects of the health-related issues of growing organisms intended for human consumption directly in wastewater, but it was considered beyond the scope of this research to attempt to address these issues in detail. For the health aspects, monitoring was restricted to the ability of the system to remove fecal coliform organisms.

In addition, laboratory studies were performed to measure the ability of the freshwater prawn to utilize water hyacinth as a substrate. Under conditions of constant temperature, the growing of the freshwater prawns was monitored while being fed a controlled diet of three combinations of water hyacinth and commercial fish food. Finally, a conceptual model of the water hyacinth system describing the dynamics of three nitrogen species (ammonia, organic, and nitrate) was developed.

RELATED RESEARCH

The process that was the focus of this study used a staged combination of water hyacinth, freshwater prawns and Azolla to remove nutrients from secondary municipal wastewater. The first-stage of the process consisted of water hyacinth to affect removal of the nutrients while producing biomass. The second-stage, consisting of Azolla and freshwater prawns, affected additional nutrient removal and produced prawn biomass while controlling ORG-N and suspended algal solids in the effluent. The freshwater prawns were intended to consume naturally occuring detritus, artificially provided quantities of ground water hyacinth and supplemental commercial pellet feed. The Azolla transformed dissolved nutrients and acted to shade the water column thus preventing algal growth.

The following review relates specific background information regarding polyculture wastewater treatment systems and the species selected for this two stage system including water hyacinth, Azolla, and freshwater prawns, Macrobrachium rosenbergii.

Polyculture Wastewater Treatment.

Polyculture wastewater treatment systems may have single or multiple aquacultural components mixed with a conventional waste-treatment system to form a process. Traditional sewage lagoons can be loosely defined as polycultural systems, because they combine many organisms in a single unit to treat wastewater; however, a true polyculture system should not work by accident, but by design. Polyculture systems are designed to operate in a particular way with a calculated result both in terms of wastewater treatment and the production of the cultured species. Only a few of these systems have been developed and operated at any scale for any length of time. These systems are reviewed in the following sections of this chapter.

Marine Polyculture. This pilot-scale system was developed at Woods Hole Oceanographic Institute, MA and was designed to remove nitrogen from secondary municipal effluent while at the same time culturing commercially viable marine organisms (Ryther 1979 and 1983). The secondary wastewater was fed into a series of shallow algae culture ponds that also received seawater. The sea water, which was diluted 10-20 percent with secondary wastewater, produced unicellular marine algae before being introduced to a series of aerated raceways that contained stacked trays of shellfish. These raceways also contained lobsters and flatfish, that preyed on the solids produced by the shellfish and lower trophic animals (polychaetes, amphipods, isopods) in the system (Ryther 1979). The shellfish preyed on the unicellular algae grown initially; however, production levels of shellfish were difficult to maintain because of seasonal changes in the algal species. Several species of molluscs were tried and the Japanese oyster, Crassotrea gigas, appeared the best. After the water passed through this second-stage shellfish culture unit, it entered a series of polishing lagoons that contained a mixed culture of red algae. This stage was intended to control any regenerated nutrients from the shellfish culture unit (Ryther 1979).

This pilot-scale system was operated continuously over a period of several months treating about 30,000 L/day, (7,926 gpd) of wastewater with 1,700 m^2 of algae ponds. Preliminary data indicated that the wastewater from 10,000 people could produce enough algae to grow 11 million market-sized (8-10 cm) oysters or 183 metric tons of oysters per year (Ryther 1979).

Overall total nitrogen removal, with all stages contributing, was 89 percent. However, another study focusing on the final seaweed or algae stage suggested that the use of this stage alone would give better effluent quality (Ryther 1983). The seaweeds cultured in the final polishing step also had a market value of about $500/dry ton in Japan (1973 price). In a staged series of tanks, the total nitrogen concentration of the influent was reduced 99 percent from 2.41 mg/L to 0.02 mg/L in the effluent (Ryther 1979).

In general, harvested bivalves from the second-stage of the combined system were no more contaminated after a 10-day clean-water purge than normal bivalves collected from unpolluted sources (Ryther 1979).

Algae-Finfish System. Finfish culture in various wastewaters has been successful for a variety of species. In Benton, AR a series of six ponds with a total surface area of 10.2-ha (24 acres) received raw wastewater at an average daily flow rate of 1,711 m^3/day (0.45 mgd) (Henderson 1979). These lagoons generated algae which were then consumed by herbivorous fish. Over eight months of operation the daily BOD and suspended solids were reduced by 96.4 and 86 percent, respectively.

Inorganic and total phosphorous were reduced 59 and 30 percent,respectively. Effluent values for NH_3-N and NO_3-N were reported to be 2.0 and 0.5 mg/L, respectively (Henderson 1979 and Ryther 1983).

Fish production, consisting of a combined harvest of silver and bighead carp, averaged 3,344 kg/ha which if sold for direct consumption at a price of $0.50-5.0/lb, would produce a gross annual return of $3,600-4,525/ha (Henderson 1979). Analysis of the fish flesh for various contaminants indicated that no samples were above guidelines established by FDA or the Arkansas Department of Health (Hendersen 1979).

Algae-Brine Shrimp-Rotifer System. Developed by Dow Chemical Company at a facility in Freeport, Tx, this system was essentially an extended aeration process designed to receive and treat tertiary effluent from hypersaline aerated waste lagoons. The 0.3-ha pilot-plant operation received 129 m^3/day (34,100 gpd) of waste effluent. The system was a series of lagoons where waste nutrients served as substrate for culturing microalgae and bacteria. The effluent from this algae-bacteria culture then flowed to a temperature controlled tank where the algae and bacteria were consumed by filter feeding brine shrimp and rotifers (Milligan et al., 1979).

The system was capable of removing 89 percent of the BOD and 88 percent of the NH_3-N while producing about 615 metric tons/ha-yr of brine shrimp and 1,333 metric tons/ha-yr of rotifers. Evaluation of the brine shrimp for various chemical contaminants indicated no significant accumulation (Milligan et al., 1979).

Watercress-Crayfish Polyculture. A pilot-plant scale watercress-crayfish polyculture system was developed to remove nutrients and produce viable by-product biomass (Rundquist, Gall and Goldman 1977 and Ryther 1979). This system was designed to treat trout hatchery effluent that contained high concentrations of total nitrogen. The treatment plant removed most of the phosphorous and nitrogen while producing biomass that could then be fed to crayfish, which were held separately and not grown in the same water source.

Analysis of the treatment efficiency of the system indicated that NO_3-N and total phosphorous concentrations were reduced by 98.6 and 92 percent, respectively. Ammonia, total kjeldahl nitrogen (TKN), and total iron increased through the system by 10, 54, and 92 percent, respectively. All concentrations of the constituents in the influent and effluent were less than 1 mg/L. The biomass of the watercress was reported to be present at 500 gm/m^2 (dry wgt), but growth rates of the plant were not determined. The results of the crayfish growth studies indicated that better growth was achieved with the watercress diet heavily supplemented with trout chow. However, the investigators concluded that the watercress did help to increase growth by providing increased habitat and supplying needed vitamins (Rundquist, Gall and Goldman 1977).

Chara-Grass Carp-Freshwater Prawn System. A pilot-scale system located in central Florida used the aquatic algae, *Chara*, to remove nutrients from a secondary municipal wastewater source (Ryther 1983 and Ryther, Williams and Kneale 1977). Grass carp and freshwater prawns were grown in nonconnected adjacent ponds using the *Chara* as the feed stock.

The ponds were 25,000 L (6,650 gal) each and the *Chara* pond received an undiluted flow of 500 L/day (132 gpd) of secondary wastewater from a local activated-sludge treatment plant. Influent total inorganic nitrogen and phosphorous averaged 40 mg/L and 6.2 mg/L, respectively. With a 50-day detention time the *Chara* system was capable of removing 83 and 87 percnet of the nitrogen and phosphorous, respectively. Effluent concentrations of total nitrogen and phosphorous were 6.9 mg/L and 0.8 mg/L, respectively (Ryther, Williams and Kneale 1977).

The results of growing freshwater prawns and grass carp in the second pond using the *Chara* as feed were mixed. The grass carp were fed 170 kg of *Chara* over 161 days and grew from 21 to 180 gm/fish or a total biomass increase of 4.5 kg with a food conversion efficiency of 2.6 percent. As food conversion efficiencies for grass carp can usually be expected in the 10-20 percent range, 2.6 percent was very low. In another test, freshwater prawns and carp were grown together over a period of 146 days with a yield of 5.0 and 1.3 kg of grass carp and shrimp, respectively. This yield was equivalent to a 6-month production of 1,910 kg/ha or 3,819 kg/ha on a two crop per year basis (Ryther, Williams and Kneale).

Problems associated with this system included fragmentation of the *Chara* when handling and harvesting the algae and phytoplankton blooms in the *Chara* pond resulting in high effluent suspended solids. Other rooted plants were suggested as replacements for the *Chara* and beds of bivalves could have been used to control the phytoplankton (Ryther, Williams and Kneale 1977).

Hercules System. This system represents a full size wastewater-treatment facility designed to treat 1,325 m^3/d (0.35 mgd) of raw wastewater to secondary treatment standards for BOD and suspended solids (Richardson and Daigger 1984). The process consisted of a series of different types of ponds designed to remove specific components of the wastewater. These ponds included an anaerobic pond, an aerobic pond, and an aquacell. The aerobic pond was designed to remove soluble BOD by suspended and attached bacteria. Synthetic media was provided to allow for increased surface area for bacterial attachment and low volume aeration was provided for mixing. The final pond was the aquacell, which contained water hyacinth and more synthetic strips to provide growth sites for organisims that would consume the bacteial solids from the previous aerobic stage.

This system was constructed by the City of Hercules, CA using funds from the EPS and was tested through the pilot-plant and operational stages. The plant was run for two months below design flow and the effluent BOD averaged 7 mg/L and suspended solids averaged below 3 mg/L. The flow was increased to design flow and the plant promptly failed, with BOD averaging over 21 mg/L and a maximum BOD of 57 mg/L. The plant was shut down in April 1981 and has not been reopened. This treatment plant did not produce any specific aquacultural by-products (Richardson and Daigger 1984).

The systems described in this section are the best documented cases. Other systems have been used on an experimental basis in North America and around the world. These systems, unfortunately, were not rigorously studied using acceptable or comparable techniques.

Water Hyacinth

Water hyacinth, Eichhornia crassipes, is perhaps the most productive freshwater aquatic plant in the world (Dinges 1982 and Reddy and Sutton 1984). Distribution in North America is limited to temperate and tropical areas of Florida, Alabama, Mississippi, Texas, and California (Reddy and Sutton 1984). Worldwide distribution is extensive in tropical areas including Central and South America, China and Indoneasia, and parts of the Mideast including the Nile River (Dinges 1982 and Penfound and Earle 1948). Although potentially useful as a wastewater treatment tool, the plant's prolific nature generates serious management problems. Problems associated with water hyacinth stem from both blockage of navigable waterways to increased losses of water from impoundments through evapotranspiration that exceeds the normal evaporative losses. The estimated cost for control in the state of Florida alone is $20 million per year (Reddy and Sutton 1984).

Water hyacinth has been shown to be effective at removing nutrients from highly eutrophic waters as well as for treating various kinds of wastewaters, including industrial and municipal, and for polishing secondary effluent (Colt et al., 1980, Dinges 1979, EPA 1976, Hauser 1984, McDonald and Wolverton 1979 and 1980, Wolverton and McDonald 1976). While many factors such as solar radiation, plant density, ambient temperatures, and nutrient availability affect water hyacinth growth rates, specific nutrients such as nitrogen and phosphorous appear to have the greatest controllable influence on hyacinth growth rates.

Physical and Water Quality Requirements. Temperature range for the growth of water hyacinth is between 10 and 35 C with optimum conditions in the range of 25 to 28 C (McClure and Lipinsky 1981, Dinges 1979). Survival of water hyacinth is apparently limited to areas where the mean atmospheric temperature does not fall below 1 C (Colt et al., 1980, Dinges 1979, Penfound and Earle 1948, Reddy and Sutton 1984). However, the plant can survive short periods of freezing and plants grown in highly eutrophic water appear to resist the effects of freezing better than plants grown in relatively low-nutrient water (Reddy and Sutton 1984).

Salinity has also been shown to limit the growth of hyacinth with concentrations of 1.66 percent apparently toxic to the plant (Haller, Sutton and Barlow 1974, Penfound and Earle 1948).

The effect of pH on the growth of water hyacinth is apparently of little consequence as the plant grows well in the pH range of 4.0 to 10.0 with maximum growth occurring at a pH of 7.0 (McClure and Lipinsky 1981).

Growth of water hyacinth appears to increase with increasing solar radiation. Maximum growth in studies in Florida occurred during the months of average maximum solar radiation where the average monthly solar radiation reached a maximum value of 18.4 MJ/m^2-d in August (Reddy and Sutton 1984). In New Mexico the maximum average value for solar radiation in 1984 occurred in the month of July and was 25.7 MJ/m^2-d. Light intensity below 1,400 lm/m^2 apparently inhibites hyacinth growth (Nolan and Kirmse 1974). Photosynthetic efficiency of water hyacinth has been estimated between 3.2 and 4 percent, which is higher than most terrestrial plants (Dinges 1979, Reddy and Sutton 1984).

Composition, Nutrient Uptake and Productivity. Analysis of the composition of water hyacinth indicates values of total nitrogen, phosphorous, and water to range from 1.2-5.6, 0.1-0.8, and 92.9-94.7 percent, respectively (McClure and Lipinsky 1981).

Water hyacinth requires a minimum iron concentration to prevent the plant from developing a highly chlorotic condition and diminishing growth rate. Several studies indicated the need to add iron to waters with less than 0.5 mg/L iron (McClure and Lipinsky 1891, Nolan and Kirmse 1974); however, the actual limiting concentration has not been determined. Apparently, hyacinth requires higher iron concentrations when grown on wastewater containing mostly NO_3-N as opposed to predominately NH_3-N or a mixture (Reddy and Sutton 1984). Studies have also indicated that nitrogen, phosphorous, phenol, pesticides and various heavy metals could be removed using water hyacinth for wastewater treatment (Muramoto and Oki 1983, Reddy and Sutton 1984, Wolverton and McDonald 1976).

Productivity of water hyacinth is reported to vary between 12.0 and 29.0 gm (dry wgt)/m^2-d depending on season and growth media (McClure and Lipinski 1981, Reddy and Sutton 1984). Biomass concentration is generally reported to range between 650 and 2,910 gm (dry wgt)/m^2 (McClure and Lipinsky 1981). Growth rate studies based on water surface coverage have estimated that hyacinth is capable of doubling its coverage in 5 to 7 days under optimum conditions (McClure and Lipinsky 1981). Both growth rates and productivity are influenced by plant densities. Growth rates are highest when hyacinth densities are between 5 to 30 kg (wet wgt)/m^2. Beyond this point the growth rates diminish until virtual cessation of growth occurs at biomass concentration of 50 kg/m^2 (Center and Spencer 1981, Debusk, Williams, Ryther 1983, Mitsch 1976).

Wastewater Treatment.

Studies using water hyacinth for nutrient removal in wastewater treatment systems indicate that removal efficiencies for phosphorous are significantly lower than those for nitrogen (Dinges 1979, Hauser 1984, Reddy and Sutton 1984). Ortho-phosphorous removal efficiencies have been reported in the range of 40 to 61 percent (Reddy 1982, Sheffield 1967) and total phosphorous removal ranges from 20 to 40 percent (Reddy 1982, Reddy and Sutton 1984). Estimated uptake rates of phosphorous from primary and secondary wastewater were 387 kg P/ha-yr and 321 kg P/ha-yr, respectively (Reddy and Sutton 1984).

Under conditions studied by various investigators using water hyacinth for wastewater treatment, phosphorous does not appear to be a limiting nutrient nor do hyacinth systems appear to be efficient enough to be considered as a phosphorous control methodology without some modification.

Total nitrogen removal efficiences range between 70 to 80 percent with NH_3-N species being selected over NO_3-N, particularly in waters where iron concentrations are below 0.05 mg/L (Reddy and Sutton 1984). Uptake rates of total nitrogen by hyacinth have been estimated to be 1,726 kg N/ha-yr and 1,193 kg N/ha-yr for primary and secondary sewage, respectively (Reddy and Sutton 1984). Several investigators have extrapolated maximum uptake rates for phosphorous and nitrogen to range from 700 to 1,260 kg P/ha-yr and 2,500 to 5,350 kg N/ha-yr, respectively (Reddy and Sutton 1984, Sato and Kondo 1981).

Several pilot and full-scale hyacinth systems have been operated for a number of years in Florida, Mississippi, Texas, and California (Dinges 1979 and 1980, Stewart 1979, Wolverton and McDonald 1979). These systems have treated both primary and secondary municipal wastewater as well as some industrial wastewaters (Hauser 1984, Richardson and Daigger 1984, Stewart 1979). Two pilot-plant operations were of particular interest because they treated municipal wastewater and centered on nutrient removal as well as carbonceous BOD or COD removal (Hauser 1984, San Diego 1983). These pilot plants were located in San Diego, CA and Roseville, CA. The Roseville pilot plant consisted of three ponds each with a surface area of 692 m^2 (7,500 ft^2) and an effective depth of 0.46 m (1.5 ft). This plant was designed to treat secondary effluent to 10 mg/L, BOD and TSS and remove NH_3-N. Flowrates to each of the ponds averaged 106 m^3/d (0.28 mgd) giving a detention time of three days. After the hyacinth was allowed to grow and acclimate, three variations in operation were employed on each of the ponds. Pond 1 was unmanaged and received no aeration or harvesting. Pond 2 was aerated but not harvested, and pond 3 was aerated and harvested. Ponds 1 and 3 were capable of warm weather reduction of NH_3-N of 72 and 77 percent, respectively. Pond 2 removed up to 99 percent under the same climatic conditions. Using a nitrogen content of the water hyacinth of 3 percent, total nitrogen uptake was estimated to be 900 mg-N/m^2-d. In pond 3, plant uptake accounted for about half of the NH_3-N input to the system, while nitrification accounted for the other half. Pond 2 (aerated and unharvested), overall, removed more nitrogen than pond 1 (unmanaged) and pond 3 (aerated and harvested) because of disturbances caused by harvesting in pond 3 and the diminished rate of nitrification in pond 1. The actual fate of nitrogen in these systems as well as in stabilization lagoons in general is unknown and is the subject of a great deal of speculation (Ferrara and Harleman 1981, Hauser 1984, Reddy 1982, Reddy and Sutton 1984, Reed 1985).

The pilot-plant in San Diego was designed to treat raw municipal wastewater with water hyacinth biomass to be used to produce bio-gas in on-site digestors (San Diego 1983). The system consisted of six 5.68-m^3 (15,000 gal) tanks with a split flow going to the first three tanks and then recombined to go through the last three tanks in series. The average flowrate was 9.46-m^3/d (25,000 gpd) with a detention time of 2 to 4 days. Air was provided at a rate of 0.94 to 23.5 L/sec (2-50 cfm) to maintain a dissolved oxygen concentration of 1-6 mg/L. Ammonia removal from the system ranged between 97 to 99 percent with effluent BOD and TSS averaging 20 mg/L and 5 mg/L, respectively. The operation harvested 2,268 kg (wet wgt)/month and 4,536 kg (wet wgt)/month of hyacinth in winter and summer, respectively.

Azolla

The common names of Azolla include water fern, water velvet, mosquito fern, Helechito del Agua (Spanish) and others (McClure and Lipinsky 1981, Lumpkun and Plucknett 1980). In North America, three species (A. Filiculoides A. caroliniana, and A. mexicana) are widely distributed along the southeastern coast to Texas and west and northwest to California, Oregon, Washington and British Columbia. Worldwide distribution is extensive in tropical and temperate freshwaters. The plant represents a unique symbiont between the cyanobacter, Anabaena azollae, and the protective plant Azolla. Azolla has been shown capable of fixing atmospheric nitrogen and thus can grow in nitrogen-deficient waters (McClure and Lipinsky 1981, Lumpkin and Plucknett 1980, Peters and Mayre 1974). Because of this ability, interest had developed to use Azolla as a fertilizer especially for rice and other wetland crops (LeVan and Sobachkin 1963, Lu et al., 1963, Lumpkin and Plucknett 1980).

Physical Characteristics. Temperature ranges for growth appear to be specific for all species of Azolla; however, maximum growth rates generally occur between 20-30 C (Lumpkin and Plucknett 1980). Outside of this range, plant growth drops significantly until death begins to occur. A. pinnata dies at temperatures above 45 C and below 5 C (Reddy and Debusk 1983). Some species can tolerate higher or lower temperature extremes. A. filicluoides can withstand temperatures as low as -5.0 C without apparent harm while A. mexicana dies at these temperatures. But A. mexicana can survive much higher temperatures than A. filicuoides (McClure and Lipimsky 1981).

Salinity has been shown to affect growth rates of Azolla with increasing salinity resulting in decreased growth rates (McClure and Lipinsky 1981). At a salt concentration of 1.3 percent (37 percent of seawater) the growth of Azolla ceases and as salinity increases death occurs (Haller, Sutton, Barlow 1974). Other investigations indicated that TDS concentrations above 1,500 mg/L will result in the death of Azolla (LeVan and Sobachkin 1963).

Azolla can survive in a pH range of 3.5 to 10.0 but optimum growth occurs between a pH of 4.5-7.0 (Downing and Knowles 1966, LeVan and Sobachkim 1963). Apparently pH and light intensity act together to directly influence relative growth rates. The combinations of high light intensities with high pH and low light intensities with low pH conditions achieve maximum relative growth rates (Ashton 1974). In addition, at a neutral pH the rate of nitrogen fixation has been shown to decrease (Ashton 1974, Watanabe 1977).

Light intensity also affects Azolla growth rates. High light intensities increase growth rates and a decline in rates occurs with diminishing light intensities. Several authors reported growth occuring in light intensity ranges of 500-120,000 lux; however, optimum growth rates varied considerably (Lumpkin and Plunknett 1980). Growth rates for A. filicuoides were reported to be maximum at 50 percent of full sunlight or 40,000-57,500 lux (Lu, et.al. 1963). A. pinnata was reported to attain maximum growth rates in the range of 20,000-40,000 lux (Lu et al., 1963). Other studies measuring solar radiation indicated maximum biomass yields occurred in the range of 7.54-13.92 MJ/m^2-d (Reddy and Debusk 1983).

Nitrogen Fixation, Nutrient Uptake and Productivity. Azolla can supply itself with nitrogen from either the nitrogen fixation process or by absorption through the water, or both, without decreasing nitrogenase activity

(Peters and Mayne 1974). But, in most cases these studies were performed at very low NH_3-N concentrations (1 ppm) and, in fact, the regulation of nitrogen fixation in Azolla is not fully understood when NH_3-N concentrations exceed 1 ppm. Additional studies, again with low levels of NH_3-N in the aqueous solution, indicate that Azolla excretes nitrogenous compounds. The amount of excreted nitrogen was reported by several authors to vary between 2 and 21 percent of the actual amount of nitrogen fixed (Lumpkin and Plucknett 1980). Other studies indicated that small quantities or no quantities of nitrogenous compounds were released by Azolla (Peters 1977, Watanabe 1977, Watanabe and Espinas 1976). Estimates of annual rates of nitrogen fixation by Azolla vary widely between 103-1,000 kg N/ha-yr (Lumpkin and Plucknett 1980).

Because of the ability of Azolla to fix nitrogen, nitrogen has not been considered a major nutrient limiting the growth of Azolla (Lumpkin and Plucknett 1980). As previously mentioned, while nitrogen in the aqueous solution may not strictly limit the growth of Azolla, the actual uptake of nitrogen compounds may have definite boundaries that could act to turn on or off the fixation process. In general studies on nitrogen fixation, the process has been shown to be inhibited by the presence of NH_3-N (Brill 1977); however, the actual concentrations causing inhibition of the fixation process are unknown (Peters 1975, 1976, and 1977).

Phosphorous and iron have been suggested as the major limiting nutrients regulating the growth of Azolla. Productivity of Azolla with supplemental phosphorous is reported to vary between 6.7-20.0 gm (dry wgt)/m^2-d and, without supplemental phosphorous, productivity was significantly reduced to 1.6-6.9 gm (dry wgt)/m^2-d (Reddy and Debusk 1983). Actual limiting quantities of phosphorous are unknown; however, Azolla was found to thrive in waters with phosphorous concentrations of 1.1 mg/L (Olsen 1944) and plants grown in phosphorous-deficient waters developed curled roots, reddish-yellow colors and eventually died (Lumpkin and Plucknett 1980). In waters with iron concentrations of 1.0 mg/L or higher Azolla thrived, but in iron-deficient water the Azolla developed a reddish-yellow color and died (McClure and Lipinsky 1981, Talley, Talley and Rims 1977). The exact nature of the color development is not well understood; however, it appears to be stress-related in all cases.

In studies examining Azolla growth rates with and without nitrogen and including both primary and secondary wastewater effluents, investigators indicated the greatest growth rates were achieved in primary wastewater effluents which contained concentrations of NH_3-N greater than 10 mg/l (Reddy and Debusk 1983). This same study indicated that Azolla grown in media containing no nitrogen but with elevated phosphorous concentrations had lower growth rates than Azolla grown in the primary wastewater effluent. Total nitrogen recovery rates for Azolla grown in wastewater were reported in the range of 201 to 287 mg N/m^2-d and productivity was estimated to range from 8.2 to 12.9 gm (dry wgt)/m^2-d (Reddy and Debusk 1983).

Biomass densities for various species of Azolla under varying conditions have been reported to range from 47 to 278 gm (dry wgt)/m^2 (McClure and Lipinsky 1981). Analysis of the composition of Azolla with respect to nitrogen, phosphorous and water indicate ranges of 4.0 to 5.0, 0.28 to 0.49, and 92.2 to 95.0 percent, respectively (McClure and Lipinsky 1981).

Freshwater Prawns

The freshwater prawn, Macrobrachium rosenbergii, is native to Southeast Asia and Thailand where it grows in slow flowing rivers and streams. Over 100 species of Macrobrachium are reported worldwide with about 26 species occurring in the Americas (Bardach, Ryther and McLarney 1972, Fujimoto, Fujimura and Kato 1977, Hanson and Goodwin 1977). Various species have been cultured but M. rosenbergii is the culture species of choice to date (Bardach, Ryther and McLarney 1972, Hanson and Goodwin 1977). Common names for Macrobrachium rosenbergii includes prawns, freshwater prawns, blue-claw lobsters, and Malaysian prawns. The culture of freshwater prawns is a relatively recent development although freshwater prawns have been captured in the wild and consumed for centuries.

Life Cycle. The general life cycle of the freshwater prawn was first studied by S.L. Ling in 1961 (Hanson and Goodwin 1977, Hayes and Johnson 1980). Ling discovered that just-hatched larvae require brackish water in order to survive and metamorphose into juvenile prawns or post-larvae (PL's). This single discovery allowed for the development of mass rearing of larvae by Fujimura (Fujimoto, Fujimura and Kato 1977, Hanson and Goodwin 1977), that enabled the establishment of commercial hatcheries to provide mass quantities of larvae for stocking.

In the wild, freshwater prawns mature strictly in freshwater rivers and streams. As the females come into season or begin to develop eggs, they migrate downstream towards more brackish water and presumably are fertilized by a male along the way. Within about 21 days the eggs hatch and the larvae flow downstream with the current until suitable brackish conditions are encountered. If brackish water of at least 12 to 15 ppt is not encountered, the larvae perish. The larvae are planktonic at this stage and will go through about 11 larval stages in 18-45 days. After this time the larvae drop to the bottom and begin traveling upstream into freshwater. At this point the larvae are called post-larvae (PL's) and weigh about 0.1 gm. While they are energetic swimmers they are considered pelagic, somewhat opportunistic feeders (Hanson and Goodwin 1977, Hayes and Johnson 1980).

In managed prawn cultural systems a degree of genetic selection is attained by selecting large females to mate with large males. This breeding stock is used to produce the young which are sold. Female freshwater prawns may spawn several times per year producing 60,000 to 100,000 eggs per spawn (Hanson and Goodwin 1977, Hayes and Johnson 1980. Fertilized eggs are retained for about 20 days until the egg mass color changes from orange to grey. This color change usually signifies that hatching will occur in 24 hrs. Unfertilized eggs begin to deteriorate and are reabsorbed by the female prawn (Hanson and Goodwin 1977).

One technique used to rear larval prawns to saleable size is the "green-water" system, which uses manure or fertilizer-induced phytoplankton blooms as the basic feed source. In some systems this "green-water" technique is also carried over to the grow-out system in which the phytoplankton serve to support development of higher food-chain organisms that become food for the prawn. The green-water systems are inexpensive but somewhat erratic because of changing phytoplankton populations and die-off resulting in depletion of dissolved oxygen in the pond (Hanson and Goodwin 1977, Hayes and Johnson 1980).

A second larval culture technique is the "clear-water" system, which uses dried feeds, chopped fish or other available feeds. These feeds are fed directly and in amounts that do not promote algal blooms. Many grow-out systems use this method. The "clear-water" system usually requires more maintenance but allows for greater control of prawn production (Hanson and Goodwin 1977).

Prawns mature in 5 to 7 months. Marketable prawns weigh 20 gm or more in fresh weight. If allowed, prawns may grow to over 120 gm (Hanson and Goodwin 1977, Shang and Fujimura 1977). The variability associated with the pond grow-out systems has caused culturists worldwide to examine factors that could be used to reduce irregularities and compensate for shorter growing seasons (Shang and Fujimura 1977, Smith, Jenkins, and Sandifer 1983). Some of these factors include stocking densities, diets, water quality, stock mix, water column use and related engineering designs, and integrated engineered systems primarily directed at water temperature control.

Water Quality Requirements. Salinity, temperature, dissolved oxygen and dissolved nitrogen are among the major physical influences on freshwater prawns (Bardach, Ryther, and McLarney 1972, Cohen, Stern, and Borut ND, Colt and Armstrong 1979, Hanson and Goodwin 1977). Brackish water is required for prawn larvae to develop to the PL stage (Hanson and Goodwin 1977). Adult prawns have been captured in the wild in salinities up to 18 ppt (Hanson and Goodwin 1977), and recent studies indicate that growth rates of prawns grown in varying salinities up to 15.3 ppt were similar to growth rates obtained from freshwater (Cohen, Stern, and Boru ND). Cohen et al., determined that Macrobrachium can survive salinities as high as 24 ppt. Growth however, was significantly inhibited at this salinity concentration (Cohen, Stern, and Borut ND). Specific ions may inhibit growth at certain stages but, the data are very inconclusive (Cohen, Stern, and Borut ND, Hanson and Goodwin 1977).

Water temperature is a major limitation to freshwater culture in temperate regions (Brody et al., 1980). The freshwater prawn requires temperatures between 16 and 33 C with optimum-growth temperatures between 27 and 31 C (Brody et al., 1980, Hayes and Johnson 1980). Because of this limitation many studies have centered on the use of heated waters for the culture of prawns, including geothermal water, hot springs, and industrial process waters (e.g., steam-electric power-plant cooling water). In addition, recently engineered systems have integrated solar heating with light-weight greenhouses to provide and maintain optimum temperatures (Arieli and Rappapot 1982, Smith, Sandifer, and Trimble 1976). In spite of promise, as yet no full-scale commercial prawn farms use water heated by any of these sources. To be cost-effective heated systems require exceptionally intensive cultural techniques that increase the per acre output while minimizing the actual volume or surface area required, thus reducing energy and cost imputs.

Dissolved oxygen (DO) is critical in maintaining adequate growth rates of freshwater prawns (Bardach, Ryther and McLarney 1972). Macrobrachium was reported to have a lower tolerance to oxygen depletion than most fish. When DO in the pond was low enough to cause stress, the prawns were observed to swim at the pond surface near the perimeter (Hayes and Johnson 1980). No specific oxygen concentration has been determined to cause inhibition of growth or death but below 3 mg/L appears to be a critical level (Costa-Pierce, Craven, and Laws ND).

Three forms of inorganic nitrogen can affect the growth rates of freshwater prawns. Un-ionized NH_3-N at a concentration of 0.090 mg/L NH_3-N in water with a pH of 6.8 was shown to significantly reduce growth rates of larval prawns (Colt and Armstrong 1979). Adult prawn growth rates were reduced 30 percent in water with 0.10 to 0.6 mg/L of un-ionized NH_3-N. Nitrite nitrogen at concentrations of 1.8 mg/L caused a 35 percent reduction in the growth of Macrobrachium larvae. Adult prawn growth rates were reduced 50 percent by 180 mg/L of NO_3-N. In all nitrogen species pH compounded the effects of the various concentrations of nitrogen. Suggested values for pH in prawn ponds are 7.0-8.0 (Hanson and Goodwin 1977, Hayes and Johnson 1980).

Diets. Diets of freshwater prawns have been a subject of controversy since initial culture practices began (Wiendenbach 1982). Commercial feeds are available and are used regularly by both laboratory and commercial culturists but, it remains unverified whether these formulated foods constitute the main portion of diets of prawns or if the feeds act as a substrate upon which other natural foods are derived (Smith, Jenkins, and Sandifer 1983, Wiendenbach 1982). Recent studies indicate that prawns directly ingest commercial pellets when available but that natural foods are a part of the prawn diet regardless of the presence of pelletized feed (Wiendenbach 1982). In addition, prawns will adjust to the absence of commercialized feed by increased ingestion of vegetable matter. Other studies have indicated that the omnivorous feeding habits of prawns depend solely on the provisions of the environment (Wiendenbach 1982). In comparative growth studies with prawns raised on pellets, natural foods, or a combination of natural foods and pellets the combination diet generated better growth than either the pellets or natural foods alone. Interestingly, natural foods alone produced significantly higher growth rates than pelletized feeds (Wiendenbach 1982). These studies have also indicated that prawns are selective and opportunistic feeders with unknown food preferences (Wiendenbach 1982). What foods are selected and how is basically unknown, but other detritavore arthropods contain fungal material in their guts (Martin 1979). In addition, detrital material, colonized with fungi and bacteria has a higher ratio of carbon-to-nitrogen uncultured material (Wiendenbach 1982). Other studies have also indicated that well-fed prawns exhibited less aggressive behavior than hungry or stressed prawns (McSweeney 1977). Addition of plant material to prawn-culture ponds could serve several potential functions: (1) a direct food source, (2) substrate for fungal and bacterial growth, (3) protective habitat, and (4) a nutrient source to enhance primary productivity. These concepts may hold the key to the design and management of an intensive prawn culture system both from the water quality management point of view as well as feeding methodology. At the present time the costs associated with the use of commercial feeds represents one of the most significant limitations to prawn culture worldwide (Brody et al., 1980, Hanson and Goodwin 1977).

Stocking Rates and Stategies. High stocking densities result in higher mortalities and lower harvest size (Brody et al., 1980, Hayes and Johnson 1980, McSweeney 1977). Stocking densities of 3 to 20 prawns/m^2, a common density in commercial and non-intensive operations result in mortalities ranging from 15 to 30 percent. Increasing the stocking density to over 100 prawns/m^2 increased mortalities to 60 to 80 percent. Stocking density also affects the size and weight of the harvestable crop of both males and females (Brody et al., 1980, Smith, Sandifer, and Trimble 1976). Stocking higher densities of prawns results in lower weights per individuals harvested with lower net market value due to the lower prices associated with the small prawns. Conversely, these ponds produce more total prawn biomass than ponds stocked at lower densities (Smith, Sandifer and Trimble 1976).

Initial studies of mixed stocking sizes indicate that the use of this technique did not substantially alter the final harvest size (Hayes and Johnson 1980, McSweeney 1977). But these studies focused on seasonal stocking with limited duration for growth and the results from year-round operations may be influenced by the mixed size stocking.

Use of larger initial stocking sizes result in 17 to 27 percent increase in the final harvest size compared to stocking with 0.02-0.04 gm prawns (Smith, Jenkins, and Sandifer 1983). Post-larval stage prawns (0.02-0.04 gm) are intensively cultured until reaching 1.0 to 2.0 gm sizes at which point the prawns are stocked in the grow-out facilities. Post-larval prawns reared to the 1.0 to 2.0 gm size using a modified culture method have been shown to have lower mortalities (4 to 18 percent) at stocking densities of 1,200 to 6,300 prawns/m^2 over periods of 140 days. Higher survivorship at high stocking densities was achieved with artificial habitats designed to increase the effective surface area within the water column. Production of these nursery prawns is envisioned to be an off-season, indoor activity for a temperate commercial prawn farm.

Recently investigated intensive cultural and polycultual techniques in pond grow-out systems include various artificial substrates and habitats which increase effective surface area and the use of continuous aeration to allow for increased stocking densities (McSweeney 1977, Sandifer et al., 1982). In most of these systems, water quality was managed with recirculating water treatment, and commercial feeds (25 percent protein) were fed daily (McSweeney 1977, Sandifer et al., 1982). Results generally indicate lower survivorships (66.5 percent), and lower weight gains, but an impressive gross production of over 3,000 kg/ha (Sandifer et al., 1982, USMG). Estimates from both researchers and private sources put eventual gross production at over 10,000 kg/ha with a net potential value of $42,000/ yr/ha. Polycultural methods employing Chinese carp and freshwater prawns indicate that both organisms could be produced in comparable qualtities to those achieved in monoculture (Malecha et al., 1981). Prawns attained harvestable size without the addition of commercial feeds. Swine manure apparently served as either a direct or indirect food source (Malecha et al., 1981). Additional studies suggest that Channel catfish may be grown in polycultural combination with freshwater prawns (Hanson and Goodwin 1977, USMG).

MATERIALS AND METHODS

Both field and laboratory work were required to achieve the objectives of this research. Field work included obtaining permits, construction, operation and analysis of the pilot plant process. Laboratory work involved the analyses of various water-quality parameters and detailed studies of prawn growth.

Permits

The field facility used for this study was a 225 m^2 greenhouse owned and operated by the City of Las Cruces Department of Parks and Recreation. The greenhouse was adjacent to the Las Cruces wastewater treatment plant, which is located on the west side of the City of Las Cruces, N.M. Permits were required by two state agencies to operate the facility. Initially, written permission to use the greenhouse was obtained through the city council of Las Cruces but, permits to operate the facility were required from the New Mexico Environmental Improvement Division (NMEID) and the New Mexico State Department of Agriculture (NMDA). The NMEID permit consisted of a discharge plan requiring the construction of a leachfield to dispose of the final liquid effluent from the field facility. The NMDA permit was a security agreement between the City of Las Cruces and New Mexico State University, designed to prevent water hyacinth from spreading to the Rio Grande, Burn Lake, and nearby irrigation canals.

Experimental Facility

The pilot-plant process flow diagram is shown in Figure 1. Secondary municipal effluent was provided by a pipeline extending from the Las Cruces treatment plant to the experimental facility. Wastewater flowed by gravity from the treatment plant through the pipeline to a one-third horsepower lift pump which delivered wastewater to a sand filter at a hydraulic loading rate ranging between 0.5-2.5 L/m^2-sec. The filter was employed to insure that the suspended solids concentration of the influent was always less than 30 mg/L. The filtered wastewater was stored in four plastic coated 208 L drums until it was fed through metering pumps that regulated the flow to the hyacinth tanks. Two 1249L galvanized steel stock tanks, lined with a 10-ml polyliner, were connected in series to form the first-stage water hyacinth treatment

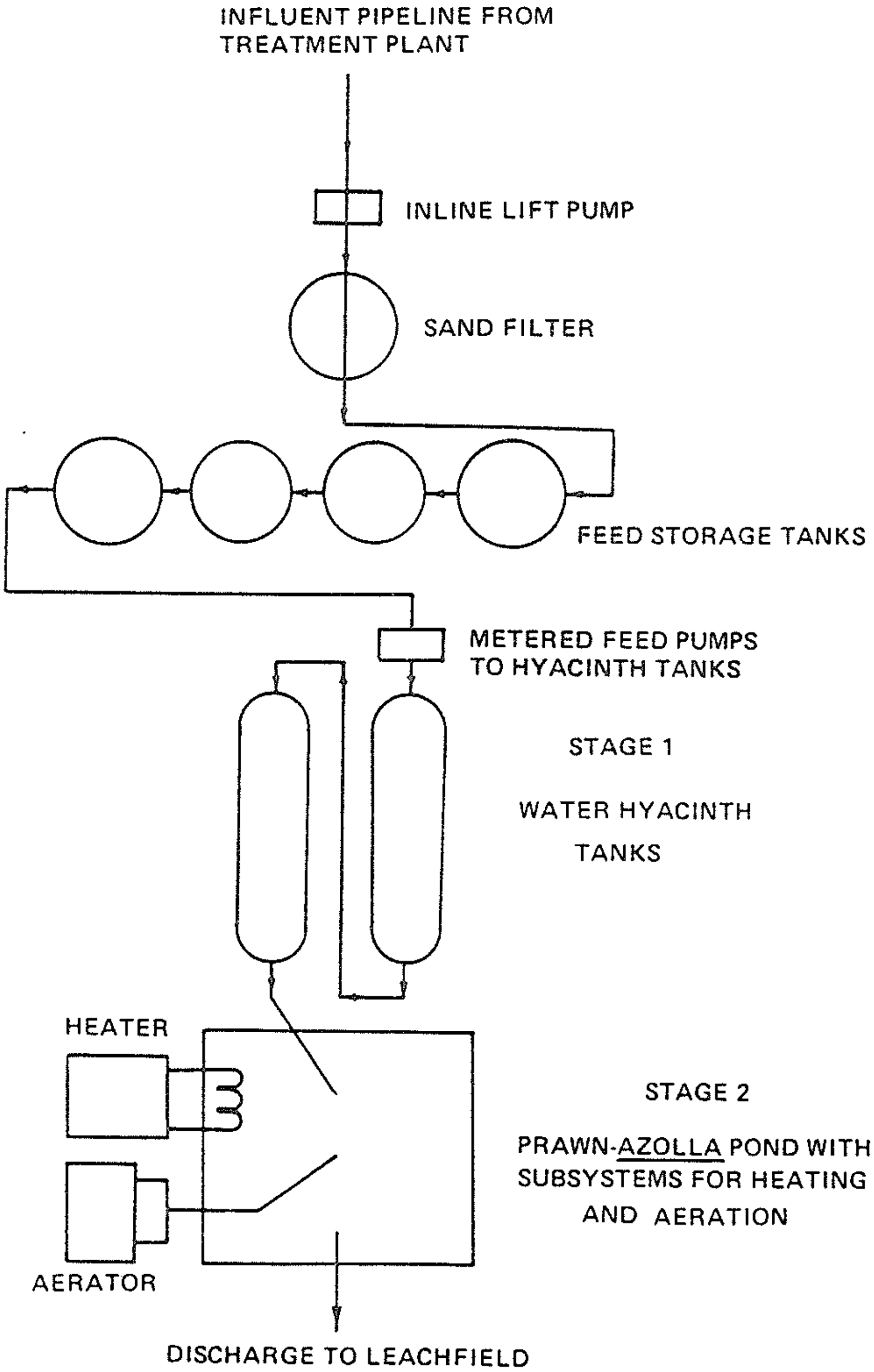

Figure 1. Schematic of the polyculture system pilot plant.

process. The tanks were enclosed in a structure made of plywood and 3.2 mm hardware cloth as required by the NMDA permit. Effluent from the first-stage flowed by gravity to the Azolla-prawn pond. The Azolla-prawn pond was an earthern pond having dimensions 3.05 m x 5.50 m x 0.92 m with side slopes 1:1. A 10-mil, clear polyliner was installed in the pond bottom to prevent infiltration of water. The liner was covered at the base with 10.2 to 15.2 cm of fine sand. The final volume of the pond, when filled to a depth of 0.76 m, was 8,270 L and the effective surface area was 15.6 m^2.

The leachfield was designed to treat a daily flowrate of 757 L. The trenches were excavated to a depth of 1.2 m and were back-filled with 5.1 cm of gravel to a depth of 15-30 cm, and to the surface with the natural soil. About 32.0 m of 10.2 cm diameter perforated PVC pipe was installed above the gravel in a 14.5 m^2 bottom trench area.

The temperature control in the greenhouse was inadequate for maintainence of desired water temperature levels in the Azolla-prawn pond, thus a pond heating system was installed. A clear 10-mil poly enclosure was installed on wooden supports over the pond to reduce nighttime surface heat loss and to maximize daily solar gain. A closed loop, thermostat-controlled electric heating system was provided to compensate for winter cloud cover and potential greenhouse heating failure. The heating system consisted of a standard electric hot water heater (AO Smith Model EES-30-910); a 30.5 m length, high temperature flexible mm tubing; a Milton Roy recirculating pump (Model No. CP-3B-1A); and an immersion-type thermostat controller (Penn Controller Series A19 variable set -29 to 38°C). The flexible tubing was coiled and placed in the Azolla-prawn pond and the thermostat was installed in a PVC pipe boot which was immersed in the water. The pond heating system effectively controlled temperature even during periods when outside temperatures dropped below -4.0°C.

Cooling the Azolla-prawn pond was a problem in the summer when temperatures in the greenhouse would exceed 40°C. Minor changes in the greenhouse were performed to increase cooling efficiency; however, under some extreme conditions, ice blocks were added directly to the pond water.

Aeration was also added to the Azolla-prawn pond subsystem. The aeration system consisted of a Montgomery Ward Heavy Duty Compressor (Model No.X346418) connected to an expansion tank consisting of three 6,425 L steel medical oxygen bottles. The expansion tank was connected to a Bridgeport tire valve regulator (0-2.07x10^6 pa), that controlled the outlet pressure to the diffusers in the pond. The air flowed through an activated carbon filter and then through tubing to two fine bubble diffusers. The airflow was measured by a Gilmont Instruments Flowmeter (Model No. E-4539) and was maintained at about 200 ml/min.

Freshwater prawns were purchased from Blue Lobster Farms in Fresno, CA. About 1,200 freshwater prawns consisting of about 200 juveniles (1.0 gm) and 1000 post-larval (<0.1 gm) prawns were stocked at a rate of 22-32 per available square meter. The prawns were fed a sinking-feed ration (Purina Catfish Chow 32 percent protein) at a rate of about four percent of the total prawn weight.

Start-up of the system was initiated upon completion of construction. The hyacinth tanks were filled with tap water and hydrostatically tested, then filled and drained several times to flush chemical residues leached from the plastic liner. Initially, tap water plus a plant fertilizer and 10 plants were introduced into each tank. The plants were allowed to acclimate under these conditions until the influent flow system was completed. The filtered secondary sewage replaced the commercial fertilizer. All systems were operated for two months prior to beginning experimentation to allow complete process stabilization and to evaluate the need of any additional environmental support-system requirements.

Experimental Pilot-Plant Operation

In order to evaluate the treatment efficiency of the proposed polyculture system, the pilot plant was operated semi-continuously over a period of eight months. Normal daily operation consisted of filling the wastewater storage tanks, adjusting pumps and flowrates, taking temperature measurements, feeding the prawns and performing several maintenance activities. These operations were performed between 8:30 and 10:00 a.m. to take advantage of lower suspended solids contained in the effluent from the treatment plant.

Analysis of the performance of the system was investigated during the eight months of operation. The experimental program was designed to evaluate the polyculture system by monitoring water quality at critical points in the system. These analyses were performed daily with some parameters determined onsite while other parameters were determined in the laboratory.

Initial operation of the system consisted of feeding the filtered secondary wastewater to the first-stage hyacinth tanks bypassing the effluent directly to the leachfield. This mode of operation was designed to evaluate the first-stage treatment efficiency without jeopardizing the survival of the prawns. Thus, water quality parameters, including NH_3-N, NO_3-N, Org-N, Orth-P, and COD, could be evaluated without potential threat to the prawns. This mode of operation also represented a potential alternative to developing a culture system that could use biomass produced from sewage but avoid the obvious public health problems associated with growing foodstuffs directly in municipal wastewater.

To evaluate the efficiency of combining the first and second-stages of the treatment process, a series of analytical experiments were initiated. The system was run continuously over this time, except for two periods when mechanical failures forced shutdowns. A series of test runs were used to evaluate the system's ability to treat various NH_3-N concentrations. Because of the long detention times through the system, NH_3-N loading (kg/ha-d) was used to evaluate the two-stage process. The criteria for each of the five load levels are presented in Table 1.

The variation in loading rates for the first and second test runs were created by increasing the flowrate. For the third and proceeding test runs, analytical-grade ammonium chloride was added to the secondary effluent to increase the NH_3-N loading rate, but the flowrate remained relatively constant at 894-922 L/day. Washout of the system through decreased hydraulic resident time could have been detrimental to system performance without necessarily reflecting the influence of the NH_3-N loading rate. Thus, the addition of ammonium chloride was used in all the remaining test runs to increase the NH_3-N loading rates without significant alteration of the flowrate.

Water Quality Analysis. The following section outlines the analytical procedures used to determine water quality parameters measured in the field and in the laboratory. Water samples for the evaluation of the two-stage polyculture system were taken at three separate locations (see Figure 1) once per day and at approximately the same time each day. The first sample

Table 1

Criteria for the analysis of the polyculture system for each of the five sample runs

		STAGE ONE			STAGE TWO		
Test Run Period	No. of Days Sampled	Average Flowrate (L/day)	Average Detention Time (days)	Average NH_3-N Loading (kg NH_3-N/ha-d)	Average Flowrate (L/day)	Average Detention Time (days)	Average NH_3-N Loading (kg NH_3-N/ha-d)
1	10	582	4.3	11.6	541	10.1	0.12
2	8	903	2.8	13.8	841	6.5	0.15
3	6	922	2.7	26.7	853	6.4	1.3
4	6	915	3.0	60.3	872	6.3	6.0
5	6	894	2.8	76.1	802	6.8	1.4

site was located at the discharge side of the metered feed pumps. The second sample location was the discharge pipe from the first-stage hyacinth system, and the third sample site was located in the discharge well of the Azolla-prawn system. Two polyethylene 2-liter acid-washed containers were used to collect each sample. Each sample container was rinsed with distilled water before being gently filled with the sample. One of the sample bottles was sealed and placed in an ice cooler for transport to the laboratory. The second sample was used for the water analyses determined on site. In analyzing the samples, duplicate analyses were performed on each sample. Because of the variation associated with COD test procedures, triplicates on COD were performed for each sample.

pH. The pH of various samples was taken daily in the field using a Hach model 19,000 pH meter.

Specific Conductance. The specific conductance of water samples was measured in microhms/cm using a temperature-compensating Hach Drel/5 conductance meter.

Water Temperature. Water temperatures of the tanks, pond and water samples were taken with Yellow Springs Instrument (YSI) combination oxygen-temperature meter with probe.

Air Temperature. Air temperatures were recorded in the field as high-low temperatures measured in °C using Sybron/Taylor (model 5458) maximum/minimum self-registering thermometer.

Ortho-Phosphorous. Ortho-Phosphorous was measured using the Vanadomolybdophosphoric Acid Colorimetric Method, as outlined in Section 425D of Standard Methods (1976).

Alkalinity. The alkalinity of various samples was determined in the field by the samples with 0.02N H_2SO_4 to the Bromcresol-methylred end point, as outlined in Section 403 of Standard Methods (1976).

Carbon Dioxide. The carbon dioxide concentration was determined in the field using the titration method, as per procedures outlined in Section 407B of Standard Methods (1976).

Nitrate Nitrogen. Nitrate nitrogen in the range of 1-20 mg/L was determined, using the DeVarda's alloy method outlined in Section 419F of Standard Methods (1976) modified for use with a Bucchi Brinkman 420 distillation unit. Low concentrations of NO_3-N (<1.0 mg/L) were determined using the Hach low range cadmium reduction reagents.

Ammonia Nitrogen. Ammonia nitrogen was determined on samples collected in the field and analyzed the same day using the titrimetric method for high concentrations (1-50 mg/L) and the Nesslerization method for low concentrations (<1 mg/L), as per procedures outlined in Section 418D and Section 418B, respectively of Standard Methods (1976). For each method, distillation was achieved using the Buchi Brinkman model 420 distillation unit.

The procedures outlined in Standard Methods (1976) were modified by:

(1) Placing a 25-ml sample in the distillation flask with two boiling beads.

(2) Adding 5 mls of borate buffer and 2 drops of phenolphthalein indicator.

(3) Placing a collecting flask containing 10 mls of indicating boric acid solution on the distillate receiving tube.

(4) Distilling about 75-100 mls of sample.

(5) Running either the high or low level NH_3 method, depending on concentration expected.

Organic Nitrogen. Organic nitrogen was determined from the difference between total kjeldahl nitrogen (TKN) and NH_3-N. TKN was determined in accordance with the procedures outlined in Section 421 of Standard Methods, (1976) modified for use with the Bucchi Brinkman model 420 distillation unit and digestor.

Chemical Oxygen Demand. Both total and soluble COD were determined for each sample. Soluble COD was measured on a sample that had been filtered through a 0.45-micron glass fiber filter. COD determination was measured on a Hach micro digestor unit using procedures outlined by Gibbs (1982) and Hach Chemical Co. All reagents were prepared in accordance with the ratios described for the micro digestor. All samples were digested for two hours at a temperature of 150 °C. Colorimetric measurements were determined using a Bausch and Lomb Spectronic 21 spectrophotometer set at a wavelength of 620 nm. A distilled water blank was carried through the digestion and used to set the transmittance at 100 percent.

Dissolved Oxygen. The dissolved oxygen content of water samples from the pond and tanks were measured using a YSI Dissolved Oxygen Meter and Probe Model 57. The probe was standardized by saturating distilled water with bubbled air and measuring the dissolved oxygen concentration by the modified Winkler Method procedures, as outlined in Sections 422B and 422F of Standard Methods (1976).

Suspended Solids. Suspended solids concentrations were measured using Gooch crucibles and 2.1 cm glass fiber filters. The filters were dried at 103°C and weighed, as outlined in Section 208B of Standard Methods (1976).

Total Coliform. Total coliform bacteria were determined using the Standard Total Coliform MPN test as outlined in Section 908A of Standard Methods (1976).

Flowrates. Flowrates were determined in the field for the influent to the hyacinth system, the effluent to the hyacinth system, the effluent from the hyacinth system, and the discharge from the prawn pond using a graduated cylinder and a hand-held stopwatch. Triplicate measurements were averaged tc obtain each reading.

Biomass. Plant biomass and productivity were determined from sample quadrants, or sections, with known areas, that were installed in the hyacinth tanks and in the Azolla-prawn pond. Biomass was weighed with an Ohaus Toploading Balance model B1500 and an Ohaus Triple Beam balance. Water hyacinth biomass was weighed at about two week intervals. Azolla was weighed every three to five days. All weights were measured as wet weights, which was the weight of the collected biomass after draining the excess water for five minutes. Plant dry weights were calculated from net weights assuming a 95 percent water content. Plant productivity, on a biomass basis, was measured by determining the differences between the biomass measurements and averaging over the time span between measurements. Productivity was recorded on a kg or gm (dry wgt)/m^2/day basis. Plant-N uptake was determined by assuming the percent nitrogen content of Azolla and water hyacinth to be 4.5 and 3.5, respectively (McClure and Lipinsky 1981).

Biomass and productivity measurements of the freshwater prawns were determined in the field and in the laboratory. The prawns were collected and set on absorbent toweling for about two minutes and then weighed in a tarred beaker of water and returned to the pond. Nitrogen content of the prawns was calculated using a protein content of about 60 percent, or 9.6 percent nitrogen, and a water content of 90 percent. Excretion values of nitrogen for prawns were available; however, calculation indicated these values to be of little consequence for prawns sized 10 gms or less (fresh wgt).

Prawn Growth Study

Prawn growth studies were performed in the laboratory and in the field. The field measurements were obtained from the prawns growing in the second-stage Azolla-prawn system and were used to determine the amount of nitrogen fixed in the system by the prawn population. The laboratory prawn growth studies were performed in an environmental chamber to assess the value of ground-up water hyacinth as a feed source for freshwater prawns. These studies were designed to determine whether processed water haycinth could provide 100 percent of the prawn diet, or some lower percentage when mixed with a commercial feed. These studies were carried out in aquaria located in an environmental control chamber (Forma Scientific, Inc. Model B8-128R Walk-In Environmental Room). Temperature in the aquaria was maintained at about 28 °C. The aquaria were 75.7 L in volume, all glass equipped with single air stones delivering about 1.4 L/min of air. Each aquarium was covered with plastic mesh to prevent the escape of juvenile prawns and each aquarium contained about 2.5 cm of fine washed river sand for bottom substrate.

The prawns were stocked at a rate of 4 to 8 prawns per aquarium, or 17 to 35 prawns/m^2, depending on the size of the prawns. Small juveniles, less than 1.0 gm, were stocked at 7 to 8 per aquarium while larger (1.0 gm and greater) prawns were stocked at 4 to 6 per aquarium. The final stocking rate was determined on a trial and error basis three to four days prior to the beginning of the experiment. Too many prawns in the aquarium would cause death in excess of 30 to 40 percent within two days. Once the stocking rate was ascertained and the prawns were acclimated, initial weights were determined. In all aquaria, total prawn weight was used rather than individual prawn weights; thus statistically each aquarium was a treatment. After 10 to 12 days, final weights were determined and were used to evaluate the diets. For each of the diets, the amount of pellet or ground hyacinth was weighed daily and the amounts fed were adjusted for increases in prawn biomass. During the study period the prawns were fed daily and distilled water was added to keep aquaria water levels at a constant mark.

Two complete experimental runs were performed with each run being replicated and within each replicate there were five aquaria per treatment.

The first experiment was operated as a nonrandomized block design with three treatments and 30 observations in the data set. Average prawn size over the study period ranged from 2.13 gm to 3.45 gm (fresh wgt). Each aquarium contained about five prawns or a stocking density of 22 prawns/m^2.

For the first experiment, the prawns in Treatment A received the 100 percent commercial pellet diet (Purina Catfish Chow) at a rate of 25 percent of body weight. The Treatment B diet consisted of 50 percent commercial pellet and 50 percent dried ground hyacinth each fed at a level of 25 percent of body weight. The Treatment C diet consisted of 100 percent dried ground hyacinth fed at a rate of 25 percent of total body weight.

The second experiment used a randomized block design with 3 treatments and 30 observations. The randomized block design was employed in an effort to increase the sensitivity of the statistical analysis.

Average prawn weight over the experimental period varied from 0.4 to 0.9 gm and each aquaria averaged about seven prawns, or a stocking density of 30 prawns/m^2. The prawns in the second study were smaller and were stocked at a higher rate; however, the biomass in each aquarium was greater in experiment 1 than in experiment 2. On the average, an aquarium in experiment 1 contained about 11.7 to 18.1 gm and in experiment 2 an aquarium contained 3.5 to 4.6 gms of prawn biomass.

In experiment 2, a lower feed rate of 15 percent of total body weight of prawn was used. In addition, the combined feed did not double this rate as in experiment 1. Treatment A received a 100 percent commercial pellet diet throughout the two replication periods. Treatment B received a 50 percent commercial pellet, 50 percent ground hyacinth diet with each constituent fed at 7.5 percent of body weight. Treatment C received 100 percent ground hyacinth throughout the study period. The results of the laboratory prawn growth studies were analyzed statistically for comparative purposes using the Least Significant Difference (LSD) technique assessed by the Statistical Analysis System (SAS).

In the field, freshwater prawns were stocked at a density of 24 prawns/m^2. Mortality at stocking was estimated at less than two percent and no further mortalities were observed over the first 24 hours. Five hundred, 0.1 gm prawns, were stocked and after mortality the <u>Azolla</u>-prawn system was estimated to contain 48.5 gm (fresh wgt) of prawns.

RESULTS AND DISCUSSION

The System

This section presents the results and discussion of the performance evaluation of the polyculture system including the nitrogen balance. This evaluation is presented in two sections, the first-stage water hyacinth system and the second-stage *Azolla*-prawn system.

First-Stage Water Hyacinth System. Variation of NH_3-N loading was the major variable in the analysis of the treatment performance of the first stage of the polyculture system. The nitrogen loadings were determined by measuring the influent concentration and flowrate to the hyacinth system. The NH_3-N loading rates were varied over five sample periods in order to evaluate the systems capability to remove NH_3-N, NO_3-N, ORG-N, and total-N.

Nitrogen Loadings and Removal. Flowrates served to produce the mass loading data used to evaluate the system performance and gave accurate measurements for the water budgets of the first-stage water hyacinth system. Figure 2 shows the influent and effluent flowrates for the hyacinth system. Influent flowrates ranged from 582 to 922 L/day over the sample periods (table 1 and fig. 2). While effluent flowrates varied from 541 to 872 L/day (fig. 2). Losses of water in the hyacinth system were due primarily to evapotranspiration, since no seepage occurred in the hyacinth tanks. From the average influent and effluent flowrates the net water lost by the hyacinth system was calculated to be 41, 62, 69, 43, and 92 L/day for sample runs 1-5, respectively. These evapotranspiration rates appeared to fluctuate with flowrate into the system, but many other factors such as plant density, relative humidity, plant growth rates, and wind velocity, water and air temperatures can contribute to evapotranspiration making any simple correlation between factors unlikely.

The average NH_3-N loading rates for each of the five sample run periods are presented in Table 2 and illustrated in Figure 3. As shown in Table 2, the influent NH_3-N loading rates ranged from 4,742 to 31,214 mg-N/day for sample periods 1-5, respectively. The effluent discharge ranged from 180 to 16,161 mg-N/day for these same periods. As shown in Table 2, NH_3-N removal efficiency progressively decreased from 96 to 48 percent for sample periods 1-5, respectively. While these removal efficiencies could be considered excellent for some wastewater treatment applications, it is doubtful that

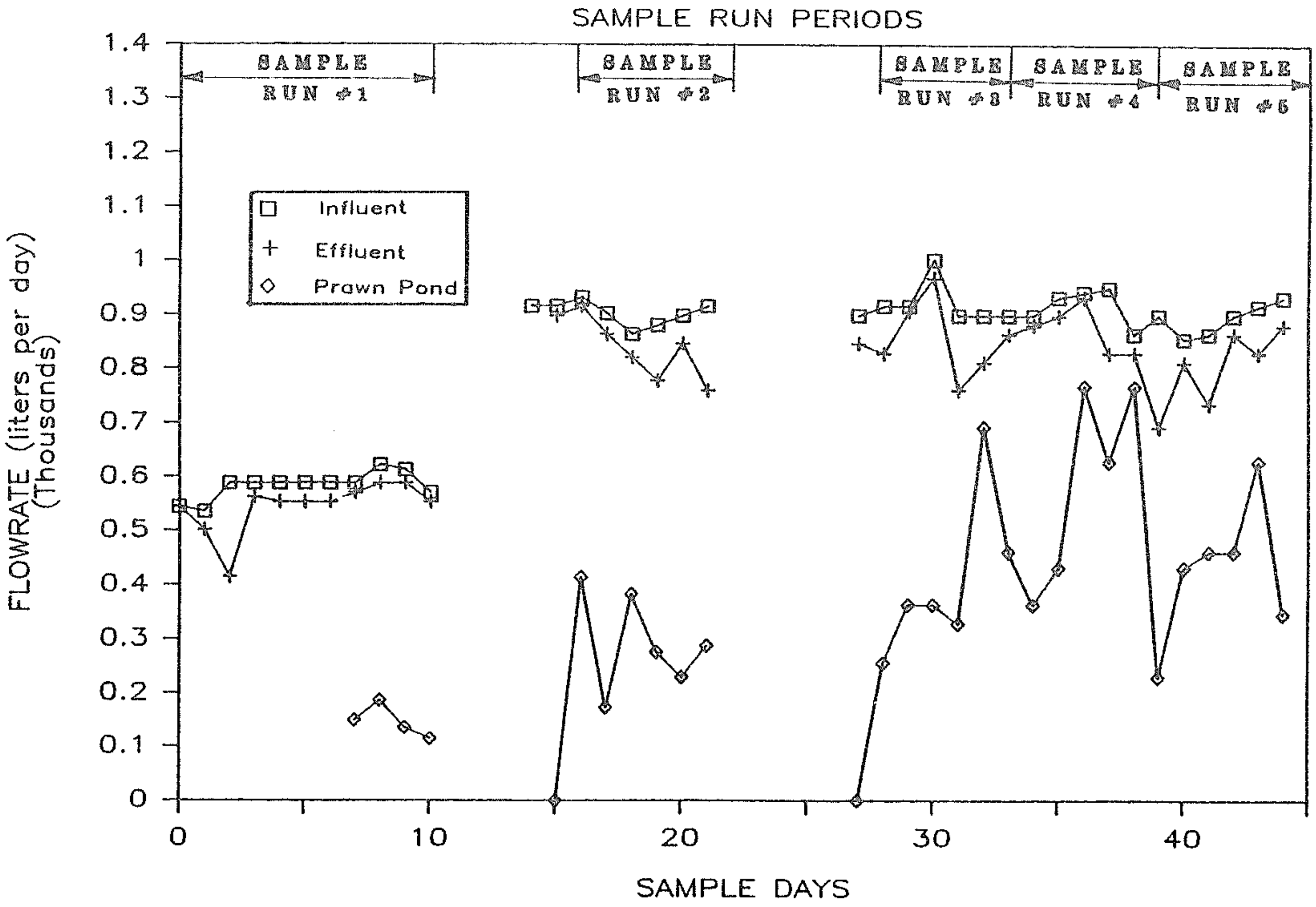

Figure 2. Flowrates for the polyculture system

Table 2
Nitrogen mass balance data for the first-stage water hyacinth system

Sample Run Period	Influent (mg-N/day)			Effluent (mg-N/day)			Percent Removed		
	NH_3-N	NO_3-N	ORG-N	NH_3-N	NO_3-N	ORG-N	NH_3-N	NO_3-N	ORG-N
1	4742	945	1944	180	307	494	96	68	75
2	5659	2644	4556	234	522	496	96	80	89
3	10964	760	8922	1984	418	496	82	45	94
4	24714	3105	5665	9317	541	720	62	83	87
5	31214	2065	5638	16161	1562	283	48	24	95

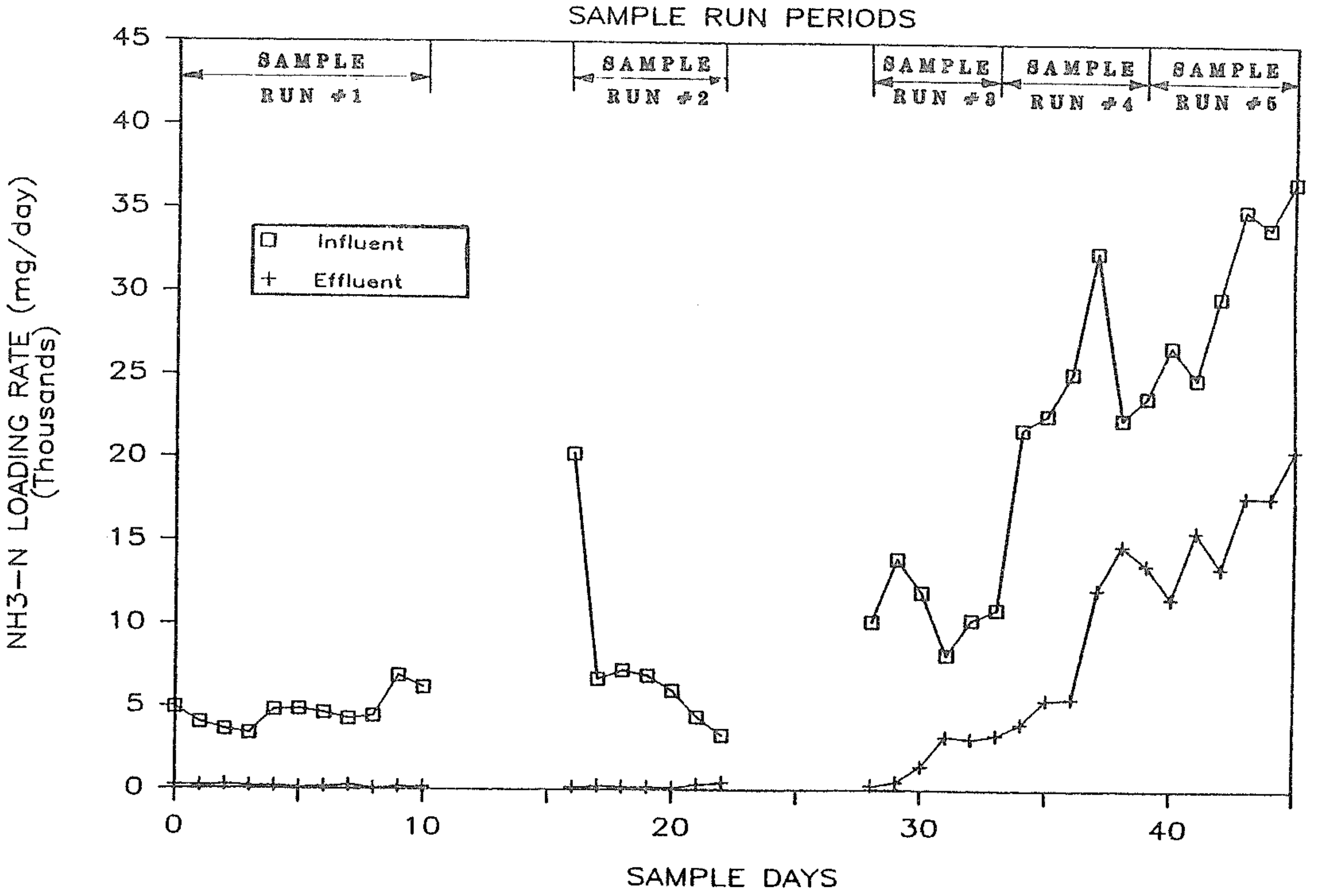

Figure 3. Ammonia nitrogen loading rates for the first-stage hyacinth system

many aquaculture systems would be adequately served by these NH_3-N removals, particularly at high pH values where the concentration of un-ionized NH_3 could exceed lethal concentrations.

The highly variable loading rates and concentrations of NO_3-N and ORG-N in the influent were caused by a variation in the natural load discharged by the Las Cruces treatment plant. The influent loads for NO_3-N and ORG-N were not altered by supplemental additions of NH_4Cl. Loadings rates for influent and effluent NO_3-N are presented in Table 2. Loading rates of NO_3-N fluctuated between 945 to 3,105 mg-N/day. Effluent values for the five sample periods ranged from 307 to 1,562 mg-N/day, resulting in NO_3-N removal efficiencies ranging from 24 to 83 percent (table 2). The variations in removal efficiencies indicate that either NO_3-N removal was inconsistent or that NO_3-N was highly dynamic, representing the transient form for the major removal pathways of nitrogen in the system.

The influent ORG-N loading ranged from 1944 to 8922 mg-N/day while the effluent discharge ranged from 283 to 720 mg-N/day (table 2). Removal efficiencies fluctuated between 75 to 95. Removals efficiencies were all above 75 percent, indicating high retention of ORG-N in the system.

Figure 4 shows the influent and effluent NH_3-N concentrations for the first-stage water hyacinth system. Table 3 summarizes the average concentrations of the various nitrogen species over the five sample periods. The average influent NH_3-N concentration for sample periods 1 and 2 was 8.4 mg/L. The NH_3-N concentration was then sequentially increased through the next three sample periods from 12.0 to 34.8 mg/L. Throughout the five sample periods, the effluent NH_3-N concentration progressively increased from 0.3 to 20 mg/L. Removal efficiencies for NH_3-N, on a concentration basis, were similar to those based on mass balance and ranged from 43 to 97 percent.

Concentrations of NO_3-N for the first-stage water hyacinth system are shown in Figure 5. Average NO_3-N concentrations for each sample period in Table 3. Average influent NO_3-N concentrations varied between 0.8 and 3.4 mg/L, while effluent NO_3-N concentrations were generally below 2.0 mg/L throughout the five sample periods.

The concentrations of ORG-N for the hyacinth system are shown in Figure 6. Average concentrations for each of the five sample periods are presented in Table 3. Influent concentrations varied widely, and depended highly on the

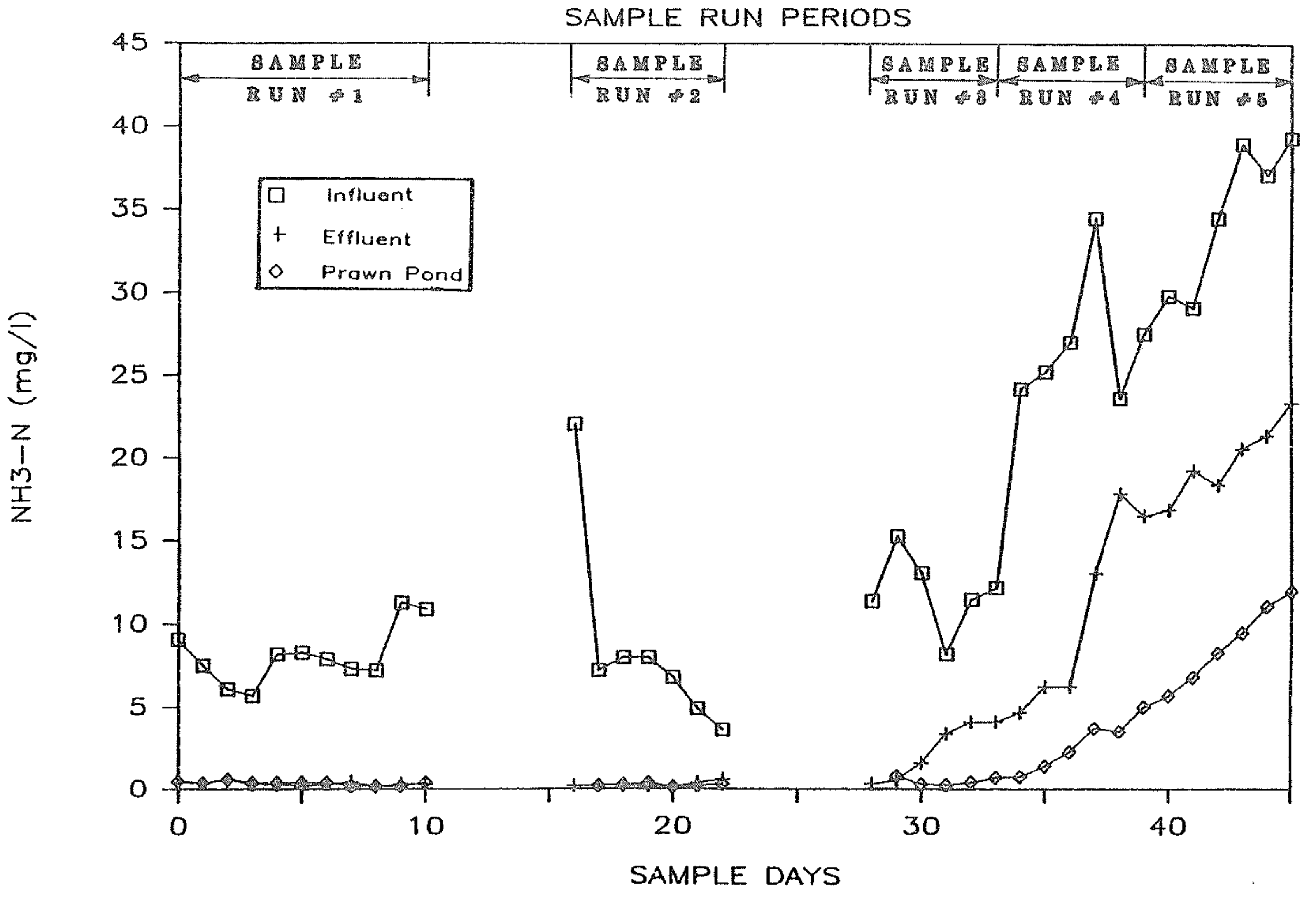

Figure 4. Ammonia nitrogen concentrations in the polyculture system

Table 3
Average nitrogen species concentration data for the first-stage water hyacinth system

Sample Run Period	Influent mg/l			Effluent mg/l			Percent Removed		
	NH_3-N	NO_3-N	ORG-N	NH_3-N	NO_3-N	ORG-N	NH_3-N	NO_3-N	ORG-N
1	8.1	1.6	3.4	0.4	0.6	0.9	95	63	73
2	8.7	2.9	5.1	0.3	0.6	0.6	97	79	88
3	12.0	0.8	8.0	2.4	0.5	0.5	80	38	94
4	27.0	3.4	6.3	10.8	0.6	0.8	77	77	87
5	34.8	2.3	6.6	20.0	2.3	0.3	43	0.0	96

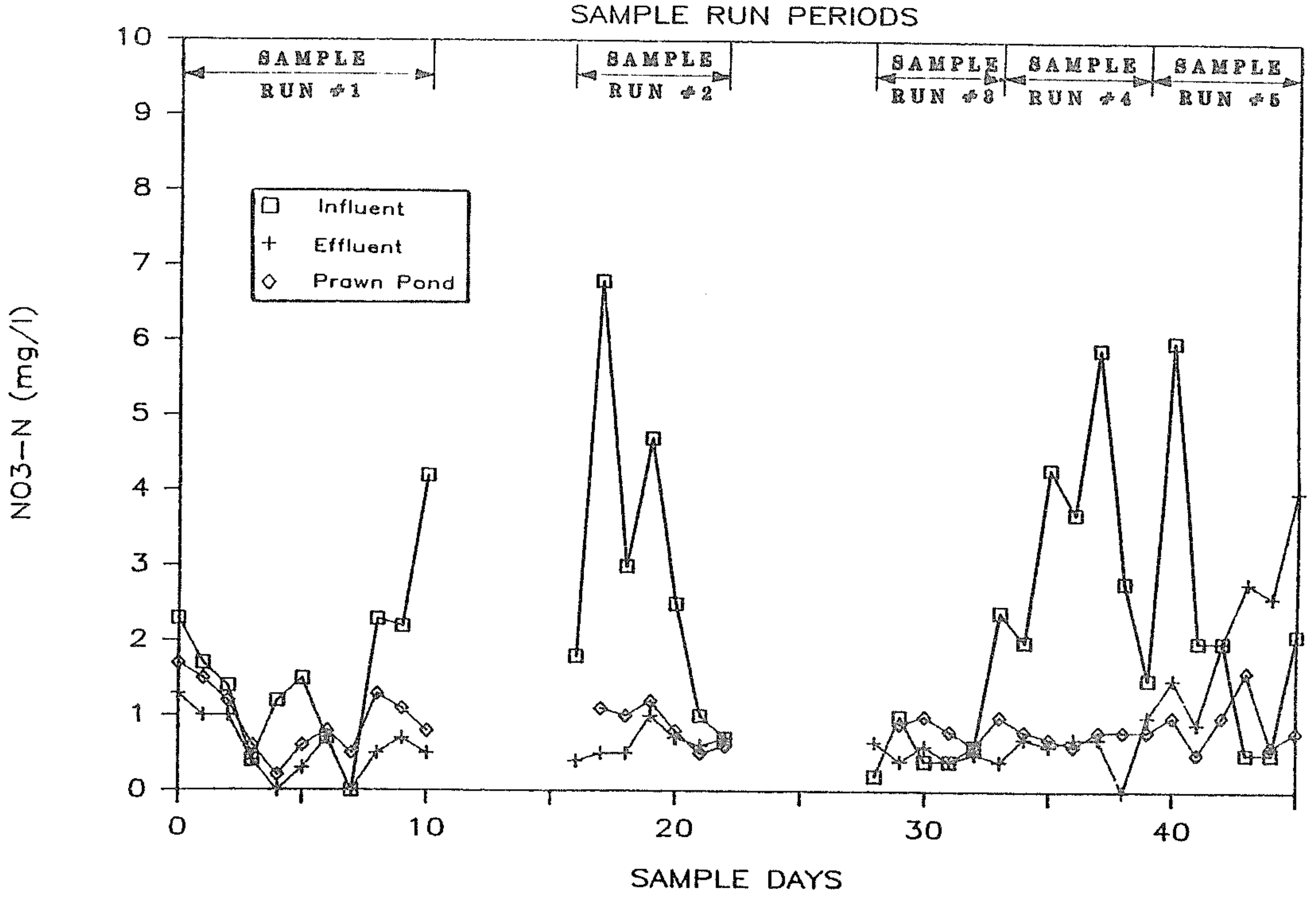

Figure 5. Nitrate nitrogen concentrations in the polyculture system

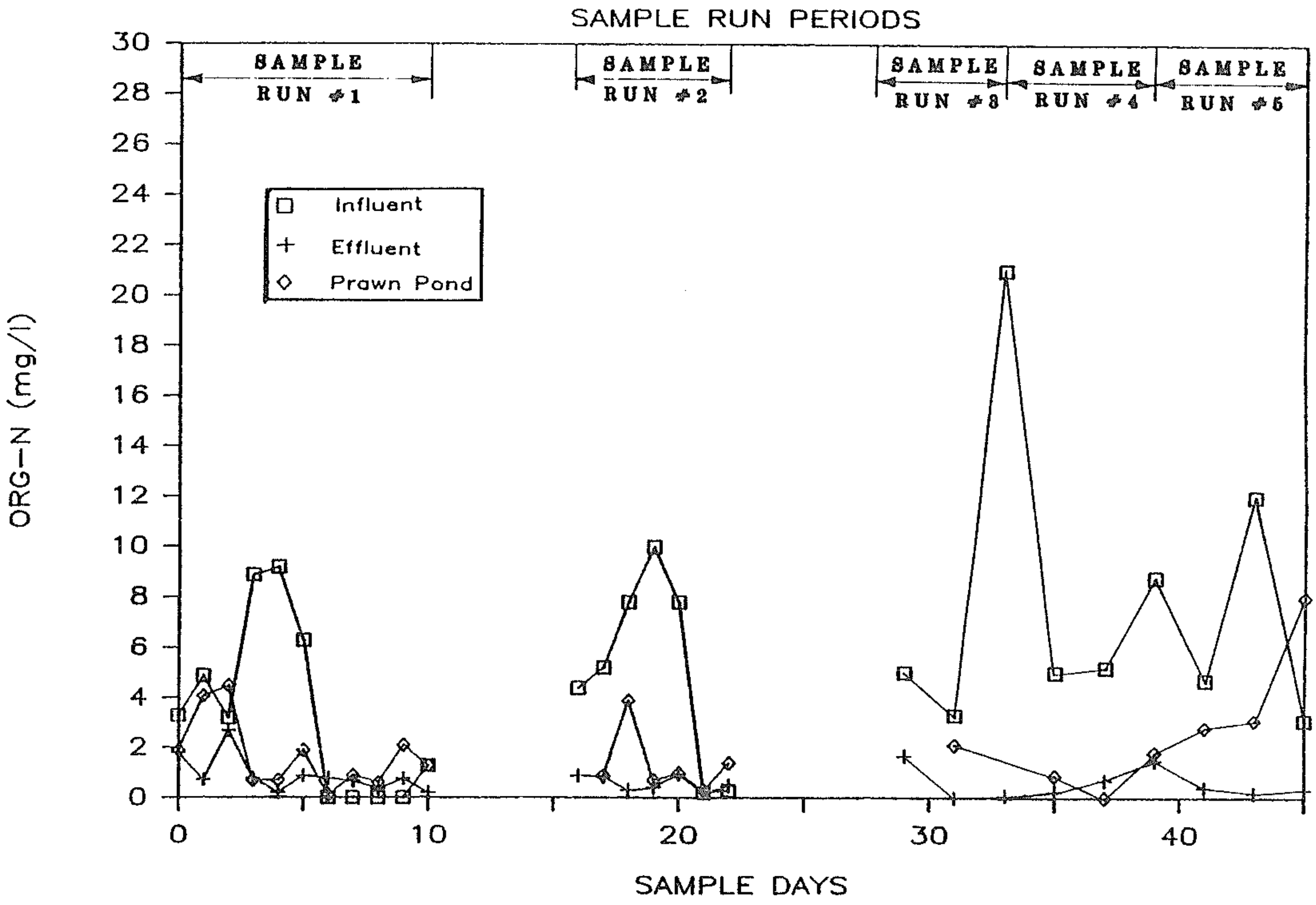

Figure 6. Organic nitrogen concentrations in the polyculture system

quality of the treatment plant effluent. Good quality secondary effluent from the Las Cruces treatment plant was only produced during the latter part of the first sample run period. In general, the treatment plant performance was poor and the solids in the influent to the hyacinth system were only partially controlled by the preceeding sand filter. Thus, contributions of ORG-N to the system were higher than expected; however, they were consistently high throughout the study.

Nitrogen Mass Balance. Evaluation of the nitrogen mass balance data in the hyacinth system is presented in Table 4. This data was developed to identify the potential mechanisms for nitrogen flow into and out of the hyacinth system. The total-N flow to the system varied progressively over the five sample periods from 7,631 to 38,917 mg-N/day, while the outflow ranged from 981 to 18,006 mg-N/day. These values represent total-N removal efficiencies ranging from 54-90 percent. Several mechanisms of nitrogen removal have been suggested for water hyacinth systems; including plant uptake, nitrification, denitrification, simple sedimentation, and NH_3-N volatilization (Ferrara and Harleman 1978, Reddy 1982, Reed 1985).

Plant-N uptake, assumed to be equal to the amount of growth by the plant each day in terms of the plants percent nitrogen, is shown as an average value for each of the five sample periods in Table 4. Figure 7 compares the plant-N uptake with total-N lost to the system over the five sample periods. For sample period 1, plant-N uptake accounted for more than 100 percent of the nitrogen removed by the system. Thus, the nitrogen loading in the first sample period was 100 percent taken up by the plant. In the second sample period, plant-N uptake dropped to 44 percent of the total-N lost to the system, leaving 56 percent of the nitrogen unaccounted. In the third sample period, plant-N uptake accounted for only 26 percent of the total-N removed, leaving about 74 percent of the nitrogen unaccounted. In the fourth and fifth sample periods, this trend continued with plant-N uptake accounting for only 20 and 22 percent, respectively, of the net nitrogen lost to the system. The nitrogen unaccounted for in the fourth and fifth sample periods was 80 and 78 percent, respectively. For the five sample periods, plant-N uptake accounted for an average of 42 percent of the total-N removed by the system. Other investigators have reported that plant uptake has accounted for 35 to 40 percent of the total inorganic-N removed by a hyacinth system (Reddy, 1982). These studies did not consider ORG-N and, thus, may underestimate the actual total-N percentages.

Table 4
Fate of nitrogen mass in the first-stage water hyacinth system

Sample Run Period	Influent Total-N (mg-N/day)	Effluent Total-N (mg-N/day)	Net Loss[a] to the System (mg-N/day)	Total-N Uptake[b] by Plant (mg-N/day)	Unaccounted[c] for Total-N (mg-N/day)	System Total-N Removal %
1	7631	981	6650	7413(111)	-763(11)	87
2	12859	1253	11606	5130(44)	6476(56)	90
3	20646	2898	17748	4535(26)	13213(74)	86
4	33484	10578	22906	4535(20)	18371(80)	69
5	38917	18006	20911	4535(22)	16376(78)	54

[a] Net loss to the system = Influent - Effluent.

[b] Number in parenthesis = % TN uptake by plant.

[c] Unaccounted for TN = net lost to system - plant uptake.

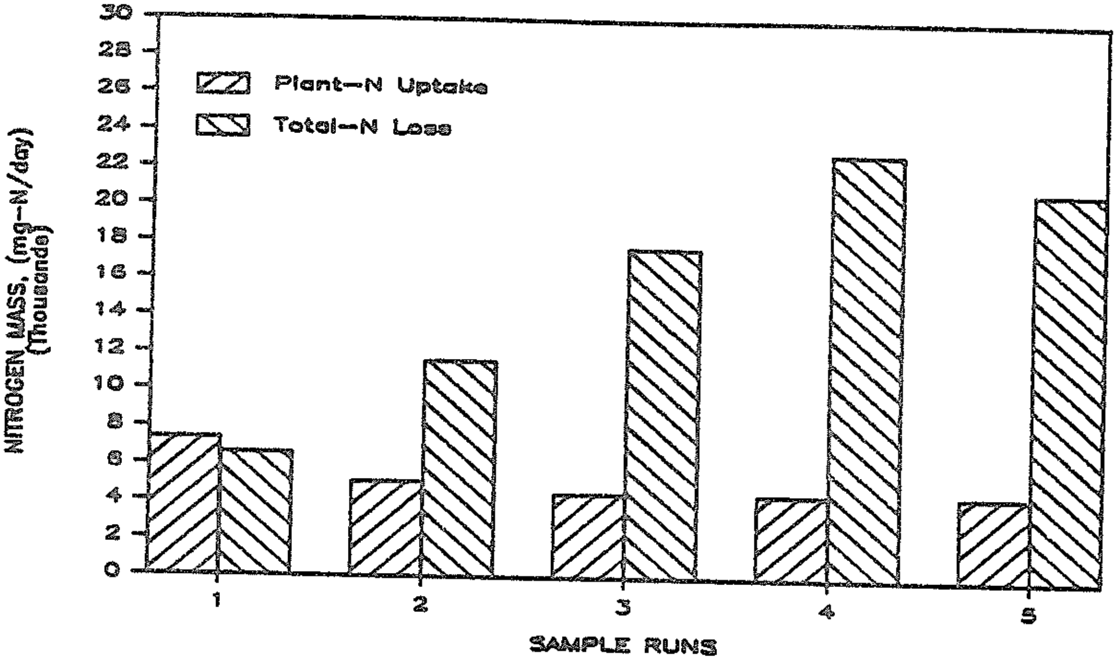

Figure 7. Comparison of Plant-N uptake and Total-N loss in the first-stage water hyacinth system

It was apparent that as nitrogen loading increased, the role of plant-N uptake decreased, and other mechanisms of nitrogen removal were enhanced by the presence of the water haycinth and the environment it creates.

Nitrification-denitrification has been suggested by several investigators as the major pathway for nitrogen removal in aquatic-plant based systems. Reddy (1982) surmised that in hyacinth systems 45 to 52 percent of unaccounted for inorganic-N was lost through nitrification-denitrification. Nitrification and denitrification are the major processes which directly affect the alkalinity of the first-stage water hyacinth system. Thus, the quantification of the alkalinity in the system could estimate the relative activity of these processes. The alkalinity data for the hyacinth system, shown in Figure 8, indicates that the effluent alkalinity was consistently lower than the influent, with an average reduction in alkalinity through the hyacinth system of 14.6 percent on a concentration basis. Calculated values of alkalinity on a mass balance basis through the system indicated an average reduction of 21.1 percent. Since alkalinity is a conservative property, the difference between the influent mass and effluent mass could be considered alkalinity consumed by the system. It would thus appear that processes resulting in the consumption of alkalinity such as nitrification and denitrification, are active in the first-stage water hyacinth system.

The process of nitrification can proceed at O_2 concentrations as low as 0.3 mg/L, but is considered to be slowed because each mg of NH_3-N oxidized to NO_3-N requires 3.84 mg of O_2 (Barnes and Bliss 1983). Measurements of the DO profile in the hyacinth system were highly variable but indicated that near the top of the roots, at the water surface, the DO varied between 1.3 and 2.8 mg/L. As the depth increased to 15 cm, the DO dropped to 1.0 mg/L, and the DO just above the pond bottom (61 cm) was 0.70 mg/L. The root mass penetration of the water column was variable, but generally extended 15-50 cm. Thus rates of nitrification could have been limited in the non-aerated hyacinth system. This suggestion is substantiated by the findings of Weber and Tchobanoglous (1983), that indicated the NH_3-N oxidation rates were almost doubled in aerated hyacinth systems over non-aerated systems. Aerated hyacinth systems were shown to remove 99 percent of influent NH_3-N compared to 70 percent removals in non-aerated hyacinth systems at a pilot scale wastewater treatment facility (Hauser 1984). In the pilot scale system studied under the present research, not only

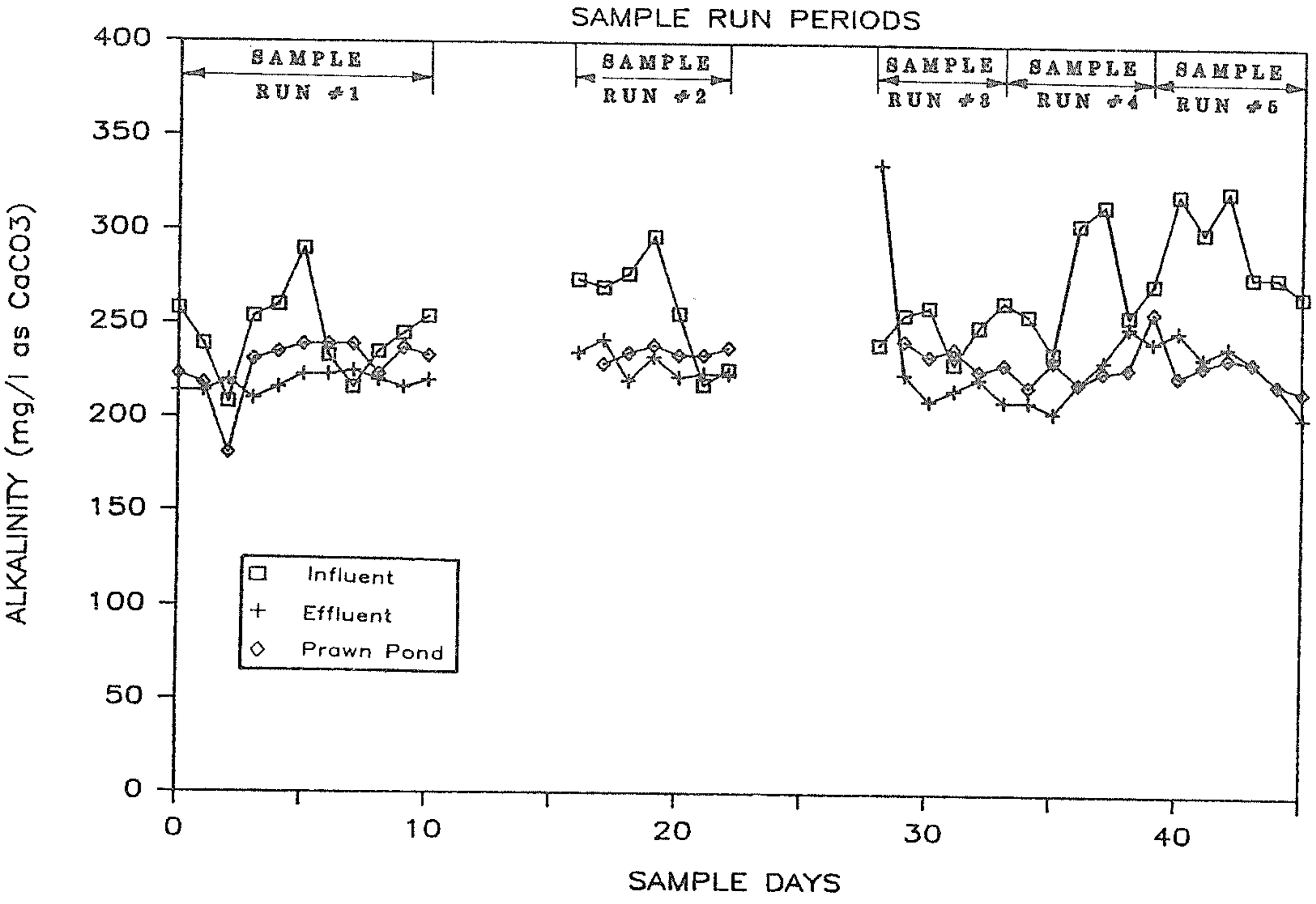

Figure 8. Alkalinity (as $CaCO_3$) for the polyculture system

was the removal of NH_3-N high, but detention times were about only three days, indicating that the process of nitrification was rapid.

In non-aerated hyacinth systems, such as the one studied in this research, the process of nitrification can be envisioned as a stop-start process, which may waiver as the DO profile in the system rises and falls from the top of the root mass to the bottom of the pond. The environment of the hyacinth roots could provide ready sites for nitrifier attachment. Nitrifiers are obligate aerobes, they are not drastically affected by O_2 changes, but would cease converting NH_3-N when O_2 is unavailable. If nitrification was limited, then there should be larger proportions of NH_3-N than NO_3-N in the system, or NO_3-N removal efficiencies would be highly variable. In the effluent of the first-stage water hyacinth system, NO_3-N concentrations were quite unpredictable with the fifth sample period having the highest NH_3-N loading rate and the worst NO_3-N removal efficiency of 24 percent. However, sample period four with the next lowest NH_3-N loading rate, was able to achieve 83 percent NO_3-N removal. Nitrate removal followed no obvious pattern in this system.

Denitrification, the coupling reaction for complete removal of nitrogen from the first-stage system, is carried out by various facultative microbes. When O_2 is available, these microbes use it as a terminal electron acceptor and when O_2 is absent NO_3-N is used as the terminal electron acceptor. Oxygen is preferred energetically, but whether O_2 is present or not, these microbes are active. Denitrification is inhibited by O_2 fluxes, but can take place in a hyacinth system that has anaerobic zones varying from within the roots of the floating plants to the bottom of the pond to a zone just within the surface layer of the pond sediments. Under these fluctuating conditions, denitrifier populations could rapidly remove NO_3-N while it is present and O_2 is absent. As O_2 levels rise, denitrification rates would slow down without destruction of the denitrifying populations. In a recent study investigating the maximum kinetic rates of denitrification in eutrophic lake sediments, parameters were developed that indicated a maximum NO_3-N transformation rate of 1.09 gm NO_2-N formed per gm of NO_3-N consumed per gm of wet sediment per hr (Messer and Brezonik 1984). Other studies of water hyacinth systems indicated that as much as 3-5 gm NO_3-N/m^2/day could be removed via denitrification (Debusk, Williams, and Ryther 1983).

In a hyacinth system, the coupled nitrification-denitrification process may be limited by the nitrification step along with the rate of diffusion of

NO_3-N to the anaerobic regions of the pond. However, the environment of the root mass could provide sites of attachment which, when the prevailing conditions are correct, could enhance either nitrification or denitrification. And while these rates may stop and start, when they are functioning they may be near optimum levels of substrate removal, thus, affecting nutrient removal at a rapid rate.

Volatilization, which is the physical transport of NH_3-N (gas) from the aqueous pond to the atmosphere, has been suggested as a major nitrogen removal pathway in some algal based lagoon systems (EPA 1975, Ferrara and Avci 1982, Reed 1985). This process is regulated by the total NH_3-N concentration, temperature, pH and the partial pressure of NH_3-N in the air adjacent to the water. Although the actual importance of volatilization as a removal mechanism has recently been questioned, the process has been suggested to remove between 35 and 85 percent of the NH_3-N in raw wastewater lagoons (EPA 1975). Reed (1985) has suggested that the pH variability and the long detention times of 20 to 80 days or more in algal based lagoons provide sufficient environments for volatilization to account for a large percentage of total-N removed in these systems. Volatilization as a mechanism in the hyacinth system studied by the present research probably plays a minor role at best. The detention time through the system was three to four days and the pH averaged 6.9. At a pH of 6.9 and a temperature of 25°C, the percent un-ionized NH_3-N is only 0.45 percent, which would not result in a large loss of NH_3-N from the system.

Examination of the sedimentation process indicates that it is probably a much more complex process in a hyacinth system then in algal based systems. Small particles may settle, according to Stokes' law, in the traditional mode of discrete sedimentation theory. These particles could also be joined by larger, more irregular pieces of roots, dead macro-organisms or other debris generated within the system and, instead of settling to the bottom, all or part of the particles could be retained or enmeshed in the haycinth root mass. Thus, the root mass would consist of a mass of living and dead material. The significance of this process is that the material suspended in the aerobic zone of the pond could easily decompose two or three times faster than the particles that settle to the anaerobic zone of the pond.

The root mass in this tertiary treatment system was not necessarily anaerobic and the aerobic zones may fluctuate up and down in the root mass depending on the BOD of the waste load, the surface wind velocity, the velocity of water through the ponds, and other criteria which may affect the DO profile in the system. Upon examining the nitrogen mass balance data in Table 4 and using the ORG-N mass loading data in Table 3, it becomes apparent that the removal of ORG-N by the system contributed between 21 to 47 percent of the total-N lost to the system. Thus, actual enmeshment-sedimentation removal processes can contribute more to total-N removal than plant-N uptake especially at the higher NH_3-N loading rates. For the five sample periods, the enmeshment-sedimentation pathway averaged 30 percent of the total-N removed by the first-stage hyacinth system.

Other Water Quality Parameters. Additional water quality parameters, besides nitrogen species, were monitored over the course of the experimental period. Othro-phosphorous concentrations are presented in Figure 9. The Ortho-P concentrations in the influent to the hyacinth system were highly variable, ranging from less than 10 mg/L to 60 mg/L. The highest concentrations occurred during the third, fourth, and fifth sample runs, where influent concentrations increased from 8 to 12 mg/L to 40 to 60 mg/L. The increasing concentrations of Ortho-P in the influent to the system could not be explained. The reagent-grade ammonium chloride added to supplement NH_3-N in sample periods 3-5 did not contain sufficient PO_4 (<0.0001%) to account for the increase. Thus, these high Ortho-P concentrations were due to the natural loading from the city treatment plant. The effluent concentrations of Ortho-P from the hyacinth system were consistently less than the influent; however, the effluent concentrations did increase as the influent increased. Over the study period, influent Ortho-P averaged 22 mg/L, and effluent Ortho-P averaged 17 mg/L, which represented a removal efficiency of 22 percent.

Specific conductance and pH of the hyacinth system were also determined. The data is presented in Figures 10 and 11, respectively. Influent levels for specific conductance increased from less than 1,100 to over 1,300 micromhos/cm. This increase of specific conductance was attributed to the spiked additions of NH_3Cl in the last three sample periods. First-stage effluent levels of specific conductance ranged from less than 1,000 to over 1,100 micromhos/cm over the five sample periods. The influent specific conductance averaged 1,206 microhoms/cm and the effluent specific conductance averaged 1,114 micromhos/cm, which indicated an eight percent reduction in specific conductance through the first-stage system. Values for pH in the

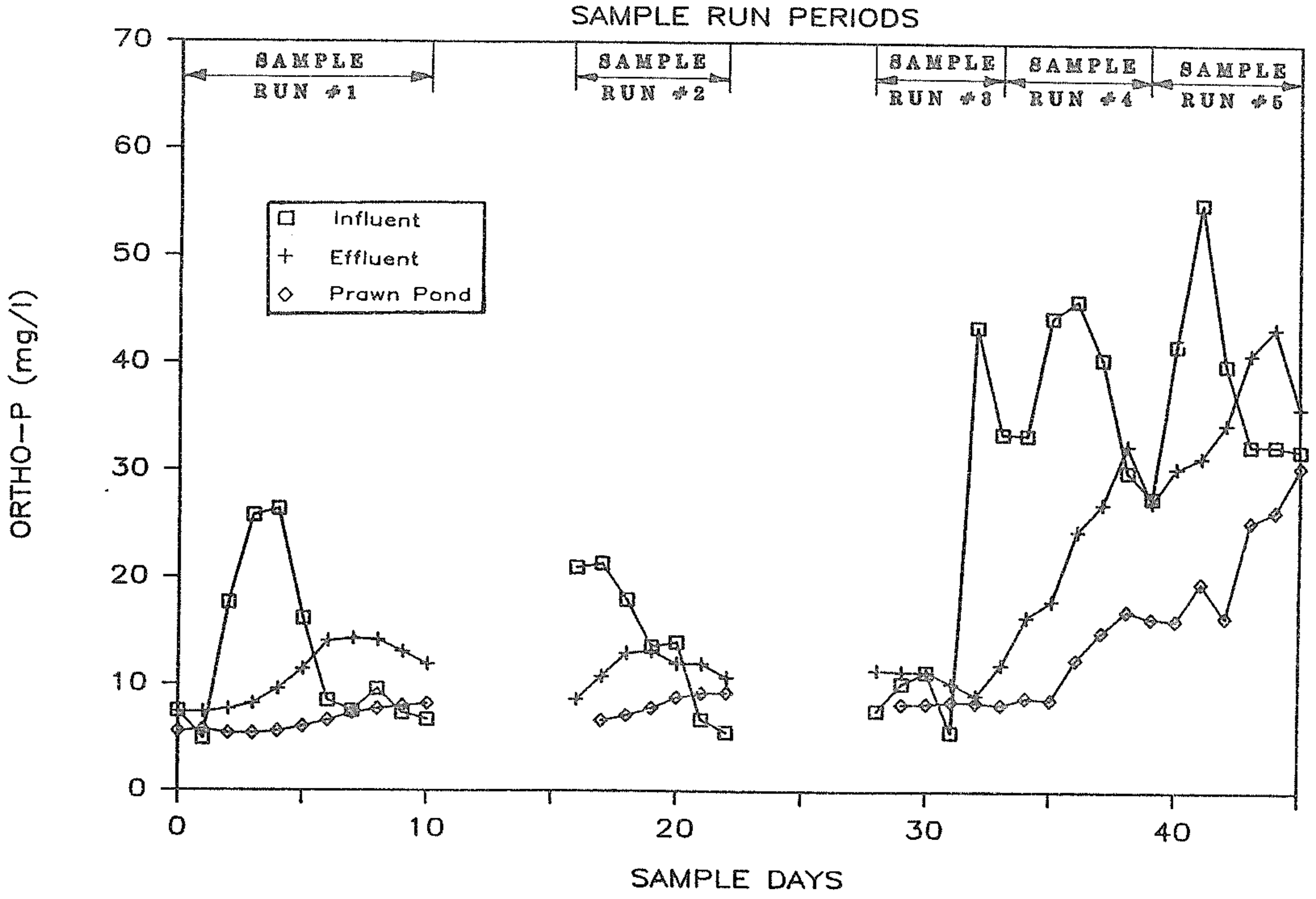

Figure 9. Ortho-phosphorous concentrations in the polyculture system

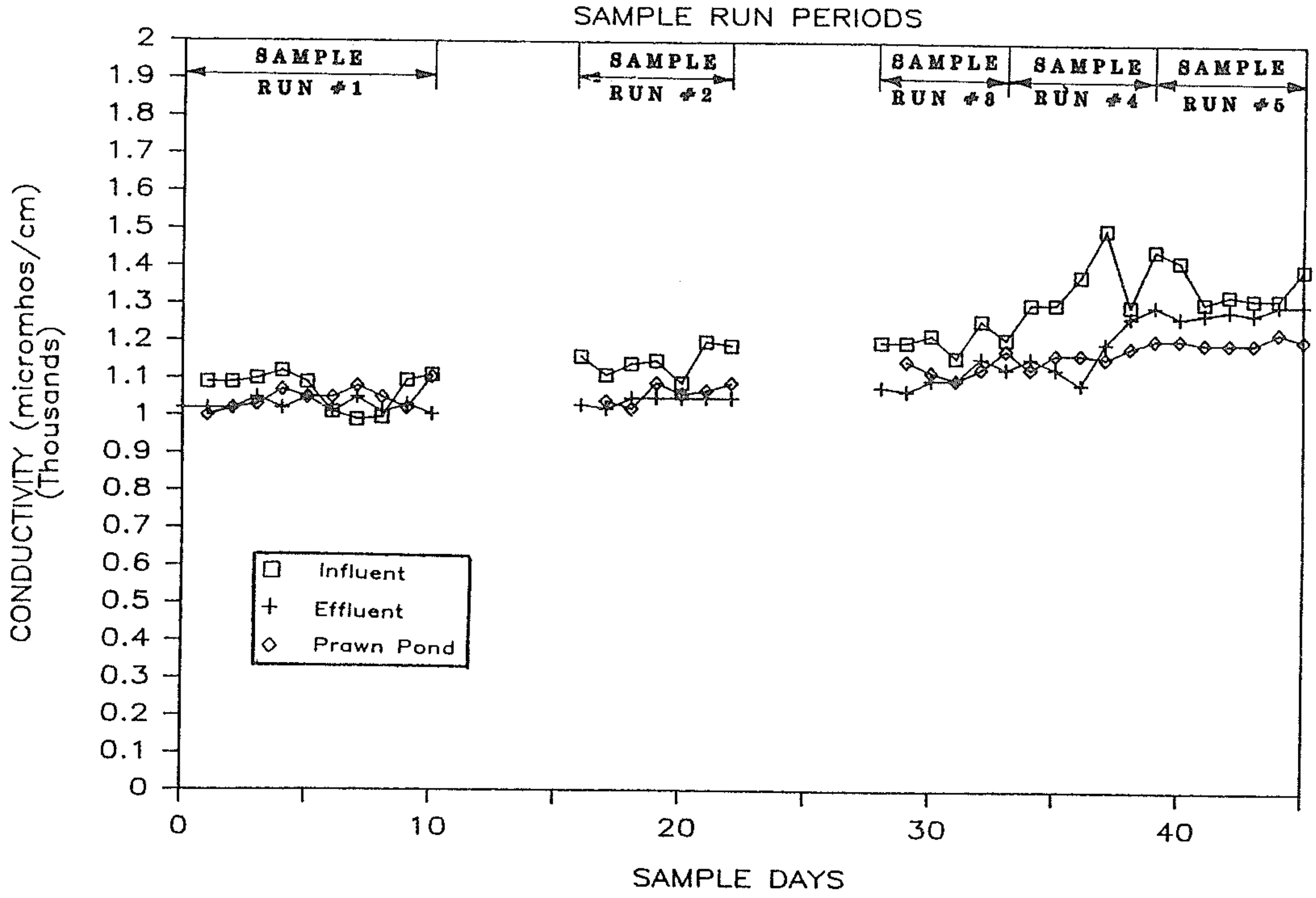

Figure 10. Specific conductance in the polyculture system

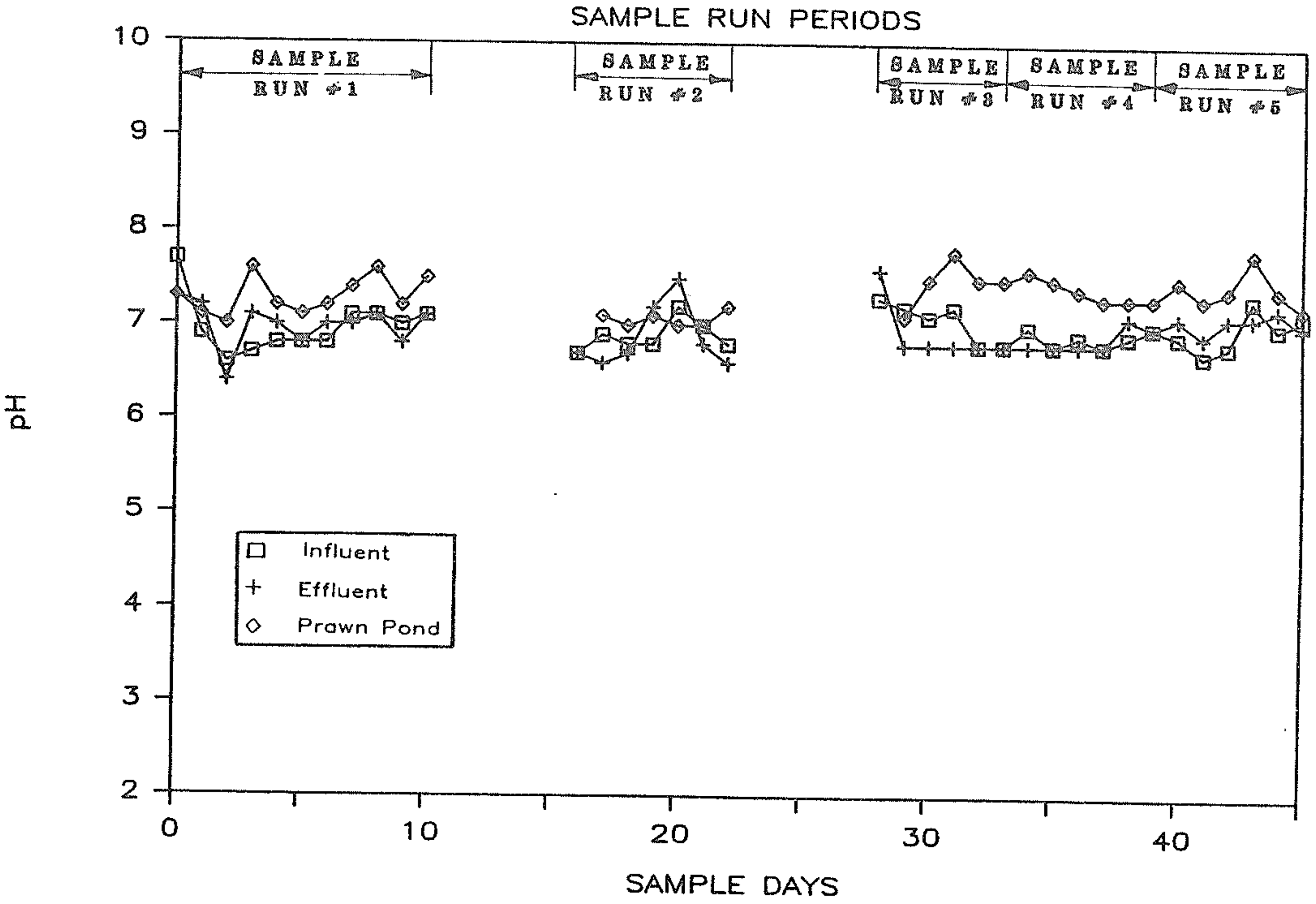

Figure 11. pH of the polyculture system

influent and effluent of the hyacinth system ranged from 6.7 to 7.8 and 6.5 to 7.8, respectively. The average pH for the influent was 7.0 and the average effluent pH was 6.9.

Additional water quality parameters were evaluated on an occasional basis. These parameters included total COD, TSS, total coliform (MPN), and turbidity. The results of this data are presented in Table 5. For the influent total COD, about 43 percent was calculated to be filterable suspended solids with the remaining fraction due to soluble materials. On a total COD basis, the hyacinth system removed an average of 53 percent of the COD. Comparisons of effluent total COD and soluble COD concentrations indicated very little difference in the two parameters. It was apparent that heterotrophic activity responsible for the reduction of COD was minimal in the water hyacinth system.

Total suspended solids removal through the system was excellent, averaging 93 percent, resulting in average effluent concentrations of TSS below 10 mg/L. Total coliform and turbidity removals were 99 and 97 percent, respectively. While total coliform removal was high, even at these levels the MPN counts were well above surface water criteria and drinking water standards (Salvato 1972). Turbidity removal was excellent and the effluent from the first-stage system appeared clear and free of color or particulate matter.

Second-Stage Azolla-Prawn System. Ammonia nitrogen loading also formed the basis for the evaluation of the second-stage Azolla-prawn system. The NH_3-N loading rates to the Azolla-prawn pond varied from 115 gm NH_3-N/ha-d for the first sample period, to over 10,360 gm NH_3-N/ha-d for the fifth sample period (table 1). No additions or alterations were made to increase or decrease either NH_3-N concentrations or flow rates.

Nitrogen Loading and Removal. Combining the effect of flow and concentration to determine mass loadings to evaluate the second-stage Azolla-prawn system presented difficulties because of the variation in the effluent flowrate as shown in Figure 12. This variability was compounded by two factors: (1) the outlet structure prevented good replication of effluent flowrate data, and (2) environmental conditions in the greenhouse made evapotranspiration rates difficult to assess.

Fluid lost by the system was lost by either evapotranspiration or seepage. In a completely mixed system, with a high rate of seepage loss, the concentration of soluble material should theoretically be in the seepage flow as the

Table 5
General wastewater characteristics for the polyculture system

Parameter	First-Stage Influent	First-Stage Effluent	Second-Stage Prawn-Pond
Total COD (mg/L)	132	62	55
TSS (mg/L)	130	10	3
Total Coliform MPN (per 100 ml)	76×10^5	51×10^3	2.5×10^3
Turbidity (NTU)	42	1.2	2.5

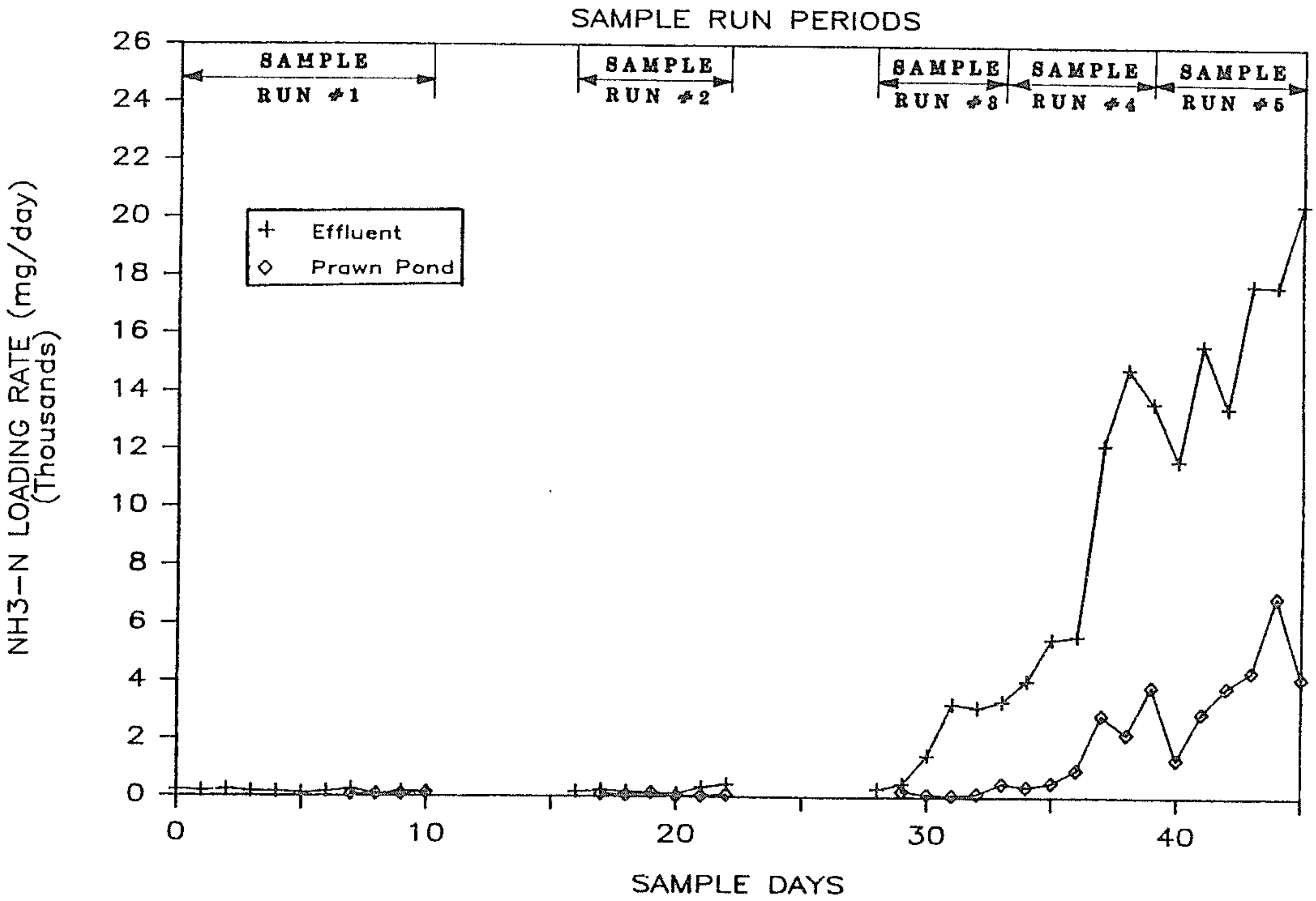

Figure 12. Ammonia nitrogen loading rates for the second-stage Azolla-prawn system

concentration in the effluent of the system. Thus, even though seepage results in a reduction of mass in the effluent due to reduction in the flowrate, it is not a legitimate process removal mechanism. Neglecting seepage can result in an over-estimate of the performance of the system. Evaporation or evapotranspiration concentrates the materials by removing the water but retaining the nutrients.

Table 6 shows the average hydrodynamic data of the sample periods for both the measured and estimated hydraulic losses. The influent and effluent flowrates were field measurements and the resulting net loss assumed that evapotranspiration removed most of the water and any seepage that occurred was negligible. Based on local conditions, the evapotranspiration rate was determined to be 2.65 cm/day. The following is considered to be a "best case" situation. In many stabilization lagoon systems with long detention times, a 50-75 percent water loss through the system is not unusual (Ferrara and Avci 1982, Ferrara and Harleman 1978). In most of these cases, seepage losses were considered minor when compared to evapotranspiration. But these were large systems treating in excess of 693 m^3/day and calculated seepage losses were only a fraction of the total volume of the system. In the Azolla-prawn system, a large seepage could negate the treatment ability of the system and thus, it was imperative to account for seepage losses.

In an attempt to estimate evapotranspiration from the pond surface, a model developed by Deadorff (1978) was employed. In the greenhouse environment many variables interacted to make the model over-predict the actual evapotranspiration losses. The best estimate of evapotranspiration obtained, using Deardorff's model, at the greenhouse site resulted in a predicted value of 5.0 cm/day. This was still twice as high as the evapotranspiration rate that resulted in the original flow data. To resolve this problem two scenarios were used to generate the mass flow data to evaluate the system. The first scenario used the measured data and assumed most of the losses were due to evapotranspiration and that seepage was minor. The second scenario, which was intended to represent a realistic "worse case" situation, assumed that the daily evapotranspiration losses were about equal to average pan evaporation data obtained from the local weather station. The average evapotranspiration rate for the study period was about 1.04 cm/day which was equivalent to a loss of 162 L/day from the second-stage Azolla-prawn system. Based on this average evapotranspiration rate, seepage rates varied between 53 and 70 percent of the

Table 6

Second-stage *Azolla*-prawn hydrodynamics for actual and estimated water losses

Sample Run Period	Measured Flowrate (L/day)		Measured Net Loss (L/day)[a]	Estimated Losses	
	Influent	Effluent		Evaporation[b]	Seepage
1	541	147	394	162	232
2	841	294	547	162	385
3	853	400	453	162	291
4	872	570	302	162	140
5	802	426	376	162	214

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[a] Assumes 100 percent loss through evapotranspiration, (+2.65 cm/day), and that seepage loss is negligible.

[b] Evaporation loss = 1.04 cm/day.

total loss from the system. Using the original measured flow data and the estimated flow data, removal efficiencies of nitrogen species based on mass loading to the system were generated and compared.

Table 7 summarizes the influent and effluent mass loadings of the nitrogen species based on the measured flowrate data (first scenario). Figure 12 shows daily fluctuations of NH_3-N loading rates. Average NH_3-N removal efficiencies ranged from 44 to 90 percent (table 7). The NO_3-N removal efficiencies for sample periods fluctuated between 16 to 75 percent. These removal efficiencies for NO_3-N indicated an inconsistent pattern of removal which may suggest that NO_3-N was a dynamic element in the major pathway for ultimate nitrogen removal from the second-stage system. Organic-N concentrations resulted in a 26 percent increase, or a 62 to 89 percent removal. Aside from sample run 1, all removal efficiencies were high, consistently above 60 percent. Calculated total-N removal efficiencies for the second-stage Azolla-prawn system ranged from zero to 83 percent. Overall, the system removed in excess of 73 percent of the total-N even at the higher loading rates.

The nitrogen species mass loading data generated for the estimated evapotranspiration rate of 1.04 cm/day (second scenario) are shown in Table 8. The removal efficiencies based on the date are shown in Table 9. For sample periods two through five, NH_3-N removal efficiencies ranged from 26-83 percent. Aside from sample period one, which showed a 14 percent increase, all the sample periods had removals in excess of 25 percent. This level of removal is reasonable, considering the low level of nitrogen entering and interacting in the system. The removal efficiency values for NO_3-N for sample periods one through five showed either a 15 to 32 percent increase, or a zero to 63 percent removal (table 9). These percentages were considerably less than the removal efficiencies for the hyacinth system; however, it is apparent that NO_3-N was being generated in the second-stage Azolla-prawn system. Organic-N removal efficiency in the second-stage system for the estimated water losses, resulted in either a 70 percent increase or a 43 to 79 percent removal. The numbers shown in parentheses are the removal efficiencies for the system not including the addition of nitrogen from the prawn feed table 9). As can be seen, the addition of the feed-N contributes significantly to removal efficiencies.

Comparison of the first and second scenarios described for the two differing flow regime assumptions indicated that removal efficiencies were

Table 7
Measured nitrogen mass balance data for the second-stage *Azolla*-prawn system

Sample Run Period	Influent (mg-N/day)			Effluent (mg-N/day)			Percent Removed			
	NH_3-N	NO_3-N	ORG-N	Feed-N	NH_3-N	NO_3-N	ORG-N	NH_3-N	NO_3-N	ORG-N+ Feed-N
1	180	307	494	230	100	140	982	44	54	-26[a]
2	235	522	496	2754	75	272	347	68	33	89
3	1984	418	496	2754	200	354	500	90	16	84
4	9317	541	720	2754	1783	435	569	81	19	84
5	16161	1562	283	4498	3930	383	1802	76	75	62

[a] -sign indicates net increase

Table 8
Estimated nitrogen mass balance data for the second-stage *Azolla*-prawn system

Sample Run Period	Influent mg/N/day				Effluent mg-N/day				Seepage mg-N/day			
	NH_3-N	NO_3-N	ORG-N (Feed-N)[a]	TN[b]	NH_3-N	NO_3-N	ORG-N	TN	NH_3-N	NO_3-N	ORG-N	TN
1	180	307	494 (230)	1211	100	140	982	1222	158	221	1550	1928
2	235	522	496 (2754)	4007	75	272	347	694	982	356	454	909
3	1984	418	496 (2754)	5652	200	354	500	1054	129	257	366	767
4	9317	541	720 (2754)	13332	1783	435	569	2787	438	108	142	684
5	16161	1562	283 (4498)	22504	3930	383	1802	6115	1974	194	913	3072

[a] Parenthesis indicates Feed-N.

[b] Total-N includes Feed-N.

Table 9
Second-stage *Azolla*-prawn system nitrogen removal efficiencies for estimated water losses

Sample Run Period	Percent Removal			
	NH_3-N	NO_3-N	ORG-N	Total-N
1	-14[a]	-15	-80 (-70)[b]	-61
2	26	-17	-38 (75)	60
3	83	-32	-43 (73)	68
4	76	0	-1 (79)	74
5	63	63	-89 (43)	43

[a] Sign indicates net increase

[b] Excluding nitrogen contributed by the prawn food

better for all nitrogen species under the "best case" scenario. In the first scenario, NH_3-N, NO_3-N and ORG-N removal efficiencies were 72, 39, and 59 percent, respectively. The second scenario, using the estimated evapotranspiration losses, resulted in poorer removal efficiencies; however, NH_3-N, NO_3-N, ORG-N and total-N removal efficiencies still averaged 47, -0.2, 40 and 37 percent, respectively.

The average concentrations of the nitrogen species for the five sample periods are shown in Table 10. Figure 4 shows the influent and effluent concentrations of NH_3-N for the Azolla-prawn system. The influent concentration of NH_3-N for the five sample periods progressively increased from 0.3 to 20.0 mg/L, while the effluent concentration also progressively increased from 0.3 to 8.9 mg/L. The removal efficiencies of NH_3-N for sample periods three through five ranged from 56 to 79 percent, while removal efficiencies for the first two sample periods were both zero. The best removal effieciencies of NH_3-N appeared to occur between average influent concentrations of 2.3 mg/L to 10.8 mg/L. Low influent NH_3-N concentrations were not removed to any significant degree, and influent concentrations of NH_3-N of 20 mg/L were reduced 56 percent.

Concentrations of NO_3-N in the Azolla-prawn system are shown in Figure 5. Average influent NO_3-N concentrations (table 10) ranged from 0.5 to 2.3 mg/L. Average effluent NO_3-N concentrations ranged from 0.8 to 0.9 mg/L. With the exception of the fifth sample period, NO_3-N appeared to increase through the Azolla-prawn system, but all effluent NO_3-N concentrations remained below 1.0 mg/L.

Variations of influent and effluent ORG-N concentrations for the second-stage Azolla-prawn system are shown in Figure 6. Average ORG-N concentrations, (table 10) varied from 0.3 to 0.9 mg/L. To simplify the situation, feed-N was considered as ORG-N in the analysis of the system nitrogen mass balance. Averaged concentrations of ORG-N in the effluent ranged from 0.9 to 4.6 mg/L. Again, as with NO_3-N, the concentrations of ORG-N increased in the second-stage Azolla-prawn system. Without feed-N, ORG-N generated through the system was in excess of the input ORG-N. This ORG-N generation could have come from the natural productivity in the pond which could include phyto and zooplanktors such as Desmids, copepods, and amphipods, plus a variety of water mites and spiders. When feed-N was considered, the average influent ORG-N concentration increased by 0.1 to 4.3 mg/L.

Table 10
Average nitrogen species concentration data for the second-stage *Azolla*-prawn system

Sample Run Period	Influent (mg/L)			Effluent (mg/L)			Percent Removed (%)		
	NH3-N	NO_3-N	ORG-N	NH_3-N	NO_3-N	ORG-N	NH_3-N	NO_3-N	ORG-N
1	0.4	0.6	0.9	0.4	0.9	1.8	0	-33[a]	-50
2	0.3	0.6	0.6	0.3	0.9	1.4	0	-33	-57
3	2.4	0.5	0.5	0.5	0.9	2.1	79	-20	-76
4	10.8	0.6	0.8	2.8	0.8	0.9	74	-25	-11
5	20.0	2.3	0.3	8.9	0.9	4.6	56	61	-94

[a] -Sign indicates net increase.

Nitrogen Mass Balance. The fate of nitrogen in the *Azolla*-prawn system presented in Table 11 shows total-N in and out of the system, and the net loss to the system. The net loss is the total-N removed by the system and should be accounted for by prawn-N uptake, plant-N uptake or other processes. The net loss of nitrogen to the system varied between -11.0 to 16,389 mg-N/day over the five sample periods. Nitrogen uptake by *Azolla* ranged from 6084 to 7934 mg-N/day. Prawn-N uptake was calculated to be constant throughout the five sample periods at 158 mg-N/day. Nitrogen unaccounted for in the *Azolla*-prawn system varied from -6,253 mg-N/day (negative sign indicates plant-N plus prawn-N uptake exceeded actual net nitrogen inflow to the system), to 8,379 mg-N/day. The role of plant-N uptake as a mechanism for N-removal in the *Azolla*-prawn system was difficult to assess. The nitrogen removed, or taken up by the plant, remained somewhat constant throughout the five sample periods. However, the unaccounted for nitrogen changed from a highly negative value of -6,253 mg-N/day to a positive value of 8,379 mg-N/day. The negative unaccounted total-N indicated that the plant had to generate its required nitrogen through fixation, and the change to a positive indicated that the plant-N uptake was, at least partially, satisfied by aqueous nitrogen. The extent to which *Azolla* takes up nitrogen from an aqueous media is not well understood. (Reddy and Debusk 1983).

Figure 13 shows the change in plant-N uptake of and net total-N lost to the system over the five sample periods. The net nitrogen loss to the *Azolla*-prawn system increased from -11 to 16,389 mg-N/day over the five sample periods, while plant-N uptake was relatively constant around 6,000 to 8,000 mg-N/day over the same period. It would appear that during the first three sample periods, *Azolla* had to fix 42 to 100 percent of its nitrogen requirements. For the fourth and fifth sample periods, plant-N uptake appears to be satisfied by nitrogen lost to the sytem. However, these results do not support the contention that *Azolla* was removing aqueous nitrogen in any form. But as discussed in the previous section (table 9), NH_3-N was being removed from the system even under the "worse case" situation. Considering these difficulties, and in spite of the high productivity of *Azolla*, it would appear that the use of a non-nitrogen fixer, such as *Lemna minor* (duckweed), would be a greater asset to the second-stage system. This conclusion is true only if there is a market demand for products that could be generated from *Azolla*, or unless the *Azolla* could be provided to agricultural enterprizes that could use

Table 11
Fate of nitrogen mass in the second-stage Azolla-prawn system

Sample Run Periods	Influent Total-N (mg-N/day)	Effluent Total-N (mg-N/day)	Net Loss[a] to the Sytem (mg-N/day)	Total-N Uptake (mg-N/day) by Plant	Total-N by Prawn	Unaccounted[b] Removal (mg-N/day)	System Total-N (%)
1	1211	1222	-11	6084	158	-6253	0.0
2	4007	694	3313	7934	158	-4779	83
3	5652	1054	4598	7852	158	-3412	81
4	13332	2787	10545	7852	158	2532	79
5	22504	6115	16389	7852	158	8379	73

[a] Net loss to the system = Influent = Effluent

[b] Unaccounted Total-N removal = net system loss - (plant uptake + prawn uptake).

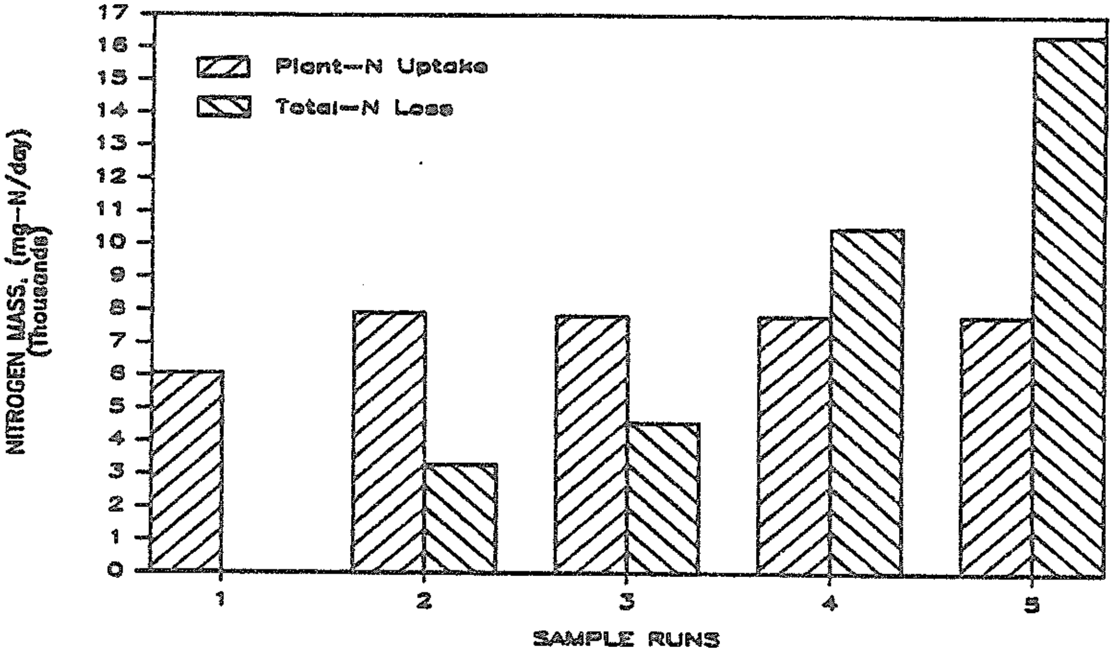

Figure 13. Comparison of the Plant-N uptake and Total-N loss in the second-stage *Azolla*-prawn system

the plant for fertilizing. This situation is not a likely case for North America, but certainly a possibility in subtemperate to tropical regions where Azolla is used and an overwintering stock can be difficult to obtain (McClure and Lipinsky 1981).

The freshwater prawn contributed to a small portion of the actual nitrogen removed from the system. However, as prawns grow larger, towards marketable sizes of over 20 gm, their value as a nitrogen sink may also increase. The second-stage Azolla-prawn system was never operated with prawns in this size category, and it could be expected that different modes of operation would have to be employed to adequately deal with the increased carrying capacity.

Total-N removal for the Azolla-prawn system varied from about zero percent for the first sample period to over 70 percent for the last four sample periods, with an average percent removal for the last four sample periods of 79 percent.

Other processes that could account for the nitrogen losses in the second-stage system are similar to those in the first-stage water hyacinth system. However the Azolla-prawn system was different because it was aerated and was aerobic throughout the water column. Thus, while nitrification was enhanced, denitrification may have been limited. Ammonia removal, even under the "worse case" scenario, was good; however, NO_3-N removals were highly variable under all conditions. Alkalinity data presented in Figure 8 indicates that the alkalinity of the Azolla-prawn effluent may have been slightly higher than the influent; however, the differences were marginal, therefore it is difficult to make a determination of the activity of the nitrification-denitrification processes in the second-stage system.

Sedimentation could not have accounted for ORG-N losses to any large degree. However, processes such as ammonification would have also been active in the highly aerobic environment resulting in the rapid conversion of ORG-N to NH_3-N.

Volatilization could have contributed to NH_3-N removals because detention times through the system were over five days and the system was actively aerated. The pH of the system was below 7.6, which probably restricted the rate of volatilization.

Plant-N uptake should have removed a large amount of the nitrogen at the low NH_3-N concentrations and long detention times encountered in the Azolla-prawn system. If the fixation process of the Azolla merely switches on and off, depending on the aqueous concentration of NH_3-N, then the plant may be an excellent candidate for the system by producing large quantities of biomass and removing low concentrations of NH_3-N. More information detailing the contributions of aqueous nutrients to the growth of Azolla is required for optimum utilization of Azolla in a nutrient removal capacity.

Other Water Quality Parameters. Additional water quality parameters were measured during the analysis of the second-stage Azolla-prawn system. Ortho-P, shown in Figure 9, remained below 15.0 mg/L for both influent and effluent to the second-stage system for the first and second sample periods. Effluent Ortho-P concentrations were generally 12 to 43 percent lower than corresponding influent concentrations. Throughout the third, fourth and fifth sample periods, influent Ortho-P increased from near 10 mg/L to over 35 mg/L. During these sample periods, effluent Ortho-P concentration increased from under 10.0 mg/L to over 25.0 mg/L. Percent removal of Ortho-P during these three sample periods varied from 0 to 45 percent.

The specific conductance of the Azolla-prawn system, shown in Figure 10, indicated that levels for both influent and effluent were similar and ranged between 1,000 to 1,300 micromhos/cm. The average influent and effluent specific conductance measurements were 1,114 and 1,116 micromhos/cm, respectively, which indicate a negligible overall rise through the system.

The alkalinity and pH of the Azolla-prawn system are shown in Figures 8 and 11, respectively. Both influent and effluent pH varied between 6.4 to 7.6, and 7.0 to 7.6, respectively. In general, throughout the sample periods, pH appeared higher in the effluent than in the influent with the average values for influent and effluent pH at 6.9 and 7.3, respectively. This rise could have been due to the loss of CO_2 since the system was actively aerated. Alkalinity of the Azolla-prawn system, in both the influent and effluent, ranged from about 175 to 250 mg/L (as $CaCO_3$). The average alkalinity for the influent and effluent of the Azolla-prawn system was 223 and 230 mg/L (as $CaCO_3$), respectively. This slight increase was less than three percent and was not significant.

Other water quality parameters were also evaluated for the Azolla-prawn system. COD, TSS, MPN, and turbidity were shown in Table 5. Influent and effluent COD concentrations were 62 and 55 mg/L, respectively, giving a general removal of 12 percent. Average total suspended solids were 10 and

3 mg/L for the influent and effluent, respectively, giving a average removal efficiency for TSS of 66 percent. MPN samples, which were quite variable for the system, gave valued of 51 x 10^3 and 2.45 x 10^3 coliform/100 ml for the influent and effluent, respectively. This is a total coliform removal efficiency of 95 percent. The value of 2.45 x 10^3 MPN/100 ml approaches surface water quality criteria standards of 2,000 MPN/100 ml for non-contact waters (Salvato 1972). Turbidity in the Azolla-prawn pond, shown in Table 5, increased through the system from an average of 1.2 NTU in the influent to 2.5 NTU in the effluent. Although the results indicated an increase of turbidity through the second-stage system, overall combined system turbidity reductions were 94 percent. A potential source of turbidity and ORG-N generation could have been exudates from Azolla decomposition as it mounds up on the water surface as biomass. The lower layers cease to be photosynthetic and begin to decompose. A faint yellowish-green exudate was observed in the drain water when the plants were periodically harvested.

System Design Criteria. Based on the operation of the two-staged polyculture system and the analysis of the data, general design criteria were developed for both the first-stage water hycinth system and the second-stage Azolla-prawn system. This criteria applies to allowable system detention times, flowrates, NH_3-N loading rates, basin configurations and biomass concentrations. These criteria are presented in the following sections.

Detention Time. The detention time for the first-stage system ranged from 2.7 to 4.3 days with an average detention time over the study period of 3.1 days. Other investigators have reported using detention times of between three and 20 days (Hauser 1984). Certainly no more than a three-day detention time would be adequate to achieve the percent NH_3-N removals as demonstrated in this study. However, if lower effluent concentrations of NH_3-N were required, particularly for aquacultural applications, the detention time would be a variable to consider to help lower effluent NH_3-N concentrations to acceptable levels. Non-aerated hyacinth lagoons have used detention times of over 30 days to treat raw municipal wastewaters. Obviously, for a tertiary treatment system, trade-offs in land cost versus energy costs for aeration or other nitrogen removal methods would be a major consideration.

In the second-stage Azolla-prawn system, the detention times ranged from 6.3 to 10.1 days, with an average of 7.2 days. For treatment efficiency the shorter detention times, which produced higher NH_3-N loading rates gave the highest NH_3-N removals; however, because the culture of prawns depends on a large amount of effective pond bottom area, minimizing the size of the pond to match a desired detention time only reduces the system capability of producing a high valued culture species. As long as the aquatic plant used in the second-stage system can remove NH_3-N or other compounds to levels safe for the culture organism, the detention time should not be minimized as to decrease potential production of the culture species. The use of artificial substrates could be used to increase the effective bottom area without increasing pond volume.

Flow Rate. Flow rates, which are related to detention times, are mentioned here because flow rates for either system should not be increased so drastically as to produce hydraulic sheering and turbulence in the system. The sheering and turbulences would result in increases in ORG-N in the effluents and possible loss of the nitrifying-denitrifying biomass important for ultimate removal of nitrogen.

Ammonia Nitrogen Loading Rates. Figure 14 indicates the relationship between influent NH_3-N loading rates and effluent NH_3-N concentrations in the first-stage water hyacinth system. Two standards of NH_3-N loading rates need to be developed for non-aerated systems. For wastewater applications, loading of NH_3-N up to 26.7 to 60.3 kg NH_3-N/ha-d resulted in good removal efficiencies and resulted in concentrations of NH_3-N in the effluent of less than 15 mg/L. These same loadings in an aquaculture system would have resulted in concentrations of un-ionized NH_3-N that could cause growth inhibition or death of prawns. An increase of the system detention time or the use of slow aeration could enable the NH_3-N loading rate to be extended above 60.3 kg NH_3-N/ha-d to levels suggested by Weber and Tchobanoglous (1983), of over 121 kg NH_3-N/ ha-d. However, at these higher loading rates, the affect on the second-stage system would have to be closely monitored.

Figure 15 shows the relationship of influent NH_3-N loading rates to the effluent NH_3-N concentrations for the second-stage Azolla-prawn system. The NH_3-N loading rates to this system were very low when compared to the loading rates of the first-stage system. At the maximum NH_3-N loading rates of 10.4 kg NH_3-N/ha-d, the effluent from the Azolla-prawn pond had an NH_3-N concentration ranging from 5.7 to 12.0 mg/L. At a temperature of 25°C and a pH 7.4, the un-ionized NH_3-N concentration ranged from 0.08 to 0.2 mg/L at this same NH_3-N loading rate. These concentrations are well within levels previously

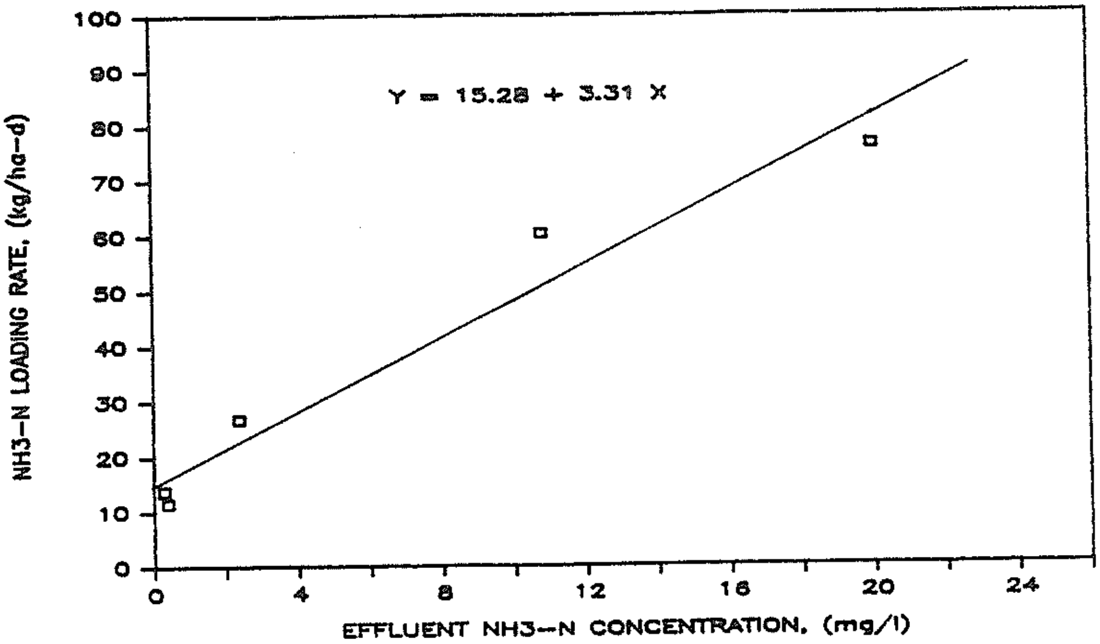

Figure 14. Relationship between ammonia nitrogen loading rates and effluent NH_3-N concentrations for the first stage of the polyculture system

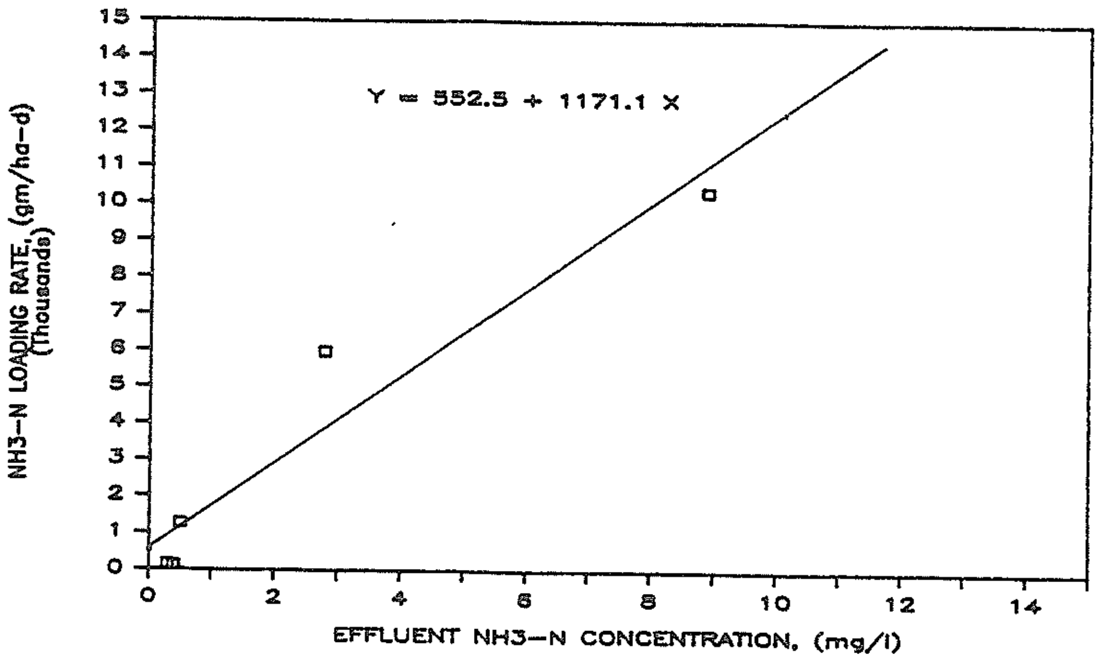

Figure 15. Relationship between ammonia nitrogen loading rates and effluent NH_3-N concentrations for the second stage of the polyculture system

mentioned as inhibiting prawn growth. Inhibition of growth of the prawn was not investigated in this study, but no deaths were observed either. It is apparent that using the Azolla in the Azolla-prawn system will limit the allowable NH_3-N loading rates to below 10.4 kg NH_3-N/ha-d.

Biomass Concentration. Biomass concentration is a major operational parameter in the first-stage system. It is important to maintain a standing crop of hyacinth between 8 and 12 kg (fresh wgt)/m^2 to optimize growth and nitrogen removal. Lower densities would result in areas of open waterin the pond that result in algal growth producing increased effluent ORG-N concentrations, while high plant densities will lower plant growth and nitrogen uptake rates. But it is desirable to leave plants in the system long enough to enable the plants to develop a large root mass. This mass becomes the site for nitrifiers and denitrifiers and helps enmesh organic material. Thus, it would appear that a system could be managed for both types of growth modes. One area of the pond harvested frequently to maintain the optimum plant density, and the other area of the pond maintained to allow for root mass development. Root mass development can be limited by the availability of nutrients; thus, the faster growing plants should be placed in the front of the system and the older plants placed towards the back of the pond where limited nutrients would enhance root development. Frequent harvesting has been shown to increase TSS in the effluent and a management scheme such as the one presented above may reduce the increased TSS caused by plant harvesting (Hauser 1984), while still producing good overall nitrogen removal.

In the second-stage system, biomass management was also an important criteria. When the Azolla densities exceeded 1,500 gm (fresh wgt)/m^2, small prawns (< 0.5 gm), which have a tendency to jump, would become trapped on the surface of the Azolla matt. The prawns were strong enough to jump through the plant mass, but did not weigh enough to drop back through and would eventually perish. Thus, Azolla had to be managed to keep plant densities below 1,500-1,600 gm (fresh wgt)/m^2 for this small prawn size category. The affect of this management limited the biomass and the nitrogen uptake of Azolla because optimal growth rates or plant densities were never determined. As the prawns grow, greater biomass densities would be allowable and greater nitrogen removal could be achieved.

Basin Configurations. The basin configurations for the first-stage system will depend a great deal on climatic conditions and management schemes.

In general, long-narrow-series-operated ponds with length to width ratios of 3 or greater would allow easy harvesting of plant biomass or seasonal use of greenhouse structures. A maximun pond width should not exceed 35 to 37 m and pond depths could vary between 0.61 and 1.5 m depending on the employment of supplementary aeration. If the system were managed, an area where plants could achieve greater densities and larger root masses could be provided and then the configuration of the basin does not need to be narrow. This high density basin would be harvested infrequently, if at all, and the configuration could be square or rectangular with length to width ratios of 1-2:1. Irregular shapes could also be used as long as short circuiting did not occur.

Basin configuration for the second-stage system should be long and narrow, similar to the rapid growth basin previously described for the first-stage system. This would allow for easy harvesting of the prawns as well as economical installation of seasonal greenhouse structures. Depth of the Azolla-prawn pond should be 0.9-1.5 m although shallower basins could be used. Deeper basins may reduce mixing of pond bottom areas with over-lying water, resulting in prawn avoidance areas due to low dissolved oxygen.

The Prawn Growth Studies. Prawn growth studies were performed in the laboratory and in the field. The summary results of the first laboratory experiment are presented in Table 12, which shows the net growth increase for each aquarium for each of the three treatments. The percentages shown in the table represents the feed percent based on the body weight of the prawns. The recorded data for the treatments for each sample period was the net weight gain in gms for each aquarium.

Treatment A (Trt.A), which received a commercial pellet-only diet, produced an average net weight gain of 3.4 gm while Treatment B (Trt.B), which received the commercial pellet and plant diet, produced an average net weight gain of 4.0 gm. Treatment C (Trt.C), which was the plant-only diet, produced an average net increase of 0.5 gm.

A statistical analysis of the three treatments of this nonrandomized block experiment indicated that there was no significant difference between Trt. A and Trt. B at the 0.005 level of significance. Both Trt. A and Trt. B resulted in better growth than Trt. C at the same level of significance. It is possible because of the experimental design that this statistical analysis was not sensitive enough to detect differences in Trt. A and Trt. B. On a straight percentage basis, Trt. B grew 15.5 percent better than

Table 12
Experiment 1, prawn growth data
using non-radomized block design

	Net Weight Gain for Each Aquarium, gms		
Sample Period	TRT. A 25% Pellet	Trt. B 25% Plant 25% Pellet	Trt. C 25% Plant
1	4.1	4.2	1.5
1	4.0	3.9	NA[a]
1	2.6	2.6	0.7
1	2.0	2.9	2.1
1	1.7	2.7	1.0
2	6.1	7.1	-1.0
2	3.8	4.1	NA
2	3.6	5.5	-0.1
2	2.3	3.1	-1.0
2	3.7	4.0	NA

[a] NA indicates no data available.

Trt. A. Treatment B also showed an 88 percent increase in growth over Trt. C, while Trt A showed an increase in net growth of 86 percent over Trt. C.

Since both Trt. A and B fed the same amount of commercial pellet to the prawns, the results of both the statistical analysis and the percentage analysis indicate that, while increased feedrate by supplementing processed hyacinth may increase growth by 15.5 percent, a reduction in commercial pellet-feed replaced by hyacinth was not statistically supported. Thus, it can be stated that the same level of commercial pellet-feed and groundwater hycinth to the diets of 2.1 to 3.5 gm prawns, did result in a 15.5 percent increase in net growth.

The data summarizing the second set of prawn growth experiments are shown in Table 13. For experiment 2, Trt. A, which received a ten percent commercial pellet diet, produced an average net weight increase of 2.52 gms, while Trt. B, which received the five percent plant and 5 percent commercial pellet diet produced an average weight increase of 1.4 gms. Treatment C received a ten percent plant diet and produced an average net weight increase of 0.2 gm. The A and B treatments were fairly consistent throughout the two sample periods; however, Trt. C began to radically show the affects of the plant-only diet with negative growth being recorded for all of sample period 2.

A statistical analysis of the three treatments of this randomized block experiment indicated that Trt. A was not the same as Trt. B at a 0.005 level of significance. Both Trt. A and Trt. B were not the same as Trt. C at the 0.005 level of significance.

Examination of the mean net weight increases indicated that Trt. A resulted in 44 percent better growth than Trt. B, which had a partially supplemented diet. Treatment C produced growth rates that were 94 and 89 percent less than Trts. A and B, respectively.

The results of this second growth study showed that hyacinth supplemented diets did not increase growth rates of prawns in the 0.4 to 0.9 gm size category. In fact, the commercial pellet diet alone resulted in growth rates that were greater than the hyacinth-pellet combination. The hyacinth feed alone resulted in significantly reduced growth and even negative weight changes.

These two experiments were performed in aquaria using ground, dried water hycinth, which was not pretreated or innoculated in any way to add to the flora and fauna that might develop as a result of its natural decomposition. Single cell protozoans, attached stalked ciliates and some fungal mycelia were

Table 13
Experiment 2, prawn growth
data using random block design

Sample Period	Net Weight Gain for Each Aquarium, gms					
	Trt.A 10% Pellet	Block	Trt.B 5% Pellet 5% Plant	Block	Trt.C 10% Plant	Block
1	2.0	1	1.0	1	0.4	1
1	2.6	2	1.1	2	0.6	2
1	2.5	3	0.9	3	0.5	3
1	2.1	4	1.3	4	0.2	4
1	2.0	5	0.7	5	0.7	5
2	2.6	1	1.7	1	-0.2	1
2	2.6	2	1.6	2	-0.2	2
2	2.7	3	1.7	3	-0.1	3
2	3.2	4	2.0	4	-0.2	4
2	2.9	5	2.0	5	-0.1	5

observed, but it was apparent that in comparing the microbial activity of these aquaria to the natural community developed in the Azolla-prawn pond, the aquaria were lower in biological activity and certainly lower in the development of higher organisms such as small crustaceans, annelids, worms, insect larvae, and small fish that might serve as prawn food in a natural pond environment.

Growth rates in the Azolla-prawn pond for prawns in the 0.1 to 2.1 gm size category averaged 34.0 mg fresh wgt/prawn/day, and in the aquaria study for prawns sized 0.4 to 0.9 gm, the growth rates averaged 22.0 mg fresh wgt/prawn/day. No statistical comparisons were made; however, this data does indicate a 34 percent lower growth rate for the prawns grown in the aquaria. The larger prawns, 2.1 to 3.5 gm, had average growth rates in the aquaria of 58 mg fresh wgt/prawn/day. No data for pond-grown prawns in this size category was available for comparison.

The Conceptual Model

The major focus of the modeling efforts of this research centered only on the first-stage water hyacinth system. The development of the conceptual model follow three of the five steps outlined by Lassiter (1975) for modeling aquatic systems:

1. Define the ecosystem in terms of the problem requiring a solution.
2. Construct a diagram of the basic ecosystem representing the system variables and their interrelationships.
3. Develop the basic mathematical descriptions of individual physical, chemical and biological processes involved with material flow.
4. Assemble the model using the subcomponents while observing the principles of conservation of mass and energy.

Additional considerations of the modeling effort related to specific application needs included:

- The model should describe nutrient transformations and processes sufficiently enough to predict water quality of regulated effluent discharge, or for use in aquacultural operations.
- The model should be capable of being used for both design purposes as well as for daily operational management.
- The model should be developed with simulation costs and hardware limitation in mind. Thus, it should be no more complex than needed to be useful onsite.

The first-stage water hyacinth system was essentially a tertiary wastewater treatment process or polishing lagoon designed to remove various forms of phosphorous and nitrogen. Some carbon removal is effected by this system, but carbon was considered an optional element to the model and not necessary to the system. Nitrogen was selected as the target nutrient because of the importance of NH_3-N to fish culture systems and because nitrogen has been considered by some authors as the limiting nutrient in wastewater lagoons (McKinney 1977). The nitrogen species of interest were NH_3-N, NO_3-N, and ORG-N. Nitrite nitrogen was considered to be relatively short-lived in the system and was measured as NO_3-N. The model as designed was envisioned to be a three-compartmental model which could use the mathematics of a completely mixed system, described by a linked system of differential equations. Each species of nitrogen was set up as a compartment and each compartment was linked by a series of mass-flow processes.

The model describing the removal of the three nitrogen species from secondary municipal wastewater was essentially the synthesis of two research studies. The first study (Ferrara and Harleman 1981, Fritz, Middleton, and Meredith 1979) developed a series of time-dependent dynamic models describing stabilization lagoons treating raw municipal wastewaters. The second study (Lorber, Mishow and Reddy 1984, Mitsch 1976) developed a discrete seasonal model to predict the biomass production of water hyacinth in lakes receiving elevated levels of nutrients. Both of these models converged to become the basis for the conceptual model developed in this research.

The nitrogen compartments from the stabilization lagoon models were used as a starting point in the development of the water hyacinth model. The model by Ferrara and Harleman (1978) was employed more directly since very little data existed for water hyacinth systems. In addition, analyses by Ferrara and Avci (1982) provided for the inclusion of a rate equation describing NH_3-N volatilization.

The first-stage water hyacinth ecosystem is defined in Figure 16. This diagram shows only the in-pond transformation processes and, for clarity, does not include the hydraulic-mass inflow and outflow to and from each compartment of the system.

The flow diagram incorporates both physical processes and various biological transformations to predict effluent parameters. Physical processes include simple mass flow in and out of the system (not shown in Figure 16),

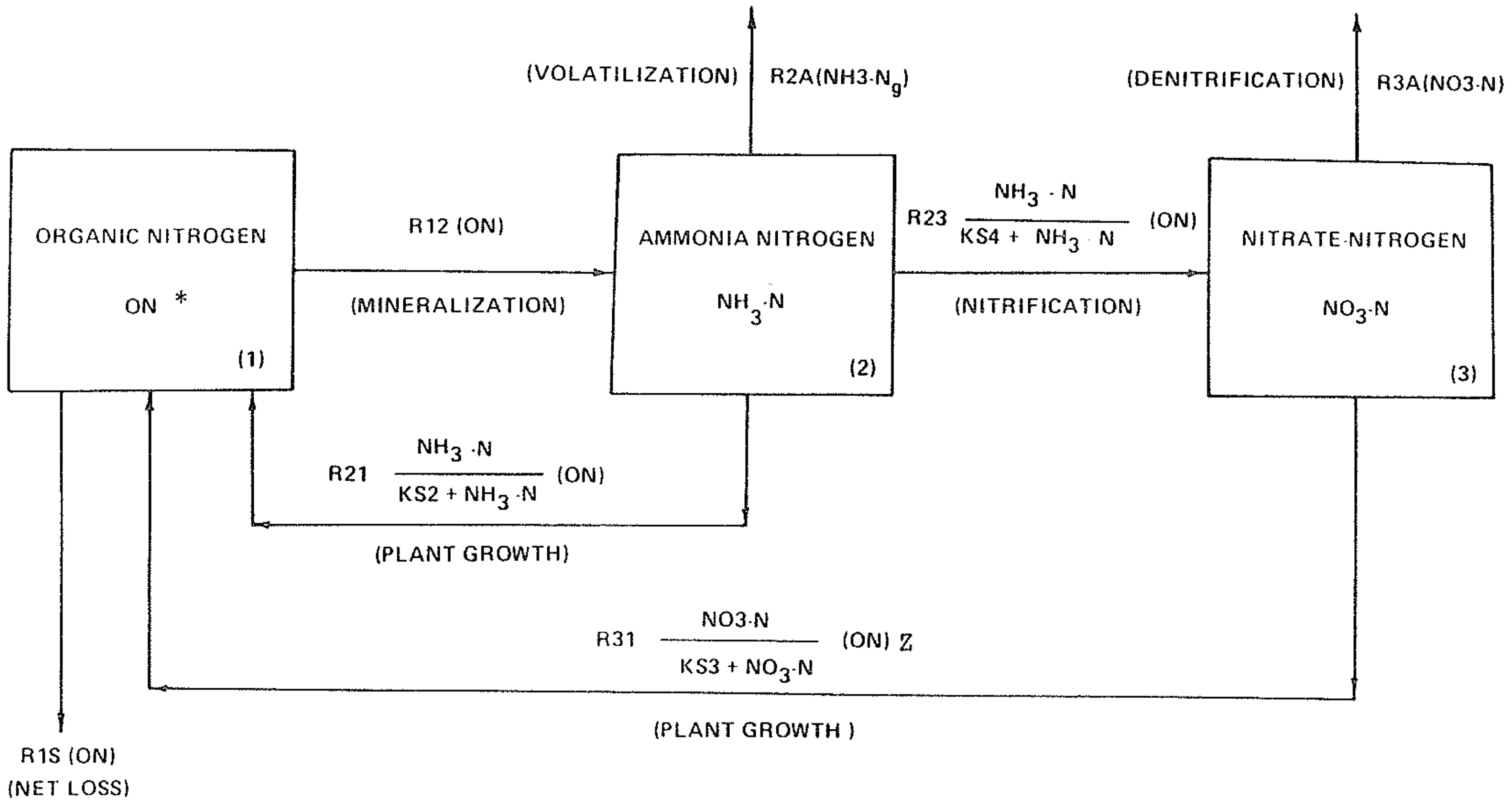

Figure 16. Three-compartment biogeochemical nitrogen model of the first-stage water hyacinth system.
*NOTE: ON = ORG-N

as well as net losses via sedimentation from the system and losses due to NH_3-N volatilization. Nitrification and plant growth processes were based on Monod-type substrate limiting equations to describe the removal of NO_3-N and NH_3-N. Other biological processes, ammonification and denitrification, were described as simplified first order reactions.

The organic-N compartment is affected by mass inflow and organic-N produced from plant growth using NO_3-N and NH_3-N as substrate. Losses from this compartment include mineralization (R12) and net loss of organic-N to the sediments (R1S). Mineralization is the loss of oganic-N via aerobic decomposition of dead organic material resulting in the release of NH_3-N. The net loss of oganic-N to the sediments combines the rate of actual loss to the sediments with the rate of material returned to the system via microbial growth or some other form of small particulate organic material. This structure simplified the detrital influences without losing the net effects.

The ammonia compartment receives inputs from mass inflow to the system and mineralization (R12) of organic material. Losses from the NH_3-N compartment include volatilization (R2A), nitrification (R23), and plant growth (R21). Volatilization is highly pH dependent and proceeds via simple diffusion through the water surface. Nitrification is mediated by microbial activity and substrate concentration. Plant uptake of NH_3-N is mediated by substrate concentration, plant density and availability of micronutrients. KS_2 and KS_4 are the half saturation constants for plant uptake of NH_3-N and nitrification, respectively. Half saturation constants are used in Monod-type equations for kinetic studies examining the relationship between substrate removal rates and substrate concentration (Monod 1949).

The nitrate compartment is affected by mass inflow and input of NO_3-N produced from nitrification (R23). Losses from this compartment include denitrification (R3A), and plant uptake of NO_3-N (R31). The rate of denitrification encompasses the losses of NO_3-N via anaerobic processes occurring both in the water column and sediments. Thus, this rate accounts for further transformations in the detritus without actually addressing the detritus compartment of the system. Plant uptake of NO_3-N is mediated by plant growth, plant density, substrate availability and an ammonia preference factor (Z). This factor takes into account the preference of water hyacinth for NH_3-N over NO_3-N. KS_3 is the half saturation constant for plant uptake of NO_3-N.

The system described, presents a detailed conceptual model of the first-stage water hyacinth system focusing on the major control nutrient; nitrogen. The model allows for the interactions of the three nitrogen species but simplifies some of the processes including the transformation of NO_2-N and the detritus compartments. The model as presented, gives some initial detail as to the mathematical formulation which could be used to represent the rates mediating the transformations and material flow between the compartments.

CONCLUSIONS

Based on the results of this investigation, the following conclusions can be drawn:

1. The results of the analysis of the first and second-stages of the polyculture system indicated that the system subcomponents were well integrated and performed complimentary functions for the removal of the three nitrogen species.
2. Based on the general wastewater characteristics, the polyculture system reduced total COD, TSS, total coliform (MPN), and turbidity (NTU), an average of 58, 98, 99.9 and 94 percent, respectively.
3. The results of the analysis of the first-stage water hyacinth system indicate that the system is capable of removing significant quantities of NH_3-N, NO_3-N and ORG-N.
4. The first-stage water hyacinth system produced effluent NH_3-N concentrations of less than 5.0 mg/L at influent NH_3-N loading rates of 10 to 35 kg/ha-d.
5. For the five sample periods, plant-N uptake accounted for an average of 42 percent of the total-N removed by the first-stage system; however, at higher NH_3-N loading rates this percentage dropped to 22 percent.
6. Calculated values of alkalinity through the system indicated an average reduction of 21.1 percent. From this result it would appear that processes such as nitrification and denitrification were active in the first-stage water hyacinth system.
7. At the average operational pH of the hyacinth system, it appeared that volatilization would not remove significant quantities of NH_3-N.
8. The removal of ORG-N by the hyacinth system averaged 30 percent of the total -N lost to the system. This loss could be attributed to the enmeshment-sedimentation removal processes occurring within the system.
9. The results of the analysis of the second-stage *Azolla*-prawn system indicated that the system was capable of providing good removal of low concentrations of NH_3-N, NO_3-N and ORG-N on a mass loading basis while allowing for the culture of the freshwater prawn, *Macrobrachium rosenbergii*.

10. Although final effluent NH_3-N concentrations were greater than 8.0 mg/L, the Azolla-prawn system achieved the best NH_3-N removals at loading rates greater than 1,272 gm NH_3-N/ha-d.
11. The role of Azolla nitrogen uptake in the second-stage system was difficult to assess because of the nitrogen fixing capabilities of the plant.
12. For the size categories of prawns used in this study, the prawns accounted for less than five percent of total-N lost to the system.
13. Prawn growth studies performed in aquaria in the laboratory indicated that, for prawns in the 2.13-3.45 gm size category, there was no statistical difference at the 0.005 level of significance in growth rates obtained from diets consisting of either commercial pellets only or a commercial pellet-hyacinth combination.
14. A comparison of mean growth rates for the 2.13-3.45 gm prawns revealed that the combination diet resulted in 15.5 percent higher growth rates than the commercial pellet-only diet.
15. For prawns in the size category of 0.43 to 0.9 gm, the commercial pellet-only diet resulted in a 44 percent higher growth rate than the combination diet at the 0.005 level of significance.
16. For both size categories the prawn growth rate using the hyacinth-only diet was 87 to 92 percent lower than the other two diets at 0.005 level of significance.
17. The conceptual model was developed for the first-stage water hyacinth system. It appears that a three compartment model should be adequate to describe the nitrogen dynamics of the water hyacinth system.

RECOMMENDATIONS

In the course of the field development and analysis of the polyculture system, several specific areas of possible future research were identified for the system.

1. Investigate the use of other native macrophytes such as duckweed (Lemna minor), water cress (Nasturtium officinale), and cattails (Typha latifoila) for use in a polyculture treatment system.
2. Investigate the inclusion of cage-grown carp, catfish or Tilapia in the second-stage system to increase saleable by-products.
3. Quantify the nitrifier population in the hyacinth system and verify if the root mass provides enhanced sites of bacterial attachment.
4. Quantify and locate the denitrifier populations in the first-stage water hyacinth system and develop a design to enhance the removal of NO_3-N.
5. Operate the system over a full season to assess areas of operation which may need further refinement.
6. Develop energy, feed and construction costs associated with the integrated polyculture system to optimize a business plan to anticipate month-to-month cash flow variations.
7. Evaluate the health-related aspects of growing cultured species in wastewater.
8. Fully develop the conceptual model for application on a microcomputer.
9. Complete testing, calibration and validation of the mathematical model.
10. Expand the first-stage water hyacinth model to integrate with a similar model for the second-stage Azolla-prawn system.

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