AMMONIA EXCRETION OF FISH AND NITRIFICATION CAPACITY OF BIOFILTERS IN RECIRCULATING FISH HOLDING FACILITIES

by

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ABSTRACT

Ammonia production of Tilapia zillii and nitrification capacity of biofilters were tested to develop data useful in designing large recirculating systems. A reliable method of predicting ammonia production by fish was supported with experimental data. The Ammonia Potential method states that ammonia production is wholly dependent upon the amount of food fed to the fish within a system, the percent nitrogen of the food and the conversion ratio maintained in the system. Fish excrete several nitrogenous waste products in addition to ammonia which will be eventually degraded to ammonia in a 100 percent recycle system. Consequently, all nitrogen excreted by fish will exert a nitrification demand and must be taken into account when designing recirculating systems.

A nitrification ratio of 18.065 mg ammonia-N/ft^2/day in biofilters was determined. Appreciable nitrification occurred from nitrifers attached to submerged walls and floors of fish holding facilities. Little, if any, nitrification occurred from suspended nitrifers.

This information is useful for designing larger recirculating systems, however consideration must be given to detrimental social interactions among fish at high densities. Extrapolation of these data to other systems must be done cautiously because of the difficulty in duplicating the chemical and biological conditions that existed in this study.

INTRODUCTION

Fish excrete nitrogenous waste products via the gills and the kidneys (Smith, 1929) and as fecal material. Ammonia is the primary nitrogenous excretion. Brett and Zala (1975) indicated that ammonia composed 63 percent of the total excreted nitrogen by sockeye salmon (Oncorhynchus nerka). In rainbow trout (Salmo gairdneri), Fromm (1963) found about 60 percent of the excreted nitrogen was ammonia. Wood (1958) found ammonia excretion was highly variable and averaged 69 percent in three marine species. Urea, trimethylamine, trimethylamine oxide, creatine, creatinine and uric acid form progressively smaller percentages of the remaining nitrogenous waste products (Wood, 1958; Fromm, 1970).

All nitrogenous wastes can be degraded to ammonia. For example, urea can be hydrolyzed to ammonium carbonate by several genera of bacteria (Buchanan and Fulmer, 1930; Breed, Murray and Smith, 1957). Additionally, the nitrogenous components of fecal material can undergo ammonification and exist as ammonia (Sawyer and McCarty, 1967). A previous attempt to design nitrification facilities for water reuse in trout hatcheries (Speece, 1973) accounted for nitrogen excreted only as ammonia, but did not take into account the ammonia produced through ammonification of urea, fecal material, trimethylamine, etc. All forms of nitrogen must be accounted for because they will eventually exert a nitrification demand upon a recirculating system.

Under typical pH's and temperatures encountered in fish culture, most aqueous ammonia exists in the ionized form (ammonium - NH_4^+). Positively charged ammonium ions combine with negatively charged ions to form harmless compounds. The small percentage of un-ionized ammonia ions, 0.85 percent at 15°C and a pH of 7.5 (Trussell, 1972), can be lethal to fish. Rice and Stokes (1975) found a 24-hour TL_m of 0.097 mg/l NH_3 for rainbow trout. Lloyd and Orr (1969) indicated "toxic levels" of NH_3 for rainbow trout were 0.39 mg/l and recommended that un-ionized ammonia concentrations remain below 12 percent of this value (i.e., below 0.0468 mg/l). Burrows (1964) reported extensive hyperplasia in salmonid gill epithelium exposed to a continuous un-ionized ammonia level of 0.006 mg/l for six weeks.

Ammonia can accumulate rapidly and become toxic to fish held in a closed system. However, ammonia can be detoxified into nitrate by using a biofilter in which the nitrification process occurs. Biofilters are effective nitrification mechanisms when properly maintained (Chu and Greene, 1967; Giudice, 1966; McCrimmon and Berst, 1966; Parker and Simco, 1974).

Parisot (1967) described his biofilter as 24 inches deep with a media volume of about 25 ft^3. At Humboldt State College, DeWitt and Salo (1960) described their filters as "a series of three 230 gpm sand-and-rock filters". Collins, Gratzek, Shotts, Dawe, Campbell and Senn (1975) used one liter of filter media for every 70 liters of aquarium water in their nitrification studies. Although reference was given in these studies to filter size, no rationale was given for the particular size used.

Information from all of these studies do not provide a basis for designing large scale recirculating systems. Data are lacking that relate the ammonia production of fish to the nitrification capacity of biofilters.

The purpose of this project was to collect data on fish and recirculating systems that could be extrapolated from pilot studies to systems of a larger design. The basic objectives were:

1. To determine the performance differences, if any, of various media depths in biofilters,

2. To develop a method of predicting the amount of ammonia produced by Tilapia zillii, and

3. To determine the nitrification capacity of biofilters.

METHODS AND MATERIALS

Laboratory experiments were conducted from February to December 1976 using *Tilapia zillii* as test fish. The fish were fed Purina Catfish Chow at approximately 1 percent total body weight every other day unless otherwise indicated. The percent nitrogen of this food was determined at EFCO Laboratories in Tucson, Arizona. Unchlorinated well water was used throughout the experiments.

Water Quality

Dissolved oxygen (DO) and temperature were determined daily with a portable YSI Model 54 meter. Hydrogen ion concentration (pH) was determined with a Leeds Northrup Model 9775 meter and total alkalinity by the potentiometric method (*Standard Methods*, 1971). Ammonia-nitrogen was measured by the distillation-Nesslerization method using a 500 ml sample unless otherwise noted. The presence of nitrites was determined intermittently with Nitriver III (R) nitrite "powder pillows" (Hach Chemical Co., Ames, Iowa). Nitrate analysis was accomplished by personnel at the City of Tucson Sewage Treatment Plant.

DO levels above 5 mg/l were maintained with compressed air. Water temperature was regulated between 15 and 20°C by room air temperature and 150 watt submersible heaters. Alkalinity and pH were adjusted to approximately 100 mg/l as $CaCO_3$ and 8.0 as needed with $NaHCO_3$.

Ammonia Excretion in Static Isolation

Aquaria used in this experiment were thoroughly cleaned before each test period to reduce the possibility of bacterial intervention. The fish were placed in holding tanks between tests and transferred to the aquaria immediately prior to testing. Three sizes of *T. zillii* were used:

A) 50 mm T.L.; 100 each @ 1.76 g = 176 g fish

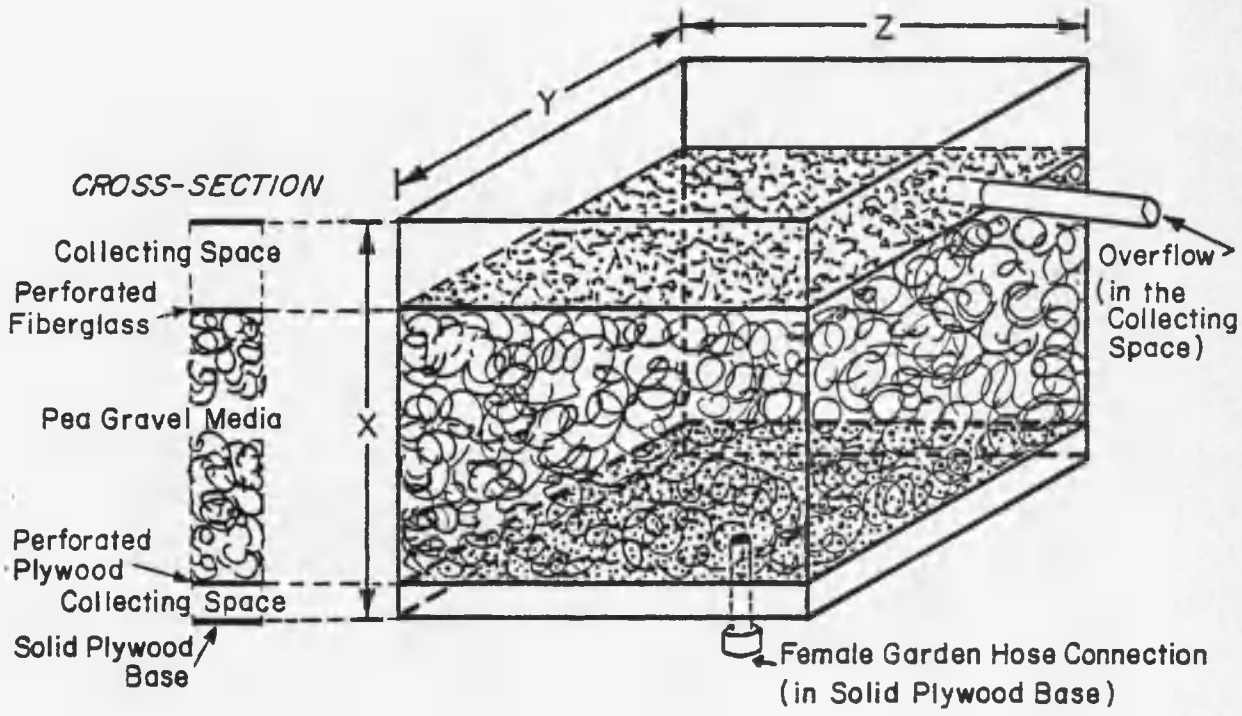
B) 75 mm T.L.; 50 each @ 7.50 g = 375 g fish

C) 100 mm T.L.; 50 each @ 15.00 g = 750 g fish

All fish were fed prior to testing and fasted during the 24-hour test period. Each group was placed in 100 liters of ammonia-free well water. At test conclusion, the ammonia concentration of a 1000 ml sample was determined and ammonia excretion per kilogram of fish per 24 hours was calculated. Three replicates of each test were conducted with several days intermission between tests.

Biofilter System Apparatus

Separate recirculating systems were operated 108 days for the various experiments. Three different filter shapes were constructed with a media capacity of one cubic foot (30,080 cm^3) each (Fig. 1). Filters were constructed of 3/4-inch (1.9 cm) plywood covered with polyester fiberglass resin, and contained a false perforated bottom to aid in drainage. To ensure even distribution of pumped water over filter media, a perforated fiberglass splash pan was placed on the media surface. An overflow tube was attached to each filter as a safeguard against the filter spilling over. A standard female garden hose



System	Dimensions in inches (cm) X	Y	Z	Media Depth
Cube	18.0" (45.7)	12.0" (30.5)	12.0" (30.5)	12.0" (30.5)
Tall	33.0" (83.8)	8.0" (20.3)	8.0" (20.3)	27.0" (68.6)
Flat	10.0" (25.4)	20.5" (52.0)	21.0" (53.3)	4.0" (10.2)

Figure 1. Illustration of basic biofilter and dimensions of filters used in experiments.

connection was attached to the bottom of each filter. Under normal operation, a drainage tube was attached to this connection that lead to the fish tank. Reverse water flow from a garden hose attached at this point aided in filter cleansing. Filters were cleaned only when they became clogged.

One quarter-inch (0.6 cm) pea gravel was used as the filter media. Measured void space and surface area per cubic foot of media were calculated by the formula in Appendix D.

The fish holding tanks were made of galvanized steel and were 4 feet (122 cm) long, 2 feet (61 cm) wide and 2 feet (61 cm) deep. One end was elevated approximately 8 inches (20 cm) to aid in feces collection by the submerged water pump. Tanks were filled with 240 liters of water and evaporation make-up water was added as needed. The tanks were covered with plastic screen to prevent fish from jumping out.

Water circulation was maintained by submersible pumps (Little Giant Model 1-MA) placed in the lowest part of the tanks. Water flow to and from filters was through 3/8-inch (1 cm) clear vinyl tubing, and flow was adjusted to four liters per minute by varying the pumping head. A system recirculation time of one hour was maintained.

A control tank, similar in all respects to the other experimental tanks except without a biofilter was used. Fish loads were periodically changed.

Ammonia Excretion and Nitrification Experiments

Additional experiments were conducted using the flat and cube systems. To ensure sufficient nutrients for the nitrifying bacteria,

systems were tested only for 24-hour periods. One cubic foot (30,080 cm^3) of mature biofilter media was used in the cube system and 0.5 cubic foot (15,040 cm^3) in the flat system.

Experiment I: Ammonia Excretion

Experiments were conducted with two systems. The cube system contained 1200 and the flat system 600 T. zillii. Average fish total length was 73 mm and each system contained 240 liters of water. To estimate ammonia excretion by these fish, the pumps were removed and biofilters disconnected. The concentration of ammonia-N in the tank water was monitored at the beginning, 1.5, 3.0, 4.5, 6.0, 7.5, 9.0, and at the end of the 24-hour test period. The filters were then connected to the tanks and allowed to recirculate.

Experiment II: System Nitrification

All fish were removed from the systems and placed in other holding tanks. The ammonia-N was increased to approximately 10 mg/l by the addition of $(NH_4)_2SO_4$. The two systems were allowed to recirculate once per hour. Ammonia-N was measured at the original concentration and at 3.0, 4.5, 6.0, 7.5 and at 9.0 hours. An additional sample from the flat system was analyzed at 21 hours. The fish were replaced and the systems allowed to normally recirculate.

Experiment III: Tank Nitrification

After several days the fish were again removed and the concentration of ammonia-N adjusted to approximately 5 mg/l by $(NH_4)_2SO_4$. The pumps were removed and the biofilters were disconnected from the tanks.

In this manner, only nitrification which occurred inside the fish holding tank was measured. Initial, 4.5, 9.0 and 24.0 hour ammonia concentration was measured. The fish and the pumps were then replaced and the systems allowed to normally recirculate.

RESULTS

The systems with flat and cube filters operated for 108 days and the systems with the tall filter and no filter operated for 67 days. Various experiments were conducted with the systems within this time period. Data are presented for individual experiments.

Performance of Biofilters with Different Media Depths

Ammonia-N concentrations remained very low in each of the biofiltered systems during the 16-day experiment (Table 1). Concentrations varied from 0.17 to 0.08 mg/l in the filtered systems and steadily increased to 2.00 mg/l in the no filter system.

Ammonia Excretion in Static Isolation

Measured ammonia-N excretion varied considerably (Table 2). The variation in ammonia excretion increased as fish size decreased. Daily ammonia-N excretion per kilogram of 100 mm fish varied between 86.67 and 106.67 mg, while the 50 mm fish excreted 79.56 to 224.43 mg ammonia-N per kilogram of fish per day.

Alkalinity Change

Increasing alkalinity was correlated with increasing ammonia-N in the no filter system until a bacterial bloom apparently occurred near day 33 (Fig. 2). Ammonia was then rapidly nitrified which decreased alkalinity at a steady rate. Increasing alkalinity with increasing ammonia also

Table 1. Ammonia-N concentrations in systems with various depths of biofilter media, equal volumes (1 ft^3) and different fish loads.

System	Media depth	Number of fish: 120	240	360
Flat	4" 10.2 cm	0.14	0.08	0.11
Cube	12" 30.5 cm	0.12	0.09	0.08
Tall	27" 68.6 cm	0.17	0.12	0.09
No Filter*	0" 0.0 cm	0.37	1.21	2.00

Ammonia-N (mg/l)

5 10 16

Time in Days

*Number held constant (120)

Table 2. Ammonia excretion in static isolation by Tilapia zillii.

Fish size (T.L.)	(mg ammonia-N/kg fish-24 hr^{-1}) Exp. 1	Exp. 2	Exp. 3	Exp. 4
50 mm	105.68	79.56	224.43	107.95
75 mm	160.00	82.67	168.00	93.34
100 mm	100.00	91.34	106.67	86.67

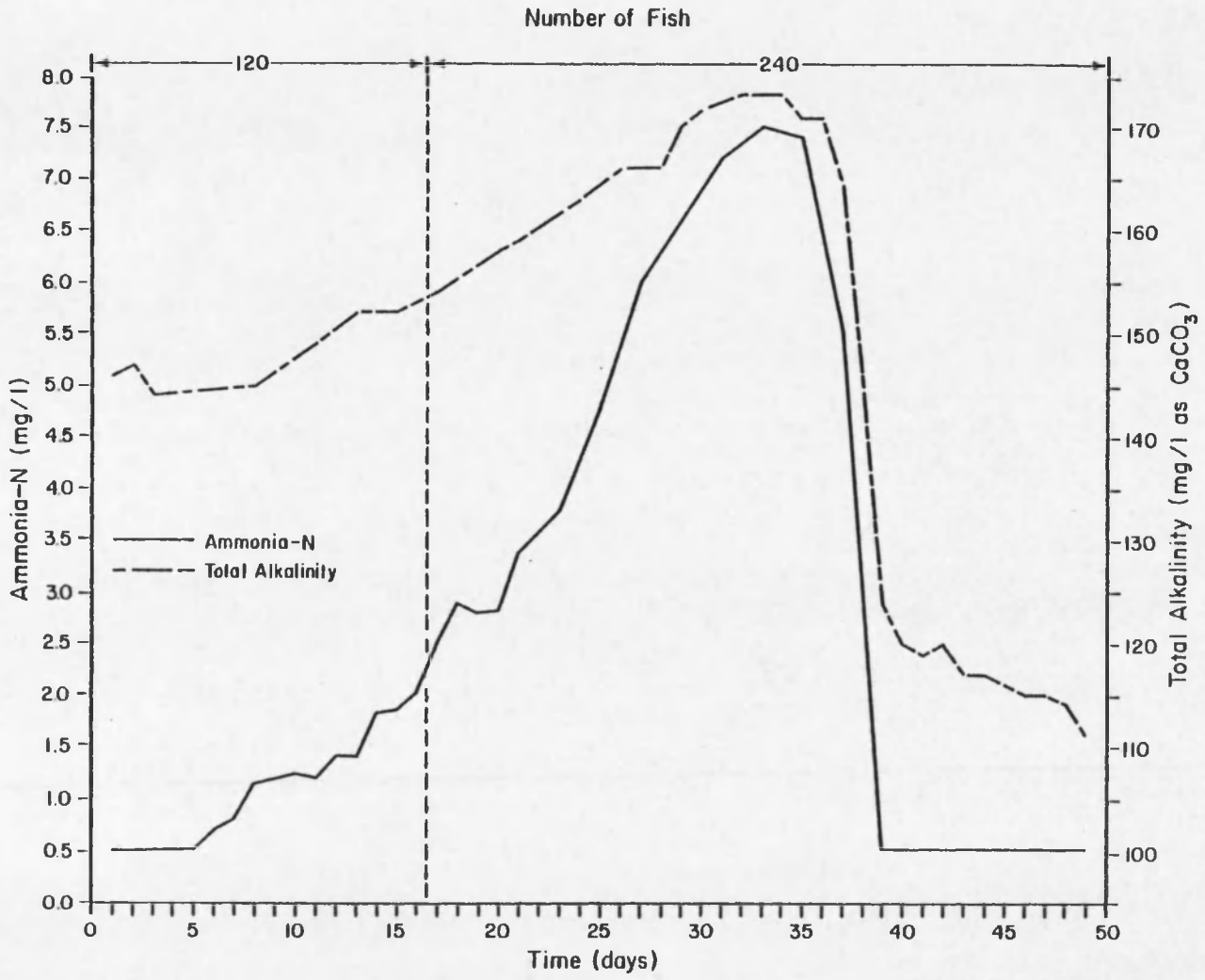


Figure 2. Ammonia-N and alkalinity changes in the no filter system.

occurred when the flat system was loaded with fish above nitrification capacity during days 94 through 97 (Fig. 3).

Nitrate Analysis

Nitrate concentrations increased as nitrification occurred and were not correlated with filter size.

System	Nitrate-N (mg/l) Day 1	Day 67	Food fed in 67 days (g)
No Filter	<1	97	170
Flat	<1	124	225
Cube	<1	257	440
Tall	<1	314	485

Nitrate concentrations at day 67 were nearly proportional to the total amount of food fed to the fish held in each system.

Ammonia Excretion and Nitrification Experiments

All changes in ammonia-N concentrations were nearly linear (Appendices A, B and C).

Experiment I: Ammonia Excretion

The concentration of ammonia-N increased at a faster rate in the tank with 1200 fish than with 600 fish. In 24 hours, the ammonia-N increased 5.25 mg/l in 240 liters of water which equaled 1260 mg ammonia-N excreted by the 1200 fish in the cube tank (Table 3). The 600 fish in the flat tank excreted a total of 559.2 mg ammonia-N in 24 hours, or an increase of 2.33 mg/l in the tank water.

Table 3. Ammonia excretion and nitrification experiments.

System	Amm-N conc. (mg/l) t = 0 hour	t = 24 hour	Change in amm-N conc. (mg/l)	X 240 liters =	Total change in entire system	Notes
EXPERIMENT I: Ammonia Excretion						
Cube	0.55	5.80	5.25	X 240 L =	1260.0 mg	1200 fish fed 50 g Total weight-7.17 kg
Flat	1.07	3.40	2.33	X 240 L =	559.2 mg	600 fish fed 25 g Total weight-3.49 kg
EXPERIMENT II: System Nitrification						
Cube	9.40	0.00*	17.625*	X 240 L =	4230.0 mg	
Flat	10.20	3.00	7.20	X 240 L =	1728.0 mg	
EXPERIMENT III: Tank Nitrification						
Cube	5.90	1.88	4.02	X 240 L =	964.8 mg	
Flat	4.80	2.00	2.80	X 240 L =	672.0 mg	

*Amm-N would have been zero at t = 12.8 hrs.; 17.625 mg/l was the calculated 24-hr. nitrification rate (See Appendix B).

Experiment II: System Nitrification

Ammonia-N steadily decreased when the fish were removed from the systems. In the flat system (0.5 ft^3 filter media) ammonia-N concentration decreased 7.20 mg/l in 240 liters of water. A total of 1728 mg ammonia-N was nitrified in 24 hours (Table 3). In the cube system (1 ft^3 filter media) ammonia-N decreased at a faster rate than in the flat system. In nine hours, 6.80 mg/l or 1632 mg ammonia-N was nitrified. Ammonia-N concentration would have been zero at 12.8 hours (Appendix B). Using these data, 4230 mg ammonia-N per 24 hours was calculated to be the nitrification capacity of this system.

Experiment III: Tank Nitrification

The amount of ammonia nitrified in the tanks without the filters was appreciable. In the flat system tank, ammonia-N decreased 2.80 mg/l which equaled 672 mg nitrified in 24 hours (Table 3). Ammonia-N decreased 4.02 mg/l in the cube system tank in 24 hours. A total of 964.8 mg ammonia-N was nitrified in this tank (Table 3). All conditions were as equal as possible in the two tanks and the difference in nitrification rates was not readily explainable.

The same tanks were used in Experiments I, II and III. To derive a true value of ammonia excretion by T. zillii, the amount nitrified in the tank (Experiment III) must be added to the excreted amount measured (Experiment I). Therefore, the 1200 fish tested in the cube system excreted a total of 2224.8 mg ammonia-N and the 600 fish tested in the flat system excreted 1231.2 mg ammonia-N per day (Table 4).

Table 4. Total ammonia-N excretion by *Tilapia zillii* in 24 hours.

System	Ammonia excretion from Exp. I, Table 3	+	Tank nitrification rate from Exp. III, Table 3	=	Total excretion
CUBE					
(1200 fish fed 50 g)	1260.0 mg		964.8		2224.8 mg
FLAT					
(600 fish fed 25 g)	559.2 mg		672.0		1231.2 mg

Nitrification Capacity of the Cube System

One cubic foot of biofilter media nitrified the ammonia produced by 1200 fish (Fig. 3). When the fish load was increased to 1400, the ammonia-N concentration increased until the fish were not fed (day 107). By the next day (108) the ammonia concentration had decreased appreciably, indicating a strong relationship between feeding and ammonia excretion by *T. zillii*.

Nitrification Capacity of the Flat System

One cubic foot of biofilter media nitrified all the ammonia excreted by 450 fish (Fig. 4). The media volume was reduced to 0.5 ft^3 and 100 fish were added to the system. The ammonia concentration remained very low indicating the capacity of the filter had not been reached. The fish load was increased to 1100 and the ammonia concentration rapidly increased continuously indicating that the nitrification capacity of the system had been exceeded. The fish load was then reduced to 600 and the ammonia concentration remained relatively constant. The ammonia-N excreted by 600 fish approximated the nitrification capacity of the system. The ammonia-N rapidly declined when the fish load was reduced to 340 fish.

Data presented in Figure 3 show a rapid use of alkalinity when the system was not loaded above nitrification capacity. Increasing alkalinity was correlated with increasing ammonia-N when the system was overloaded (days 94 through 97).

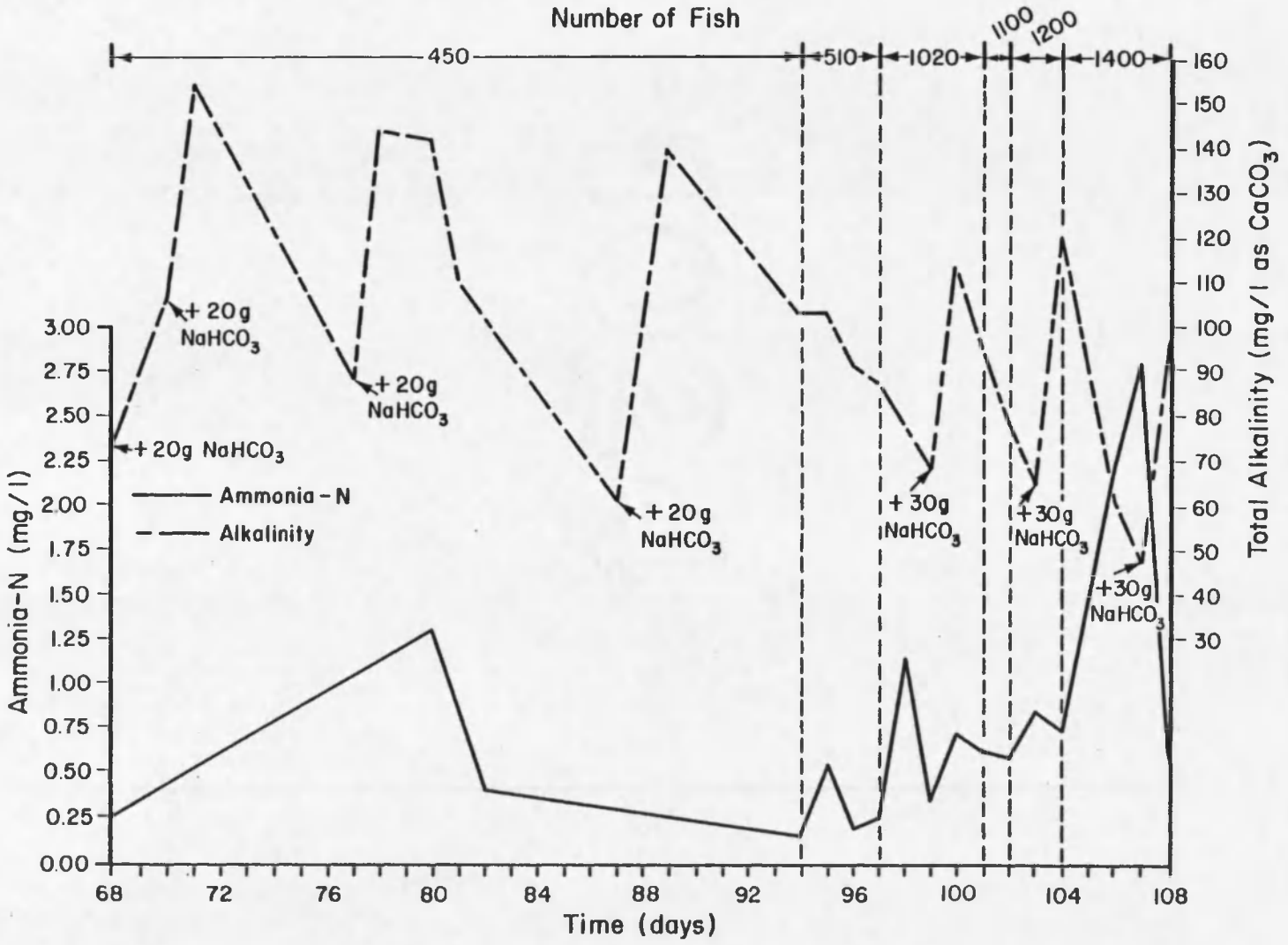


Figure 3. Nitrification and alkalinity changes in the cube system.

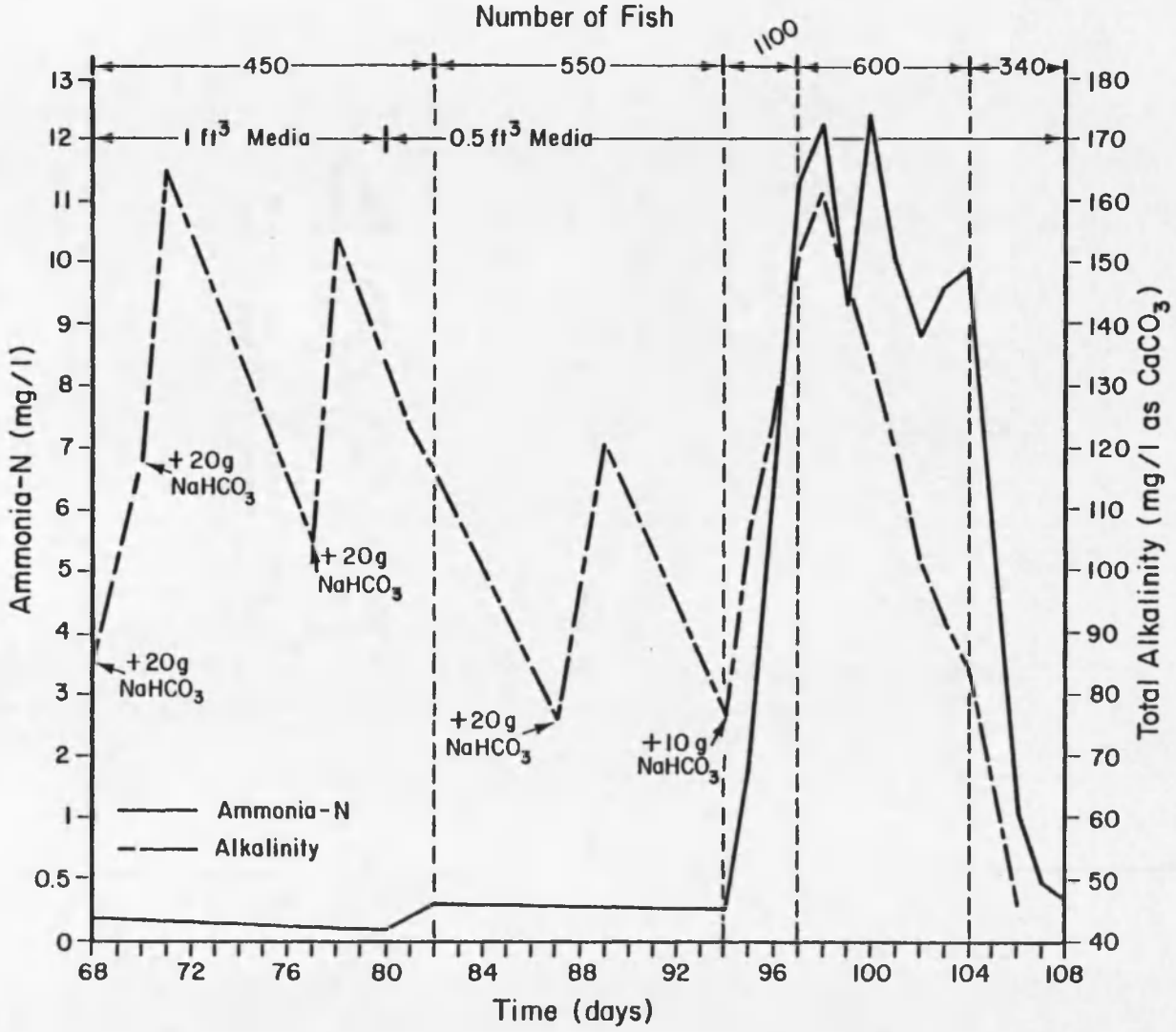


Figure 4. Nitrification and alkalinity changes in the flat system.

DISCUSSION

Evaluation complexities arose because of overlap of data in the series of experiments; therefore, to facilitate discussion, interpretive analyses follow the sequence set forth in the study objectives.

Comparison of Performance of Biofilters with Different Media Depths

Previous investigators (Parisot, 1967; Burrows and Combs, 1968; Chu and Greene, 1967) monitored different biofilters that varied in depth, size and shape. They did not present data that justified using their particular filters. In this study, three different filters with equal volumes and different media depths were used and their performance compared.

In the control tank with no filter and 120 fish, ammonia-N increased to 2.00 mg/l after 16 days. However, in each of the biofiltered systems with loads up to 360 fish per system, ammonia-N remained below 0.18 mg/l (Table 1). Although ammonia-N concentrations varied slightly among the three filtered systems, the data indicated that the three filters nitrified similar amounts of ammonia. This similarity in nitrification rates suggested that the volume rather than depth of the media was the controlling factor in nitrification capacity. Further experiments with 0.5 ft^3 and 1 ft^3 filter media revealed that the larger biofilter nitrified approximately twice the ammonia nitrified in the smaller filter (Table 3).

The flat and cube biofilters were more serviceable than the tall filter because they were easier to clean and required less maintenance. Most suspended particles in the pumped water were filtered out in the uppermost five cm of filter media. The tall filter had the least amount of surface area (64 in^2) of the three filters and consequently required more frequent cleaning.

Estimation of Ammonia Excretion in Static Isolation

The procedure of placing fish in ammonia-free water and measuring the ammonia increase with time was used by several investigators (Brett and Zala, 1975; Fromm, 1963; Gerking, 1955; Savitz, 1969; Wood, 1958). The published works from these studies did not cite specific data on ammonia excretion but apparently gave only average excretion. The present study revealed highly variable ammonia excretion rates by T. zillii (Table 2). Wood (1958, p. 1237) found: "The amount excreted in 24 hours varied greatly between fish of any one species . . . ." Transferring the fish from a holding facility to the test tanks immediately prior to testing in the present study may have interfered with their ammonia excretion as Savitz (1969) hypothesized, but no explanation was obvious for this variability in production. However, substantially higher ammonia excretion rates were obtained from experiments where the fish were not transferred to a new tank prior to testing (Table 3). This method of ammonia production measurement was determined to be unreliable and a more accurate method was desired.

Without sound data on ammonia excretion of T. zillii, measurement of nitrification capacity would be insufficient to calculate carrying

capacity. Nitrification of ammonia to nitrate changes several water quality parameters. This prompted a thorough review of the water quality data which occurred in the recirculating systems in search of a reliable and predictable, although less direct method of ammonia excretion estimation.

Estimation of Ammonia Excretion Using Water Quality Parameters

Several water quality parameters were influenced by the nitrification process. Many of these parameters were monitored daily and an effort to indirectly estimate ammonia excretion was made using these parameters.

Dissolved Oxygen

Each gram of ammonia nitrogen that was nitrified required 4.6 grams of oxygen (Sawyer, Wild and McMahon, 1973). However, many additional processes occurred in the recirculating systems which also required oxygen. Fish respiration accounted for a large and unmeasured use of dissolved oxygen. Other complicating parameters were uncontrolled absorption of oxygen from the atmosphere and aeration of tank water with compressed air. This method of ammonia excretion estimation was beyond the scope of the project and was dropped from further consideration.

Alkalinity Change

Nitrification may lower the pH of the water through the release of nitric acid (HNO_3). The capacity of the water to buffer acidity and resist pH change depends on its concentration of alkalinity. Continual input of acidity (as occurs in a recirculation system) will eventually

reduce the alkalinity in the water to a point where its buffering capacity is low. The pH value of the water will then begin to rapidly decrease.

Low pH is undesirable for many reasons in a nitrification system. The nitrifying bacteria have certain optimal pH requirements. Breed, Murray and Smith (1957) stated this range for Nitrosomonas monocella is 8.0 to 9.0. Sawyer, Wild and McMahon (1973) demonstrated that maximum nitrification occurred at a pH of 8.5 $\pm$ 0.3. Additionally, this pH range is within the normally accepted range for fish cultural purposes.

Lower pH may be beneficial in certain instances. A low pH may be considered desirable due to its effect of lowering the toxicity of a given ammonia solution, through reducing the percentage of the toxic un-ionized form, NH_3 (Trussell, 1972).

The process of nitrification uses alkalinity from the water according to the following equations (Sawyer, Wild and McMahon, 1973):

A) $2\ NH_4HCO_3 + 4\ O_2 \longrightarrow 2\ HNO_3 + 4\ H_2O + 2\ CO_2$

B) $2\ HNO_3 + Ca(HCO_3)_2 \longrightarrow Ca(NO_3)_2 + 2\ CO_2 + 2\ H_2O$

(A+B) $2\ NH_4HCO_3 + 4\ O_2 + Ca(HCO_3)_2 \longrightarrow Ca(NO_3)_2 + 4\ CO_2 + 6\ H_2O$

Therefore, one ammonium ion (NH_4^+) uses two bicarbonate ions (HCO_3^-). In terms of $CaCO_3$, each mg of ammonium nitrified to nitrate uses 7.14 mg total alkalinity. Since the main source of alkalinity in each recirculating system was added $NaHCO_3$, it was theorized that an estimation of ammonia excretion was possible using the alkalinity data presented earlier.

The most appropriate data existed between day 35 and day 39 in the no filter system, where a bacterial bloom apparently occurred very rapidly and a large amount of ammonia was quickly nitrified (Fig. 2). The ammonia decrease between days 35 and 37 was 1.88 mg/l. Stoichiometrically, 13.4 mg/l of alkalinity as $CaCO_3$ should have been used; however, a measured alkalinity reduction of only 6 mg/l occurred. Additionally, the ammonia nitrified from day 35 to 39 was 7.4 mg/l which should have utilized 52.8 mg/l alkalinity as $CaCO_3$. Again the measured decrease in alkalinity was lower than that predicted, only 47 mg/l. It was evident that an input of alkalinity occurred in the system.

Several sources of alkalinity are plausible. Input of alkalinity would occur from the process of denitrification if it occurred in the systems (Jeris and Owens, 1975). However, denitrification is generally an anaerobic process requiring an organic source of carbon, probably not available in sufficient quantity in these systems.

Fish excrete appreciable amounts of urea in addition to ammonia (Brett and Zala, 1975; Fromm, 1963, 1970; Wood, 1958) which could be hydrolyzed to two ammonia molecules and one carbon dioxide per molecule of urea. This CO_2 in addition to respired CO_2 may have contributed to the alkalinity (Stanier, Doudoroff and Adelberg, 1964). The ammonia released through the hydrolysis reaction would have resulted in an increase in alkalinity. An input of ammonia greater than the nitrifying capacity of the system could lead to the permanent formation of ammonium hydroxide and/or ammonium bicarbonate as illustrated earlier. These ammonium compounds may lead to an increase in hydroxide or bicarbonate alkalinity.

This concept is supported by data presented in Figure 2. The alkalinity increased from about 145 mg/l at day 1 to 174 mg/l at day 33, coinciding with increasing ambient ammonia. Additional supporting data is presented in Figure 4 (days 94 through 97). In systems with negligible nitrifying capacity, Konikoff (1975) reported increasing alkalinity with increasing ammonia content while testing nitrite toxicity to channel catfish. Considering the unpredictability of these sources of alkalinity, the data suggested that this method of ammonia excretion estimation also was not reliable.

Nitrate Back-calculation

In a mature aerobic nitrification system, all excreted ammonia should have been nitrified to nitrate. Consequently the amount of ammonia excreted by the fish should be calculable through nitrate analysis of the water and back-calculating to ammonia. However, 20 to 30 percent of the total nitrogen excreted by fish is excreted as urea (Brett and Zala, 1975; Fromm, 1963; Wood, 1958). This urea was probably hydrolyzed to ammonia and carbon dioxide in the biofilter by several genera of bacteria capable of producing the enzyme urease (Breed, Murray and Smith, 1957). Additionally, all of the various nitrogenous waste products excreted by fish may undergo ammonification as part of the naturally occurring nitrogen cycle (Sawyer and McCarty, 1967; Stanier, et al., 1964). Therefore, all nitrogenous waste products of fish will exist, at some point in time, as ammonia.

This concept is applicable to 100 percent recycle systems since the excretory products remain in the system with small amounts of

nitrogen removed through backflushing. Cooke (1959) found the nitrifying bio-film in sewage treatment plant trickling filters to consist of only one percent nitrogen.

Back-calculation from nitrate to ammonia will measure all ammonia nitrified by the biofilter. Since all of the nitrogenous waste products excreted by fish may undergo ammonification, nitrate back-calculation will not lead to an accurate estimation of ammonia excretion by fish. The nitrate to ammonia back-calculation procedure was discarded; however, a closer look at the nitrate analysis of the water in the systems revealed a direct correlation between food fed and nitrate concentrations. The concept of ammonification of all nitrogenous fish waste products and their origin was explored further.

Ammonia Production via Nitrogen Input

Fish probably can not manufacture elemental nitrogen and assuming a negligible amount of nitrogen-fixing algae exist in the system, all the nitrogen within the system must be derived from the proteinaceous component of the food fed to the fish. Brett and Zala (1975) illustrated a direct correlation between food fed and the timing of ammonia excretion in sockeye salmon (*Oncoryhyncus nerka*).

Some of the nitrogen fed into the system will be assimilated into bacterial cell growth. With time the bacterial population will stabilize at some level and the assimilation of nitrogen will essentially balance the nitrogen yielded from dead cells.

In a production facility, substantial amounts of nitrogen will be assimilated into fish growth and will not contribute to the overall

ammonia load on the biofilter. However, with only a maintenance diet and negligible fish growth, it appeared that all nitrogen fed into the system as food would exert an eventual ammonia load upon the system. Therefore, under these conditions of negligible growth, a method of predicting ammonia production and nitrification capacity could be tested.

This "Ammonia Potential" method (AP) was investigated and supported further in this work. The mechanics of predicting the AP of a given amount of food are depicted in Appendix E. To test the AP, nitrification in the tanks and filter must be taken into account.

Appreciable nitrification occurred in the fish holding tanks in addition to that which occurred in the biofilters (Table 3). Stone, Parker and Cotteral (1975) concluded that nitrifying bacteria needed to be attached to aerobic surface area in order to nitrify ammonia present in sewage lagoons. This information combined with preliminary tests in this study indicated that the vast majority of the nitrification which occurred in the fish tanks was a result of nitrifers attached to the walls and floors of the tanks and not suspended nitrifers. The cube and flat systems investigated herein consisted of mature biofilters and tanks.

Ammonia Potential and Nitrification Capacity in the Cube System

When 1400 73 mm fish were fed 60 g of food per day, the nitrification capacity of the system was apparently surpassed since the ammonia concentration steadily increased (Fig. 3). The calculated nitrification capacity of the cube system was 4230 mg ammonia-N per day. However, the AP of the 60 g of food fed to the fish each day was only 3916.1 mg ammonia-N. This suggested that the nitrification capacity of the system

had not been surpassed. An excess or reserve nitrification capacity of 313.9 mg ammonia-N per day existed in the cube system. The system should have been able to hold fish sufficient to consume 64.81 g of food per day or an AP of 4230 mg.

Nitrifying bacterial populations in biofilters probably adjust to the nutrient supply. Hypothetically, an increased nutrient input will lead to an increased bacterial population size provided sufficient aerobic surface area exists to attach upon. The bacterial population had adjusted to the ammonia input from 1200 fish. When the fish load was suddenly increased to 1400, the bacterial population started to increase and adjust to the larger nutrient inflow. However, the ammonia input from 1400 fish was instantaneously greater than the present population's nitrification capacity, thus the ammonia concentration started increasing. The response time of the bacterial population accounted for the ammonia increase. The fish were not fed on the third day of the experiment (day 107) and in 24 hours the ammonia-N had decreased dramatically which indicated a strong relationship between feeding and ammonia excretion in T. zillii and also indicated a high nitrification capacity in the system. If the system had operated for more than four days, the ammonia-N concentration should have eventually decreased.

Fourteen hundred fish in 240 liters of water (5.8 fish per liter) resulted in detrimental social interactions among the tilapia. Severe fin-nipping and occasional mutilation required premature termination of the experiment after only four days. The problem was alleviated by reducing the fish density to five fish per liter (1200 fish), where no detrimental social interactions were noted.

Ammonia Potential and Nitrification Capacity in the Flat System

Eleven hundred 73 mm T. zillii fed 50 g per day far exceeded the nitrifying capacity of the 0.5 ft^3 biofilter in the flat system (Fig. 4). The concentration of ammonia-N and alkalinity increased sharply. Measured system nitrification capacity was 1728 mg ammonia-N per day. The AP of 50 g food was 3263.4 mg ammonia-N, which far exceeded the theoretical limit of the system.

Figure 4 indicates that the flat system was near its capacity with 600 fish fed 25 g per day. A high ambient ammonia concentration (9.0 to 12.4 mg/l) very slowly and erratically decreased while the alkalinity steadily decreased. This indicated a maximum rate of nitrification was occurring in this system. The nitrification capacity of this system was 1728 mg ammonia-N per day and the AP of 25 g of food was 1631.7 mg ammonia. Consequently, a reserve capacity of only 96.3 mg ammonia per day existed (the AP of 1.48 g of food). This small reserve capacity explained the slowly decreasing ambient ammonia in this system. The flat system was theoretically able to nitrify the AP of 26.48 g food, or roughly one half the capacity of the cube system.

Additional Support of the Ammonia Potential Method of Ammonia Production

The concept of the Ammonia Potential method is additionally substantiated from other information presented in Tables 3 and 4. Ammonia excretion is approximately 60 to 70 percent of the total nitrogen excreted by fish. Therefore, the ammonia-N excreted and measured in Experiments II and III (Table 3) should be approximately 70 percent of the total nitrogen excreted. With negligible growth, 70 percent of the ammonia

potential of the food fed to these fish should have been excreted as ammonia-N. In the cube tank, 1200 fish were fed 50 g food per day before the experiment. The AP of 50 g of food was 3263.4 mg ammonia-N. Measured excreted ammonia in 24 hours was 2224.8 mg or 68.2 percent of the total expected nitrogen excreted.

Additionally, 600 fish were fed 25 g of food per day in the flat system and the AP of the food was 1631.7 mg ammonia-N. Measured ammonia excretion was 1231.2 mg. Therefore, 75.5 percent of the theoretical nitrogen excreted by these fish was excreted as ammonia. These percentages are very similar to results from other studies and substantiate the concept of the AP method of ammonia excretion.

The major drawback in recirculating fish holding facilities has been the lack of predictability of ammonia excretion of fish and the nitrification capacity of biofilters. Without this information, estimation of carrying capacity is not possible.

An effective and reliable method of predicting ammonia excretion or production by fish has been presented and supported with experimental data. The Ammonia Potential method of ammonia production estimation states that ammonia production is wholly dependent upon the amount of food fed to the fish within a system, the percent nitrogen of the food and the conversion ratio maintained in the system.

The nitrification capacity of fish holding facilities was shown to be relatively extrapolatable. A nitrification ratio (mg amm-N/ft^2-day^{-1}) is presented in Table 5. This information is useful for designing larger systems, however, consideration must be given to detrimental social interactions among fish at extremely high densities. Additionally,

Table 5. System comparison.

Parameter	Cube (1 ft^3 media)	Flat (0.5 ft^3 media)
Total nitrifying area of system	205 ft^2	111.5 ft^2
% Area of media	91.2%	83.9%
% Area of tank	8.8%	16.1%
% Nitrifying capacity of media	77.2%	61.1%
% Nitrifying capacity of tank	22.8%	38.9%
System Nitrification Capacity:		
- As nitrogen input (g/day)	3.285	1.342
- As food input (31.68% protein-g/day)	64.81	26.48
- As ammonia (mg/day)	4230	1728
Nitrification Ratio (mg Amm-N/ft^2/day)	20.63	15.50
Average Ratio	18.065	

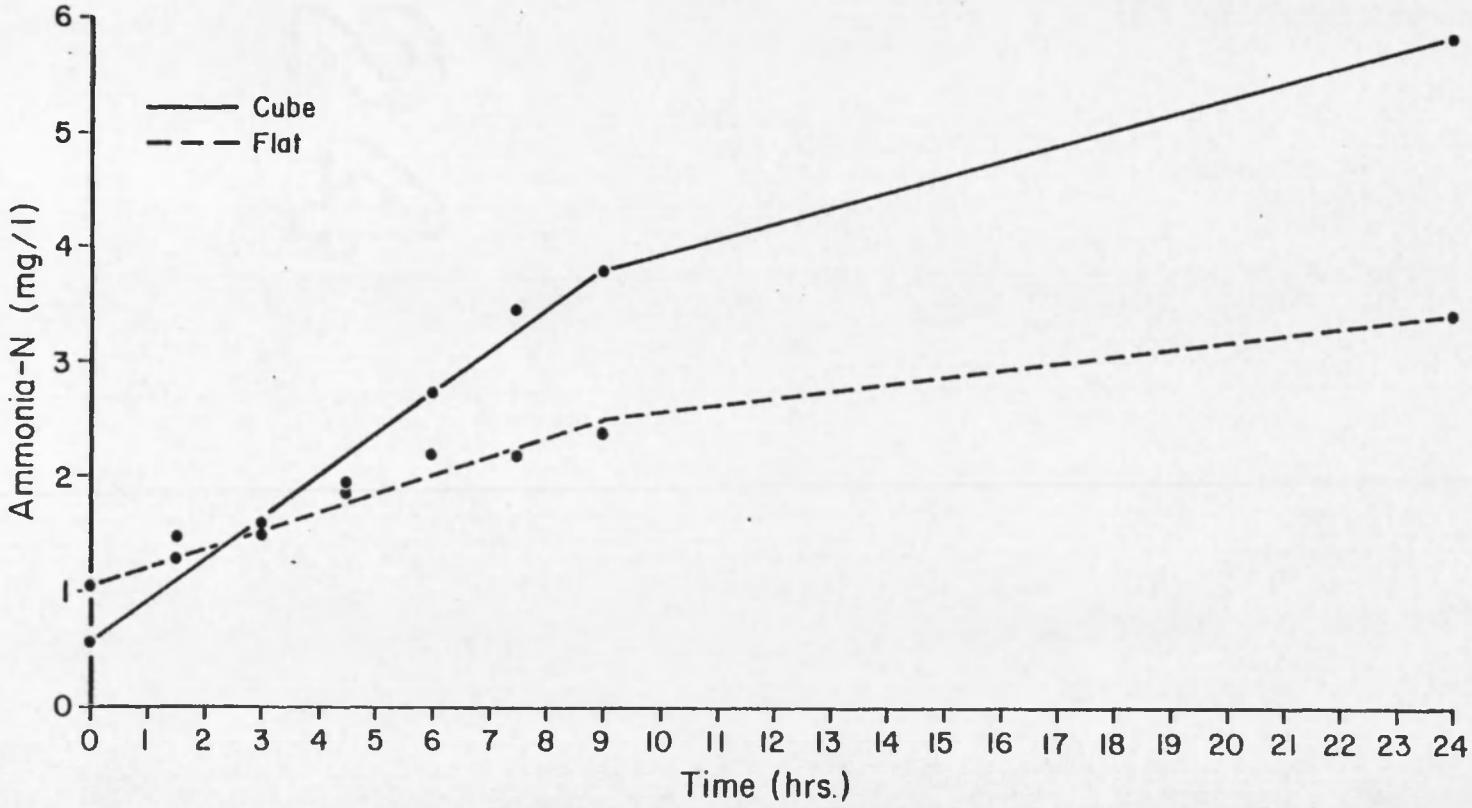
extrapolation of these data to other systems should be done cautiously because of the difficulty in duplicating the chemical and biological conditions that existed in this study.

CONCLUSIONS

1. Volume of filter media rather than depth is the most important factor controlling nitrification capacity of biofilters.
2. Ammonia excretion of fish can be predicted and estimated using the Ammonia Potential method. The AP method states that ammonia production is wholly dependent upon the amount of food fed to the fish within a system, the percent nitrogen of the food and the conversion ratio maintained in the system.
3. An average nitrification ratio of 18.065 mg ammonia-N/ft^2/day was derived from experimental data.
4. Large scale recirculating fish holding facilities can be designed using the data presented.
5. Appreciable nitrification occurs on the submerged tank walls. Experiments confirmed that nitrifying organisms are attached to the tank walls as well as the media and a very negligible amount of nitrification occurs from suspended organisms.
6. When a system is overloaded, alkalinity will increase along with ammonia-N concentration.
7. Consideration must be given to social interactions among fish at high loading densities.

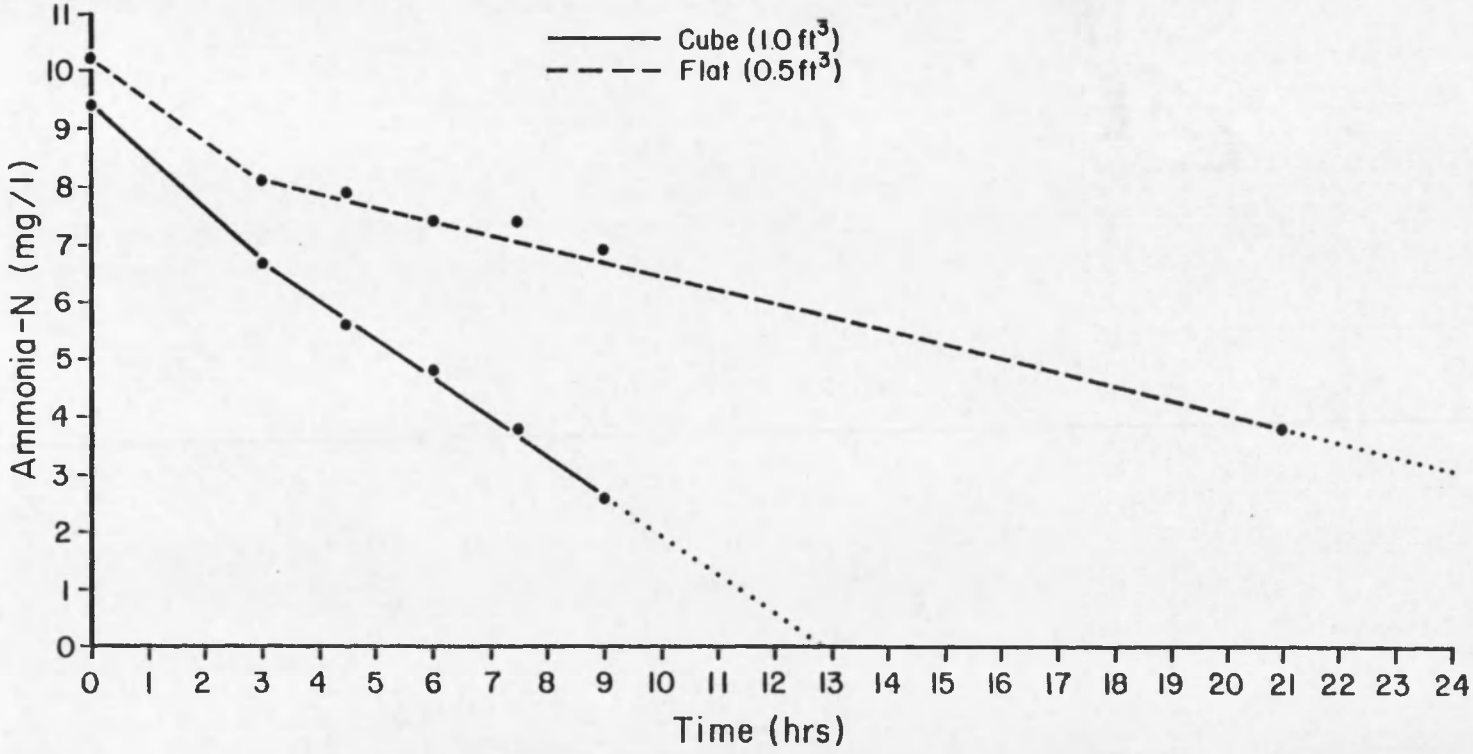
APPENDIX A

EXPERIMENT I. AMMONIA EXCRETION


Cube
Flat
Ammonia-N (mg/l)
Time (hrs.)
0
1
2
3
4
5
6
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24

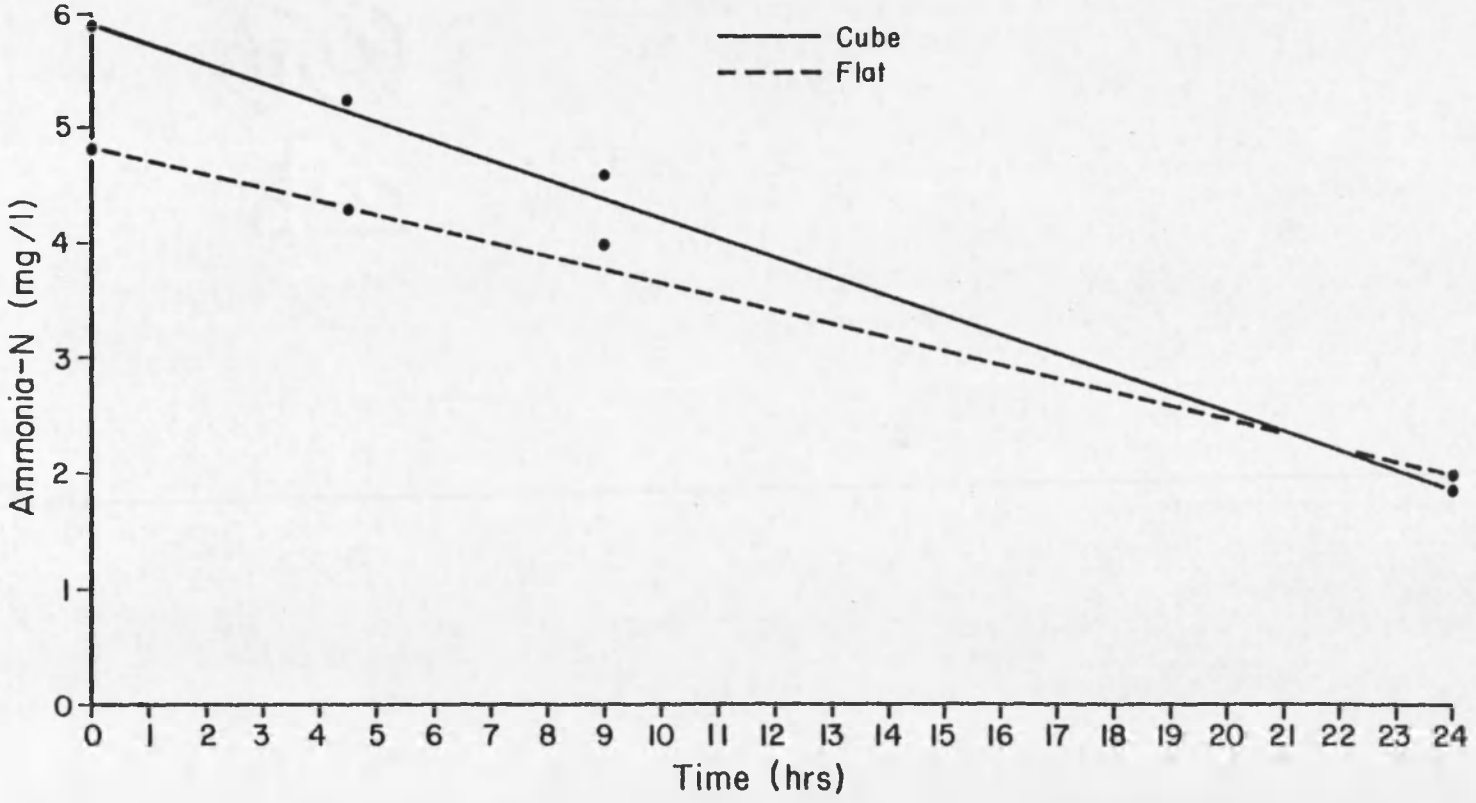
APPENDIX B

EXPERIMENT II. SYSTEM NITRIFICATION


Cube (1.0 ft³)
Flat (0.5 ft³)
Ammonia-N (mg/l)
Time (hrs)
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
0 1 2 3 4 5 6 7 8 9 10 11

APPENDIX C

EXPERIMENT III. TANK NITRIFICATION


Cube
Flat
Ammonia-N (mg/l)
Time (hrs)
0
1
2
3
4
5
6
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24

APPENDIX D

DETERMINATION OF SURFACE AREA OF MEDIA

Average diameter of pea gravel was estimated at 1/4 inch (0.6 cm). The void space was determined by filling a 1000 ml graduate cylinder with pea gravel and adding water. The cylinder was then inverted several times and rock and water were removed to precisely the 1000 ml mark. The remaining water was then poured into another graduated cylinder and measured. The void space was equal to the amount of water in the second cylinder. Measured water was 350 ml or 35 percent void space.

Steps:

1. Assume spherical shape of "peas".
2. Volume = $4/3\ \pi R^3$

$$= \frac{(4)(3.1416)\ [(0.125\ \text{in})^3]}{3}$$

$$= 0.0081812\ \text{in}^3$$

3. $1\ \text{ft}^3 = 1728\ \text{in}^3$

Assume no void space, then;

$$\frac{1728\ \text{in}^3}{0.0081812\ \text{in}^3} = 211{,}215.95\ \text{"peas"}/\text{ft}^3 \text{ gravel at zero void space}$$

4. But a 35 percent void space existed;

$$211{,}215.95\ \text{"peas"}/\text{ft}^3 \text{ gravel at zero void space}$$
$$\underline{\quad \times\ 0.65}$$
$$137{,}290.36\ \text{"peas"}/\text{ft}^3 \text{ gravel at 35\% void space}$$

5. Determine area of one 1/4" diameter "pea";

$$A = 4\pi R^2$$

$$= (4)(3.1416)\left[(0.125\ \text{in})^2\right]$$

$$= 0.19635\ \text{in}^2/\text{"pea"}$$

6. $137{,}290.36$ "peas"/ft^3 gravel (from 4)
$\times\ 0.19635$ in^2/"pea" (from 5)
$26{,}956.962$ in^2/ft^3 gravel

7. $$\frac{26{,}956.962\ \text{in}^2/\text{ft}^3}{144\ \text{in}^2/\text{ft}^2} = 187.2\ \text{ft}^2/\text{ft}^3$$

APPENDIX E

DETERMINATION OF AMMONIA POTENTIAL

The Ammonia Potential (AP) method of ammonia production by fish assumes that all nitrogen excreted by fish in a 100 percent recycle system will exist as ammonia at some point in time. To calculate the AP, the nitrogen component of the food must be known and related to the ammonium ion (NH_4), the predominant form. This information may be derived from the following formula:

$$AP_{(mg)} = \frac{\text{(Amount food in grams) X (\% Protein) X (16\% Nitrogen) X (18.04 Molec. wt. of } NH_4\text{) X (1000 mg/g)}}{\text{14.01 (Molec. wt. of Nitrogen)}}$$

For the food used in this study the formula was:

$$AP_{(mg)} = \frac{\text{(Amount food in grams) X (0.3168) X (0.16) X (18.04) X (1000 mg/g)}}{14.01}$$

$$AP_{(mg)} = \text{(Amount food in grams) X (65.268488)}$$

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