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REMOVAL OF NITROGEN BY AN AQUATIC PLANT, *ELODEA DENSA*, IN RECIRCULATING *MACROBRACHIUM* CULTURE SYSTEMS

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ABSTRACT

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Submerged aquatic plants, *Elodea densa*, were included in recirculating *Macrobrachium rosenbergii* culture systems to examine their ability to remove nitrogenous metabolites excreted by the shrimp. Shrimp were placed at two stocking densities in aquaria with new subgravel filters, with and without plants, and water quality (NH_4-N, NO_2-N, NO_3-N and pH) was monitored for 28-day periods during the activation phase of the biological filters. Ammonia and nitrite concentrations were typically an order of magnitude less in systems with *Elodea* than without when shrimp were stocked at equal densities. Peak ammonia and nitrite concentrations reached 4.0 mg NH_4-N/l and 5.7 mg NO_2-N/l in systems without plants, but were only 0.2 and 0.4 mg/l, respectively, in systems with plants.

INTRODUCTION

An important and often limiting factor in the choice of a site for aquaculture facilities is the availability of a water supply of appropriate quantity and quality. In areas where the water supply is inadequate, recirculating and reconditioning the existing water can make the site suitable for aquaculture. Technology to accomplish this has developed rapidly in recent years, making it possible to expand aquaculture into areas where formerly it could not have succeeded.

Recirculating systems, however, present culturists with difficulties not experienced in flow-through systems. Recirculated water must be purified prior to returning it to a culture system, since metabolic and excretory processes of the cultured animals cause deleterious alterations of various water quality parameters (Liao and Mayo, 1971; Hirayama, 1974). Accumulation of inorganic nitrogen compounds is one of the most critical alterations of water quality that must be controlled in recirculating systems. Ammonia is the principal form of nitrogen excreted by aquatic animals, and its toxicity to both crustaceans (Wickins, 1976; Armstrong et al., 1978) and fish (Merkens and Downing, 1957; Colt and Tchobanoglous, 1976, 1978) is well documented.

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Ammonia may be removed from culture water by physical, chemical, or biological means, but the most frequent application has been biological filtration systems utilizing nitrifying bacteria. An intermediate product of this process is nitrite which is also highly toxic (Armstrong et al., 1976; Russo et al., 1974), but is oxidized to nontoxic nitrate in the nitrifying process.

During start-up or activation phases of biological filters, both ammonia and nitrite can reach high, though transitory, concentrations as populations of the different nitrifying bacteria increase (Hirayama, 1974). Lethally toxic concentrations of both compounds can occur during filter activation (Collins et al., 1975), and so introduction of culture species must be delayed until full nitrification is achieved. An additional component of culture systems that might reduce such delays is aquatic plants. In addition to serving as refuge and food, plants reduce ammonia levels by direct absorption (Stephenson et al., 1980; Nelson et al., 1981) and probably serve as an inoculum of nitrifying bacteria. In this study we investigated the usefulness and practicality of including the aquatic plant *Elodea densa* as a component of the biological filtration system to attenuate inorganic nitrogen levels in *Macrobrachium rosenbergii* culture tanks.

MATERIALS AND METHODS

Experiments were done in 12 37-l glass aquaria, each equipped with standard undergravel filters covered by 3 l of quartz gravel and 2 l of dolomite gravel, 3- 5 mm in diameter. The dolomite served as a pH buffer to prevent rapid acidification of the culture water, a hazard commonly observed in closed systems (Spotte, 1970). Each aquarium was supplied with an airstone for additional circulation and a fiberglass cover to minimize water evaporation and loss of shrimp. Besides these 12 tanks, three aquaria were included that did not contain gravel or filters. Temperature was maintained at 27- 28°C in all aquaria by placing them in a 25 cm deep water table. Illumination was provided 16 h a day by banks of cool-white fluorescent lights suspended 43 cm above the aquaria, that delivered 162 microeinsteins m^{-2} s^{-1} (1 microeinstein = 6.02×10^{17} photons). All 15 aquaria were filled with 31 l of tapwater 2 days before the introduction of shrimp and plants to allow residual chlorine to dissipate, and to allow pH and temperature to stabilize.

Treatment variables studied in these experiments (Table I, three replicates per treatment) were two stocking densities of shrimp (20 or 40 per tank), and presence or absence of live plants and/or undergravel filters. Since the surface area of submerged aquatic macrophytes is colonized by bacteria and algae (Forsberg, 1965; Trust and Bartlett, 1976), we used artificial plastic plants as a control for comparable surface areas and epiphytic populations. This was achieved by keeping plastic plants, *E. densa*, and *M. rosenbergii* one week in the same large recirculating holding tank. This gave bacteria and algae equal opportunity to colonize both live and plastic plants. At the beginning of experiments, both plant groups were rinsed with fresh tapwater, trimmed

TABLE I

Experimental conditions of different treatments in shrimp culture systems

Characteristic	Treatment*				
	1A	1B	2A	2B	2C
Shrimp stocking density					
(Number/tank)	40	40	20	20	20
(g dry wt/l)	0.60	0.60	0.31	0.32	0.31
(Standard deviation)	0.03	0.01	0.01	0.01	0.01
Live plant stocking density					
(Number/tank)	0	60	0	60	0
(g dry wt/l)**	0	0.38	0	0.38	0
Plastic plant stocking density					
(Number/tank)	60	0	60	0	60
Undergravel filter	Yes	Yes	Yes	Yes	No

*Each treatment had three replicate tanks. Number—letter headings refer to figure numbers.
**To calculate wet weight of plants, *E. densa* has a mean water content of 92.9 ± 0.2%, n = 6.

to a length of 20 cm, and anchored in plastic egg crating (such as is used with fluorescent light fixtures) to maintain them in an upright position and distribute them evenly throughout each aquarium. Nine tanks each received 60 plastic plants and six tanks each received 60 live *E. densa* (0.38 g dry weight/l, based on 31 l total volume; the mean water content of *E. densa* is 92.9 ± 0.2%, n = 6, for conversion of dry to wet weight).

In order to determine appropriate ratios of plant : shrimp biomass for experiments designed to study nitrogen control during filter activation, preliminary experiments of ammonia uptake by *E. densa* were conducted. In a first test, nine 90-l aquaria were filled with water and NH_4Cl to give an initial ammonia concentration of about 2.8 mg NH_4-N/l (see Table II) at a water pH of about 8.8. *Elodea densa* were added to aquaria at two stocking densities of about 0.13 and 0.26 g dry wt/l, and the ammonia uptake rate was calculated based on the reduction in total ammonia over 31 h. Prior to these tests, *E. densa* were kept in carp tanks with sufficiently high ammonia levels to ensure that plants were not nitrogen-limited at the beginning of uptake tests (Fitzgerald, 1968, reported that ammonia uptake by nitrogen-limited algae and aquatic macrophytes may be 2—5 times greater than by plants reared in sufficient nitrogen). In a second experiment, a fairly equal biomass of *E. densa* (about 1.9 g dry wt/l) was added to 1000-ml beakers of different initial ammonia concentrations from 0.15 to 3.48 mg NH_4-N/l (Table II). Based on rates of ammonia uptake by *E. densa* (Table II) and published excretion rates for *M. rosenbergii* (Nelson et al., 1977a; Armstrong et al., 1981), plant : shrimp biomass ratios were selected that fell within or outside the theoretical ratio required to control ammonia during filter-activation experiments.

TABLE II

Uptake of ammonia by *Elodea densa*

Experiment	n	Test duration (h)	Plant stocking density (g dry wt/l)	Mean initial substrate concentration (mg NH_4-N/l)	Mean uptake rate (mg NH_4-N/g dry wt plant h^{-1} ± SD)
1. NH_4-N uptake in aquaria	2	31	0.0*	2.78	0.0
	3	31	0.27	2.82	62 ± 13.0
	3	31	0.13	2.95	110 ± 21.4
2. NH_4-N uptake in beakers	3	6	1.92	0.15	5 ± 0.7
	3	6	1.89	0.29	14 ± 3.4
	3	6	1.10	1.10	46 ± 7.4
	3	6	1.96	3.48	117 ± 7.7

*Two control tanks had no plants. Initial ammonia concentrations did not change over 31 h, indicating no loss by volatilization. Similar controls for the beaker experiments confirmed this result.

Juvenile *M. rosenbergii* used in experiments were obtained from the Marine Resources Research Institute of South Carolina (supplied by T. Smith and P. Sandifer), and additional animals were contributed by Anuenue Fisheries Research Center of Hawaii (S. Malecha) and purchased from Aquatic Farms, Hawaii. For experiments shrimp were weighed in groups of 5- 10 and placed in the aquaria for an initial stocking density of either 20 (1.25 g wet wt/l; 0.31 g dry wt/l) or 40 (2.39 g wet wt/l; 0.60 g dry wt/l) shrimp per tank. Measurements of carapace length, wet weight, and dry weight were taken on a subsample of 20 remaining shrimp. Mean carapace length of these shrimp was 13.2 ± 1.2 mm, average wet weight was 1.91 g/shrimp, and average dry weight was 0.45 g/shrimp.

Shrimp were fed Purina Experimental Marine Ration 20 at a daily rate of 2% of their initial wet weight (1.5 or 0.78 g of food per tank). This feeding rate was low (Smith and Sandifer, 1979), but chosen to prevent overfeeding and fouling of the water during the period of filter activation. Dead shrimp observed during experiments were removed so that decomposition would not contribute excessive amounts of ammonia to the culture water.

Water samples (250 ml/tank) were collected from each tank every two days and analyzed immediately for temperature, pH, ammonia, nitrite, and nitrate. Dissolved oxygen, hardness, and alkalinity were analyzed biweekly. Water removed for sampling was replaced with an equal volume of fresh tapwater, so that a constant volume of 31 l was maintained in all tanks.

Concentrations of ammonia, nitrite, and nitrate were determined spectrophotometrically by the phenate, sulfanilamide, and brucine methods, respectively, of Standard Methods (1976). The pH was determined with a Corning pH meter, and dissolved oxygen with a Yellow Springs Instrument Co. oxygen meter. Hardness was determined by the EDTA titrimetric method, and alkalinity by potentiometric titration to pH 4.5 (Standard Methods, 1976). The

terminology for nitrogen species used in this report is as follows: NH_3-N will be un-ionized ammonia-nitrogen, NH_4^+-N will be ionized ammonia-nitrogen, and NH_4-N will be total ammonia-nitrogen (the sum of NH_3-N and NH_4^+-N); NO_2-N will refer to total nitrite-nitrogen; and NO_3-N will be total nitrate-nitrogen. All tests results were examined for significance by analysis of variance according to SPSS Breakdown and ANOVA procedures (Nie et al., 1975).

RESULTS

Reduction of inorganic nitrogen by Elodea densa

Shrimp culture tanks that included *E. densa* had significantly lower concentrations of all three inorganic nitrogen species during 28-day tests, regardless

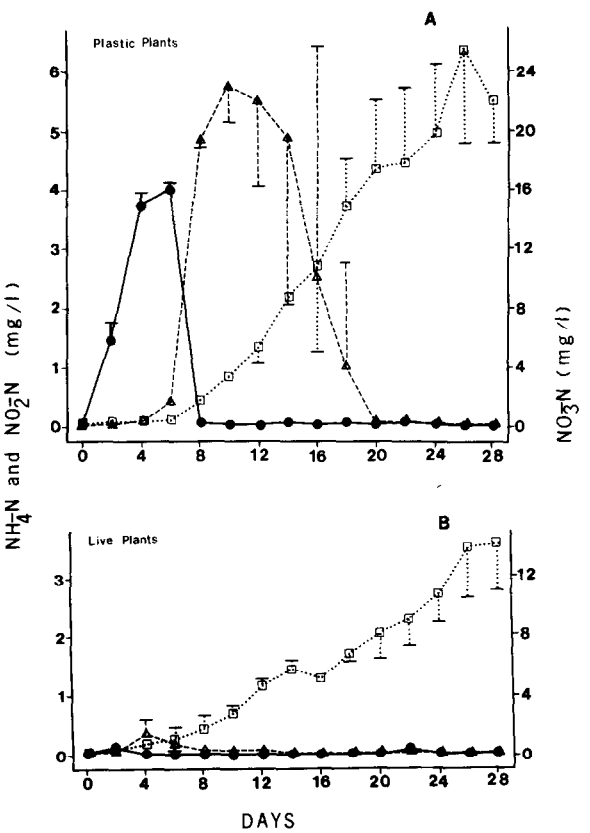

Fig. 1. Mean concentrations in mg/l of NH_4-N (solid circles), NO_2-N (open triangles), and NO_3-N (open squares) in (A) filtered treatments stocked with 40 shrimp and plastic plants, and (B) filtered treatments stocked with 40 shrimp and 60 live plants. Vertical bars represent one standard deviation from the mean, and are only included when values are significant enough to be presented visually. Symbols for NH_4, NO_2, and NO_3 are standard for all figures.

of shrimp stocking density (NH_4-N, $P < 0.001$, F = 19.219; NO_2-N, $P < 0.001$, F = 24.746; NO_3-N, $P < 0.001$, F = 22.324).

Filtered systems stocked with 40 shrimp (about 0.6 g dry wt/l) and plastic plants showed typical sequential peaks of NH_4-N, NO_2-N, and NO_3-N (Fig. 1A). Ammonia reached a peak of 4 mg NH_4-N/l on day 6, then declined to almost zero by day 8. Nitrite rose sharply between days 6 and 10, reached a peak of about 5.7 mg NO_2-N/l on day 10, and dropped to zero by day 20. Nitrate increased steadily from day 6 and accumulated to a peak of about 22- 25 mg NO_3-N/l by the end of the experiment. In contrast, tanks stocked with the same biomass of shrimp (about 0.6 g/l), but with 60 live plants per tank (0.38 g dry wt/l) showed virtually no measurable concentrations of ammonia or nitrite during the entire 28-day experiment (Fig. 1B). Nitrate only accumulated to a concentration of 15 mg NO_3-N/l, almost half the level reached in tanks without plants.

Filtered systems stocked with 20 shrimp (about 0.3 g/l) and plastic plants also had sequential peaks of the three nitrogen species (Fig. 2A), although not as pronounced as systems stocked with 40 shrimp. Ammonia reached a peak of 0.8 mg NH_4-N/l on day 4, then declined to near-zero by day 8. Nitrite reach-

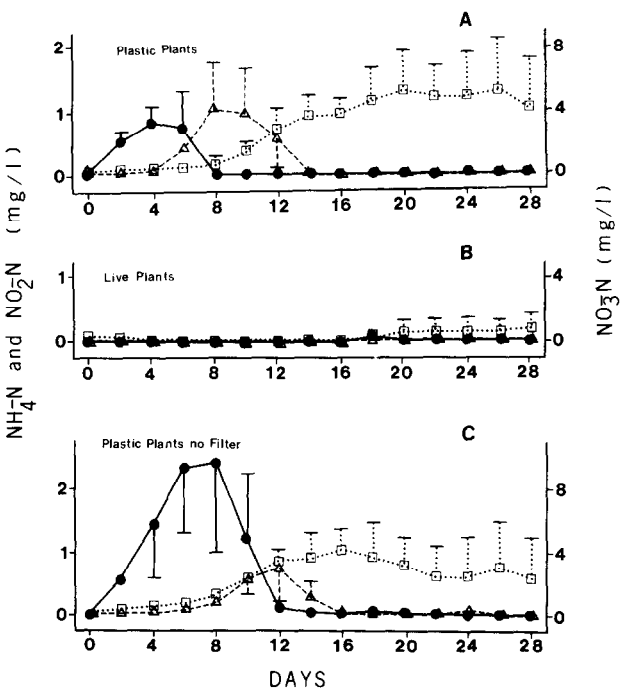

Fig. 2. Mean concentrations in mg/l of NH_4-N (solid circles), NO_2-N (open triangles), and NO_3-N (open squares) in (A) filtered treatments stocked with 20 shrimp and plastic plants, (B) filtered treatments stocked with 20 shrimp and 60 live plants, and (C) non-filtered treatments stocked with 20 shrimp and plastic plants. Vertical bars represent one standard deviation from the mean, and are only included when values are significant enough to be presented visually.

ed a peak of about 1.1 mg NO_2-N/l by day 8 and declined to near-zero by day 14. Nitrate increased steadily from day 8, peaked at about 5.2 mg NO_3-N/l, and remained at that level for the rest of the experiment. Systems stocked with 20 shrimp and 60 live plants per tank showed almost no measurable quantities of ammonia, nitrite, or nitrate during the entire experiment (Fig. 2B). Nitrate reached a peak of only 0.27 mg NO_3-N/l in these systems, about 20 times less than in systems without plants.

Unfiltered systems stocked with 20 shrimp and plastic plants accumulated three times as much ammonia (Fig. 2C) as did filtered systems with the same stocking density of shrimp, and reached a peak of 2.4 mg NH_4-N/l on day 8. Nitrite took 12 days to reach a peak of 0.76 mg NO_2-N/l, and declined to near-zero by day 16. Nitrate accumulated steadily until day 16, reaching a peak of 4 mg NO_3-N/l, and declined to 2.3 mg NO_3-N/l by the end of the 28-day experiment.

Changes in other water quality parameters

Measurements were made of other water quality parameters on days 12 and 26 of the experiments. Dissolved oxygen did not differ significantly between any treatments and averaged about 7.40 mg/l, close to saturation. Alkalinity and hardness did not change appreciably between measurements in any treatment, but both factors were substantially lower in systems that did not contain undergravel filters (Table III). The pH was monitored every 2 days and found to be lower in tanks without undergravel filters (7.4, versus 8.0 for other treatments) (Fig. 3). Because pH affects the relative concentrations of un-ionized (NH_3) and ionized (NH_4^+) ammonia in culture water, and because NH_3 is considered more toxic than NH_4^+, the proportion of total ammonia as NH_3-N was calculated as described by Armstrong et al. (1978) (pKa = 9.19 at 27°C). Calculations were made only with data from treatments that lacked *E. densa* since these were the tanks with highest and therefore potentially most toxic concentrations of un-ionized ammonia. The greatest levels of NH_3 measured

TABLE III

Values of alkalinity and hardness in recirculating systems

Treatment	Figure No.	Alkalinity (mg/l as $CaCO_3$)		Hardness (mg/l as $CaCO_3$)	
		Day 12	Day 26	Day 12	Day 26
40 shrimp, plastic plants, filtered	1A	54.0	34.7	87.3	111.3
40 shrimp, 60 plants, filtered	1B	58.7	48.0	73.3	99.3
20 shrimp, plastic plants, filtered	2A	60.3	58.0	70.7	73.3
20 shrimp, 60 plants, filtered	2B	61.0	61.3	58.0	60.7
20 shrimp, plastic plants, unfiltered	2C	10.3	16.7	36.7	40.7

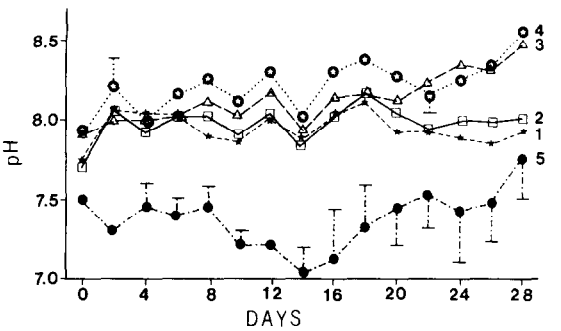

Fig. 3. Mean pH in (1) filtered treatments stocked with 40 shrimp and plastic plants (solid stars), (2) filtered treatments stocked with 40 shrimp and 60 live plants (open squares), (3) filtered treatments stocked with 20 shrimp and plastic plants (open triangles), (4) filtered treatments stocked with 20 shrimp and 60 live plants (starred circles), and (5) non-filtered treatments stocked with 20 shrimp and plastic plants (solid circles). Vertical bars represent one standard deviation from the mean and are only included when values are significant enough to be presented visually.

reached 0.25 mg NH_3-N/l in systems with 40 shrimp each (Fig. 4; refer to Fig. 1A), but this was well below levels reported as toxic to juvenile *M. rosenbergii* by Wickins (1976) (1.43 mg NH_3-N/l resulted in an LT_{50} of 28.3 h).

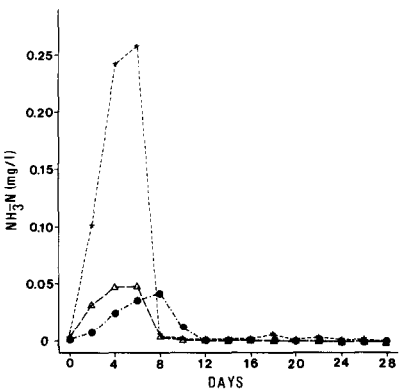

Fig. 4. Mean concentrations in mg/l of un-ionized ammonia (NH_3-N) in (1) filtered treatment stocked with 40 shrimp and plastic plants (solid stars), (2) non-filtered treatments stocked with 20 shrimp and plastic plants (solid circles), and (3) filtered treatments stocked with 20 shrimp and plastic plants (open triangles).

Mortality and growth of shrimp

Mortality was high in all treatments and ranged from 31.7% to 49.2% by day 28 of experiments but did not seem to be related to water quality or presence or absence of *E. densa*. In tanks stocked initially with 40 shrimp each, mean mortality was 49.2% and 47.5% in replicates with and without plants, respectively. Mortalities were somewhat less in tanks stocked at 20 shrimp each

but in these treatments too, plants did not attenuate mortality, which averaged 38.3% in systems with and without *E. densa.* Most of the mortality during the 28-day experiment was attributed to cannibalism, behavior commonly observed among juvenile *M. rosenbergii* (Ling, 1962; Smith and Sandifer, 1975), during the molt process when shrimp are most vulnerable to predation. Juvenile *M. rosenbergii* molt every 20–40 days (Ling, 1962), and many molted during the course of these 28-day studies, although this event was not routinely recorded because of dense plant cover in the tanks. In the close confines of small aquaria, a shrimp that molts has very little chance to escape predation and shrimp were often observed with few or no appendages remaining.

Growth among surviving shrimp averaged about 0.21 g dry wt/shrimp, from an initial weight of 0.45 ± 0.01 g/shrimp (water = 76% of total wet weight) to a final weight of 0.65 ± 0.07 g/shrimp, an increase of 47%. Carapace length increased 1.5 ± 0.4 mm from an initial length of 13.2 ± 1.2 mm. There were no significant differences in weight or length between treatments. Although shrimp grew during these experiments, total biomass per tank did not increase over 28 days because of high mortalities; in fact, biomass actually decreased slightly from the initial values presented in Table I.

Growth of Elodea densa

The biomass of *E. densa* increased in tanks of all treatments, but particularly those stocked with only 20 shrimp (Table IV). Stem length of plants increased an average of 45% from 20.3 cm to 29.4 cm/plant. Total dry weight biomass of plants increased 83% and 93% in tanks with 40 and 20 shrimp, respectively (Table IV). Although *M. rosenbergii* does not grow well on a diet composed entirely of aquatic plants, it does feed on them (Stern et al., 1976). It is as-

TABLE IV

Growth of *Elodea densa* in shrimp culture systems during a 28-day experiment

Treatment	n (tanks/ TMT)	Mean weight of plants* (g/tank)					Mean stem length (cm/plant)	
		Wet		Dry				
		Initial	Final (± SD)	Initial	Final (± SD)	In-crease (%)	Initial	Final (± SD)
40 shrimp, 60 plants, filtered	3	169.2	381 (23)	11.7	21.4 (0.9)	83	20.3	29.3 (0.5)
20 shrimp, 60 plants, filtered	3	169.2	435 (47)	11.7	22.6 (2.7)	93	20.3	29.5 (1.5)

*Sample size for initial weights: n = 20; final weights: n = 80 for both treatments.

sumed that shrimp cropped plants in all treatments, and a higher initial stocking density caused reduced growth of plants measured in treatments with 40 animals.

DISCUSSION

Elodea densa significantly lowered inorganic nitrogen concentrations during activation of biological filters in shrimp culture systems. Systems without plants developed sequential peaks of ammonia (4.0 mg NH_4-N/l), nitrite (5.7 mg NO_2-N/l), and nitrate (up to 24 mg NO_3-N/l) that are typical of successional development of nitrifying bacterial populations (Hirayama, 1974; Collins et al., 1975). Although concentrations of ammonia and nitrite did not reach levels reported as toxic to larvae and juveniles of *M. rosenbergii* (Armstrong et al., 1976, 1978; Wickins, 1976), high transitory concentrations serve to remind culturists of the threat of nitrogen toxicity inherent in filter activation. Collins et al. (1975) reported 100% mortality of channel catfish during filter activation caused by high levels of ammonia, and particularly nitrite. The results of the present study indicate that aquatic plants can improve water quality to the extent that such occurrences can be prevented. Hydroponic culture of terrestrial plants in new recirculating systems appears to be less successful in reducing ammonia and nitrite levels than is use of submerged aquatic plants. Bisordi (1981) grew lettuce and rye in a trout culture system, which prevented accumulation of ammonia, but not nitrite, during filter activation. Pierce (1980) and Naegel (1977) successfully grew hydroponic plant crops in fish culture water, but inorganic nitrogen concentrations were not reduced by such growth.

In order to evaluate further the usefulness of aquatic plants in recirculating aquaculture systems, a partial nitrogen budget for the treatments was calculated based on input as food and removal as increase in plant biomass. Although preliminary determinations of nitrogen input were based primarily upon shrimp ammonia excretion rates, it was not possible to use this method to evaluate the results of the final experiment because shrimp biomass could not be accurately determined after the experiment had begun. Significant mortality occurred in all treatments, which resulted in a decline in total shrimp biomass by the end of the 28-day trial. Since the nitrogen content of Purina Ration 20 is known (4.42% nitrogen; Nelson et al., 1977b) and was a constant daily input to each treatment (2% of initial wet wt = 1.50 or 0.78 g food per tank per day), this value rather than the ammonia excretion rate of shrimp was used to estimate the amount of nitrogen added to tanks during the course of experiments (Table V). The amount of nitrogen removed by plants was calculated from data on nitrogen content from Gerloff and Krombholz (1966) who found that 2—3% of the dry weight of *Elodea* spp. was nitrogen. The increase in dry weight of plants at the end of 28 days (Table IV) was multiplied by 0.03 to determine the increase in plant nitrogen over the entire experiment. The concentrations of ammonia, nitrite, and nitrate on day 28 as mg/l were multiplied

TABLE V

Nitrogen input as food and removel by *Elodea densa* in recirculating systems during 28-day experiments

Treatment (n = 3 tanks/TMT)	Total nitrogen input as food (g)	Mean nitrogen removal by plants		Total nitrogen in culture water on 28th day		Unaccountable nitrogen losses	
		(g)	(%)	(g)	(%)	(g)	(%)
40 shrimp, plastic plants, filtered	1.856	—	—	0.670	36.1	1.186	63.9
40 shrimp, 60 plants, filtered	1.856	0.292	15.7	0.434	23.4	1.130	60.9
20 shrimp, plastic plants, filtered	0.965	—	—	0.116	12.0	0.849	88.0
20 shrimp, 60 plants, filtered	0.965	0.327	33.9	0.017	1.8	0.621	64.4
20 shrimp, plastic plants, unfiltered	0.965	—	—	0.069	7.2	0.896	92.8

by 31 l to derive total dissolved inorganic nitrogen as part of the budget (Table V).

It was estimated that plants removed 15.7% of the total nitrogen introduced as food in treatments with 40 shrimp, and 33.9% in those stocked with 20 (Table V). Unaccountable losses of nitrogen were considerable for all treatments (60.9–92.8%). Algal and bacterial biomass, sediment, and dissolved organic nitrogen were all probable fates of this unaccountable nitrogen. In addition, the removal daily of dead shrimp (some only slightly cannibalized) represents some of the nitrogen introduced as food, although this loss was not quantified. Volatilization of gaseous NH_3 is probably not a significant route of nitrogen lost from tanks since only 1–2% of total ammonia was NH_3 at pH of culture water. Furthermore, no loss of ammonia occurred in control experiments (no animals, plants, or substrate) during 6- and 31-h experiments (Table II). Rakocy and Allison (1981) reported similar results in experiments with *Tilapia aurea* in recirculating fish culture systems. Floating and submerged aquatic plants removed 12.0–15.8% of the nitrogen added as feed during the experiment, fish growth accounted for 36.5–38.3%, dissolved inorganic nitrogen 11.1–16.0%, and the remainder, 37.5–40.9%, was unaccountably lost, probably to fates similar to those suggested for this experiment.

A buildup of nitrate occurred in tanks with plants, probably a result of

partial inhibition of nitrate uptake caused by ammonia. The assimilation of nitrate in plants is mediated by an adaptive enzyme, nitrate reductase, the activity of which is inhibited in the presence of ammonia (Ferguson, 1969). Thus, when ammonia and nitrate are present together in solution, as in recirculating systems, very little nitrate may be absorbed by vascular aquatic plants such as *Elodea* (Joy, 1969; Ferguson and Bollard, 1969; Nichols and Keeney, 1976; Nelson et al., 1981). In addition, it is likely that the oxidation of ammonia to nitrate by bacteria is more rapid than uptake of ammonia by plants. If so, once nitrifying bacteria become established in recirculating systems, some of the ammonia generated in the tanks is oxidized by bacteria before plants can absorb it as ammonia.

A decrease in nitrate concentrations was observed in some treatments without plants during the latter part of the experiment (Figs. 2A and 2C). This was likely due to the removal of nitrate from culture water by an algal bloom that developed during the last 10- 12 days in these treatments. The algae were predominantly *Oscillatoria* sp. and *Oedogonium* sp., which are capable of using nitrate as a source of nitrogen (McCarthy, 1980). Algal growth was suppressed in tanks containing *E. densa*, a phenomenon often observed with other vascular plants in aquatic systems (Hasler and Jones, 1949; Hogetsu et al., 1960; Goulder, 1969; Fitzgerald, 1969). This is usually attributed either to competition for nutrients or to growth-inhibiting substances produced by some aquatic macrophytes. Another possibility is that the dense *Elodea* canopy in tanks shaded and reduced light to levels too low for algal growth.

Some of the reduction of ammonia and nitrite levels observed in treatments with live *E. densa* may be due to more substantial inoculations of nitrifying bacteria carried on the plants' surface than introduced in treatments with plastic plants, despite precautions taken (see Materials and Methods). Both ammonia and nitrite had peaked and disappeared within 8 days in treatments containing live plants and 40 shrimp (Fig. 1B), whereas it took 20 days for these peaks to disappear in treatments with plastic plants and 40 shrimp (Fig. 1A). These results are similar to those of Carmignani and Bennett (1977) and Bower and Turner (1981), who seeded filters in new recirculating systems with bacteria from established filters to eliminate toxic concentrations of ammonia and nitrite. *Elodea densa* used in our experiments directly absorbed ammonia and likely provided a substantial population of nitrifying bacteria. The overall effect was virtual elimination of potentially toxic ammonia and nitrite, which speaks strongly for inclusion of aquatic macrophytes in freshwater aquaculture systems.

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